Growth and Metabolism in the Embryonic White-Spotted Bamboo Shark, *Chiloscyllium plagiosum*: Comparison with Embryonic Birds and Reptiles

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ABSTRACT

Birds and reptiles have been important models for studying the energetics of embryonic development. Studies on these groups reveal three metabolic patterns: an exponential increase in metabolism with embryo age, a sigmoidal increase with age, or a sigmoidal increase followed by a decrease before hatching. Models developed to explain avian metabolic patterns and developmental costs partition total costs between growth and maintenance. To test the generality of these models, we examined embryonic energetics of the oviparous white-spotted bamboo shark *Chiloscyllium plagiosum*. Oviparous sharks must actively ventilate during development, which could increase their development costs relative to birds and reptiles. Our results demonstrated that bamboo shark embryos have a peaked metabolic pattern and sigmoidal increase in body mass similar to ratites, crocodilians, and some turtles. The total cost of development was higher in bamboo sharks than in reptiles and many birds. However, calculations reveal that the high cost of bamboo shark development can be explained by the relatively long incubation time rather than the additional cost of muscular movement. Finally, an avian model can reasonably describe shark embryonic metabolism, suggesting that movement costs do not significantly alter the metabolic pattern during development.

Introduction

Embryonic development in vertebrates involves dramatic increases in body size and physiological complexity. Birds and oviparous reptiles have been important model systems for studying the energetics associated with developmental changes. These taxa are convenient for investigating embryonic energetics because they develop within a protective eggshell and are nourished by maternally supplied yolk, making it possible for embryos to develop independently from their parents.

Birds and reptiles exhibit three basic metabolic patterns during embryonic development. Altricial birds, snakes, and some turtles show an exponential increase in oxygen consumption during embryonic development (termed the "altricial pattern"; reviewed in Vleck et al. 1979, 1980*a*; Vleck and Vleck 1987; Vleck and Hoyt 1991). By contrast, most precocial birds and some lizards show a sigmoidal increase in oxygen consumption with age, where oxygen consumption reaches a plateau before hatching (the "precocial pattern"; Vleck et al. 1979, 1980*a*; Vleck and Vleck 1987; Thompson 1989; Vleck and Hoyt 1991; Birchard et al. 1995; Thompson and Stewart 1997). Finally, emus and other ratites, crocodilians, and some freshwater turtles show a modified precocial pattern, where oxygen consumption increases sigmoidally but then decreases before hatching (the "peaked precocial pattern" or "peaked pattern"; Vleck et al. 1980*a*, 1980*b*; Vleck and Vleck 1987; Thompson 1989; Whitehead and Seymour 1990; Vleck and Hoyt 1991; Booth 1998).

Studies of avian and reptilian embryos reveal that developmental changes in metabolism are generally correlated with distinct growth patterns (Vleck et al. 1979, 1980*a*; Vleck and Vleck 1987; Vleck and Hoyt 1991; but see Dietz et al. 1998). For example, those species exhibiting an altricial pattern of metabolism show an exponential increase in body mass. By contrast, species that show a precocial pattern of metabolism exhibit a sigmoidal increase in body mass because embryos remain in the shell for some time after attaining maximal body mass.

The observed parallel between changes in metabolism and growth rate during embryonic development has led to the hypothesis that the two variables are causally interrelated (Vleck et al. 1979, 1980*a*; Hoyt 1987; Vleck and Vleck 1987). This hypothesis has provided testable models designed to describe the change in metabolic rate over the course of development. A current model for avian embryonic metabolism partitions development costs between the costs associated with building new tissues (growth costs) and those associated with maintaining existing tissues (maintenance costs; Hoyt 1987; Vleck

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and Vleck 1987). According to this model, maintenance costs are proportional to body mass, whereas growth costs are proportional to growth rate. This model provides an elegant explanation for the observed link between growth rate and metabolic patterns (Hoyt 1987; Vleck and Vleck 1987). For example, the exponential increase in body mass seen in altricial birds should lead to an exponential increase in metabolism because growth and maintenance costs continue to increase during development. In the case of precocial birds, declining growth rates towards the end of development should lead to a decrease in growth costs, thereby causing total metabolic rate to stabilize (the precocial pattern) or even decrease (the peaked precocial pattern).

Not only does this model for avian embryonic energetics explain the pattern of embryonic metabolism with age, but it also generates predictions about the total cost of development. For example, this model predicts that for a given egg mass and incubation time, precocial birds should incur higher developmental costs than altricial birds because precocial developers must maintain a greater amount of tissue throughout development. This model also predicts that longer incubation times will increase developmental costs because an embryo must maintain a given body mass for a longer period of time. Finally, for a given developmental period, total developmental costs should increase with hatchling size because both maintenance and growth costs will increase. Empirical studies on various birds support these predictions (Hoyt 1987; Vleck and Vleck 1987). Moreover, a recent study on two lizard species suggests that the avian model may apply to oviparous amniotes in general (Thompson and Stewart 1997). The applicability of the model to nonamniote oviparous vertebrates, however, remains untested.

In this study we further test the generality of this avian model by measuring the oxygen consumption and body mass of developing oviparous shark embryos. Being nourished solely by a maternally supplied yolk, oviparous sharks develop in a fashion superficially similar to embryonic birds and reptiles. However, unlike birds and reptiles, which are amniotes and exchange gases through vascularized extraembryonic membranes, sharks are not amniotes and must, therefore, actively ventilate their egg case at all but the earliest stages of development (Diez and Davenport 1987; Ballard et al. 1993; Thomason et al. 1996*b*; Tullis and Peterson 1998; Leonard et al. 1999), although additional flow may be generated by external water currents (Koob and Summers 1996). Recently, Leonard and coworkers (1999) demonstrated that ventilatory movements increased the standard metabolic rate of developing skates (*Raja erinacea*) by approximately 20%. Thus, muscular activity associated with ventilation could add to developmental costs of elasmobranchs, resulting in higher than expected costs based on avian models. Significant costs associated with embryonic movement could also produce different metabolic patterns in developing oviparous sharks, as compared to birds and reptiles. Data from another elasmobranch, the lesser spotted dogfish (*Scyliorhinus canicula*), show that oxygen consumption increases exponentially with embryo age (Diez and Davenport 1987), as in altricial birds and snakes. However, the paucity of data on metabolism and growth of embryonic sharks makes it impossible to conclude if this pattern is common to all elasmobranchs. Additional information on the developmental energetics of elasmobranchs is important for uncovering general principles governing the ontogeny of metabolism and cost of embryonic development in oviparous vertebrates.

Among the 146 known species of oviparous sharks (Dulvy and Reynolds 1997), we chose to study embryonic energetics of the white-spotted bamboo shark, *Chiloscyllium plagiosum*, for two reasons. First, bamboo sharks breed readily in captivity, facilitating acquisition of study animals. Second, bamboo sharks are tropical and normally live at approximately 25°C, which is 10°–18°C higher than the developmental temperature of temperate species such as the lesser spotted dogfish and little skate. The higher developmental temperature of bamboo sharks will facilitate comparison with the developmental energetics of reptiles and birds, which commonly develop at 30°–37°C. We have two principle objectives in this study. First, we will describe and quantify the metabolism and growth of developing embryonic white-spotted bamboo sharks. Then, we will examine the developmental energetics of bamboo sharks in the context of the current avian model.

Material and Methods

Animals

Eggs of the white-spotted bamboo shark, *Chiloscyllium plagiosum*, were obtained from the Point Defiance Zoo and Aquarium in Tacoma, Washington. Eggs were collected once or twice per week and transported in seawater to the University of Puget Sound, where they were placed in filtered and aerated marine tanks. The developing eggs were maintained at their normal habitat temperature of 24°–26°C, with salinity at 30–34 ppt. Approximately 10% of the tank water was replaced every week and water chemistry remained consistent with the conditions at the Point Defiance Aquarium. Individual egg cases were labeled with nonpiercing plastic tags bearing the collection date and an identification number. We designated the collection date as day 1 of embryo age. This convention ensured that the largest error in estimating the date of lay was an underestimate of 5 d, equivalent to about 4% of the average developmental period. Throughout this article, "embryo" refers to a shark before hatching, and "hatchling" refers to a shark soon after eclosion. Time from laying until eclosion is referred to as "incubation period" or "developmental period."

Observations of Living Embryos

To observe morphological and ventilatory changes that occur during development, we placed several developing embryos in 100-mL glass beakers. To remove embryos from their egg case, an egg was placed in a large finger bowl containing seawater. One end of the egg case was then cut open with scissors, and the embryo and its yolk sac were then carefully decanted into the beaker. Throughout the entire process, the embryo remained submerged. After securing a permeable mesh covering over the top of the beaker, the beaker was returned to its original tank. Only embryos older than 20 d postlaying were removed from their egg case. Before day 20, a gelatinous material surrounds the embryos, preventing removal from the egg cases without rupturing the yolk sac.

Observations of embryos during development focused on morphological and behavioral aspects thought to be important for gas exchange. Specifically, we monitored (1) presence or absence of external gill filaments, (2) occurrence of buccal pumping, and (3) duration and intensity of axial movement. Respiration rate, in breaths per minute, was determined by observation after embryos began buccal pumping. We collected observational data at least two times per week for beaker-raised embryos. In addition to documenting respiratory parameters, we also recorded the time when the external yolk sac was no longer visible and when animals hatched from their egg case or beaker. Embryos hatched out of egg cases by creating an opening at one end of the egg case and forcing their way through the opening. In a similar fashion, beaker-reared sharks "hatched" by pushing the mesh covering off of the beaker and swimming out into the main tank. Hatching dates were determined unambiguously for six egg-reared embryos and four beaker-reared embryos. Only these individuals were used in the analysis of developmental time.

Oxygen Consumption

We used closed system respirometry to measure the rate of oxygen consumption ($\dot{V}O_2$) of 14 developing bamboo shark embryos enclosed within their egg cases. The respirometer was an airtight 0.59-L cylindrical Plexiglas chamber (7.5-cm diameter; 13.5-cm height) equipped with a plastic-mesh partition (mesh size 1 cm) that separated a small magnetic stir bar from the egg case. To begin each trial, the respirometer was submerged within the holding tank, allowing us to introduce the egg case–enclosed embryo into the respirometer with minimal stress. We then removed the respirometer from the tank, attached the lid, and inserted an oxygen probe (Cameron Instrument, E101 Oxygen Electrode, Port Aransas, Tex.). Insertion of the oxygen probe created an airtight seal. The decline in oxygen partial pressure within the chamber over time was measured with an oxygen meter (Cameron Instrument, OM200 Oxygen Meter). Output from the oxygen meter was continuously recorded with a MacLab/2e (ADInstruments, Mountain View, Calif.) and displayed on a PowerMac 7100/80 computer (Apple Computer, Cupertino, Calif.). Before each set of trials for a given day, we calibrated the oxygen electrode using nitrogen-saturated and air-saturated water (0% O_2 and 21% O_2, respectively).

Data were collected continuously throughout each experimental trial, which lasted at least 30 min. We measured respirometer water temperature immediately before and after each trial; these measurements revealed that water temperature within the respirometer remained at 25° ± 1°C. Because embryos within an egg case cannot effectively stir the water surrounding the egg case, we used a magnetic stir bar to slowly mix water outside of the egg case to prevent the formation of oxygen-depleted areas. Following a 5 min adjustment period, the rate of oxygen consumption remained relatively stable during each trial 30+ min, suggesting that the animals were neither oxygen limited nor respiring intermittently. We conducted control trials that were identical to the experiments described above but without an embryo or egg case (preliminary studies revealed that controls with and without empty egg cases produced identical results). These controls determined average background oxygen consumption. For the final analysis, we subtracted any background consumption from animal oxygen consumption.

All embryos included in the analysis were tested at least four times each during development, with the majority tested at least nine times. Six out of the 14 animals were tested from 2 wk postlaying until hatching. Others were tested only during a portion of their developmental period because of animal availability or because an animal completed development at a time when we were not sampling. Oxygen consumption of six recent hatchlings was also measured.

Growth

To determine the growth rates of bamboo shark embryos, it was necessary to know the mass of embryos without their attached yolk sac. Because removing yolk sacs from embryos is terminal, we could not weigh embryos used in the oxygen consumption measurements described above. To generate a curve relating age and body mass, we killed 51 additional embryos at various stages of development. After removal from the egg case, we cut through the vitelline duct to separate the embryo from its yolk sac. We blotted and weighed each animal and compiled these weights to determine the relationship between age and body mass. We also separately weighed the external yolk sac. Embryos used to determine the growth curve came from the same captive population and were maintained under identical conditions as those used in the respirometry measurements.

Data Analysis

Data were analyzed using Excel 5.0, where means are presented as ± SD. We fit curves to our data using DeltaGraph 4.0. We then calculated total metabolic costs by integrating areas under $\dot{V}O_2$ versus age curves with Mathematica. SYSTAT 5.2.1 was used to perform the multiple nonlinear regression analysis.

Results

Development Time and Observations of Embryos

Development time averaged 126 ± 9.2 d (range: 116–144 d; $n = 6$) for embryos that developed within their egg case. In comparison, the developmental time for beaker-reared embryos averaged 118 ± 7.9 d (range: 110–126 d; $n = 4$). Although embryos raised in beakers tended to hatch sooner than individuals within egg capsules, the incubation periods of these two groups did not differ significantly (Student's *t*-test, $P = 0.15$).

Embryonic white-spotted bamboo sharks shifted from respiring with external gills to internal gills. Fourteen-day-old embryos had rudimentary filamentous external gills. These gill filaments typically reached a maximum length at approximately day 24 and then decreased in size until they were no longer visible by about day 65 (i.e., halfway through embryonic development). Embryos generally began buccal pumping by about day 30 of development. Rates of buccal pumping increased rapidly thereafter, reaching an average of 75 breaths per minute at approximately day 65. Embryos continued to respire at this rate until approximately 90 d, after which respiration rates decreased until hatching. The respiration rate of newly hatched sharks was approximately 35 breaths per minute.

Movement by bamboo shark embryos changed qualitatively during development. Embryos younger than 40 d engaged in rapid and sporadic lateral undulations that appeared to be poorly coordinated. By contrast, embryos older than 50 d moved in a more controlled fashion, making rhythmic lateral undulations somewhat similar to the swimming motions of hatchlings. In addition to changes in the form of movement with increasing age, there was also a general decrease in the time spent performing axial movements. Very young embryos undulated frequently, whereas 95 d and older animals undulated very rarely, their movements being limited primarily to buccal pumping. Between these two extremes, the frequency and duration of whole-body undulations declined gradually with age.

Growth

Body mass of developing embryos increased sigmoidally with age and could be adequately described using a logistic growth function (Ackerman 1981*a*; Fig. 1*A*):

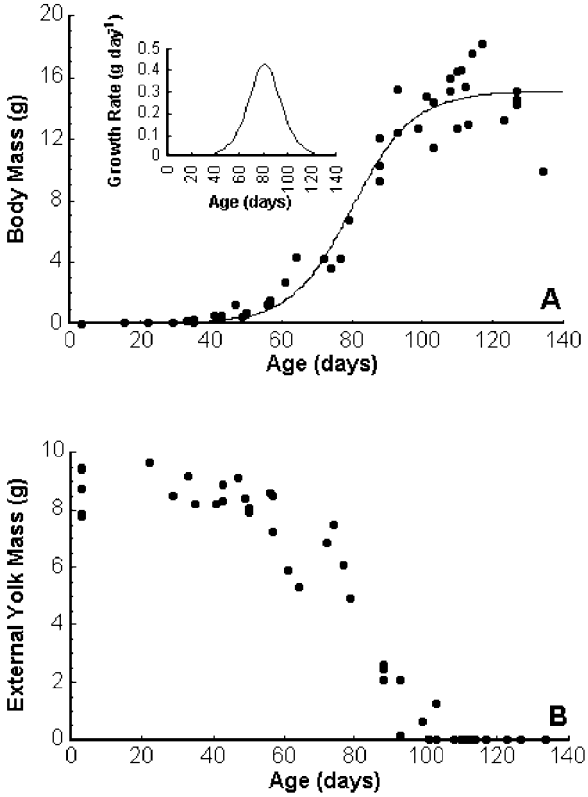

Figure 1. The change in the body mass (*A*) and external yolk mass (*B*) of developing bamboo shark embryos. Body mass is wet mass without external yolk. Body mass increased sigmoidally with embryo age and was accompanied by a decrease in external yolk mass. Each point on the graph represents one individual ($n = 51$). The line in *A* is a logistic curve fit to the data (see eq. [1] in text). The inset in *A* is growth rate in grams per day calculated by dividing the difference in mass by the difference in time for successive ages.

$$\text{body mass} = \frac{15}{\{1 + e^{[-0.12 \times (\text{age} - 80)]}\}} \qquad (1)$$

$$(r^2 = 0.95;\ n = 51).$$

Body mass of newly hatched animals averaged 14.3 ± 1.24 g (range: 12.1–16.4 g; $n = 8$). Growth rates, estimated by dividing the difference in mass by the difference in time for successive ages, peaked on approximately day 80 at about 0.4 g d^{-1} (inset in Fig. 1*A*).

The increase in body mass with age was accompanied by a decrease in external yolk mass (Fig. 1*B*). At laying (day 0), external yolk sacs weighed 8.6 ± 0.36 g ($n = 5$) and were no longer visible around day 100.

Oxygen Consumption and Age

Oxygen consumption of developing bamboo sharks first increased with age until approximately day 85 and then decreased

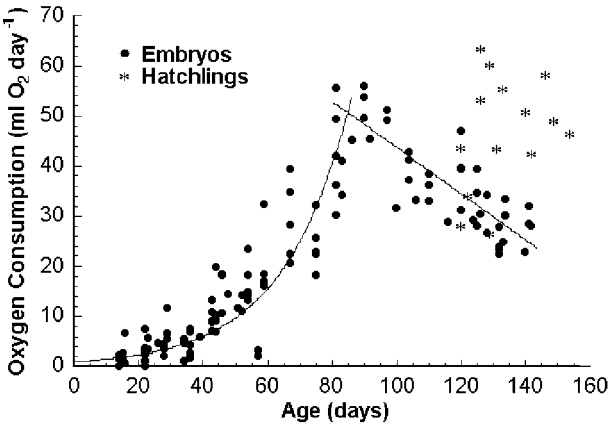

Figure 2. Oxygen consumption of embryonic bamboo sharks during development (*filled circles*; $n = 14$ individuals). Oxygen consumption was measured at 25°C using closed system respirometry. Embryonic oxygen consumption increased exponentially with age until approximately day 85 and then decreased linearly until hatching. Values for recent hatchlings are also shown for comparison (*asterisks*; $n = 6$ individuals). The oxygen consumption of each animal was measured at least four times and each point represents an individual measurement. The lines represent the regression equations fit to the data (see eqq. [2] and [3] in text).

until hatching (Fig. 2). Because of the dynamic nature of the change in $\dot{V}O_2$ with age, we chose to describe the relationship with two different functions. During the first two-thirds of development, oxygen consumption of bamboo shark embryos could be described with an exponential function (Fig. 2):

$$\dot{V}O_2 = 0.85 \times e^{0.05 \times age} \quad (2)$$

($r^2 = 0.60$; $\dot{V}O_2$, mL O_2 d^{-1}; age, days).

After approximately 85 d, $\dot{V}O_2$ decreased linearly until hatching, according to the equation (Fig. 2)

$$\dot{V}O_2 = -0.46 \times (age) + 90 \quad (3)$$

($r^2 = 0.66$; $\dot{V}O_2$, mL O_2 d^{-1}; age, days).

Peak oxygen consumption of individuals averaged 42.0 ± 7.8 mL O_2 d^{-1} (range: 34–55 mL O_2 d^{-1}; $n = 9$). By contrast, oxygen consumption just before hatching averaged 30.4 ± 3.9 mL O_2 d^{-1} (25.1–36.3 mL O_2 d^{-1}). The $\dot{V}O_2$ of hatchlings measured 5–10 d posthatching averaged 42.8 ± 10.9 mL O_2 d^{-1} (27.6–57.9 mL O_2 d^{-1}). Although posthatching $\dot{V}O_2$ values tended to be higher, pre- and posthatching $\dot{V}O_2$ measured in six different individuals were not significantly different (paired *t*-test, $P = 0.08$). Integrating the areas under the curves described by equations (2) and (3) yielded a total oxygen consumption of 3,018 mL O_2 for a 127-d developmental period.

Oxygen Consumption and Body Mass

Oxygen consumption increased with body mass until embryos weighed approximately 11 g. As embryo weight increased beyond 11 g, $\dot{V}O_2$ declined with body mass until hatching. However, analyzing the log-transformed $\dot{V}O_2$ versus mass data revealed essentially one allometric relationship, such that oxygen consumption increased with body mass according to the following equation (Fig. 3):

$$\dot{V}O_2 = 1.14 \times mass^{0.39} \quad (4)$$

($r^2 = 0.69$; $\dot{V}O_2$, mL O_2 d^{-1}; mass, g).

Discussion

This study is the first to look at developmental energetics of a tropical elasmobranch. Overall, bamboo shark embryonic development was similar to that of other oviparous elasmobranchs in terms of gross embryo characteristics and respiratory behaviors (Luer and Gilbert 1985; Diez and Davenport 1987; Ballard et al. 1993). The major difference between development of bamboo sharks and these other elasmobranchs is that bamboo sharks develop in less than half the time required for temperate species (127 vs. >300 d, respectively). This difference in

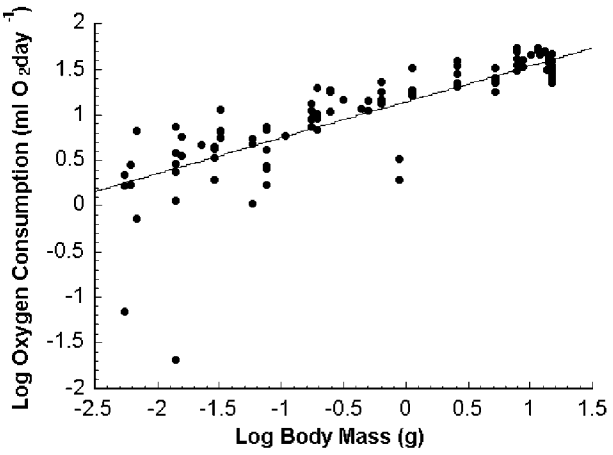

Figure 3. Log-log plot of total oxygen consumed relative to body mass for bamboo shark embryos. Body masses of animals used in the oxygen consumption experiments were predicted from the equation for age and body mass empirically derived in this study (Fig. 1*A*; eq. [1]). Each point represents an individual measurement. The line represents the regression equation fit to the data (see eq. [4] in text).

developmental period is consistent with an approximately 10°C temperature difference between temperate and tropical waters.

Pattern of Oxygen Consumption during Development

The pattern of oxygen consumption in bamboo shark embryos was clearly peaked (Fig. 2). In this respect, ontogeny of metabolism in bamboo sharks is similar to that of ratites (emus, rheas, and ostriches), crocodilians, and many turtles (Vleck et al. 1980*b*; Cannon et al. 1986; Vleck and Vleck 1987; Thompson 1989; Whitehead and Seymour 1990; Leshem et al. 1991; Booth 1998). Embryonic bamboo shark $\dot{V}O_2$ peaked approximately 70% of the way through the developmental period. This is comparable to the timing of the peak $\dot{V}O_2$ of common rhea and emu embryos (Vleck et al. 1980*b*) but somewhat earlier than the peak seen in Darwin's rhea (Cannon et al. 1986) and various turtle (Leshem et al. 1991; Booth 1998) and crocodilian (Thompson 1989; Whitehead and Seymour 1990) embryos. Comparing the metabolic pattern of developing sharks and oviparous tetrapods with that of oviparous teleosts is difficult because many teleosts still possess an external yolk sac on hatching. For example, although rainbow trout, *Salmo gairdneri*, show a peaked metabolic pattern during embryonic development (Rombough 1987), the peak $\dot{V}O_2$ occurs after hatching when the embryo is free-living but still has an external yolk sac.

In contrast to bamboo shark embryos, lesser spotted dogfish embryos show an exponential increase in $\dot{V}O_2$ until hatching (Diez and Davenport 1987) similar to that shown by altricial birds and snakes (Clark 1953; Dmi'el 1970; Vleck et al. 1980*a*; Vleck and Vleck 1987). This difference can be at least partially explained by differences in growth dynamics. Body mass of embryonic bamboo sharks increased sigmoidally, such that maximal growth rate occurred at about 65% of the developmental period (ca. day 80; Fig. 1*A* and inset), coinciding roughly with the timing of peak $\dot{V}O_2$. In the case of the lesser spotted dogfish, however, body mass and $\dot{V}O_2$ increase exponentially with age (Diez and Davenport 1987), following a pattern that is similar to altricial birds and snakes. Parallel changes in metabolism and growth during the embryonic development of birds, reptiles, and elasmobranchs supports the hypothesis that growth costs are a major determinant of the total cost of embryonic development across diverse taxa (Vleck et al. 1980*a*; Hoyt 1987; Vleck and Vleck 1987). In addition, studies on two species of shark (Diez and Davenport 1987; this study) suggest that oviparous elasmobranchs, like oviparous reptiles, exhibit at least two metabolic patterns during embryonic development. It is noteworthy that the different metabolic patterns seen in developing reptiles and sharks are not correlated with altricial or precocial development because all members of these two orders are functionally precocial.

The peaked metabolic pattern of developing precocial birds, crocodilians, and some turtles has been linked to synchronous hatching of the young, with the plateau phase of oxygen consumption representing a "waiting" stage (Vleck et al. 1980*b*; Cannon et al. 1986; Whitehead and Seymour 1990). We did not observe any alterations in developmental period that would have produced synchronous hatching of bamboo sharks. Notably, however, synchronous hatching may occur in the lesser spotted dogfish (Thomason et al. 1996*a*), a species that shows an exponential increase in oxygen consumption during embryonic development.

Although declining growth rate likely accounts for most of the decrease in $\dot{V}O_2$ during the latter part of bamboo shark development, additional factors could be involved. For example, our observations on living embryos revealed that the amount of axial movement decreased with embryo age. Thus, the decline in $\dot{V}O_2$ during the latter stages of development may have been partially caused by a decrease in energy expended for movement. However, lesser spotted dogfish embryos exhibit an exponential increase in oxygen consumption with age even though movement declines as development proceeds (Diez and Davenport 1987; Thomason et al. 1996*b*). Regardless of exactly how movement contributes to total developmental costs, this additional cost (Leonard et al. 1999) sets elasmobranchs apart from birds and reptiles, which do not exhibit whole body ventilatory movements during development. It is somewhat surprising, therefore, that elasmobranch embryos exhibit the same metabolic patterns shown by birds and reptiles despite the added cost of muscular movement.

Oxygen Consumption and Total Cost of Embryonic Development

Bamboo shark embryos consumed 30 mL O_2 d^{-1} (1.3 mL O_2 h^{-1}) just before eclosion (Fig. 2). This value is almost five times greater than the oxygen consumption of lesser spotted dogfish (*Scyliorhinus canicula*) at the same point in development (Diez and Davenport 1987). However, bamboo shark hatchlings are over four times larger than lesser spotted dogfish hatchlings (approximately 14 g vs. 3.3 g, respectively) and develop at temperatures 10°C warmer (15°C for the dogfish and 25°C for the bamboo shark). When expressed on a mass-specific basis and adjusted for temperature, the metabolic rate of bamboo shark embryos is about half that of lesser spotted dogfish (1.4 vs. 3.0 μL O_2 g^{-1} min^{-1}, respectively; $Q_{10} = 2.1$; Butler and Taylor 1975). This difference is consistent with data showing that metabolic rate scales with body mass to the 0.6–0.9 power (Withers 1992).

The $\dot{V}O_2$ of embryonic bamboo sharks just before eclosion is similar to that of comparably sized reptiles (11–16 g) at the same developmental stage. When adjusted for temperature, reptiles consume between 1 and 2 mL O_2 h^{-1} just before hatching compared to 1.3 mL O_2 h^{-1} consumed by the bamboo sharks (snapping turtle, Birchard and Reiber 1995; soft-shelled turtle, Leshem et al. 1991; snakes, *Vipera* and *Spalerosophis*, Dmi'el

1970). Measured values (summarized in Bushnell et al. 1989) and those calculated from allometric equations (Bennett 1982) indicate that similarly sized adult sharks and reptiles also have comparable metabolic rates.

We calculated the total cost of bamboo shark development by integrating oxygen consumption as a function of age from laying to hatching (eqq. [2] and [3]). These calculations yielded a total cost of 3,018 mL O_2, equivalent to approximately 59.3 kJ (using a conversion factor of 19.64 kJ L O_2^{-1}; Hoyt 1987; Schmidt-Nielsen 1990), which is within the values reported for reptiles and birds (Table 1). Dividing the total cost of development by hatchling mass yielded a mass-specific developmental cost of 4.23 kJ g^{-1} wet mass for bamboo shark embryos. This value is much higher than mass-specific costs estimated for most reptiles and for many birds (Table 1). It is also higher than the values reported for lesser spotted dogfish and two teleosts (Table 1).

Avian models of embryonic energetics predict that long incubation times will increase the overall cost of development by increasing maintenance costs (Vleck et al. 1980*a*; Hoyt 1987; Vleck and Vleck 1987). This prediction is supported by studies on both birds and reptiles (Ackerman et al. 1980; Vleck and Kenagy 1980; Vleck et al. 1984; Birchard et al. 1995; Thompson and Stewart 1997). Our results on bamboo sharks, which take substantially longer to develop than do reptiles and birds (Table 1), further support this prediction. Dividing total developmental costs (kJ g hatchling^{-1}) shown in Table 1 by the corresponding incubation period (days) indicates that the daily cost of development (kJ g hatchling^{-1} d^{-1}) for bamboo sharks is about the same as many reptiles and substantially lower than that of all birds examined (Table 1). This comparison reveals two important points related to the developmental energetics of sharks. First, movement costs do not contribute to developmental costs so as to increase the daily cost of development of bamboo sharks relative to birds and reptiles. Second, our results are consistent with observations that long incubation times will lead to higher total costs of development.

Comparisons with the lesser spotted dogfish indicate that the high total developmental cost seen in bamboo shark embryos is not the rule for elasmobranchs. The total cost of development of bamboo sharks was nearly six times higher than the 10 kJ used by the lesser spotted dogfish during embryonic development (Diez and Davenport 1987; Table 1). Dividing total costs by hatchling body masses shows that it costs almost 1.5 times as much to build each gram of bamboo shark hatchling relative to each gram of dogfish hatchling (4.2 vs. 3.0 kJ g hatchling^{-1}, respectively). Lesser spotted dogfish take over twice as long to develop as do bamboo sharks. However, the much larger size and higher incubation temperature of the bamboo shark embryos are likely to overwhelm any contribution from increased incubation time and lead to higher overall developmental costs in bamboo sharks relative to lesser spotted dogfish. Differences in the hatchling size, and therefore cost, between bamboo sharks and lesser spotted dogfish suggest that these two shark species may have distinct reproductive strategies. Additional information on adult female size and laying schedule is needed to evaluate this idea.

Partitioning the Total Cost of Shark Development according to an Avian Model

The current model for avian embryonic energetics partitions developmental costs into growth costs and maintenance costs according to the following equation (Hoyt 1987; Vleck and Vleck 1987):

$$\dot{V}O_2 = A \times \text{mass}^B + C \times \text{growth rate}, \tag{5}$$

where parameter A represents metabolic intensity (mL O_2 g^{-1} d^{-1}), B is the scaling of maintenance costs with body mass, and C is the metabolic cost of growth (mL O_2 g^{-1}). The units of the remaining terms are as follows: $\dot{V}O_2$ (mL O_2 d^{-1}); body mass (g); growth rate (g d^{-1}). To determine if this model can be applied to an anamniote, and to gain insight into how growth and maintenance costs are partitioned in developing sharks, we fit our data to this model with multiple nonlinear regression and our empirically determined relationships. Briefly, we first calculated input values for body mass and $\dot{V}O_2$ using equations (1), (2), and (3). We then used equation (1) to calculate growth rate as a change in mass per unit time for each successive age. Multiple nonlinear regression analysis was then used to fit our values for $\dot{V}O_2$, body mass, and growth rate to the above equation. Results of this analysis yielded the following parameter values: $A = 10$ mL O_2 g^{-1} d^{-1}, $B = 0.48$, and $C = 45.5$ mL O_2 g^{-1}. To compare parameter A to values found for birds and reptiles, we repeated the analysis using embryonic dry mass (Hoyt 1987; Thompson and Stewart 1997) estimated at 27% of wet mass (Diez and Davenport 1987). This manipulation increased parameter A to 18.6 mL O_2 g^{-1} d^{-1} but did not change parameters B or C.

Our results indicate that embryonic sharks have a lower metabolic intensity (parameter A) than do developing birds and reptiles. Parameter A was lower in bamboo sharks (18.6 mL O_2 g^{-1} d^{-1}) than in birds (150–217 mL O_2 g^{-1} d^{-1}) and reptiles (28.91 mL O_2 g^{-1} d^{-1}) subjected to the same analysis (Hoyt 1987; Thompson and Stewart 1997). Even if our calculated metabolic intensity for sharks is corrected for developmental temperature, it is still only 25% of the lowest bird value and 74% of the reptile value. Data on five species of birds indicate that metabolic intensity increases with body mass (Hoyt 1987). Mass-dependence of metabolic intensity would complicate comparison between results because the mass of bamboo shark hatchlings exceeded that of the birds and reptiles for which these data are available. However, the same multiple regression analysis on published data from the lesser spotted dogfish (Diez and Davenport 1987) yielded an even lower value for metabolic

Table 1: Comparison of the embryonic developmental costs of oviparous reptiles, birds, and oviparous sharks

Species	Study Temp. (°C)	Hatchling Mass (g ww)	Incubation Period (d)	Total Oxygen Consumed (mL O_2)	Total Energy Used (kJ)	Mass-Specific Development Costs (kJ g Hatchling^{-1})	Daily Development Costs (kJ g Hatchling^{-1} d^{-1})	Source
Echis colorata	30	6.2	43	445.20	8.74	1.41	.033	Dmi'el 1970
Cerastes cerastes	30	6.5	62	864.50	17.0	2.61	.042	Dmi'el 1970
Vipera xanthina	30	10.7	41	588.80	11.6	1.08	.026	Dmi'el 1970
Spalerosophis cliffordi	30	16.3	60	1,328.50	26.2	1.60	.027	Dmi'el 1970
Natrix tessellata	30	5.1	37	529.90	10.4	2.04	.055	Dmi'el 1970
Python molurus	30	116	68	4,969.4	97.6	.84	.012	Black et al. 1984
Sceloporus virgatus	30	.38	35.7	82.0	1.61	4.24	.119	Vleck and Hoyt 1991
Varanus komodoensis	29	98	235	16,772	329.4	3.36	.014	Birchard et al. 1995
Eumeces anthacinus	27	.24	27	42.8	.8	3.50	.130	Thompson and Stewart 1997
Eumeces fasciatus	27	.285	24.8	46.6	.9	3.21	.130	Thompson and Stewart 1997
Trionyx triunguis	27	11.83	81.8	1,283.00	108.4	2.13	.026	Leshem et al. 1991
Chelydra serpentina	24	7.5	70.4	764.00	15.0	2.00	.028	Birchard and Reiber 1995
Emydura signata	24	5	78	464.00	9.1	1.82	.023	Booth 1998
Caretta caretta	30	18.1	50	1,939.00	107.1	2.10	.042	Ackerman 1981*b*
Chelonia mydas	30	30.8	63	2,739.00	88.9	1.75	.028	Ackerman 1981*b*
Chelonia mydas	30	19.9	65	3,142.00	157.9	3.10	.048	Ackerman 1981*b*
Alligator mississippiensis	30	44	68	5,295.32	59.3	2.99	.012	Thompson 1989; Vleck and Hoyt 1991
Crocodylus johnstoni	30	47.2	91	6,206.80	121.9	2.58	.028	Whitehead and Seymour 1990
Crocodylus porosus	30	74.2	91	9,267.60	182.0	2.45	.027	Whitehead and Seymour 1990
Anser anser	37	88.5	28	12,046.91	236.6	2.67	.095	Romanoff 1967; Vleck et al. 1980*a*
Gallus gallus	37	32	21	4,861.41	95.5	2.98	.142	Romanoff 1967
Sterna maxima	37	42	28	5,351.81	105.1	2.50	.089	Vleck et al. 1980*a*
Casmeridius albus	37	28.4	27	2,750.53	54.0	1.90	.070	Vleck et al. 1980*a*
Bulbulcus ibis	37	14.7	23	1,641.79	32.2	2.19	.095	Vleck et al. 1980*a*
Eudocimus albus	37	27.6	22	2,324.09	45.6	1.65	.075	Vleck et al. 1980*a*
Columbia livia	37	9.7	17	1,364.61	26.8	2.76	.162	Romanoff 1967; Vleck et al. 1980*a*
Melopsittacus undulatus	37	1.44	18	255.86	5.0	3.49	.194	Bucher as in Vleck et al. 1980*a*

Table 1 (*Continued*)

Species	Study Temp (°C)	Hatchling Mass (g ww)	Incubation Period (d)	Total Oxygen Consumed (mL O_2)	Total Energy Used (kJ)	Mass-Specific Development Costs (kJ g Hatchling^{-1})	Daily Development Costs (kJ g Hatchling^{-1} d^{-1})	Source
Parus major	37	1.2	13	213.22	4.2	3.49	.268	Mertens as in Vleck et al. 1980*a*
Troglodytes aedon	37	.83	12	191.90	3.8	4.54	.378	Kendeigh 1940
Paephila guttata	37	.58	14	100.21	2.0	3.39	.242	Vleck et al. 1979
Puffinus pacificus	37	32	52	7,950.00	156.1	4.88	.094	Ackerman et al. 1980
Leipoa ocellata	34	98	62	31,211.81	613	6.26	.101	Vleck et al. 1984
Alectura lathami	34	98	49	24,134.42	474	4.84	.099	Vleck et al. 1984
Gadus morhua	5	.00030	36	.020	.00048	1.65	.046	Davenport and Lonning 1980
Salmo gairdneri	6	.028	107	20.50	.403	1.97	.018	Rombough 1987
Scyliorhinus canicula	15	3.3	300	502.00	10.0	2.99	.010	Diez and Davenport 1987
Chyloscyllium plagiosum	25	14	127	3,018.00	59.3	4.23	.033	This study

Note. Hatchling masses for birds and most reptiles represent yolk-free hatchlings as reported in the literature. The masses given for shark, teleost, *C. johnstoni*, *C. porosus*, *T. triunguis*, and *E. signata* hatchlings include any residual yolk. To express energy use in common units, the following conversion factors were sometimes employed: 1J = 0.239 kcal (Randall et al. 1997) and 1 L O_2 = 19.64 kJ (Hoyt 1987). If only dry mass for reptiles or birds was provided in the original article, wet mass was calculated as 4 × dry mass and 5 × dry mass, respectively. Temperature for bird development averaged from data compiled in Zonneveld and Kooijman (1993). ww = wet weight.

intensity than that obtained for the bamboo shark (analysis not shown), suggesting that developing sharks do have a lower metabolic intensity than reptiles and birds. The greater metabolic intensity of amniotes may result from additional energy spent on maintenance of extraembryonic membranes. The much higher metabolic intensity of avian embryos relative to shark and reptile embryos may be due to the suite of developing physiological features that distinguish endotherms from ectotherms. Determining the physiological basis for the differences in metabolic intensity requires additional cellular and biochemical studies on developing sharks, reptiles, and birds.

Maintenance metabolism of embryonic bamboo sharks scaled with body mass to the 0.48 power (parameter *B* in eq. [5]), a value which falls within the range reported for developing birds (0.45–0.55; Hoyt 1987) but is lower than that reported for a developing reptile (0.77; Thompson and Stewart 1997). The value of 0.48 is larger than the factor of 0.39 found for the scaling of total metabolic rate with body mass (eq. [4]). If the scaling relationship is determined for bamboo shark embryos weighing ≤11 g (i.e., while $\dot{V}O_2$ is still increasing with body mass; see "Results"), the exponent increases to 0.45. It has been argued that maintenance costs of embryos should scale isometrically with body mass (scaling factor of 1), even though the resting metabolic rate of adult animals scales, on average, with the 0.7 power of body mass (Brody 1945 in Vleck et al. 1980*a*). This argument is based on observations that percent body water decreases and muscular movement increases during development (Vleck et al. 1980*a*), factors which would tend to increase the 0.7 scaling exponent of adult maintenance costs. However, data on various birds, reptiles, and now bamboo sharks do not support the hypothesis that embryonic maintenance costs are directly proportional to body mass.

According to our analysis, the cost of growth in bamboo shark embryos (parameter *C*) was 45.5 mL O_2 g^{-1}, which is lower than values found for a reptile (68 mL O_2 g^{-1}; Thompson and Stewart 1997) and for various birds (ranging from 89 to 284 mL O_2 g^{-1}; Hoyt 1987), suggesting that the cost of growth may be lower in elasmobranchs than in oviparous amniotes. The higher growth costs in birds and reptiles may be partly due to the synthesis of extraembryonic membranes (Ar et al. 1987; Dietz et al. 1998).

Using our values for parameters *A*, *B*, and *C* and equation (5), we determined total developmental costs and the total amount of energy devoted to growth and maintenance during bamboo shark development (Fig. 4). Integrating under the growth and maintenance curves revealed that bamboo shark embryos used about 2,157 mL O_2 for maintenance and 679 mL O_2 for growth, yielding a total cost of development of 2,836 mL O_2. This value is only 6% lower than the 3,018 mL O_2 calculated from our empirically derived oxygen consumption versus age equations. Dividing the individual costs by the total cost indicates that developing bamboo sharks use 76% of total

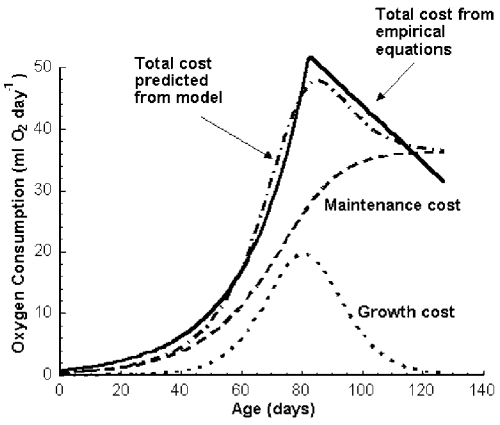

Figure 4. The cost of embryonic development in bamboo sharks. The solid line represents the cost predicted from empirical equations in the present study (eqq. [2] and [3]). The remaining three curves are costs predicted using an avian model of embryonic energetics (Hoyt 1987). The dotted-dashed line shows the total cost predicted from the avian model, the dashed line represents predicted maintenance costs, and the dotted line represents predicted growth costs. We obtained these three later curves by using multiple nonlinear regression to fit our data to the avian model.

energy for maintenance and 24% for growth. These percentages are within the ranges for avian embryos (70%–80% of total costs on maintenance and the remainder on growth; Hoyt 1987) but differs from that of a small lizard (90% for maintenance and 10% for growth; Thompson and Stewart 1997). Data from three physiologically different vertebrates classes, an anamniote, an ectothermic amniote, and an endothermic amniote, suggest that maintenance costs greatly exceed growth costs during embryonic development.

In summary, tropical white-spotted bamboo sharks showed a peaked precocial pattern of oxygen consumption during embryonic development much like that exhibited by some precocial birds, crocodilians, and some turtles. The total cost of development was high in bamboo sharks as compared to reptiles and many birds. However, the relatively long incubation times of the sharks adequately explain the high developmental cost. We have also demonstrated that the ontogeny of metabolism in bamboo sharks can be adequately described by an avian model of developmental energetics, despite the additional cost associated with ventilatory movements.

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Literature Cited

Ackerman R.A. 1981*a*. Growth and gas exchange of embryonic sea turtles (*Chelonia*, *Caretta*). Copeia 1981:757–765.

———. 1981*b*. Oxygen consumption by sea turtle (*Chelonia*, *Caretta*) eggs during development. Physiol Zool 54:316–324.

Ackerman R.A., G.C. Whittow, C.V. Paganelli, and T.N. Pettit. 1980. Oxygen consumption, gas exchange, and growth of embryonic wedge-tailed shearwaters (*Puffinus pacificus chlororhynchus*). Physiol Zool 53:210–221.

Ar A., H. Girard, and P. Dejours. 1987. Oxygen consumption of the chick embryo's respiratory organ, the chorioallantoic membrane. Respir Physiol 68:377–388.

Ballard W.W., J. Mellinger, and H. Lechenault. 1993. A series of normal stages for development of *Scyliorhinus canicula*, the lesser spotted dogfish (Chondrichthyes: Scyliorhinidae). J Exp Zool 267:318–336.

Bennett A.F. 1982. The energetics of reptilian activity. Pp. 155–199 in C. Gans and F.H. Pough, eds. Biology of the Reptilia. Vol. 13. Academic Press, New York.

Birchard G.F. and C.L. Reiber. 1995. Growth, metabolism, and chorioallantoic vascular density of developing snapping turtles (*Chelydra serpentina*): influence of temperature. Physiol Zool 68:799–811.

Birchard G.F., T. Walsh, R. Rosscoe, and C.L. Reiber. 1995. Oxygen uptake by Komodo dragon (*Varanus komodoensis*) eggs: the energetics of prolonged development in a reptile. Physiol Zool 68:622–633.

Black C.B., G.F. Birchard, G.W. Schuett, and V.D. Black. 1984. Influence of incubation water content on oxygen uptake in embryos of the Burmese python (*Python molurus biovittatus*). Pp. 137–145 in R.S. Seymour, ed. Respiration and Metabolism of Embryonic Vertebrates. Junk, Dordrecht.

Booth D.T. 1998. Incubation of turtle eggs at different temperatures: do embryos compensate for temperature during development? Physiol Zool 71:23–26.

Bushnell P.G., P.L. Lutz, and S.H. Gruber. 1989. The metabolic rate of an active, tropical elasmobranch, the lemon shark (*Negaprion brevirostris*). Exp Biol 48:279–283.

Butler P.J. and E.W. Taylor. 1975. The effect of progressive hypoxia on respiration in the dogfish (*Scyliorhinus canicula*) at different seasonal temperatures. J Exp Biol 63:117–130.

Cannon M.E., R.E. Carpenter, and R.A. Ackerman. 1986. Synchronous hatching and oxygen consumption of Darwin's rhea eggs (*Pterocnemia pennata*). Physiol Zool 59:95–108.

Clark H. 1953. Metabolism of the black snake embryo. II. Respiratory exchange. J Exp Biol 30:502–505.

Davenport J. and S. Lonning. 1980. Oxygen consumption in developing eggs and larvae of the cod, *Gadus morhua* L. J Fish Biol 16:249–256.

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

Diez J.M. and J. Davenport. 1987. Embryonic respiration in the dogfish (*Scyliorhinus canicula* L.). J Mar Biol Assoc UK 67:249–261.

Dmi'el R. 1970. Growth and metabolism in snake embryos. J Embryol Exp Morphol 23:761–772.

Dulvy N.K. and J.D. Reynolds. 1997. Evolutionary transitions among egg-laying, live-bearing and maternal inputs in sharks and rays. Proc R Soc Lond Ser B Biol Sci 264:1309–1315.

Hoyt D.F. 1987. A new model for avian embryonic energetics. J Exp Zool 1(suppl.):127–138.

Kendeigh S.C. 1940. Factors affecting length of incubation. Auk 57:499–513.

Koob T.J. and A. Summers. 1996. On the hydrodynamic shape of little skate (*Raja erinacea*) egg capsules. Bull Mt Desert Isl Biol Lab 35:108–111.

Leonard J.B.K., A.P. Summers, and T.J. Koob. 1999. Metabolic rate of embryonic little skate, *Raja erinacea* (Chondrichthyes: Batoidea): the cost of active pumping. J Exp Zool 283:13–18.

Leshem A., A. Ar, and R.A. Ackerman. 1991. Growth, water, and energy metabolism of the soft-shelled turtle (*Trionyx triunguis*) embryo: effects of temperature. Physiol Zool 64: 568–594.

Luer C.A. and P.W. Gilbert. 1985. Mating behavior, egg deposition, incubation period, and hatching in the clearnose skate, *Raja eglanteria*. Environ Biol Fishes 13:161–171.

Randall D., W. Burggren, and K. French. 1997. Eckert Animal Physiology: Mechanisms and Adaptations. W. H. Freeman, New York.

Romanoff A.L. 1967. Biochemistry of the avian embryo. Wiley, New York.

Rombough P.J. 1987. Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead, *Salmo gairdneri*. Can J Zool 66:651–660.

Schmidt-Nielsen K. 1990. Animal Physiology: Adaptations and Environment. Cambridge University Press, Cambridge.

Thomason J.C., W. Conn, E. Le Comte, and J. Davenport. 1996*a*. Effect of temperature and photoperiod on the growth of the embryonic dogfish, *Scyliorhinus canicula*. J Fish Biol 49:739–742.

Thomason J.C., J. Davenport, and E. Le Comte. 1996*b*. Ventilatory mechanisms and the effect of hypoxia and temperature on the embryonic lesser spotted dogfish. J Fish Biol 49:965–972.

Thompson M.B. 1989. Patterns of metabolism in embryonic reptiles. Respir Physiol 76:243–256.

Thompson M.B. and J.R. Stewart. 1997. Embryonic metabolism

and growth in lizards of the genus *Eumeces*. Comp Biochem Physiol A 118:647–654.

Tullis A. and G.M. Peterson. 1998. Developmental changes in the metabolism of embryonic bamboo sharks, *Chiloscyllium plagiosum*. Am Zool 37:121. (Abstr.)

Vleck C.M. and D.F. Hoyt. 1991. Metabolism and energetics of reptilian and avian embryos. Pp. 285–306 in D.C. Demming and M.W. Ferguson, eds. Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles. Cambridge University Press, Cambridge.

Vleck C.M., D.F. Hoyt, and D. Vleck. 1979. Metabolism of avian embryos: patterns in altricial and precocial birds. Physiol Zool 52:363–377.

Vleck C.M. and G.J. Kenagy. 1980. Embryonic metabolism of the fork-tailed storm-petrel: physiological patterns during prolonged and interrupted incubation. Physiol Zool 53: 32–42.

Vleck C.M. and D. Vleck. 1987. Metabolism and energetics of avian embryos. J Exp Zool 1(suppl.):111–125.

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

Vleck D., C.M. Vleck, and D.F. Hoyt. 1980*b*. Metabolism of avian embryos: ontogeny of oxygen consumption in the rhea and emu. Physiol Zool 53:125–135.

Vleck D., C.M. Vleck, and R.S. Seymour. 1984. Energetics of embryonic development in the megapode birds, mallee fowl *Leipoa ocellata* and brush turkey *Alectura lathami*. Physiol Zool 57:444–456.

Whitehead P.J. and R.S. Seymour. 1990. Patterns of metabolic rate in embryonic crocodilians *Crocodylus johnstoni* and *Crocodylus porosus*. Physiol Zool 63:334–352.

Withers P.C. 1992. Comparative Animal Physiology. Saunders, Fort Worth, Tex.

Zonneveld C. and S.A.L.M. Kooijman. 1993. Comparative kinetics of embryo development. Bull Math Biol 55:609–635.