Mechanism and cost of synchronous hatching

Paul L. Colbert¹, Ricky-John Spencer*² and Fredric J. Janzen¹

¹Department of Ecology, Evolution, and Organismal Biology, Iowa State University, 253 Bessey, Ames, Iowa 50011, USA; and ²Native and Pest Animal Unit, School of Natural Sciences, College of Health & Science, University of Western Sydney, Locked Bag 1797, Penrith South DC, New South Wales 1797, Australia

Summary

1. Synchrony in the timing of births is thought to have evolved as a general predator avoidance strategy. In turtles, synchronous hatching may facilitate group emergence from the nest, which in turn, may limit predation by diluting an individual’s risk of predation or by simply swamping predators upon emergence. However, synchronizing hatching should not be easily achieved in natural nests because of thermal gradients affecting developmental rates.

2. We evaluated pipping synchrony in the painted turtle (Chrysemys picta), where the drive to hatch synchronously may be reduced, because in many populations, hatching and emergence are dissociated through hatchlings overwintering within a nest.

3. We also assessed developmental mechanisms through which synchrony occurs and explored potential trade-offs between pipping synchrony and individual fitness by determining potential short and long-term neuromuscular developmental costs to hatching prematurely. These data were also used to develop a theoretical model to determine how differential embryonic maturation rates affect hatching synchrony.

4. Underdeveloped embryos pipped much earlier than expected and at similar times to their more advanced sibs. In addition, a trade-off between hatching synchronously and neuromuscular development (motility) was evident several days after hatching and up to 9 months later, after the overwintering period.

5. Synchronous hatching is an ancestral trait in turtles, but its relevance today is not solely for predator avoidance in all species.

6. Abiotic factors during incubation (e.g. temperature regime and moisture) have long-term effects on reptiles during ontogeny but it is also clear that incubation behaviour is major factor for the persistence of these developmental costs.

Key-words: Chrysemys picta, painted turtle, behaviour, early development, long-term developmental costs, trade-off, incubation, synchronous hatching

Introduction

Synchrony in the timing of births has evolved in many species (O'Donoghue & Boutin 1995), but development in oviparous species is primarily dependent on micro-environmental conditions, essentially making synchrony of hatching between all sibs an apparent improbability. Many factors promote intra-clutch variation in incubation period, including differences in egg size, order of ovulation and disparate thermal microenvironments of eggs (Andrews 2004). Despite these potential sources of developmental asynchrony, synchronous hatching through alteration of incubation period are known in many oviparous taxa, including invertebrates (Frechette & Coderre 2000), fishes (Bradbury et al. 2005), amphibians (Sih & Moore 1993; Warkentin 1995, 2000), crocodilians (Ferguson 1985), squamates (Vitt 1991), turtles (Doody et al. 2001; Spencer, Thompson & Banks 2001) and birds (Lack 1968; Vince 1969; Davies & Cooke 1983).

Any shortening of the incubation period is likely to incur some developmental costs; hence it is curious that hatching synchrony occurs at all. Although organisms that exhibit plasticity in developmental periods are diverse, the impetus is often considered the same: predator avoidance. In amphibians, shortened developmental periods and synchronous hatching occur during predation on egg masses by cat-eyed snakes (Lepidoeira septentrionalis, Warkentin 1995) and social wasps (Polybia rejecta, Warkentin 2000). In addition, perceived predation risk to neonates following hatching may induce some amphibian embryos to prolong development and hatching (Sih & Moore 1993; but see Anderson & Petran-
ka 2003). For most other taxa, group formation as a means of predator avoidance has been proposed to promote hatching synchrony (Lack 1968; Clark & Wilson 1981). For chelonians in particular, synchronous hatching may facilitate group emergence and mass migration from nests to water (Carr & Hirth 1961; Spencer, Thompson & Banks 2001), reducing risks of encountering predators through swamping or the per capita dilution of predation risk (Arnold & Wassersug 1978; Dehn 1990). Hence, turtle species that do not emerge until well after the last neonate in a clutch hatches should not exhibit hatching synchrony if there are associated costs (e.g. Andrews 2004; Peterson & Kruegl 2005).

Freshwater turtles ovulate clutches of eggs simultaneously, and embryonic development is arrested within the oviducts at the gastrula stage until oviposition (Ewert 1985). However, because eggs are generally deposited in several layers within a shallow nest, environmental gradients alter developmental rates (Maloney et al. 1990; Gyuris 1993; Thompson 1997). Specifically, eggs near the top of a nest experience higher temperatures (up to 6 °C higher in nests of the Murray River turtle, Emydura macquarii), which results in increased developmental rates and shortened incubation periods relative to eggs near the bottom of the chamber (Thompson 1988, 1989; Booth & Thompson 1991). Thus hatching synchrony should not occur within a freshwater turtle nest because incubation times should differ significantly between eggs at the top and bottom of the nest. However, an Australian freshwater turtle (E. macquarii) hatches synchronously through a mechanism whereby less developed siblings within a nest simply hatch earlier or increase development rates (independent of temperature) in response to siblings hatching (Spencer, Thompson & Banks 2001). This result concurs with the predator avoidance theory because the Australian turtle emerges from the nest during rain, which may be immediately after hatching or within 24 h (R.-J. Spencer, unpublished data). However, if predator avoidance has broadly driven the evolution of synchronous hatching in turtles, then we would not expect to observe this phenomenon in species that do not emerge from the nest shortly after hatching because hatching prematurely is likely to incur both short- and long-term costs (Bhutta et al. 2002). The acute short- and persistent long-term costs to performance and fitness is not known in any species, but in populations where hatching turtles remain in the nest for an extended period after hatching, the connection between selection for group emergence from the nest to avoid predation and hatching synchrony should not be linked. To test this hypothesis, we evaluated pipping synchrony in the painted turtle (Chrysemys picta; Emydidae), a freshwater species in which many populations of neonates remain in the nest over winter and emerge the following spring (c. 7 months after hatching; Gibbons & Nelson 1978; Weisrock & Janzen 1999).

We investigated whether pre-term pipping and subsequent hatching has potential short- and long-term effects on neuromuscular development. Turtles and precocial birds have similar developmental metabolic profiles during incubation, whereby the last stages of development are characterized by a reduced rate of oxygen consumption, which is thought to be associated with maturation of the neuromuscular system (Vleck, Hoyt & Vleck 1979; Ricklefs & Starck 1998; Peterson & Kruegl 2005). Hence any shortening of this period may have deleterious effects on agility and performance.

Materials and methods

STUDY SPECIES AND FIELD SITE

The painted turtle is one of the most common and widespread turtles in North America. The species ranges from coast to coast in the northern United States and southern Canada, and is found contiguously from the southern Great Plains to the mid-Atlantic seaboard and into parts of the South (Ernst, Lovich & Barbour 1994). Chrysemys picta inhabits freshwater environments and deposits c. 6–20 eggs (c. 10 in our study population; Morjan 2003) in relatively shallow terrestrial nests (c. 9 cm at our study site; Morjan 2003) (Fig. 1). Temperature differentials between the top and bottom of natural nests may be as much as 8 °C in July (F. Janzen, unpublished data), thus developmental asynchrony in the field is likely the norm.

Eggs were obtained from natural, newly constructed nests (<1 day old) at the Thomson Causeway, Thomson, Illinois, USA (41°57′28″N, 90°55′56″W). From 27 May to 1 June 2004, eggs from 16 nests were collected, labelled according to clutch and egg number using a blunt pencil (HB), and placed in moist vermiculite for transport to a laboratory at Iowa State University. The majority of hatchlings from this population overwinter within the nest (Filorama & Janzen 1999).

INCUBATION METHODS

To evaluate hatching synchrony, we followed protocols similar to Spencer, Thompson & Banks (2001). Specifically, we induced developmental asynchrony among clutch mates and then reunited eggs at a common incubation temperature and monitored their hatching times (Fig. 2). Ninety-six eggs were used in two experimental and two control groups that contained four clutches of six eggs each. Experimental treatments were designed to determine how synchronous hatching occurred, whether by less developed eggs hatching early (the

![Fig. 1. Hatchling painted turtles (Chrysemys picta) overwintering within a nest.](image-url)
demonstrates whether turtles were altering their developmental rate.

Les et al. (2001) performed incubation was carried out at 26°C for 11 days (Spencer, Thompson & Banks 2001). Throughout incubation, all egg boxes were re-hydrated weekly to maintain a water potential of −150 kPa in the vermiculite substrate, as well as rotated clockwise among shelves, and turned 180° within environmental chambers twice weekly to counteract possible thermal gradients.

After this period, half clutches (three eggs) held at 30°C were removed from their containers (30°C moved; 30NM) and placed next to their clutch mates held at 26°C (26°C not moved; 26NM). Incubation was then carried out at 26°C until hatching (Fig. 2). The control was treated similar to the experimental group, but the half-clutches were held at the same temperature (26°C) for the first 11 days of incubation (26°C control moved; 26CM; and 26°C control not moved; 26CNM).

Essentially, in the ‘catch-up’ experiment, eggs were incubated at 26°C except for a group of ‘stimulus’ eggs that were accelerated by an initial 11-day period at 30°C. After this period, the stimulus group was then reunited and incubation resumed at 26°C until hatching. To determine the incubation period, eggs were visually inspected for signs of pipping (the initial breaking of the egg shell by the caruncle) at least three times daily, beginning at day 40 of incubation. Incubation period was measured as the amount of time (days) from initial egg collection until pipping. Pipping (when the eggshell is first slit) is better than hatching as an index of the end of the incubation period, because it shows less variability than hatching (Gutzke et al. 1984; Les et al. 2007). Moreover, pipping is most relevant in this study because it is the first observable sign that individuals have actively begun the hatching process. To test the catch-up hypothesis, we compared incubation periods of less advanced embryos from the experimental treatment to those of the controls. This comparison demonstrates whether turtles were altering their developmental rate or pipping prematurely (26NM vs. 26CNM).

Experimental group 2: ‘wait’

A similar methodology was used to evaluate the wait hypothesis, except that in the ‘wait’ experiment all eggs were incubated at 30°C except the ‘stimulus’ group that was delayed by an initial period at 26°C. After developmental asynchrony was established, the stimulus group was reunited (26°C moved; 26M) with their clutch mates held at 30°C (30°C not moved: 30NM) and incubation continued at 30°C until hatching. The control group was also divided into half-clutches for the first 11 days of incubation, but held at 30°C (30°C control moved: 30CM; and 30°C control not moved: 30CNM). Control clutches were then reunited and incubation resumed at 30°C until hatching (Fig. 2). In this case, comparison of the incubation periods of the more advanced embryos from the experimental treatment to those of the control tested whether more advanced embryos postponed pipping in the presence of less advanced sibs (30NM vs. 30CNM).

PERFORMANCE TRIALS

Righting trials were performed to assess whether a developmental cost was associated with the alteration in incubation periods, which will indicate the mechanism by which synchrony was achieved. Righting ability requires balance and coordination, traits that may be adversely affected when development time is shortened. In addition, righting trials are a good indicator due to their possible ecological importance in turtles: hatchlings that are unable to right themselves during the terrestrial migration to water may be more susceptible to avian predation (Burger 1976; Freedberg, Ewert & Nelson 2001; Steyermark & Spotila 2001).

Righting trials were performed on neonates within 12 h of hatching because emergence from the nest of some turtle species occurs within this period (R.-I. Spencer, unpublished data). Following temperature acclimation at 22°C for 15 min, hatchlings were placed on their carapace and we recorded the length of time required for an individual to right itself (i.e. flip over). If no movement occurred within 90 s, the trial was terminated and that individual was excluded from analyses. All individuals that attempted movement within 90 s eventually righted themselves prior to trial termination, thus this time constraint did not excessively influence differential treatment effects. Neonates were then placed directly on the bottom of large closed plastic cups and overwintered in 3 mL of distilled water. Laboratory incubators were maintained at 5°C (temperature reduced from 22°C by 3–4°C daily over the first 5 days) until the following spring. Turtles were checked and re-hydrated (with distilled water) every 2 weeks. Environmental temperatures were slowly (over a 3-week period) raised to 18°C at the second week of April 2005 and hatchlings were maintained at 22°C for 48 h before righting trials were again performed.

DATA ANALYSES

Three factors could affect hatching times in this experimental design: initial incubation temperature (26 or 30°C), movement of eggs (i.e. whether an egg was left in a container or placed into a different container after the first 11 days of incubation) and developmental stage of neighbouring eggs (i.e. different in developmental stage as in experimental groups or the same developmental stage as in control groups). A general linear model was used to evaluate the influence of these factors and their interactions on incubation period. Response variables in each experiment were then analysed using independent t-tests to determine whether synchrony occurred and by what mechanism (catching-up or waiting).

We examined the impacts of initial incubation temperature, movement of eggs, developmental stage of neighbouring eggs and their interactions on righting ability of neonates after hatching and in the
spring, using general linear models. Response variables in the autumn and spring were also analysed using paired t-tests to determine whether the method of achieving synchrony was consistent with altering developmental rates (no performance cost predicted) or incubation periods (reduced performance expected). In the spring righting time analyses, we include data from eight additional clutches to compensate for sample size reductions resulting from post-hatching mortality and non-performing individuals. The additional eight clutches were half of a replication of the synchrony experiment, the other half of which was lost due to incubator failure. Although incubation periods and autumn righting times were not recorded for these individuals from the failed experiment, all 24 clutches were treated in an otherwise identical manner and are therefore included.

In addition to analysing righting times, we also assessed reductions in neuromuscular function by examining the treatment-specific likelihood of performance using chi-squared tests. A full (i.e. eight parameters) contingency table was used to first determine whether heterogeneity in the likelihood of performance (righting time) based on treatment existed, followed by several two-parameter contrasts to ascertain the developmental mechanism underlying synchronous hatching. For all evaluations of hatching synchrony and righting ability, the specific contrasts performed are presented alongside their results. All analyses were performed using JMP 5.1.2 (SAS Institute 2004).

A potential complication in analysis of righting times was sex. Chrysemys picta exhibits temperature-dependent sex determination in which males are produced at low temperatures (typically <28.5 °C) and females at high temperatures (Ewert & Nelson 1991; Janzen & Paukstis 1991). Temperatures used in this study would confound comparisons of righting times with the addition of sex. However, developmental asynchrony was established before the temperaturesensitive period of sex determination (the middle-third of incubation; Bull & Vogt 1981; Janzen & Paukstis 1991). Consequently, all hatchlings from the relevant treatment and control comparisons should be of the same sex. Hence, no between-sex comparisons are made in this study.

**Results**

**INCUBATION PERIOD AND SURVIVAL**

Incubation periods ranged from 48 to 62 days, depending on treatment (Fig. 3a,b). Treatment-specific incubation periods followed the expected pattern as eggs held at 30 °C for the entire period pipped first (30CM, 30NM and 30CNM respectively), eggs held at 26 °C pipped first (26CM, 26CNM and 26MN respectively), and temperature-switched eggs had intermediate incubation periods (26M and 30M respectively). Hatching success of eggs among treatments ranged from 83.3% to 100%, and winter survival of neonates from 66.7% to 100%.

**HATCHING SYNCHRONY**

Initial incubation temperature, treatment group and most interaction terms had a significant effect on incubation period (Table 1). However, movement per se of eggs did not impact incubation period (Table 1). To test for synchrony and its causes, we compared incubation periods between particular

**Fig. 3.** Mean incubation periods (mean + SE) of eggs from the experimental treatments: (a) catch-up and (b) wait. In the catch-up treatment, group abbreviations along the x-axis correspond to: eggs initially incubated at 30 °C and moved to 26 °C (30M), eggs initially incubated at 26 °C and not moved (26NM), control eggs initially incubated at 26 °C and moved to 30 °C (26CM) and control eggs initially incubated at 30 °C and not moved (30CNM). In the wait treatment, group abbreviations along the x-axis correspond to: eggs initially incubated at 26 °C and moved to 30 °C (26M), eggs initially incubated at 30 °C and not moved (30NM), control eggs initially incubated at 30 °C and moved to 30 °C (30CM), and control eggs initially incubated at 30 °C and not moved (30CNM). *Significant difference in incubation times between treatments NS, no significant differences in incubation times between treatments.

**Table 1.** Hatching synchrony general linear model results

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (experimental and control)</td>
<td>1</td>
<td>37.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Movement</td>
<td>1</td>
<td>2.1</td>
<td>0.156</td>
</tr>
<tr>
<td>Initial temp</td>
<td>1</td>
<td>51.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment × movement</td>
<td>1</td>
<td>0.8</td>
<td>0.380</td>
</tr>
<tr>
<td>Treatment × initial temp</td>
<td>1</td>
<td>14.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Movement × initial temp</td>
<td>1</td>
<td>34.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment × movement × initial temp</td>
<td>1</td>
<td>63.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>84</td>
<td>1</td>
<td>91</td>
</tr>
</tbody>
</table>

Bold indicates significance below 0.1.
treatment groups. Specifically, we tested the hypotheses that the mean incubation periods of eggs in the catch-up and wait #262 experimental groups were not significantly different (Fig. 3a,):

\[ H_0 : 30M = 26NM \text{ (Fig. 3a)} \]
\[ H_0 : 26M = 30NM \text{ (Fig. 3b)} \]

Hatching did not occur synchronously in either treatment \((t_{22} = 12.1, P < 0.001 \text{ and } t_{22} = 2.6, P < 0.009 \text{ respectively})\); nevertheless, incubation periods of eggs in the catch-up treatment support the hypothesis that less developed embryos in the presence of more developed sibs (stimulus group), pip earlier than expected (Fig. 3a).

\[ H_0 : 26NM < 26CNM \text{ (Fig. 3a)} \]

Eggs incubated at 26 °C with the stimulus group next to them (26NM) pipped up to 6 days earlier than eggs kept at 26 °C with sibs of the same developmental stage next to them (26CNM; \(t_{22} = 6.4, P < 0.001\)). In contrast, the wait hypothesis was not supported. Incubation periods of eggs held at 30 °C with the less advanced stimulus group (30NM) did not differ from eggs held at 30 °C with sibs of the same developmental stage (30CNM; \(t_{22} = 0.5, P = 0.3\)), indicating that incubation is not prolonged in the presence of less developed eggs.

**DEVELOPMENTAL COSTS**

Initial incubation temperature, treatment group and their interaction had a significant effect on righting ability of neonates following hatching (Table 2a). However, neither movement of eggs nor any interaction with movement significantly impacted righting ability (Table 2a). To test the underlying mechanism of shortening incubation periods, we investigated whether a developmental cost (i.e. long righting times) was apparent in groups known (26NM) or thought (26M) to have pipped early. Specifically, we tested the hypotheses that the mean incubation periods of neonates with shortened incubation periods were not significantly different from their sibs and/or corresponding controls (Fig. 4a, b):

\[ H_0 : 26NM = 26CNM, 30M \text{ (Fig. 4a)} \]
\[ H_0 : 26M = 30NM \text{ (Fig. 4b)} \]

**Table 2.** Righting trial general linear model results: (a) following hatching and (b) following winter

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Movement (experimental and control)</td>
<td>1</td>
<td>13 0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Movement</td>
<td>1</td>
<td>0.9</td>
<td>0.340</td>
</tr>
<tr>
<td>Initial temp</td>
<td>1</td>
<td>54.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment × movement</td>
<td>1</td>
<td>0.2</td>
<td>0.661</td>
</tr>
<tr>
<td>Treatment × initial temp</td>
<td>1</td>
<td>21.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Movement × initial temp</td>
<td>1</td>
<td>0.1</td>
<td>0.731</td>
</tr>
<tr>
<td>Treatment × movement × initial temp</td>
<td>1</td>
<td>0.8</td>
<td>0.384</td>
</tr>
<tr>
<td>Residual</td>
<td>80</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>87</td>
<td></td>
</tr>
</tbody>
</table>

Bold indicates significance below 0.1.

-Tests showed that turtles incubated at 26 °C with the advanced stimulus group next to them (26NM) took significantly longer than their more advanced sibs (30M; \(t_{22} = 4.6, P < 0.001\)) and controls (26CNM; \(t_{20} = 63.9, P < 0.001\)) to right themselves (66.69 s vs. 1233 and 1556 s respectively; Fig. 3a). Similarly, turtles initially incubated at 26 °C and placed next to their stimulus group (26M) took significantly longer than their sibs (30NM) to right themselves (60 s vs. 11 s; \(t_{22} = 3.0, P < 0.004\); Fig. 3b).

Failure to perform during autumn Righting trials was noted solely among early hatching treatments (Table 3), and resulted in significant heterogeneity in the likelihood of hatch-

\[ H_0 : 26NM = 26CNM, 30M \text{ (Fig. 4c)} \]
\[ H_0 : 26M = 30NM \text{ (Fig. 4d)} \]

Developmental costs were still apparent following overwintering, albeit somewhat reduced; early hatching treatments shortened righting times by c. 20 s in both cases. Yet, in the catch-up treatment, individuals known to have pipped prematurely (26NM) still took significantly longer than their sibs (30M; \(t_{22} = 4.76, P < 0.001\)) and controls (26CNM; \(t_{22} = 4.2, P < 0.001\)) to right themselves (47 s vs. 10 and 9 s respectively; Fig. 4c). In the wait treatment, individuals thought to have pipped prematurely (26M) also took longer than their sibs (30NM) to right themselves (40 s vs. 18 s), although this difference was but marginally nonsignificant \((t_{13} = 1.67, P = 0.066\); Fig. 4d). Retrospective power analysis of the t-test comparison estimated power at 0.38, indicat-
ing that the sample size for this contrast \((n = 15; \text{Table 3})\) was insufficient given our observed effect size \((\delta = 11.02)\).

Significant heterogeneity existed among treatments in the likelihood of hatchlings to perform during spring righting trials \((\chi^2 = 27.88, P < 0.001)\). Five of eight treatments exhibited higher proportions of non-performers than expected (higher than 30%), including both early hatching experimental treatments (26NM and 26M) and all warm incubation treatments (30NM, 30CNM and 30CM; Table 3). Treatment-specific contrasts provided additional support for the view that altering incubation periods elicited chronic reductions in neuromuscular function. Neonates that pipped early in the catch-up experiment (26NM) were significantly less likely to perform than their sibs (30M; \(\chi^2 = 13.41, P < 0.001\)) and corresponding controls (26CNM; \(\chi^2 = 7.58, P = 0.007; \text{Table 3}\)). In the wait treatment, suspected early-hatching individuals (26M) were substantially less likely to perform than their sibs (30NM; Table 3), yet this difference was not statistically significant \((\chi^2 = 1.89, P = 0.17)\).

**Discussion**

**Hatching synchrony**

Contrary to expectations based on current adaptive theory, we detected a similar catch-up mechanism in our population of *C. picta* to freshwater turtles that emerge from the nest shortly after hatching (Spencer, Thompson & Banks 2001). Failure of less advanced embryos to fully catch-up to their sibs in this study is probably due to the length of time over which developmental asynchrony was established. Spencer, Thompson & Banks (2001) established developmental asynchrony over a 7-day period; however, we allowed 11 days before reuniting clutch mates. The resulting difference in developmental stage between more and less advanced *C. picta* embryos appears to have been beyond a critical point where synchronous hatching can still occur. Developmental rate is highly dependent on incubation temperature in general (Deeming & Ferguson 1991; Thompson 1997), but the strength of the temperature effect on developmental rate may be dependent on developmental stage (Birchard & Reiber 1995; Birchard 2000; Shine & Elphick 2001; Andrews 2004). Development time necessary for neurulation, organogenesis and early growth decreases with increasing temperature, while the length of the late growth interval is independent of temperature (Andrews 2004). In the context of our study, this pattern means that less advanced embryos from the catch-up (26NM) and wait (26M) treatments were even farther behind in development, and had even less chance to catch-up, than
predicted if temperature and developmental rate varied linearly.

We used pipping instead of actual hatching emergence from the egg to test for synchronous hatching in *C. picta*. Although pipping coincides with various physiological changes to prepare the neonate for life outside the egg, the time interval between pipping and hatching is subject to variability both among and within clutches. So pipping may actually signify a willingness for an individual to hatch; in reality, turtles that pipped prematurely may take significantly longer to emerge from the egg than their more advanced sibs.

Means of communication of developmental stage between embryos could not be discerned in this study, and is poorly known for turtles. Avian and crocodilian embryos may communicate developmental stage by means of audible clicks (Driver 1965; Wolf, Bixby & Capranica 1976; Ferguson 1985; Nicolai, Sedinger & Wege 2004). Additional cues might come from vibrations of eggs in close contact at hatching or other means of physical disturbance, such as a predator opening a nest or disturbing a clutch (Vitt 1991; Warkentin 1995, 2000). However, vocalization is not known in turtles, and there was no contact between eggs during incubation in our experiment. Spencer, Thompson & Banks (2001) note that the auditory sounds produced in pipping could be a potential cue, as well as the changes in oxygen consumption, carbon dioxide production and heart rate that are characteristic of the overall decrease in metabolic rate before hatching (Birchard & Reiber 1995; Birchard 2000; Peterson & Kruegl 2005).

**Costs of Altering Incubation Periods**

The autumn righting trials (after hatching) clearly show that hatching early comes at an immediate developmental cost to coordination and movement, which is consistent with the hypothesis that shortened incubation periods were achieved by hatching prematurely rather than accelerating developmental rates. This undermines current theory for a single, predator avoidance-driven origin for synchronous hatching in turtles. In addition, the long righting times of less advanced sibs in the wait treatment is evidence that these individuals also pipped prematurely in the presence of more advanced sibs (Fig. 4b). This finding is consistent with late-developmental patterns of precocial birds: which shorten developmental times (Cannon, Carpenter & Ackerman 1986) and subsequently have reduced motor skills (Vince & Chinn 1971).

Incubation temperature can have profound long-term effects on vital life history parameters of reptiles. Lower incubation temperatures reduced hatching performance in two other emydid turtles (Freedberg, Ewert & Nelson 2001), and Spencer, Janzen & Thompson (2006) demonstrated the effect of incubation temperature on growth of wild Australian turtles until the ages of 4–5. However, incubation temperature alone could not account for the variation (early hatching = slower righting times) observed in righting ability. The long-term effect observed in this study suggests that some deeper physiological or neurological adjustment has occurred. The lack of statistically significant differences in both spring righting times and performance probabilities between early-hatching turtles and their sibs from the wait treatment (26M vs. 30NM) is probably due to a combination of reduced sample size and temperature effects. Turtles from warm incubation treatments exhibited high incidence of non-performers, thus making distinctions between groups less apparent than in catch-up comparisons. Such temperature effects become more clear when comparing the frequency of non-performers among control treatments only; hatchlings that experienced warm incubation conditions were significantly less likely to perform than those produced from low temperatures (57-9% vs. 15-4% non-performers; \( \chi^2 = 11.14, P < 0.001 \)). As such, we believe that in both cases, persistent performance reductions were significantly linked to the alteration in incubation periods, a result that does not support the theory that synchronous hatching is a general response to predation in turtles (Spencer, Thompson & Banks 2001).

**Possible Significance of Hatching Synchrony in *C. picta*\)**

It is not understood why synchronous hatching occurs in our population of *C. picta*, but we do know that it can have significant long-term effects on performance and co-ordination. Group formation theory for the evolution of hatching synchrony is not applicable when hatching and emergence are substantially decoupled because formation of groups is intrinsic. Instead, in species like *C. picta*, and other species that can also overwinter in the nest, hatching early may be adaptive prior to emergence from the nests. Position within the nest has profound implications for winter survival, thus, individuals hatching first may have a distinct advantage in securing optimal overwintering sites within the nest (i.e. surrounded by clutch mates near the bottom of the nest). Winter conditions within nest chambers can present a serious challenge to survival, even for a cold-adapted species such as the painted turtle (Lindeman 1991; Packard et al. 1997; Packard & Packard 2004). Nest thermal gradients that differentially affect developmental rates of clutch mates in the summer may also have differential effects on freezing mortality in the winter. Costanzo et al. (1995) reported that minimum temperatures experienced at depths of 5 cm vs. 10 cm (the typical range for *C. picta* nest chambers) were as much as 2°C lower during two winters in Garden County, Nebraska, USA. Furthermore, the cumulative amount of time of potential freezing events experienced at 5 cm was from 98 to 504 h longer than at 10 cm. In addition, hatchlings on the periphery of the nest may be at greater risk of freeze mortality as they are more likely to come in contact with soil, ice and ice nucleating agents (Hotaling, Wilhoff & McDowell 1985; Costanzo et al. 2000). Hence, synchronously hatching may be a response to compete for overwintering positions, but whether hatchlings actually ‘jockey’ for position within the nest remains a question for future investigation.
Although shortening the developmental period had significant long-term negative effects on coordination and movement of neonatal painted turtles, whether these behavioural differences are selected against in nature is unknown. Synchronous hatching may represent a primitive trait in this species retained through phylogenetic inertia. In some populations, the majority of C. picta hatchlings emerge from the nest prior to winter, indicating that autumn nest emergence is possibly an ancestral trait (Costanzo, Lee & Ultsch 2008). The potential ubiquity of synchronous hatching within Testudines is supported by the presence of this phenomenon in two species as distantly related as C. picta (megaorder Cryptodira, hidden-necked turtles) and E. macquarii (megaorder Pleurodira, side-necked turtles; Ernst & Barbour 1989). Such a scenario does not rule out a single, group formation-driven origin of synchronous hatching in turtles. However, group emergence of neonates, even in classic sea turtle examples, may not be as prevalent as commonly perceived (Houghton & Hays 2001).

THEORETICAL CONSIDERATION OF SYNCHRONOUS HATCHING IN TURTLES

We developed a general model to describe a possible mechanism behind synchronous hatching and provide a theoretical framework of interspecific and intraspecific comparisons. The constant-temperatures synchrony (CTS) model (Fig. 5) makes four primary assumptions. First, physical developmental rates differ significantly between the top and bottom eggs in a nest, only up to a period of late growth, at which point they do not differ. Second, plasticity in hatching time must also occur whereby a minimum embryonic stage exists at which stage, hatching can also occur. Similarly, a maximum embryonic stage exists at which hatching must occur, and these stages are independent of temperature. Third, the minimum and maximum hatching stages correspond to minimum (early-hatching) and maximum (full-term) incubation periods, respectively, the lengths of which are dependent on temperature. Temperature-dependent incubation periods result in the potential for hatching asynchrony. Finally, a characteristic synchrony potential exists in the form of a right triangle, the area of which is determined by the lengths of the intervals between the minimum and maximum developmental stages for hatching and the resultant minimum and maximum incubation periods.

The CTS model demonstrates clearly that hatching synchrony occurs when synchrony potentials overlap, a situation that would arise when temperature differentials within a nest are small, or when synchrony potentials are large. In addition, synchrony potentials could be used to derive incubation plasticity indices for any given species under standard conditions, and thus a means for comparisons. Although fluctuating nest temperatures can be converted to constant temperature equivalents (CTEs; Georges 1989), differences in egg size, composition and metabolic rate may affect the CTS model. However, the CTS model is useful for visualizing the trade-off between the advantages of hatching synchronously and the costs of altering incubation periods, and leads to several predictions regarding patterns in synchrony potential.

In conclusion, synchronous hatching in turtles comes at a developmental cost, but whether these laboratory tests translate into reduced fitness in the field is unknown. It is likely that synchronous hatching is an ancestral trait in turtles, but its relevance today is not solely for predator avoidance in all species.

Acknowledgements

We thank R. Paity and N. Fry for assistance in nest location and egg collection, Y. Ortiz and K. Birk for their help in setting up and maintaining experimental treatments, K. Bowen and S. Titterington for their aid in measurements of hatching morphology and performance, and the rest of the Janzen Lab and Turtle Camp crews for their support. As always, we are indebted to the U.S. Army Corps of Engineers and the U.S. Fish and Wildlife Service for permitting us access to lands under their purview. K. Bowen, J. Costanzo, multiple anonymous reviewers and numerous Janzen Lab members offered constructive comments on early drafts of this manuscript. Eggs were collected under Illinois DNR permit NH04.0073 and experiments were performed in accordance with COAC protocol 6-04-5684-DJ from Iowa State University. This research was supported by a National Science Foundation grant DEB-0089680 awarded to F.J.J.

References

Bhutta, A.T., Cleves, M.A., Casey, P.H., Cradock, M.M. & Anand, K.J.S.
Birchard, G.F. (1990) An ontogenetic shift in the response of heart rates to tem-
perature in the developing snapping turtle (Chelydra serpentina). Journal of
those of megapode birds. Egg Incubation: Its Effects on Embryonic Develop-
Cannon, M.E., Carpenter, R.E. & Ackerman, R.A. (1986) Synchronous hatch-
Clark, A.B. & Wilson, D.S. (1981) Avian breeding adaptations: hatching asyn-
379.
temperature on embryonic development in reptiles and birds. Egg Incubation:
Doodly, J.S., Georges, A., Young, J.E., Pauza, M., Pepper, A.L., Alderman,
Driver, P.M. (1965) ‘Clicking’ in the egg-yong of nidifugous birds. Nature,
268, 315.
tion Press, Washington, USA.
and Canada. Smithsonian Institution Press, Washington, DC.
Fili, 2001, 1050–1057.
temperatures inside lizard nests, and on the phenotypic traits of hatching liz-
ards. Biological Journal of the Linnean Society, 72, 555–565.
Spencer, R.J., Janzen, F.J. & Thompson, M.B. (2006) Counterintuitive den-
sity-dependent growth in a long-lived vertebrate after removal of nest preda-
Spencer, R.J., Thompson, M.B. & Banks, P.B. (2001) Hatch or wait? A
dilemma in reptilian incubation. Oikos, 93, 401–406.
Steyermark, A.C. & Spottis, J.R. (2001) Body temperature and maternal iden-
Further dilemmas in reptilian incubation


Received 6 August 2008; accepted 22 May 2009
Handling Editor: Raoul van Damme