Respiration and energetics of embryonic development in a large altricial bird, the Australian pelican (*Pelecanus conspicillatus*)

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Summary

We examined whether the previously reported low cost of embryonic development in pelicans could be attributed to a more efficient conversion of egg energy to hatchling tissues as a result of high initial egg water content, low embryonic metabolic rate and growth later in incubation than in more precocious species. We therefore determined egg and hatchling composition and the development of embryonic respiration in the Australian pelican *Pelecanus conspicillatus*, which lays one of the largest eggs (140–210 g) with an altricial developmental mode. The small yolk fraction (21%) is typical of all pelecaniforms; however, we found that intraspecific variability in fresh egg mass was related to water content (principally in the albumen), but independent of yolk mass (mean 13 g dry mass). *P. conspicillatus* eggs have, on average, 635 kJ of energy, irrespective of egg mass across the whole range of egg mass.

The embryonic developmental pattern of O_2 consumption and CO_2 production showed clear plateaus lasting 2–3 days immediately prior to internal pipping, resembling the typical precocial pattern. However, the rate of pre-internal pipping O_2 consumption was low in comparison with that of precocial species of similar egg mass. There is no evidence to support the hypothesis that the observed plateau in rates of O_2 uptake is due to a diffusion limitation of the eggshell gas conductance in this species. Embryonic metabolic rate nearly doubled during the pipping period, but the mass-independent metabolic rate of the hatchling was low in comparison with that of the resting adult. The total O_2 consumed (11 063 ml) is equivalent to 217.3 kJ (or 34% of egg energy) based on indirect calorimetry and the observed respiratory exchange ratio of 0.71. Thus, the cost of development (direct calorimetry) was 0.29 kJ J^{-1} in the egg (mean egg mass 168 g), which is one of lowest reported values. As a result, the production efficiency of pelican embryonic development was 61.6%, higher than the average for birds in general (56.9%) and, in particular, of seabirds that have prolonged incubation periods on the basis of egg mass. High efficiency in embryonic development in this species was attained as a result of rapid embryonic growth later in incubation, low hatchling energy density (23.6 kJ g^{-1} dry matter) and dry matter content, low embryonic metabolic rate throughout incubation and a shorter than expected incubation period of 33 days (predicted 36 days).

Key words: respiration, embryo, egg, Australian pelican, *Pelecanus conspicillatus*, development.

Introduction

Birds vary in the size of the eggs they lay, clutch size and the energy they allocate to them for embryonic development. Altricial modes of development have favoured both a reduction in the size of the egg as a fraction of the adult female mass (Rahn et al., 1975), and a reduction in the amount of the main egg energy source, the yolk lipids (Sotherland and Rahn, 1987; Vleck and Vleck, 1987). However, it is not certain whether tissue production in altricial embryos is more efficient than in species with precocial modes. The recent review of available data by Vleck and Bucher (1998) confirms that species with more active or mobile hatchlings, namely precocial types, hatch from eggs that contain yolk fractions of 30–65% on average (with a few exceptions), whereas the eggs of nest-bound, poorly developed semi-altricial and altricial types contain as little as 16–27% yolk. Consequently, average egg energy densities are less than 5 kJ g^{-1} wet mass in altricial eggs, but higher in semi-precocial and precocial ones (up to 12 kJ g^{-1}).

The eggs of altricial pelecaniforms are a small fraction of the female's body mass, but nevertheless very large. The dozen species of pelecaniforms investigated to date have, on average, yolk fractions of 22% and egg energy densities of 5.1 kJ g^{-1} wet mass, similar to those of the smallest altricial passerines (Vleck and Bucher, 1998). Previous investigations of egg composition

in *Pelecanus onocrotalus* and *P. erythrorhynchos* found some of the lowest known egg energy densities among the altricial phyla (Bugden and Evans, 1997; Jones, 1979; Williams et al., 1982), with the exception of a high energy density for the smaller pelican *P. occidentalis* (Lawrence and Schreiber, 1974) that may be erroneous (Bugden and Evans, 1997). The allometric comparisons of cost of development (total energy consumption per gram of egg) reported by Bucher and Bartholomew (1984) suggest that the metabolic cost of producing *P. occidentalis* hatchlings may be lower than that of precocial species.

Embryos of altricial species, and pelicans in particular, can only achieve a low cost of development over long incubation periods if they hatch significantly earlier than predicted on the basis of egg mass, are more efficient at producing hatchling tissues than more precocial species and/or hatch with lower than expected tissue energy densities. Precocial hatchlings, in general, have higher true hatchling energy densities (yolk-free dry mass) than other developmental modes, in which energy densities appear to vary little (Ar et al., 1987; Pearson, 1999). To date, the efficiency of egg energy conversion to hatchling tissues, defined as production efficiency, has not been determined for any pelecaniform or large altricial species.

How avian embryos utilise energy during incubation has been the subject of debate since it was first demonstrated that embryonic respiration and growth patterns differed among avian species after taking into account egg size (Hoyt et al., 1978; Hoyt and Rahn, 1980; Vleck et al., 1979, 1980a,b; Bucher, 1983; Bartholomew and Goldstein, 1984; Bucher and Bartholomew, 1984; Bucher et al., 1986; Hoyt, 1987; Vleck and Vleck, 1987). Rates of O_2 consumption increase rapidly throughout incubation in both precocial and altricial species, followed by a period when O_2 consumption plateaus from approximately 80% of incubation until pipping in precocial species. There was no evidence for an O_2 consumption plateau in altricial embryos using discontinuous sampling protocols. However, a few studies subsequently demonstrated, from continuous recordings, that O_2 consumption plateaus in some altricial species, although for a shorter duration than in precocials (Prinzinger and Dietz, 1995; Prinzinger et al., 1995, 1997a).

The physiological meaning of the respiration plateau that predominates in the development of precocial species has proved controversial. Since O_2 uptake by the embryo is mediated by diffusion through the porous eggshell and its membranes, gas exchange rates may become limited by the fixed gas conductance of the eggshell. This might occur after the inner surface of the eggshell membranes are completely covered by the respiratory chorioallantoic membranes, at approximately 60% of incubation in precocial species (galliforms at least). Some researchers consider that the rate of O_2 consumption of precocial embryos approaches this limitation before internal pipping (IP) because they achieve a significantly greater amount of their embryonic growth earlier in incubation than in altricial species and because incubation is prolonged in some species (Rahn et al., 1974). In contrast,

in galliform species, the plateau metabolic rate of embryos does not restrict high growth rates because synthetic efficiency probably increases during late incubation (Dietz et al., 1998). Thus, it remains unclear how avian embryos balance their energy budgets during incubation and whether the plateau in O_2 consumption of some avian embryos represents a diffusive limitation to O_2 uptake imposed by the conductance of the eggshell.

The Australian pelican *P. conspicillatus*, like *P. onocrotalus*, lays one of the largest known eggs (140–210 g) with an altricial mode of development and has one of the longest incubation periods among pelicans (33–34 days; see Vestjens, 1977). We have determined the changes in material, energy and water content of fresh eggs and hatchlings of *P. conspicillatus* to evaluate production efficiency and hatchling maturity. We have also measured O_2 and CO_2 exchange throughout incubation to confirm production efficiency, establish the respiratory exchange ratio and test for the occurrence of a plateau in respiration in late development.

Materials and methods
Egg composition and energy content of eggs and hatchlings

Pelican *Pelecanus conspicillatus* (Temminck) eggs were collected from an unnamed island (latitude 34°46′S, longitude 138°29′E) at Outer Harbour, Adelaide, on the southern coast of Australia from March to October 2000. Fresh eggs were selected from both first- and second-laid eggs of known lay dates (daily inspection of nests) to represent the range of egg masses observed and then taken back to the laboratory. These eggs were then used to determine fresh egg composition after weighing to the nearest 0.001 g and measurement for length and maximum width with a vernier caliper. Other fresh eggs (not collected) were weighed in the field (to the nearest 0.01 g) with a portable electronic balance to estimate population variability in egg mass. The collected eggs were opened through a small hole in the eggshell, the contents were gently poured into plastic dishes and the albumen was separated from the intact yolk with a 1 ml tuberculin syringe. The wet mass of the eggshell (including shell membranes) and yolk were determined immediately, and the wet albumen mass was assumed to be the difference between the combined eggshell plus yolk mass and the mass of the whole egg. Yolk and albumen samples were dried to constant mass in an oven at 50°C, reweighed to determine dry mass and then stored in a desiccator until samples were combusted.

An additional 30 eggs that were found to be actively incubated were collected and transported to the laboratory for respirometry studies. These eggs were placed within an artificial incubator (approximately 1 h after collection). In each case, the collected eggs were replaced with artificial 'transmitter' eggs or fresh abandoned eggs that were presumed to be fertile. The transmitter eggs were then used to determine incubation temperatures and nest humidities prior to the respirometry study to ascertain suitable conditions for artificial incubation (methods described in Wagner and Seymour, 2001).

In brief, transmitter eggs were prepared from the eggshells that remained after determination of egg composition and filled with agar to maintain heat transfer characteristics similar to those of the egg contents according to the methods of Wagner and Seymour (2001). To ensure that at least one egg was allowed to hatch in each nest, fertile abandoned eggs or second eggs from other nests were replaced in the nests under investigation when the original eggs were incubated to hatching in the laboratory and the embryos later killed.

Mean egg temperature determined by the transmitter eggs in the field was $35.3\pm0.3°C$ (mean ± 95% confidence interval; range $32.0–37.6°C$, N=88 intermittent recordings from 15 nests, ≤14 day periods). All eggs were incubated in the laboratory between 35.0 and 36.0°C under 45–55% relative humidity in a still-air incubator with an automatic turning mechanism. Eggs were removed from the incubator daily for mass determinations and respirometry measurements described below. After the final measurements, young embryos were chilled for 3–4 h at 9–10°C to stop development, whereas hatchlings and late embryos were killed by CO_2 and then dissected to remove the residual yolk. After determining the wet mass of residual yolk, they were oven-dried with the yolk-free hatchlings, and dry mass was determined as for fresh eggs. Subsamples of dried eggs and hatchlings were ground in a mill, pressed into pellets (approximately 0.1–0.2 g) and their energy content determined with a semi-micro oxygen combustion bomb calorimeter (Parr 1261, IL, USA) calibrated against certified standard benzoic acid (M & B Laboratory Chemicals, UK). The energy determinations of all samples were corrected for ash-free mass.

Respirometry

28 pelican eggs were incubated for the respirometry measurements; however, some embryos were killed during the last week of incubation for another study of morphometric analyses of chorioallantoic membranes, heart and lung tissues. Thus, the sample sizes varied with incubation age. The O_2 consumption and CO_2 production rates of embryos and hatchlings were determined by open-flow respirometry at a chamber temperature of 36°C. Compressed air flowed sequentially through a pressure regulator, a series of three tubes containing Drierite, soda lime and Drierite (to remove water vapour, CO_2 and water vapour, respectively), and then into one of three mass-flow controllers (Sierra Instruments Mass-Trak, CA, USA) to flow separately into three translucent animal chambers (800 ml) held within a constant-temperature cabinet (±1°C) under dim light. Excurrent air from the chambers and a fourth channel for dry, CO_2-free air passed through a solenoid gas-flow controller, which directed the samples in turn to a pump (Charles Austen Pumps, Surrey, UK), a tube of Drierite and, finally, a combination O_2 and CO_2 analyser (David Bishop Instruments 280/0427 Combo, UK) thermostatted to 36°C. Outputs from the mass-flow controllers and both gas analysers were recorded as voltages on a personal computer *via* an A/D converter (Sable Systems Universal Interface, USA). Sable Systems DATACAN v5.2 data-acquisition software was used to sample every 0.5 s. Air flow rates used during measurements varied from $100\,ml\,min^{-1}$ for early embryos to $350\,ml\,min^{-1}$ for hatchlings.

Calibration of the analysers was routinely achieved by passing dry compressed air (approx. 21% O_2), pure N_2 and a precision gas (0.586% CO_2, remainder N_2) through the respirometry system. However, since the response of the CO_2 analyser was non-linear when fractional CO_2 changes were less than 1%, a further *post-priori* calibration of CO_2 production relative to O_2 consumption was made using an alcohol flame (Young et al., 1984). Pure ethanol was burnt with a very small flame (using an inflammable asbestos wick) inside a clean metal paint can outside the cabinet. Compressed air was supplied at high flow rates to the can, and the majority of the excurrent air was vented to the outside. A small subsample of the burner gas was diluted with compressed air from a blank animal chamber before entering the analysers. Further dilution of the CO_2 content of the burner air was made by increasing the air flow into the burner can and reducing the subsample mixed with compressed air.

As the respiratory exchange ratio of ethanol combustion is 0.667, the non-linearity of the CO_2 analyser output was corrected over the range of fractional CO_2 changes of 0.05–0.70%, which was similar to the range for CO_2 production rates of pelican embryos and hatchlings in this study, using a third-order polynomial equation ($y=4101.2x^3+29.489x^2+0.677x–0.00001$, r^2=0.998, where y and x are the corrected and observed fractional CO_2 contents of excurrent air, respectively). After this correction, the final rates of CO_2 production and O_2 consumption were calculated according to Withers (1977) with iterative fitting assuming an initial respiratory exchange ratio of 0.8.

Artificially incubated embryos were measured once a day for approximately 5 min each after an equilibration period of 40 min, until internal pipping was observed (candling of the air-cell and vocalisations). Thereafter, embryos were sometimes measured several times a day until hatching. Hatchling metabolic rates were recorded for at least 14–15 min to establish minimal resting values under dim light. Total O_2 consumed during incubation (assuming 33 days of incubation) was estimated from the area under the curve of mean rate of O_2 consumption against incubation age. An energetic equivalent of $19.64\,J\,ml^{-1}\,O_2$ (Vleck et al., 1980b) was used to convert total oxygen consumed to total energy consumed during incubation.

Water vapour conductance of eggs

The water vapour conductance of pelican eggs was measured in the laboratory to investigate whether O_2 uptake becomes limited during late incubation. Eggs were removed from the routine incubator and placed in sealed desiccators (containing anhydrous silica gel) maintained at 36°C within a constant-temperature cabinet. The mass change of the developing eggs was determined 24 and 48 h later, and the eggs were returned to the routine incubator (45–55% relative humidity). Water vapour conductance was determined for

Statistical analyses

Linear regression was used to examine relationships between variables by the method of least squares. Regression statistics are presented with the standard error of the regression coefficient (s_b). Mean values are presented with 95% confidence intervals, unless stated otherwise, and sample size (N). Student's t-tests were performed on untransformed data. The significance of differences was accepted at the 5% level.

Results

Egg and hatchling size and composition

Maximum egg length (L) and width (W) averaged 90.6±0.5 mm and 57.6±0.2 mm, respectively, for 250 eggs sampled from 12 nesting sites temporally and spatially separated within the colony, including eggs with advanced embryos and eggs used for egg composition and respirometry experiments. Fresh egg mass (M_e, g) was significantly related to L and W ($M_e=0.00056LW^2-2.6474$; $r^2=0.949$, $s_b=0.000017$, $N=64$). According to this relationship, the estimated mean M_e based on this sample, including 186 eggs for which M_e on lay date was not known, from the Outer Harbour pelican colony was 166 g. A small, but significant, difference in estimated M_e was found between first- and second-laid eggs of two-egg clutches (166.9 g *versus* 163.3 g; paired t-test, $t=3.014$, $P<0.0016$, $N=101$ pairs). The mass of pelican hatchlings (M_h, g) measured in the field on the day of hatch was significantly related to the estimated initial egg mass ($M_h=0.868M_e-24.4$; $r^2=0.705$, $s_b=0.110$, $F_{1,27}=62.084$, $P<0.0001$) but, on average, was 116.5±2.2 g ($N=56$) or 71.2±0.9% ($N=29$) of M_e. Furthermore, the mass of the whole hatchling as a fraction of M_e was independent of M_e ($r^2=0.052$, $F_{1,27}=0.234$).

The composition of freshly laid pelican eggs is presented in Fig. 1 for 18 eggs ranging in mass from 140.6 to 190.4 g. Both the wet and dry yolk content of eggs were independent of fresh egg mass, so most of the variation in egg composition was attributable to significant increases in water content of albumen, and to a lesser extent eggshell, with increasing egg mass (Table 1). Allometric analyses did not provide a better fit for any of the regression models of egg and hatchling composition and are therefore omitted. Wet yolk mass accounted for 21% of fresh egg contents in this study. This value is less than the 28% reported for smaller *P. occidentalis* eggs (Lawrence and Schreiber, 1974), but a little higher than the 17.5% found in *P. erythrorhynchos* eggs of similar fresh mass (Bugden and Evans, 1997). Further, our mean wet yolk mass is similar to the 22% for pelecaniforms in general (Vleck and Bucher, 1998). The total energy content of both the egg contents ($r^2=0.043$) and the yolk were independent of fresh egg mass ($r^2=0.005$), while albumen energy content was weakly correlated with egg mass ($r^2=0.249$, $P<0.036$). The mean total egg energy content was 635±26 kJ ($N=18$) in this study.
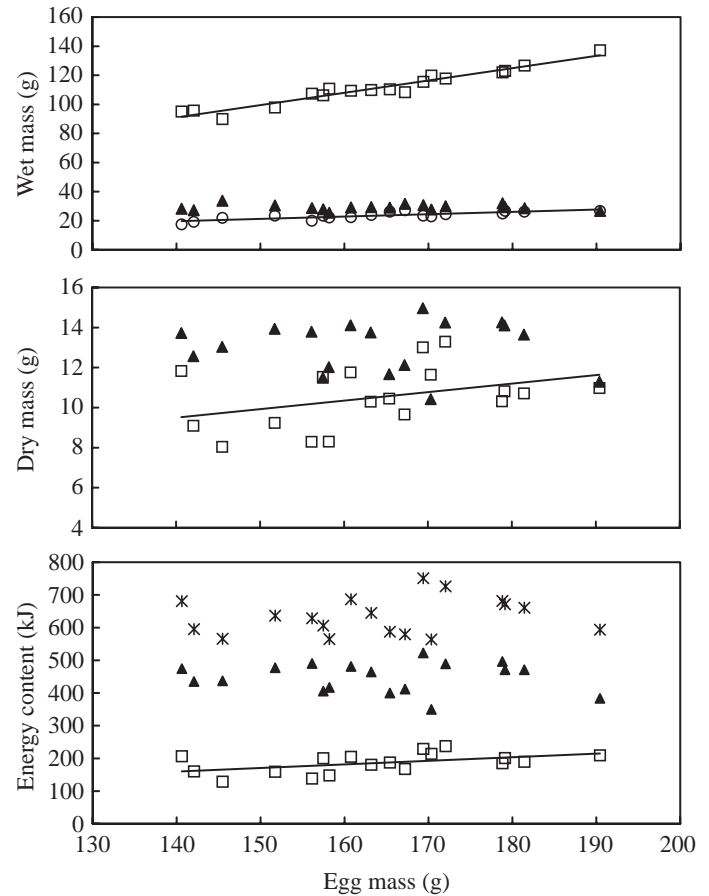

Fig. 1. The composition (wet and dry mass, g) of pelican eggs ($N=18$) and the energy content (kJ) of albumen and yolk in relation to fresh egg mass (g). Albumen is indicated by squares, yolk by triangles, eggshell by circles and total energy content by asterisks. Solid lines represent significant linear regressions summarized in Table 1.
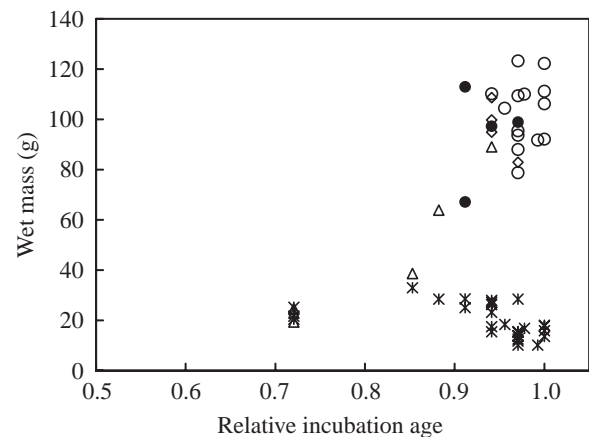

Fig. 2. Changes in yolk-free wet mass (g) of pelicans and in yolk mass (g) with respect to relative incubation age, where 1.0 is the hatchling on day 33 of incubation. Asterisks refer to wet yolk, triangles to pre-internal pipping embryos ($N=6$), filled circles to internally pipped embryos ($N=4$), diamonds to externally pipped embryos ($N=4$) and open circles to hatchlings ($N=14$).

Table 1. *The composition of freshly laid Australian pelican (*Pelecanus conspicillatus*) eggs and hatchlings*

Variable	Albumen	Yolk	Eggshell	Yolk-free hatchling	Residual yolk
Wet mass (g)	111.24±6.02	29.19±1.00	23.44±1.35	102.59±6.56 (14)	14.57±1.39 (14)
Fraction of wet egg contents (%)	79.0±2.54	21.0±2.54			
Dry mass (g)	10.52±0.69	12.96±1.16	Not measured	12.73±1.12 (4)	4.95±0.40 (14)
Linear relationships between contents and egg mass ($y=a+bx$, where egg mass is x)					
Wet contents (g)	$y=-27.387+0.846x$ $r^2=0.939, F=244.9$ $P<0.001, s_b=0.054$	NS	$y=-2.834+0.160x$ $r^2=0.675, F=33.2$ $P<0.001, s_b=0.028$		
Dry contents (g)	$y=0.745+0.046x$ $r^2=0.232, F=4.851$ $P<0.05, s_b=0.021$	NS	Not measured		
Energy density (kJ g^{-1})					
Dry mass	22.30±0.21	34.33±0.20		23.61±0.32 (4)	29.82±0.59 (14)
Linear relationships between energy content (kJ) and egg mass (where egg mass is x)					
	$y=6.844+1.092x$ $r^2=0.247, F=5.235$ $P<0.036, s_b=0.477$	NS			
Mean energy content (kJ)					
	176±14	435±22		300.5±24.7 (4)	147.3±11.7 (14)

Eggs, $N=18$; hatchlings, N indicated in parentheses.
Mean values are presented with 95% confidence intervals of the mean.
NS, not significant; s_b, standard error of the regression coefficient.

Yolk-free embryo mass was, on average, 22.3 g ($N=3$) on day 24, or 72% of the incubation period, and increased in a curvilinear fashion with age (Fig. 2). The wet mass of yolk-free pipped embryos overlapped considerably with that of the yolk-free hatchlings. Whole hatchling masses of pelicans hatched in the laboratory were 68.2±2.7% ($N=13$) of initial egg mass. Residual yolks represented an average of 17.5% ($N=4$) of whole embryo mass in externally pipped embryos and 12.6±1.0% ($N=14$) of whole hatchling mass. The residual yolk of both pipped embryos and hatchlings consisted of 65.9±2.0% ($N=18$) water, significantly more than found in the fresh yolk (55.2±1.8%), but the water content of whole hatchlings was similar to the total initial egg water content (84.2 *versus* 87.8±0.8%). The energy contents of yolk-free hatchlings and their residual yolks were 301 kJ and 147 kJ, respectively (Table 1). Energy consumed during incubation (including energy remaining as the meconium and that transferred to the chorioallantois during development) was therefore 635 kJ (egg) minus 448 kJ (whole hatchling), or 187 kJ according to direct calorimetry.

Pipping and incubation times

Eggs incubated artificially (>75% incubation) at a mid-egg temperature of 35°C internally pipped (IP, piercing of inner shell membrane and breathing in the airspace) on day 32, externally pipped (EP, breaking of the eggshell and breathing of external air) on day 33 and hatched on day 34. In contrast, eggs incubated at 36°C pipped and hatched one day earlier. In the field, embryos from the same colony were internally pipped (vocalizations heard) on day 30–31, externally pipped on day 31–33 and hatched on most occasions on day 32 or 33 (87 eggs determined to within ±1 day, mean 32.8±0.2 days, range 31–36 days).

Development of respiration

The rate of O_2 consumption of embryos increased in an approximately exponential fashion with incubation age for the first 25 days, then increased at a lower rate and plateaued for 2–3 days before IP (Fig. 3). As the O_2 consumption rates of embryos incubated at 35°C were not significantly different from those incubated at 36°C, the data were combined for the pre-pipping period (mean $M_e=171.3±18.9$ g). The greatest increases in rates of O_2 consumption to approximately 700–1200 ml day^{-1} occurred after IP. The development of O_2 consumption during the pipping period was quantitatively similar at both temperatures; the O_2 consumption rates of combined group means were 1052±266 ml day^{-1} ($N=8$) for IP eggs, 1531±170 ml day^{-1} ($N=30$) for EP eggs and 1697±176 ml day^{-1} ($N=18$) for hatchlings.

Similar patterns of CO_2 production were also found for both temperature groups as the respiratory exchange ratio (RER) was generally stable throughout the incubation period (Fig. 4). Mean RER was 0.71±0.01 for embryos ($N=30$) over the whole incubation period and 0.68±0.04 for hatchlings ($N=14$). Total O_2 consumed during incubation was 11063 ml O_2 over 33 days. Assuming 19.64 J ml^{-1} O_2 consumed, pelicans consumed
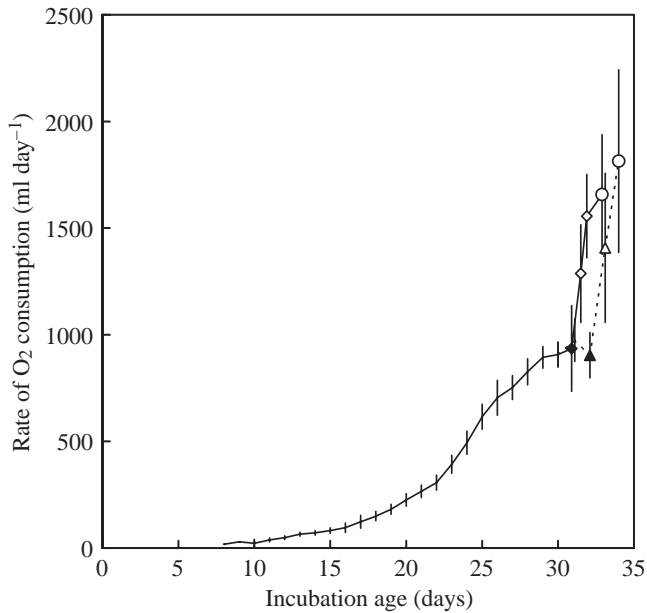
Fig. 3. Rates of O₂ consumption (ml day⁻¹) of pelican embryos and hatchlings in relation to incubation age (days). Embryos incubated at 35°C pipped and hatched 1 day later than those incubated at 36°C, but data were not significantly different and were therefore combined up to day 30 (solid lines). Data for pipped embryos and hatchlings from the 35°C group are indicated by dashed lines on days 32–34. Internally pipped embryos are represented by filled symbols (triangles and diamonds for 35°C and 36°C groups, respectively), externally pipped embryos by open symbols (as for internal pipping) and hatchlings by open circles. Error bars represent 95% confidence intervals of the mean on each day.

217.3 kJ according to indirect calorimetry. The latter estimate is equivalent to 34.2% of the initial egg energy, or 1.29 kJ g⁻¹ egg. Lack of variability in the egg mass of our sample used for respirometry, however, prevented us from analysing whether the total O₂ consumed by individual eggs was related to initial fresh egg mass.

Eggshell water vapour conductance determined for respirometry eggs and fertile eggs temporarily removed from the field was 166.2±7.4 mg day⁻¹ kPa⁻¹ (N=45, age 1–33 days), after correcting to 25°C. This conductance is lower than predicted by Ar and Rahn (1978) on the basis of egg mass (mean M_e=172 g, 95% CI 164.2–222.5 mg day⁻¹ kPa⁻¹), although not significantly so.

Discussion

Size and composition of egg and hatchling

Pelican eggs are invested with relatively small yolks, which is typical of altricial types in general. The mean yolk fraction of 22% of initial egg mass in pelecaniform species is larger than that of altricial passerines (Vleck and Bucher, 1998). *P. conspicillatus* egg contents are 83% water, similar to all other altricial species (Sotherland and Rahn, 1987), although the energy densities of the yolk and albumen solids (Table 1) are
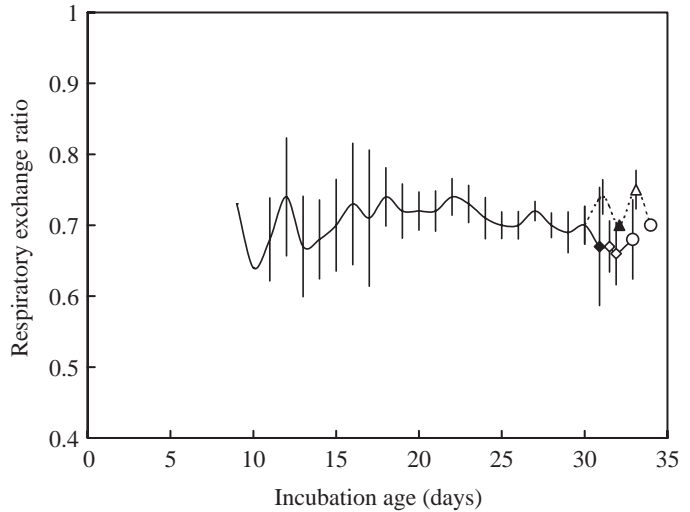

Fig. 4. Respiratory exchange ratio of pelican embryos and hatchlings in relation to incubation age (days). Mean values are presented with 95% confidence interval bars on each day. Symbols and lines are the same as in Fig. 3.

identical to the means reported for all hatchling types (Ar et al., 1987; Sotherland and Rahn, 1987) and for other pelicans (Bugden and Evans, 1997). In spite of the large size of *P. conspicillatus* eggs, they hatch after 33 days, earlier than the 36 days predicted for birds in general on the basis of egg mass (Rahn and Ar, 1974). The hatchling contains a large residual yolk (12.5%), which is no doubt important for survival during the first posthatching days, when sibling rivalry may limit food intake.

All avian eggs necessarily lose water because of the porous eggshell. This allows the shell membranes to dry and provides a diffusive path for gas exchange, as well as preventing the accumulation of water that is produced as a byproduct of embryonic metabolism (Ar and Rahn, 1980). Not surprisingly, the water content of the *P. conspicillatus* hatchling at the end of incubation is a high 84.2%, similar to the initial egg water fraction. With so few solids in the egg contents and in the hatchling tissues, it is not surprising that the pelican hatchling's energy density is a low 23.6 kJ g⁻¹ dry mass. This value is similar to the average of many other seabird species, although higher than some extremely low values (three species <22 kJ g⁻¹ and one exceptional report for *Gallus domesticus*). All other species reportedly have higher energy densities (mean for all species 25.3 kJ g⁻¹, N=34, 95% CI=24.3–26.2 kJ g⁻¹) (from Ar et al., 1987; Pearson, 1999).

Total production efficiency of converting egg energy (minus residual yolk) to yolk-free hatchling is 61.6%, higher than the mean of 56.9% for all hatchling types (data from Ar et al., 1987; Pearson, 1999). Thus, *P. conspicillatus* appears to be as efficient as columbiform, anseriform and galliform species and significantly more efficient than the three pelagic-feeding seabird species (44.8–52.5%) that were reported with low yolk-free hatchling energy densities, which is probably attributable to the prolonged incubation periods and higher degree of

hatchling physiological maturity of the latter species in terms of mobility and thermogenic capacity.

Regression analyses of egg and hatchling composition in the present study suggest that the cost of development in pelicans varies very little, irrespective of egg size. Jones (1979) reported that fresh yolk content increases significantly with egg mass (a 3.4 g yolk increase over a 74 g increase in egg mass) in *P. onocrotalus*, although the relative size of the yolk decreases, as seen in other species (cited in Jones, 1979). We found that *P. conspicillatus* eggs differing by as much as 50 g in initial mass contained nearly the same yolk mass (both wet and dry matter), so most of the mass difference in egg size is attributable to increases in water content of the albumen fraction with increasing egg mass (Fig. 1; Table 1). Precocial species are commonly reported to invest disproportionately larger yolks and lipid stores in larger eggs, which generally hatch with higher hatchling energy densities and yolk reserves (Ricklefs et al., 1978; Birkhead, 1984; Alisauskas, 1986; Rohwer, 1986; Arnold, 1989; Østnes et al., 1997). Greater energy reserves in precocial species convey survival advantages to the mobile hatchlings. In contrast, altricial species show only weak correlations between yolk content and egg mass and generally no correlation between lipid content and egg mass (Ricklefs and Montevecchi, 1979; Ricklefs, 1984). An exception is *Molothrus ater*, which invests more yolk and energy in larger eggs (Ankney and Johnson, 1985).

P. erythrorhynchos lays significantly larger first eggs than second eggs, when present, and hatchlings from first eggs are larger than those from second eggs (Evans, 1997). In this species, which is an obligate brood-reducing species, there is considerable evidence that the reduced investment in the second young conserves reproductive effort whilst providing viable insurance against the loss of the first young during incubation or the early nestling period. In contrast, the first-laid eggs of *P. conspicillatus* (not necessarily a brood-reducing species) in this study were slightly larger than the second-laid eggs ($P<0.0016$), but it is uncertain whether the 3 g difference confers any advantage to first-hatched chicks since initial yolk energy is independent of egg mass in first and second eggs.

Development of respiration

The development of a clear plateau in O_2 consumption after the period of exponential increase during early incubation in the Australian pelican resembles the pattern usually reported for large precocial species (Hoyt et al., 1978; Vleck et al., 1979, 1980b; Ancel and Visschedijk, 1993; Booth and Sotherland, 1991; Prinzinger et al., 1997b). The 2–3 day plateau in metabolic rate of *P. conspicillatus* is the longest reported so far for an altricial species (Fig. 3). Similar metabolic plateaus have been reported for some semi-altricial penguin species (Bucher et al., 1986), but the results appear inconclusive for other penguin species (Adams, 1992; Brown, 1988) and the only other pelican species investigated, *P. occidentalis* (Bartholomew and Goldstein, 1984). The results for very large altricial species lend further support to the hypothesis that the development of embryonic metabolism is

not inherently different between hatchling maturity types (Prinzinger and Dietz, 1995; Prinzinger et al., 1995, 1997b), as first believed during the period of the pioneering comparative studies (Hoyt et al., 1978; Vleck et al., 1979, 1980a,b).

The most noticeable divergence in the developmental patterns of metabolism between phyletic groups is the level of metabolic rate attained immediately before IP and the initiation of pulmonary respiration. To normalise interspecific comparisons of O_2 consumption, 80% of incubation time is routinely used to provide the pre-internal pipping rate. On this basis, the 729 ml day^{-1} at 26.5 days in *P. conspicillatus* is only 71% of the predicted uptake rate (95% CI=851–1220 ml day^{-1}) according to the allometric relationship for all hatchling types (Rahn and Paganelli, 1990). Even on day 30, the last day of pre-pipping incubation, the rate is only 907 ml day^{-1} or 89% of the predicted rate. Since both altricial and precocial taxa appear to be represented by species with less than predicted pre-pipping rates as frequently as by species with higher than predicted rates (Ackerman et al., 1980; Pettit et al., 1982a,b; Rahn and Paganelli, 1990), pre-pipping O_2 consumption rate appears not to be strictly related to the degree of cellular differentiation and maturation of tissue function that varies between developmental modes.

The most suitable convention for analysing the physiological maturity of avian embryos and hatchlings is to compare the mass-independent metabolic rate (MIM) of the young of any species with that of an adult of the same species or genus (Bucher, 1986, 1987). We assume a similar metabolic rate for adult *P. conspicillatus* and *P. onocrotalus* of similar body mass (Shmueli et al., 2000). The hatchling-to-adult MIM ratio of *P. conspicillatus* is 0.348 [mean minimal RMR (resting metabolic rate) of hatchling 3.23 ml g$^{-0.67}$ day^{-1} divided by adult BMR (basal metabolic rate) of 9.27 ml g$^{-0.67}$ day^{-1}], which suggests that *P. conspicillatus* hatches with a very low degree of physiological maturity, like most other altricial species and in contrast to more precocial developmental modes (range of ratios 0.2–0.6 *versus* 0.4–1.0; see Bucher, 1987). This is not unexpected considering the high hatchling water content (84.2%), similar to the mean of 83.8% reported for many small altricial species (Ar and Rahn, 1980). As in other avian species, a high hatchling water content correlates with a low metabolic activity (total rate of O_2 uptake). Although large hatchling size confers the advantage of lower heat loss per gram body mass in precocial species, the acquisition of a high metabolic rate in pelican species at hatching would be energetically disadvantageous since the parents do not construct insulative nests and hatchlings have essentially no insulative down to minimise the rate of heat loss when not parentally brooded. However, high hatchling water content and the more immature (proliferative) state of tissues might benefit pelicans that achieve more rapid postnatal growth than in precocial species, since there is a trade-off between mature function and growth rate in birds (for a review, see Ricklefs et al., 1998).

Embryonic growth and production efficiency

The pattern of embryo growth in *P. conspicillatus* conforms

well to the results described for *P. occidentalis* (Bartholomew and Goldstein, 1984). On day 24 of the 33 day incubation, yolk-free embryo wet mass is approximately 20% (22.3/102.6) of the final hatchling wet mass (Fig. 2). More than 40% of embryo growth occurs between the 85th and 95th percentiles of incubation, at the end of pre-pipping development, which suggests that in pelicans growth is very rapid over the shorter than expected incubation period (predicted 36 days according to Rahn et al., 1974) and, more importantly, that most of growth is relatively later in incubation. Rapid cell proliferation during the pipping phase of incubation is apparent from the increases in wet embryo mass; however, we do not know how the rate of solid accumulation in embryonic tissues changes during incubation. We can therefore only speculate that the high total production efficiency of *P. conspicillatus* hatchlings; at 61.6%, might be achieved by a low rate of increase in tissue energy density per gram dry matter, since the hatchling energy density is low. Further, Ricklefs (1987) found that the rate of dry matter accumulation itself is low in *P. occidentalis* and other altricial species.

The average cost of development in *P. conspicillatus* is $1.11\,kJ\,g^{-1}$ egg mass according to direct calorimetry (mean M_e=170 g), but $1.29\,kJ\,g^{-1}$ according to respirometry (mean M_e=171 g). Both estimates are among the lowest costs of development reported to date in birds (Bucher and Bartholomew, 1984). However, if the same amount of energy is invested in all *P. conspicillatus* eggs, then a larger egg might produce a larger hatchling at a lower cost of development per gram of egg than a smaller egg, unless there is intraspecific variability in production efficiency. Unfortunately, our sample of eggs for respirometry is not sufficiently variable in egg mass to be able to determine whether the total O_2 consumed is related to fresh egg mass.

Is O_2 uptake limited during late incubation?

Most of embryonic growth in *P. conspicillatus* occurs during the last 25% of incubation (Fig. 2), resulting in a significant reduction in available yolk energy. Embryonic metabolic rate plateaus before IP, during the period of greatest embryonic growth and increasing maintenance energy requirements, as in precocial species (Vleck et al., 1979). The present study supports the view of Bucher et al. (1986) and suggests several reasons why an O_2 uptake limitation imposed by the fixed eggshell gas conductance is unlikely to cause the plateau in metabolic rate observed in *P. conspicillatus*. Soon after IP, when pulmonary and chorioallantoic respiration modes are functional, there is no significant increase in O_2 uptake rate in spite of the removal of the diffusion limitation. The calculated O_2 partial pressure gradient across the eggshell at pre-IP is 5.46 kPa (41 mmHg), identical to the average gradient of avian embryos in general (Rahn et al., 1974). Therefore, gas conditions within the air-cell of *P. conspicillatus* eggs are not exceptionally hypoxic. Both *P. conspicillatus* and semi-altricial *Pygoscelis adeliae* eggs have a lower than expected water vapour conductance on the basis of egg mass, but the plateau rate of O_2 uptake prior to IP in the former is much lower (80% of predicted, 95% CI 540–1542 ml day^{-1}) than is predicted by water vapour conductance (Rahn et al., 1974) and in the latter species much higher (829 ml day^{-1}=123% of predicted, 95% CI 340–809 ml day^{-1}; see Bucher et al., 1986) than predicted. It remains to be determined whether the synthetic efficiency of embryonic tissue production increases during late incubation in altricial species, as reported for galliforms (Dietz et al., 1998), which is an alternative explanation for the plateau in metabolic rate.

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