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Hatch or wait? A dilemma in reptilian incubation

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Animals often form groups to reduce the risk of predation through the per capita dilution of their individual predation risk. The advantages of grouping also influence the timing of reproduction in many species. In particular, synchrony in the timing of births may have evolved as a predator-avoidance strategy as it dilutes the risk of predation upon vulnerable newborn and naive young. Eggs of an Australian freshwater turtle, *Emydura macquarii*, can hatch synchronously despite developmental asynchrony among eggs of a clutch and hatchlings have a reduced predation risk by emerging from the nest as a group. Developmental asynchrony within clutches was induced to reflect natural nests by dividing clutches and incubating them at either 25°C or 30°C. Some eggs were then reunited with their clutch-mates and hatching occurred synchronously in some of these groups. In groups where synchronous hatching did not occur, less advanced eggs still hatched earlier than the normal incubation period. Synchrony occurred because the less advanced eggs hatched up to five days earlier than the control embryos. We conclude that the less advanced embryos within a clutch either accelerate their development or hatch prematurely to ensure synchrony of hatching and hatchling group formation may facilitate emergence from the nest and dilute predation risk.

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Juvenile turtles usually emerge from a nest together, despite the high probability of different rates of development within single nests of turtles (Gyuris 1993). Development of embryonic turtles is more rapid at warmer than at cooler temperatures within the normal range of incubation temperatures (Thompson 1997) and the temperature of incubation in nature depends, in part, on the position of eggs within a nest (Maloney et al. 1990). Chelonian nest structure is consistent across many species, with eggs deposited in more than one layer in a flask-shaped, air-filled nest chamber (Packard et al. 1993). The vertical distance between the eggs at the top of the nest and those at the bottom results in different temperatures of incubation and hence different rates of development (Thompson 1988a). The influence of different rates of development on incubation period is inconsistent with neonates emerging from the nest as groups, unless hatchlings remain in the nest for a period of time or eggs within a clutch hatch synchronously regardless of development rate. In this paper we address this inconsistency by investigating mechanisms of hatching synchrony.

Synchronous hatching and group emergence of young turtles should have several selective advantages. For example, the survivorship of neonatal sea turtles is likely to be higher if more than ten attempt to dig to the surface at one time (Carr and Hirth 1961). Additionally, predation on young turtles is very high (Herman et al. 1995), so the emergence of many young turtles may swamp predators (Arnold and Wassersug 1978) because the emergence of more neonates decreases the chance of predation through the per capita dilution of individual predation risk (Dehn 1990). Indeed, synchrony in the timing of births has evolved as a predator-avoidance strategy in many species (O'Donoghue and Boutin 1995).

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We used the Australian pleurodiran turtle, *Emydura macquarii*, as a model to investigate possible hatching synchrony in turtles because the effects of temperature on incubation period (Thompson 1997) and temperature differentials within natural nests (Thompson 1989) are well known. Eggs at the top of a nest of *E. macquarii* may be 6°C warmer than the eggs at the bottom of the same nest, yet emergence of hatchlings is synchronous (Thompson 1989). Thus, we postulated that, for hatching to occur synchronously, 1) warmer, more advanced eggs must delay emergence or slow their developmental rate, or 2) cooler, less advanced eggs speed their rate of development or hatch at a developmentally earlier stage than their sibs. We refer to these two possibilities as the waiting and catch-up hypotheses, respectively, and in nature, both mechanisms could operate. Possible advantages of synchronous hatching and group formation are also discussed.

Methods

Developmental temperature and hatching synchrony

Female *Emydura macquarii* were captured using funnel traps (Legler 1960) in a lagoon near to Albury (146° 90′ E, 36° 90′ S), New South Wales. Between 24 October and 8 November 1997 turtles were held in large water-filled, holding drums in a protected shed, and fed twice a week with yabbies (*Cherax destructor*) and European carp (*Cyprinus carpio*). Water was cleaned within 24 h of feeding. At the end of the trapping period, female turtles were given a subcutaneous intramuscular injection of 2 ml of oxytocin (Syntocin, Ilium™) in the thigh (Ewert and Legler 1978) and placed in enclosed cardboard containers. Most females began to oviposit within 30 min. Eggs were uniquely marked using a HB graphite pencil and placed into a mixture of two parts vermiculite to one part water by weight in foam containers (1000 mm × 400 mm × 350 mm).

Turtle eggs were transported to the University of Sydney within 24 h of collection. Twelve eggs were selected randomly from each clutch. Six eggs were placed in two rows, so that eggs were not in contact with each other, in two plastic containers (75 mm × 200 mm × 55 mm). Eggs were incubated in vermiculite approximating −370 kPa on the basis of similar vermiculite calibrated using thermocouple psychrometry and a Wescon C52 sample chamber connected to a Wescon KR33T micro voltmeter. Distilled water was added twice weekly to maintain water potential.

A total of 24 clutches and 288 eggs were used in two experimental and two control groups that contained six replicate clutches. For both experimental groups (Fig. 1) clutches were evenly divided into two separate containers. Each half clutch was either incubated at 25°C or 30°C for one week to establish developmental asynchrony. After a week, the eggs that were held at 30°C in the first experimental group were removed from their containers and placed next to the eggs from the same clutch that were held at 25°C (Fig. 1a). The container was then re-weighed before being incubated at 25°C until hatching. Conversely the eggs held at 25°C in the second experimental group (Fig. 1b) were removed from their containers and placed next to the eggs from the same clutch that was held at 30°C, and the clutch was incubated at 30°C until hatching.

The control groups (Fig. 2) were initially treated the same as the experimental groups, but the entire clutch was held at the same temperature (each half in separate containers) for the first week of incubation, either at 25°C (control group one) or at 30°C (control group two). The eggs from one of the containers were then placed next to the eggs from the same clutch in the other container and held at the same initial temperature (either 25°C or 30°C) until the end of the incubation period.

From day 40 of the incubation period, containers were checked at 09:00 and 18:00 each day for hatchlings. The time of pipping (initial breaking of the egg

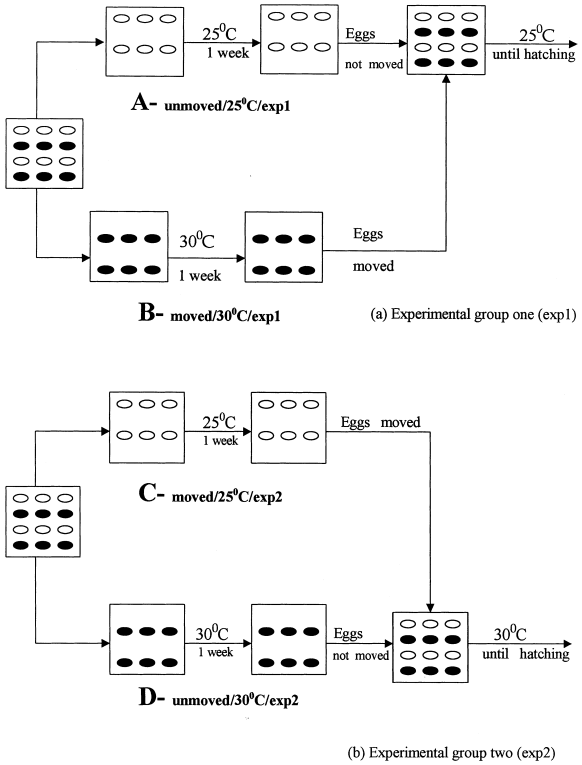

Fig. 1. Experimental design (a) Experimental group one; eggs from a clutch were incubated at either 25°C or 30°C for a week, and then held together at 25°C until completion. (b) Experimental group two: a similar design to (a); however, the clutch was held together at 30°C until completion.

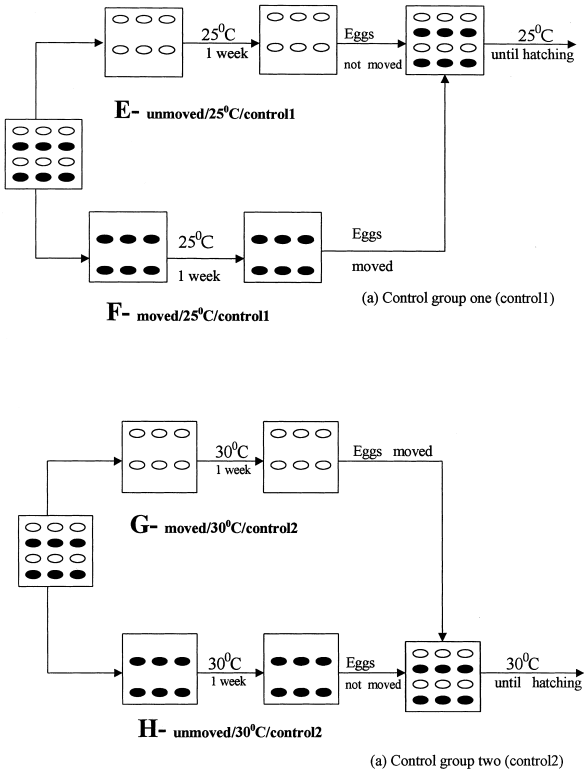

Fig. 2. Experimental design (a) Control group one; eggs from a clutch were incubated separately at 25°C for a week, and then held together at 25°C until completion. (b) Control group two: a similar design to (a); however, the clutch initially incubated separately at 30°C and then held together at 30°C until completion.

shell by the hatchling), as well as the time of hatching were recorded, and emerged hatchlings were examined for an external yolk sac and then removed from the containers. For statistical analyses, the time until emergence from the egg constituted the incubation period.

There are three factors in this experimental design; initial incubation temperature (either 25°C or 30°C), movement of eggs (i.e. whether an egg was left in the same container or placed into a different container after the initial week of incubation) and developmental stage of the neighboring egg (i.e. different in development as in experimental groups or the same as in control groups). A general linear model was used to test whether movement affects incubation time, whether clutches in the experimental groups hatched synchronously, and the mechanisms of synchronous hatching (catch-up or waiting). We then analyzed the response variables individually using independent paired t-tests.

For synchrony to occur, eggs within a clutch with different developmental stages should have similar incubation periods (experimental groups). The mechanism by which synchrony occurs (catching up or waiting), will be revealed by comparing the incubation periods of eggs maintained at the same temperature throughout the experiments, but with eggs placed next to them of either different or the same developmental stage. The catch-up hypothesis predicts that, from experiment 1 (Fig. 1a), less developed eggs (at 25°C) with advanced eggs (at 30°C) placed next to them will hatch prematurely and have shorter incubation periods than all other eggs at 25°C either moved or unmoved (Fig. 1a).

In contrast, the waiting hypothesis predicts that advanced eggs (incubated at 30°C), from experimental group two, with less advanced eggs (25°C) placed next to them (Fig. 1b) should hatch significantly later than all other eggs incubated at 30°C. The experimental group would retard development or hatching until less advanced embryos reach hatching stage.

Emydura macquarii embryos do not compensate for changes in incubation temperature (Booth 1998), thus embryos with altered temperature regimes in the experimental groups will have different incubation periods to their sibs (incubated at a constant temperature) if they were incubated separately. Also, sex determination in *E. macquarii* is not temperature dependent (TSD) but genetically determined (Thompson 1988b), thereby excluding differential incubation periods between hot and cold temperature regimes due to sex effects.

Group size and nest emergence

A further 88 eggs collected in October 1998 were held at 30°C for the duration of incubation. In January 1998, the hatchlings from all clutches were randomly allocated into two group sizes of either one or ten animals, and placed in previously excavated turtle nests, between 10 m and 20 m from the water located around two lagoons near Albury, NSW. At each lagoon, four groups of ten turtles and four individuals were placed in nests. Hatchlings were buried with surrounding soil to simulate an actual nest of recently hatched turtles. Nests were observed between 20 m and 50 m from the nest using binoculars (Tasco 10 × 21) or a night vision monocular (Starlazer). Time until emergence of the first turtle was recorded. An ANOVA was used to test for differences in emergence times between groups and individuals.

Results

Hatching synchrony and developmental temperature

Main treatment effects of initial temperature, movement of the eggs and treatment group and each interaction terms were highly significant (Table 1). Moved and

unmoved eggs within control groups did not differ in their incubation periods suggesting movement alone did not influence hatching times. To test for synchrony and its causes, we were concerned with the interactions of particular treatments and not the main effects per se, on incubation times. For synchrony to occur we tested the hypotheses that the incubation period of eggs in the first and second experimental groups were not significantly different.

H_a: unmoved/25°C/exp1 = moved/30°C/exp1 (A vs B; Fig. 1)
H_a: unmoved/30°C/exp2 = moved/25°C/exp2 (C vs D; Fig. 1)

Paired *t*-tests showed that synchronous hatching occurred in the second experimental group (Fig. 1b) where less developed eggs were moved next to advanced eggs and development proceeded at 30°C ($t_5 = 0.72$, $p = 0.50$). However, advanced eggs moved next to less advanced eggs at 25°C, hatched significantly earlier than their less advanced sibs in the same nest ($t_5 = 8.86$, $p < 0.001$; Fig. 3a).

Nevertheless, there were indications that synchronous hatching may occur as predicted by the catch-up hypothesis.

H_a: unmoved/25°C/exp1 < unmoved/25°C/control1 (A vs E; Figs 1, 2)

Eggs incubated at 25°C with advanced sibs next to them (experimental group one) hatched up to 5 d earlier than eggs kept at 25°C with sibs of the same development next to them (control group one) ($t_5 = 4.99$, $p < 0.001$; Fig. 3a). In contrast, predictions from waiting hypothesis were not supported; the incubation periods of eggs at 30°C with less advanced sibs (from the second experimental group) were the same as eggs at 30°C with sibs of the same development history ($t_5 = 1.14$, $p = 0.28$; Fig. 3b). These results suggest that there is no delay in time of hatching in the presence of less developed eggs.

Hatchlings in groups emerged from the nest on average 13 min earlier than individuals ($F_{1,15} = 15.09$, $p = 0.002$), and on all occasions at least seven hatchlings emerged from the nest within 30 s of the first hatchling to emerge.

Discussion

During our manipulations of embryonic developmental stages within a nest, hatching synchrony occurred only in nests where less advanced eggs were moved to the higher temperature and next to more advanced sibs. Nevertheless, the presence of more advanced embryos (initially incubated at 30°C), appeared to stimulate the less advanced eggs (in experimental group 1) to hatch earlier than the eggs at the same stage in the control groups (Fig. 3a), suggesting they were catching up to their more advanced sibs. Failure to hatch synchronously may have occurred because our temperature regimes meant these less advanced eggs were still too premature to hatch when the advanced eggs hatched despite the stimulus to hatch early from the hatching of advanced eggs. Nevertheless, that they hatched significantly earlier than the controls suggests that their development may have been accelerated by the presence of advanced eggs earlier in incubation. Similarly, eggs of bobwhite quail (*Colinus virginianus*) hatch early when placed next to more advanced eggs, but the more advanced quail eggs are also held back by the least developed eggs (Vince 1968). However, we found no evidence of embryos delaying hatching (waiting hypothesis) as more advanced eggs with less developed eggs placed next to them did not hatch earlier than the control group eggs.

Neonatal reptiles that hatch early often have incompletely internalized yolk sacs (Packard and Packard 1988). Surprisingly, there were no differences in the number of hatchlings with external yolk sacs in any of our experimental groups. Thus, despite the imposition of shorter incubation periods, embryonic development seems to have been accelerated rather than neonates hatching prematurely. Under-developed hatchlings that emerge early may be disadvantaged, in terms of agility and performance, compared to the more developed turtles, and hence any benefits of group emergence could be outweighed. For example, Japanese quail chicks (*Coturnix coturnix japonica*) with accelerated development stand 1–2 h later than normal chicks (Vince and Chinn 1971). If the nervous system of early hatching turtle embryos is similarly less developed than full-term hatchlings, their relative co-ordination and fitness compared to the full-term embryos may be re-

Table 1. Results from the general linear model.

	Degrees of freedom	*F*-value	*p*-value
Treatment (Experimental and Control)	1	143	<0.001
Movement	1	14	<0.001
Initial temperature	1	2162	<0.001
Treatment × Movement	1	19	<0.001
Treatment × Initial temperature	1	1454	<0.001
Movement × Initial temperature	1	938	<0.001
Treatment × Movement × Initial temperature	1	984	<0.001
Error	40		

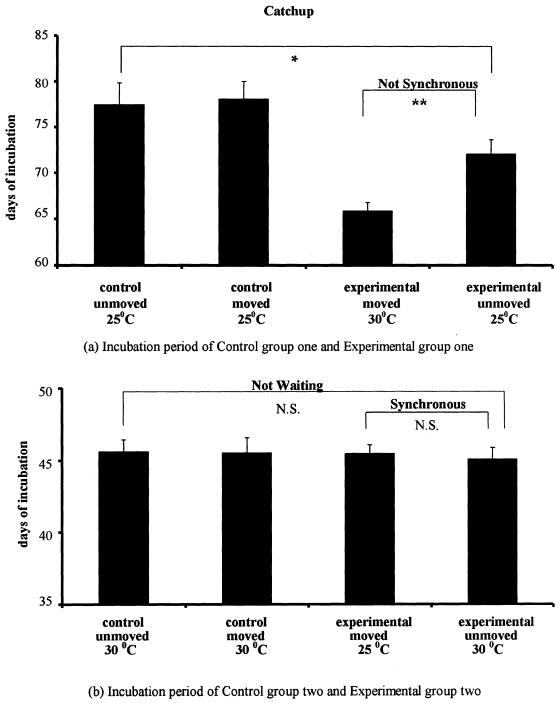

Fig. 3. (a) Incubation periods of eggs in control and experimental group one. Synchronous hatching did not occur; however, less advanced eggs hatched significantly earlier than its control, supporting the 'catch-up' hypothesis. (b) Incubation periods of eggs in control and experimental group two. Synchronous hatching occurred in experimental group two but the more advanced eggs were not delaying hatching, thus rejecting the 'waiting' hypothesis.

duced and the risk of predation to both land and aquatic predators could be greater. Thus, there may be a trade-off between the advantages of synchronous hatching and possible disadvantages of premature hatching in some eggs, which warrants further investigation.

It is possible, however, that the less advanced embryos hatched prematurely without trading off development. Oxygen consumption of embryonic *E. macquarii* increases to a peak during development followed by a decline of up to 25% from peak consumption (Thompson 1989). A similar pattern of oxygen consumption occurs in ratite birds where embryonic growth is essentially complete at the peak of oxygen consumption, several days before hatching (Vleck et al. 1979). The less advanced embryos within a clutch shorten or eliminate the phase of development after the metabolic peak and hatch at the same time as more advanced embryos without trading off development (Vleck et al. 1979). Regardless of whether there are disadvantages to early hatching by some eggs in a clutch, the occurrence of hatching synchrony in clutches of *E. macquarii* suggests that there is some advantage.

Synchronous hatching of common rhea (*Rhea americana*) eggs occurs when later-laid eggs are in contact with earlier-laid eggs throughout the incubation period and some event late in incubation, possibly acoustic signals between eggs, allows early hatching of later-laid eggs (Faust 1960). However, there are no reports of vocalisation in turtles and the eggs in this study were not in contact with each other, but the auditory sounds from physically pipping the shell may stimulate synchronous hatching. Very little is known about the stimuli of synchronous hatching, but the decelerating embryonic metabolic rate late in development may signal to less advanced eggs, through a combination of changes in oxygen consumption, carbon dioxide production, and heart rate, that hatching is imminent. Three patterns of acceleration of the heart rate are unique to the external pipping period of avian embryos: irregular intermittent large accelerations, short-term repeated large accelerations and relatively long-lasting cyclic small accelerations (Tazawa et al. 1999). Mean heart rate during pipping is also maximal for many birds and is usually higher than that of hatchlings (Pearson and Tazawa 1999) and perhaps the early turtle embryos are stimulated to hatch because of fluctuating or increasing heart rates of turtles beginning to pip their egg shell. However, the mechanisms of synchronous and asynchronous hatching are far from resolved and much further research is required.

The origin for such behavior is difficult to determine; however, pressure from predation can alter the timing of reproductive events such as birth (O'Donoghue and Boutin 1995). Emergence from the nest as a large group could swamp and confuse predators or simply dilute an individual's risk of predation amongst other members of the group. Hatchling sea turtles (*Caretta caretta*) exhibit a novel terrestrial locomotor behavior, the hatchling frenzy, characterized by rapid movement from nest to surf (Dial 1987). The hatchling frenzy in *C. caretta* may have evolved to reduce their exposure time to predators while on the beach or confuse potential predators. Conversely, a large group of turtles emerging from a nest could attract predators that would have otherwise been unaware of the nest had hatchlings emerged individually.

We found that hatchlings in groups emerge from the nests much quicker than individuals, and preliminary results suggest that hatchlings in groups also have a reduced risk of predation to common avian predators. Six Australian magpies (*Gymnorhina tibicen*) attacked hatchlings emerging from nests and each bird had difficulty swallowing hatchlings. Most birds could only process one turtle before the remaining members of the group completed the journey to the lagoon, hence hatchlings may have a small degree of defense to common avian predators, at least until other turtles from the nest have reached the water. Similarly large-mouth bass (*Micropterus salmoides*) reject red-eared sliders (*Trachemys scripta*) and painted turtles (*Chrysemys*

picta) because the hatchlings claw and bite the gill apparatus or digestive tract of bass (Britson and Gutzke 1993).

Synchronous hatching facilitates quicker group emergence from the nest, which in turn can reduce the predation risk of an individual via the dilution effect (Dehn 1990). Emerging as a group can offset any disadvantages, such as reduced agility that a hatchling may incur by hatching early. Predation risk may not be diluted evenly amongst the group as, compared to the more advanced hatchlings, the increased predation risk due to reduced agility and a greater time spent on land by early hatchlings could be real. However, the likelihood of a fellow group member hatching at an earlier stage is great, and if a predator, such as the magpie, can handle only one hatchling per group, emerging from the nest with both stronger and weaker individuals will still reduce predation risk compared to emerging individually.

Emydura macquarii eggs are usually laid into a clay or soil base that becomes dense and compacted throughout the incubation period and successful emergence from the nest by hawksbill sea turtles (*Eretmochelys imbricata*) decreases with increasing soil compaction (Horrocks and Scott 1991). Synchronous hatching may facilitate quicker emergence from the nest as hatchlings in groups emerged from the nests significantly earlier than individuals. The selective advantages of behavioral traits are difficult to determine, as both the cause and effect of a particular trait can be mutually exclusive. For example, it is possible that synchronous hatching may have evolved to facilitate quicker emergence because hatchlings in groups emerged from the nests significantly earlier than individuals, but it may also dilute an individual's predation risk to avian predators, and vice versa.

Our data strongly support the catch-up hypothesis, whereby less advanced embryos either increase their developmental rate or hatch significantly earlier than normal in the presence of more advanced eggs, and also support the hypotheses that *E. macquarii* hatchlings emerge from nests quicker in groups. Synchronous hatching facilitates group emergence, which may reduce individual predation by the 'dilution' effect.

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