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Developmental allometry of pulmonary structure and function in the altricial Australian pelican *Pelecanus conspicillatus*

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Summary

Quantitative methods have been used to correlate maximal oxygen uptake with lung development in Australian pelicans. These birds produce the largest altricial neonates and become some of the largest birds capable of flight. During post-hatching growth to adults, body mass increases by two orders of magnitude (from 88 g to 8.8 kg). Oxygen consumption rates were measured at rest and during exposure to cold and during exercise. Then the lungs were quantitatively assessed using morphometric techniques. Allometric relationships between body mass (*M*) and gas exchange parameters (*Y*) were determined and evaluated by examining the exponents of the equation $Y=aM^b$. This intraspecific study was compared to interspecific studies of adult birds reported in the literature. Total lung volume scales similarly in juvenile pelicans (*b*=1.05) as in adult birds (*b*=1.02). However, surface area of the blood–gas barrier greatly increases (*b*=1.25), and its harmonic mean thickness does not significantly change (*b*=0.02), in comparison to exponents from adult birds (*b*=0.86 and 0.07, respectively). As a result, the diffusing capacity of the blood–gas tissue barrier increases much more during development (*b*=1.23) than it does in adult birds of different sizes (*b*=0.79). It increases in parallel to maximal oxygen consumption rate (*b*=1.28), suggesting that the gas exchange system is either limited by lung development or possibly symmorphic. The capacity of the oxygen delivery system is theoretically sufficient for powered flight well in advance of the bird's need to use it.

Key words: bird, lung, juvenile, development, respiration, morphometry, diffusing capacity, symmorphosis.

Introduction

Comparative respiratory and cardiovascular physiology had focussed on the coordinated and adaptive functions of the organ systems in the adult animal. However, the developmental stages of those systems have received much less attention and are sometimes considered to be inferior to the adult ideal. Physiological differences between juveniles and adults in the capacity of the systems can introduce unwanted variability into investigations, so work on juveniles is often avoided. This focus of comparative physiology on adult animals can lead to the assumption that the physiology of earlier developmental stages needs not to be particularly well adapted, but only sufficient to keep the animal alive until it reaches reproductive adulthood. However, younger stages of animals may be under as much selective pressure as older ones, and have organ systems that are as fully functional for their requirements and adapted to the conditions of their environments. Only recently have a few physiologists started to ask about the adaptability of embryonic and juvenile gas transport systems, and specifically about the timing of their appearance and developmental modification (e.g. Spicer and Burggren, 2003).

The concept of symmorphosis is that the maximum functional capacity of parts of a system should be matched; there are no over- or under-designed links in the chains. The hypothesis was named and chiefly developed by Taylor and Weibel (1981) in their consideration of the oxygen cascade in exercising mammals. Their approach was to test the hypothesis by measuring structure and function of each level in mammals and compare them interspecifically with allometric techniques. They demonstrated that the oxygen transport capacities are matched to a large extent at each level from the lung to the mitochondrion, except for the morphometric pulmonary diffusing capacity, which seemed to be somewhat over-endowed in larger species (Taylor et al., 1989). However, physiological pulmonary diffusing capacity was subsequently shown to be well matched to the maximum capacity of the cardiovascular system (Hsia, 1998). Interspecific analyses have also been carried out on pulmonary morphometry in birds (Maina, 1993, 1998; Maina et al., 1989), but never explicitly compared to maximum metabolic rates of the same species.

The symmorphosis paradigm is an ideal that may or may not be realised by natural selection. All studies addressing the question have been directed at adult animals. Whether it is evident in developmental stages is not known. Therefore we have begun to evaluate the changes in transport capacities of the gas exchange apparatus and the cardiovascular system that occur during development in birds. We are unaware of any studies that measure the development of pulmonary diffusing capacity in relation to maximum oxygen uptake rate in paranatal and juvenile birds. The ontogeny of pulmonary structure has been measured in domestic turkeys *Meleagris gallopavo*, including morphometrics of compartment volumes and surface areas (Timmwood et al., 1987), but the blood–gas tissue thickness was not provided, so diffusing capacity cannot be calculated, and there are no data on the metabolic scope of developing turkeys. As part of a larger study on the development of the cardiovascular and respiratory systems in birds of different hatchling maturity, we present data on pulmonary diffusing capacity in relation to maximum aerobic metabolism in the altricial Australian pelican. The Order Pelicaniformes produces the largest altricial hatchlings that grow to become some of the largest extant birds capable of flight. Information on the energetics and respiration of pelican embryos has been published (Pearson et al., 2002), and a fuller morphometric analysis of embryonic and post-hatching lung development is to be published elsewhere. Here we focus on the question of symmorphosis, and use the same allometric approach as Taylor et al. (1989).

Materials and methods

Source of animals

Eggs of the Australian pelican *Pelicanus conspicillatus* Temminck were collected from a breeding colony on an unnamed island in Outer Harbour, Adelaide, South Australia. They were taken to the laboratory and incubated at 35–36°C and 45–55% RH in Bellsouth 100 incubators (Narre Warren, Victoria, Australia), with automatic turning (Pearson et al., 2002). Three birds were measured on the day of hatching, two at 11 days, and two at 21 days post-hatch. These birds were maintained in the laboratory (36°C for hatchlings and 27°C for downy juveniles) and were fed water and fish-based cat food or regurgitated fish from the nests. Five juvenile birds (about 3 months old, with down and incompletely developed contour and flight feathers), and one adult male were collected from the monitored breeding colony, taken to the laboratory and measured immediately. Body mass was measured on an appropriate balance; values were fresh, yolk-free mass for hatchlings, and total live mass for the others.

Respiration

Metabolic rate was determined by open-flow respirometry, following standard techniques (Withers, 1977). Air flow rates through the systems were measured with mass-flow meters or controllers (Sierra Instruments Mass-Trak, CA, USA) and they varied from 350 ml min^{-1} in hatchlings up to 18 l min^{-1} in the largest birds. The air leaving the system was scrubbed with Drierite (W. A. Hammond, Xenia, Ohio, USA), soda lime and Drierite columns, and delivered by a sub-sampling circuit either to a fuel-cell oxygen analyser (David Bishop Instruments 280/0427 Combo, UK), thermostatted at 36°C, or to a solid-state zirconium oxygen analyser (Ametek S-3A/I, Pittsburgh, Pennsylvania, USA). Both analysers were calibrated with pure nitrogen and dry, CO_2-free air. The outputs from these instruments were recorded with A/D converters and recording software (Sable Systems Universal Interface and DATACAN v5.2, Las Vegas, Nevada, USA).

For resting metabolism, birds were placed in darkened, plastic containers of appropriate size, inside a constant temperature cabinet or room at 36°C for hatchlings or 27°C for older birds. Metabolic rate was measured until it dropped to stable values, which occurred after 20–30 min. The resulting minimum values are not basal metabolic rates, because the birds were not fasting, they were growing, and they were measured during the day.

Two approaches were used in attempts to elicit maximum aerobic metabolism, cold exposure and exercise. Neither treatment increased the metabolic rates of hatchlings significantly, because exposure to cold temperatures caused an immediate drop in body temperature and metabolic rate, and the birds were incapable of any kind of energetic movement. Birds from 11 to 21 days old (crèche phase) were also incapable of exercising, but they exhibited strong shivering and metabolic rate increase when exposed to low temperature. Respiration was therefore increased to the highest obtainable value by abruptly lowering ambient temperature to about 5°C in the respirometry chamber and measuring oxygen consumption until it peaked and body temperature (measured *via* a thermocouple about 2 cm in the cloaca) began to fall. This procedure required about 15–30 min.

Larger birds from the field produced visible shivering and large metabolic responses to cold, but did not produce maximum metabolic rate on cold exposure, including trials with 20% oxygen in helium at about 0°C, which greatly increases thermal conductance (Hinds et al., 1993). However, they were capable of running on a treadmill and swimming in a flume, and the resulting values were 1.2–3.9 times higher than the maxima derived from cold exposure. Clear plastic, flow-through masks, taped loosely around the neck, were used during exercise. Exercise was induced by running on a treadmill at speeds up to 1.4 m s^{-1} and by swimming in a flume up to 1 m s^{-1}, all at room temperature. The birds were encouraged to exercise by holding or gently pulling the feathers on the tail. Exercise periods were variable, from 2–10 min. Runs in which the respirometry values were reasonably stable for at least 2 min during continuous activity were considered successful. Values were averaged during stable periods lasting 2–6 min. The values for running and swimming were similar, and without consistent directional bias. In all cases, reported values are averages from the two exercise methods.

Pulmonary morphometry

Lung morphometry was carried out as part of a larger study on the developmental anatomy of embryonic and juvenile pelicans. The birds were killed by rapid asphyxiation with pure carbon dioxide flowing through their chamber or head mask. This technique also facilitated the infusion of fixative into the airways, because the gas was absorbed into the tissues. Lungs were fixed with 2.5% glutaraldehyde in a 0.01 mol l^{-1} phosphate buffer (pH 7.4, 350 mOsm). The birds were placed in a supine position and the fixative was introduced *via* a cannula in the trachea with the reservoir located 20 cm above the sternum. The trachea was ligated below the cannula when the flow of fixative stopped. The lungs were removed from the thorax after 2 h. The fixed lungs were sliced in the plane of the costal grooves from apex to base into 10–20 slices, depending on lung size. Lung volume (V_L), including air and tissue, was determined using the Cavalieri method of point counting on slices from the left and the right lungs (Howard and Reed, 1998). Total lung volume was obtained by summing the volumes of the two lungs.

The right lung was used for transmission electron microscopy. Ten pieces of tissue, 2 mm in diameter, were sampled using the independent area-weighted periodic sampling technique described by Cruz-Orive and Weibel (1981). Lung pieces were stained with osmium, *en bloc* stained with uranyl acetate, dehydrated in an ascending series of alcohol, cleared in propylene oxide and embedded in Durcupan embedding resin (Fluka Chemie AG; Sigma Chemicals, St Louis, MO, USA). Gold to silver sections with sides of about 1.5 mm were cut with a diamond knife with the ultramicrotome set to cut at 100 nm. Sections were collected on copper thin bar 200 square mesh grids and stained with lead citrate prior to viewing in a Joel JEM 1200-EX (Tokyo, Japan) transmission electron microscope at an acceleration voltage of 80 kV.

Ten sections from each bird lung were viewed at 2000× magnification and sampled using the systematic area-weighted quadrats subsampling technique (Muller et al., 1981). Six fields were sampled from each section, giving 60 fields per lung. Images of the sampled fields were imported into CorelDraw 9 (Corel Corporation), a Merz grid was superimposed on the image, and point and intersection counts were carried out.

The pertinent data for the present analysis are the total surface area and the harmonic mean thickness of the blood–gas tissue barrier, where the blood capillaries oppose the air capillaries. The gas exchange surface density of the blood capillaries (S_Vc) was determined by counting intersections that ran from air to blood and calculated using $S_V c=2I/L$, where I is the number of intersections counted and L is the total length of the test system. The surface area of the blood–gas barrier, S_t, was calculated from the reference volume of the exchange tissue (Maina et al., 1989).

The harmonic mean thickness of the blood–gas tissue barrier (τ_{ht},) was calculated as $2/3[n/\Sigma(1/L)]M^{-1}$, where n is the number of measured intercepts, $\Sigma(1/L)$ is the sum of the reciprocal of the intercept lengths and M is the final magnification (Maina et al., 1989). The ratio S_t/τ_{ht}, multiplied by Krogh's coefficient of oxygen diffusion, provides the anatomical (tissue) diffusing capacity of the blood–gas barrier. The value for Krogh's coefficient was assumed to be 4.1×10^{-10} cm^2 s^{-1} $mbar^{-1}$, to be comparable to the study of Maina et al. (1989).

Statistics

Statistics include linear regressions (model 1, least squares) on $\log_{10}$-transformed data, excluding the single adult bird. The units of mass are grams. Regression statistics include coefficients of determination (r^2) and 95% confidence intervals of the slope (CI), calculated in Excel with StatistiXL add-in software (statistixl.com), as were tests for relationships among residuals and for outlier points. Differences in regression slopes and elevations were tested with analysis of covariance (ANCOVA) according to Zar (1998). Where slopes were significantly different, the regions of significantly different elevations were identified with the Johnson–Neyman technique (White, 2003). Means of other values are given with 95% confidence intervals.

Results

Respiration

Four newly hatched pelicans (body mass 88–121 g), had metabolic rates of 1.5±0.3 ml min^{-1} (mean ± 95% CI) at 36°C, near the mean of 1.18 ml min^{-1} for 18 hatchlings in a previous study of Australian pelicans (Pearson et al., 2002). It was not possible to increase metabolic rate by exposure to cold, because the hatchlings were naked and had not developed any thermoregulatory ability. They were helpless and uncoordinated and thus incapable of exercise. Therefore, metabolic rates measured on the first day of hatching were used for both resting and maximal values (Fig. 1).

Resting and maximal metabolic rates diverged in birds aged 11 days or older. The increases in resting metabolic rate were not linear on a double log plot, because 11- and 21-day-old birds had relatively high values. Maximal rate, however, was linear over two orders of magnitude body mass, with a slope of 1.28±0.07 CI (Fig. 1). At the maximum body mass of 8834 g, the regression equations indicate a resting metabolic rate of 144 ml min^{-1} and a maximum metabolic rate of 436 ml min^{-1}. This indicates a total metabolic scope of 292 ml min^{-1} or a factorial scope of 3.0. There was no apparent difference in maximal rate of the adult and the juveniles of similar mass.

Pulmonary morphometry

Lung volume data were available for 13 post-hatch pelicans (Fig. 2). There was a strong, linear relationship between log-transformed lung volume and body mass, and the slope of the relationship (1.05±0.06 CI) was not significantly greater than 1.0. The value from the adult bird was not exceptional.

The surface area of the blood–gas barrier increased allometrically with body size with a slope of 1.25±0.15 CI

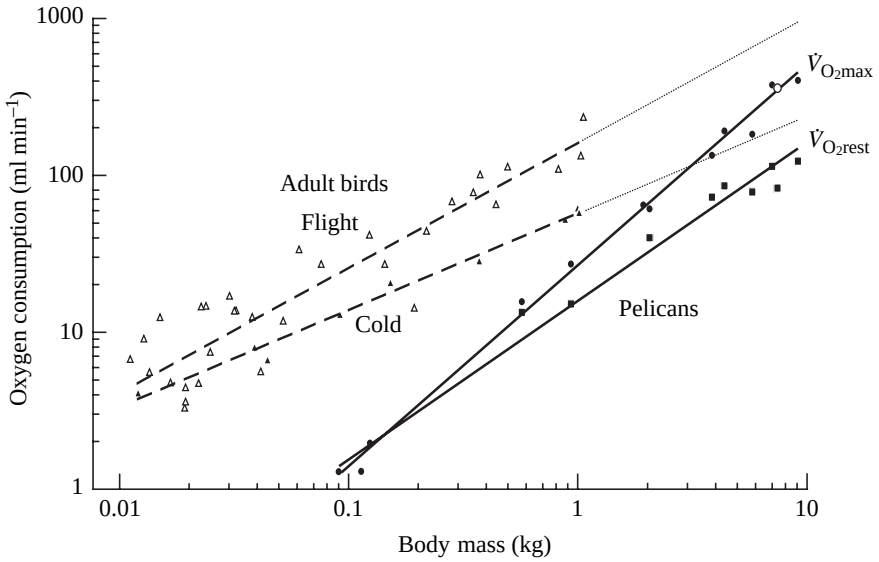

Fig. 1. Allometry of resting ($\dot{V}_{O_2rest}$) and maximum ($\dot{V}_{O_2max}$) rate of oxygen consumption of 12 Australian pelicans during post-hatching growth (filled circles), excluding one adult bird (open circle). Regression equations: $\dot{V}_{O_2rest}=0.0149M^{1.01}$ ($r^2=0.98$); $\dot{V}_{O_2max}=0.0040M^{1.28}$ ($r^2=0.99$). Data from 33 species of adult birds during flight (open triangles) are from Norberg (1996), converting W to ml min^{-1} assuming 20 J ml^{-1}, and averaging multiple data from the same species; $\dot{V}_{O_2max}=0.641M^{0.80}$ ($r^2=0.86$). Data from nine species of adult birds exposed to cold (filled triangles) are from Hinds et al. (1993); $\dot{V}_{O_2max}=0.787M^{0.62}$ ($r^2=0.99$). Dotted lines are extrapolations.

(Fig. 3). There appeared to be further increase in surface area in the single adult, and the difference was significant (Outlier test: Studentised residual 2.66 > critical value 2.23 at α=0.05).

Harmonic mean thickness of the blood–gas tissue barrier did not change significantly in the juvenile pelicans during development, the slope being 0.02±0.08 CI (Fig. 4). However, the value from the adult was significantly lower than the other juveniles of similar body mass (Outlier test: Studentised residual 4.58 > critical value 2.23 at α=0.05).

The anatomical diffusing capacity of the blood–gas tissue barrier was significantly allometric, with a slope of 1.23±0.20 CI. Because of the dramatic decrease in barrier thickness, diffusing capacity in the adult was significantly higher than values for the other juveniles of similar mass (Outlier test: Studentised residual 3.78 > critical value 2.23 at α=0.05).

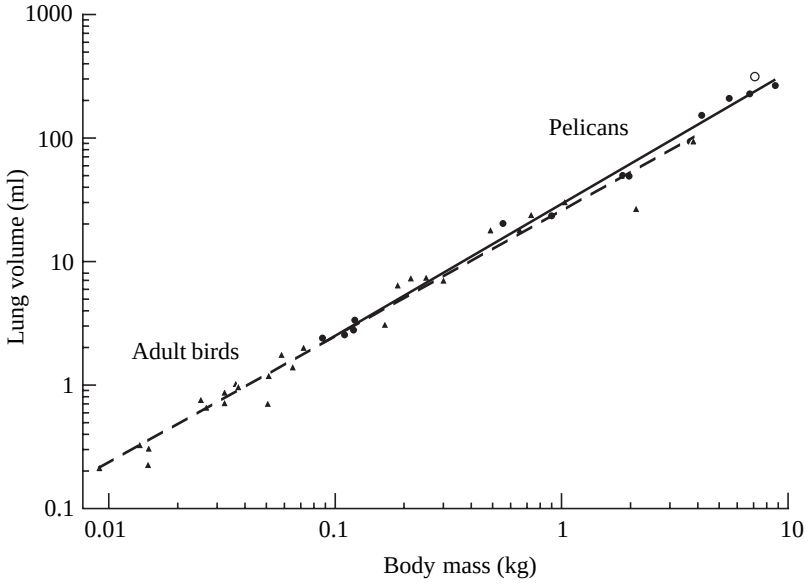

Fig. 2. Allometry of lung volume (V_L) of 13 Australian pelicans during post-hatching growth (filled circles), excluding one adult (open circle). Data from 26 species of adult birds (triangles) are from Maina et al. (1989). Regression equations: pelicans $V_L=0.018M^{1.07}$ ($r^2=0.99$); adult birds $V_L=0.022M^{1.02}$ ($r^2=0.98$).

Discussion

This study demonstrates that maximum metabolic rate and diffusing capacity of the blood–gas barrier of highly altricial pelicans increase greatly during post-hatching development, as the animals progress from helpless neonate to a state capable of flight. The scaling exponents (slopes) are greater than 1 (Figs 1 and 5). To illustrate these increases in terms of mass-specific values for an 88 g hatchling and an 8.8 kg juvenile, maximum metabolic rate increases by 357% and diffusing capacity by 291%.

The maximum metabolic rates we recorded during exercise in the 8.8 kg juvenile (402 ml min^{-1}) and the 7.2 kg adult (357 ml min^{-1}) were apparently sufficient to power flight. The cost of flight for such a large bird has not been measured, but it is estimated that the largest flying bird would weigh about 12 kg when the power required for flight is equal to the available power (Norberg, 1996). This point is 115 W, or 345 ml O_2 min^{-1}, assuming 20 J ml^{-1}. Although the cardio-pulmonary system may have the capacity to deliver enough oxygen for powered flight, it is unlikely that the oldest juveniles could sustain flight. Indeed, older juveniles (partial primary feather development) that were observed at the colony were only capable of short gliding flight, and then only after long run-ups on open ground. We judge that the juveniles we sampled had never flown sustainably. It appears that the capacity of the oxygen delivery system is developed in advance of their need to use it.

Diffusing capacity of the blood–gas barrier increases throughout most of post-hatch development by an increase of gas-exchange surface area (Fig. 3), rather than decreased blood-gas barrier thickness (Fig. 4). However, thickness decreases significantly, and surface area further increases, in the adult. This bird is the only one in this study with a barrier thickness in the range for other adult birds (Fig. 4). Although only one adult was available to us, it was a statistically significant outlier that leads to the conclusion that the gas-exchange barrier decreases in thickness and increases in area during maturation of sedentary juveniles to volant adults, although body mass and lung volume do not change.

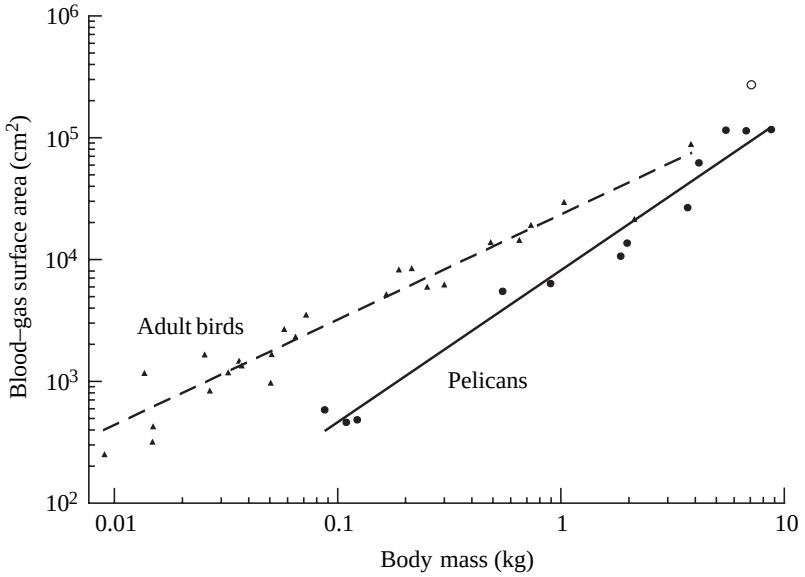

Fig. 3. Allometry of the surface area of the blood–gas tissue barrier (S_t) of 12 Australian pelicans during post-hatching growth (filled circles), excluding one adult bird (open circle). Data from 26 species of adult birds (triangles) are from Maina et al. (1989). Regression equations: pelicans $S_t=1.41M^{1.25}$ ($r^2=0.97$); adult birds $S_t=60.2M^{0.86}$ ($r^2=0.94$).

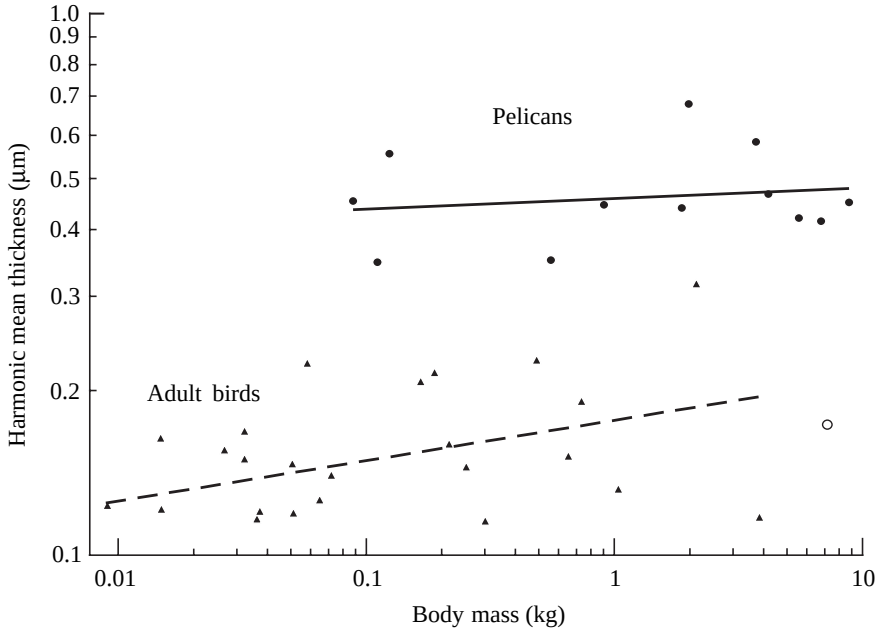

Fig. 4. Allometry of harmonic mean thickness of the blood-gas tissue barrier (τ_{ht}) of 12 Australian pelicans during post-hatching growth (filled circles), excluding one adult bird (open circle). Data from 26 species of adult birds (triangles) are from Maina et al. (1989). Regression equations: pelicans $\tau_{ht}=0.400M^{0.02}$ ($r^2=0.03$); adult birds $\tau_{ht}=0.106M^{0.07}$ ($r^2=0.18$).

It is instructive to compare the allometries of growing pelicans and adults of other species to distinguish the effects of development and inherent scaling relationships of the products of development. Fig. 1 compares maximum metabolic rate in pelicans with 33 species of flying birds (Norberg, 1996) and nine species exposed to cold (Hinds et al., 1993). Analysis of covariance reveals significant differences in slope between growing pelicans and both studies of adult birds ($P<0.0001$). While the slope for pelicans is greater than unity, it is significantly less in adult species, indicating that the mass-specific maximum rate decreases in larger species. The Johnson–Neyman technique shows that the data for developing pelicans were significantly lower than for cold-exposed adult birds at body masses less than 2.2 kg, that is, the pelicans up to and including 21 days old. The technique also showed that values from larger pelicans, including the adult, lie between extrapolated regressions for cold-exposed and flying birds, but are significantly lower than flying birds.

Fig. 5 shows a similar comparison of diffusing capacity of the blood–gas barrier between pelicans and 26 species of adult birds from a morphometric study by Maina et al. (1989). (There are slight differences between our regression equations for their data, possibly because of rounding errors in their tabled values.) Once again, ANCOVA reveals significantly different slopes ($P=0.0003$) and the Johnson–Neyman technique identifies the pelican data as significantly lower at all body masses.

The striking similarity of allometric slopes of maximum metabolic rate ($b=1.28$) and diffusing capacity of the blood–gas barrier ($b=1.23$) is indicative of a parallel development of these variables during post-hatching growth of pelicans (cf. Figs 1 and 5). These results

suggest either that pulmonary diffusion limits maximum metabolic rate or that the oxygen delivery system is to some extent symmorphic. If the cardiovascular system or processes at the cellular level limited oxygen uptake, then one would expect lower slopes for metabolic rate than for diffusing capacity. A similar congruence is apparent in adult birds in which the slope for metabolic rate during flapping flight (b=0.80) is almost identical to that of pulmonary tissue diffusion capacity (b=0.79) (cf. Figs 1 and 5). The allometry of maximum metabolic rate in birds is not known.

Our conclusion that blood–gas barrier diffusing capacity is linked to maximum oxygen uptake should be viewed with some caution, however, for three reasons. First, although similarity in allometric slopes is consistent with symmorphosis, allometry cannot prove it, because it does not explicitly measure capacity matching in the oxygen cascade, and other explanations are possible (Dudley and Gans, 1991). Secondly, the 95% confidence interval of the slope of diffusing capacity is broad (±0.20) in this study. Third, there is no relationship between the residuals of diffusing capacity and maximum metabolic rate in pelicans (r^2=0.009). If pulmonary diffusion limited oxygen uptake, one would expect birds with higher diffusing capacity to have higher maximum metabolic rate. Until there are other studies on the development of the oxygen delivery system in birds, we can only tentatively conclude that the data from Australian pelicans are consistent with the concept of symmorphosis during post-hatching development in birds.

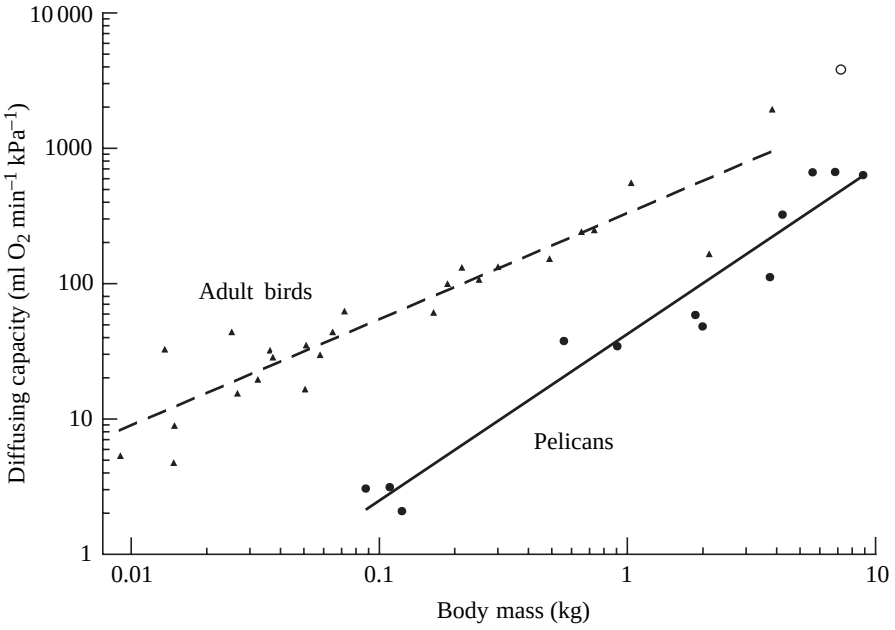

Fig. 5. Allometry of the oxygen diffusing capacity of the blood–gas tissue barrier (D_{tO_2}) of 12 Australian pelicans during post-hatching growth (filled circles), excluding one adult bird (open circle). Data from 26 species of adult birds (triangles) are from Maina et al. (1989). Regression equations: pelicans $D_{tO_2}=0.0087M^{1.23}$ (r^2=0.95); adult birds $D_{tO_2}=1.44M^{0.79}$ (r^2=0.87).

Critique

This study employed similar morphometric techniques as Maina et al. (1989), who presented a detailed critique of the methodology. They used a quadratic lattice grid, while we used the Merz grid, but a comparison of 100 random samples measured with both techniques from all pelicans revealed a mean difference of less than 1%, which was not significant (paired t-test; P=0.85). They evaluated total morphometric diffusion capacity, including individual diffusing capacities of the three barriers to diffusion, namely, the blood–gas tissue barrier, the plasma barrier and the erythrocyte. We evaluated only the tissue barrier, because it is unlikely that measurements made to determine diffusing capacity of the plasma are representative of the functional state. It is questionable whether the spatial arrangement of intravascular components, particularly the relationship of the erythrocytes to the capillary wall and to each other, is a faithful representation of the dynamic situation found *in vivo* (Maina, 1993). Our study evaluates pulmonary diffusion capacity morphometrically, not physiologically. In mammals, there are often considerable differences between the results of the two techniques (Crapo and Crapo, 1983; Weibel, 1984), but in birds, the two estimates overlap (Powell and Scheid, 1997). Regardless of small errors in measurement or differences between morphological or physiological approaches to diffusing capacity, we are interested in the allometric relationships of diffusing capacity of the blood–gas barrier, which should be comparable between studies and is the most relevant factor in oxygen delivery by the lungs.

While the morphometric techniques are fairly standard and comparable, measurements of resting and maximal metabolic rates are fraught with difficulties. How can we be certain that our measurements accurately represented the total range of aerobic metabolic rate? We did not measure blood lactate levels to determine whether the birds had exceeded their aerobic limit. However, we have other evidence that they were reaching maximum values. First, they exhibited signs of fatigue during exercise, such as slower speeds, poorer coordination, increasing reluctance to continue, and rapid breathing. These occurred after at least 2 min, which would have given time for them to achieve a steady state and the respirometry system to stabilize. Second, there was good congruence between data during running and swimming. Third, the values from the largest pelicans exceed the extrapolation from adult birds exposed to cold and converge with the extrapolation from flying species (Fig. 1). By necessity, this study involved inducing maximum metabolic rate by cold exposure and exercise. It is well known that in adult birds, exercise results in metabolic rates about three times

higher than does cold (Hinds et al., 1993). However, this difference may not occur at all developmental stages. Hatchling pelicans were not capable of sustained exercise or thermoregulation. Shivering in young juveniles produced higher metabolic rates than we could induce by exercise, but exercise became more effective in the oldest pelicans. In the five juveniles for which we have data on both exercise-induced and cold-induced metabolism, the ratio (exercise/cold) increased from 0.9 in a 4.2 kg juvenile to 3.9 in the 7.2 kg adult. Therefore our birds were reaching the threefold difference expected for adults.

The factorial metabolic scope increased from 1 at hatching to 3.0 in the largest juveniles (Fig. 1). This value is considerably lower than metabolic scopes in adult birds that range from 4.3 to 6.5 in response to cold (Hinds et al., 1993) and up to 14 during flight (Norberg, 1996). Part of the explanation may be that the oxygen delivery system is simply not as developed in the juveniles as would be expected in the adult, or that resting metabolic rate is not basal. Evidence for the latter is that the expected minimal metabolic rate for birds equals $0.149M^{0.68}$ (Frappell et al., 2001), which is 72 ml min^{-1} for a 8834 g bird. Our resting value of 144 ml min^{-1} is 2 times higher, and is doubtless influenced by the heat increment of digestion (specific dynamic action) of the birds' high protein diets and their rapid growth rate. Because resting metabolism was not the focus of the study, failure to achieve conditions for measuring basal metabolic rate does not compromise the conclusions.

We are reasonably confident that no treatment could have elicited higher metabolic rates in any of the juveniles, but we cannot dismiss the possibility that running and swimming were insufficient to elicit maximum values in the adult bird that was capable of flight. Its flight muscles were not exercised. Given that this bird had a tissue diffusing capacity significantly higher than the juveniles, but did not show a higher metabolic rate, it is quite likely that metabolic rate could be higher during flight than during running or swimming. Furthermore, aerobic metabolic rate during sustained flight is not usually maximal (Norberg, 1996). To understand the development of the gas transport systems in pelicans more fully, it would be desirable to examine flight respiration and pulmonary morphometric development between juveniles and similarly sized adults, but this would have to be done in relation to age rather than body mass.

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