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Good vibrations? Sibling embryos expedite hatching in a turtle

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A rare and remarkable animal behaviour is communication among embryos within a clutch of eggs. For example, embryonic vocalizations facilitate synchronous hatching in some birds and crocodilians. Synchronous hatching in nonvocalizing turtles suggests a different mechanism of embryonic communication: vibration-induced hatching. We addressed the idea that embryos can communicate with one another via vibrations that expedite hatching in the pig-nosed turtle, *Carettochelys insculpta*, a species that has evolved rapid hatching in response to hypoxia during nest flooding. Laboratory experiments tested the hypotheses that groups of (sibling) embryos can hatch and emerge more rapidly than solitary embryos, and that a vibration cue can expedite hatching relative to a hypoxic cue alone. We first demonstrated a vibration cue for hatching: vibration-induced hatching latency (ca. 8 min) was shorter than the hypoxia-induced hatching latency (ca. 16 min). Second, latency to both hatching and emergence from experimental nests was significantly shorter in groups of eggs than solitary eggs, when subjected to hypoxic conditions (perfusion in gaseous nitrogen or immersion in water, respectively). Although we did not directly link vibrations and the sibling effect, leaving open the possibility of embryo vocalizations, our experiments, along with a simple mathematical model, suggest that pig-nosed turtle embryos can detect and respond to sibling vibrations, and that these embryonic signals may increase the survival of siblings by reducing the latency to hatch and emerge under flood conditions. Our results are also novel in revealing multiple hatching cues in a single species within a single environmental context.

Groups of animals are capable of astonishing feats compared to solitary individuals (Krause et al. 2010). Grouping is an essential behaviour to understand because it affects the evolution of a wide range of morphological, behavioural and life history traits (Hamilton 1971; Alexander 1974; Krause & Ruxton 2002). Thus, determining the costs and benefits to grouping is fundamental to behavioural and evolutionary ecology.

Central to group living is communication. Animals generate movements, display colour patterns and emit chemicals, and these are classified as signals if they modify the behaviour of the receiver in a predictable way that has adaptive value for one or both (Hill 2008). Signals are ubiquitous across taxa and are used to coordinate mating, parental care, territoriality and defence, and numerous other intra- and interspecific interactions (Rogers & Kaplan 2000; McGregor 2005).

Animals with parental care communicate with their young, perhaps best illustrated by the example of parent birds responding to auditory and visual signals of their begging chicks (Wright & Leonard 2002). Remarkably, signalling extends to the embryo. Parent–embryo and embryo–embryo communication can facilitate synchronous hatching in some birds and crocodilians. Hatching-competent embryos in these species can produce and respond to vocalizations, bill tapping or clicking noises to solicit increased attention from the parent(s) and/or synchronize hatching within a clutch of eggs (reviewed in Brua 2002; Vergne & Mathevon 2008). Synchronous hatching also occurs in nonvocalizing animals such as turtles, despite thermal gradients in subterranean nests that should produce developmental and hatching asynchrony within a clutch of eggs (Ewert 1985). Hatching in turtles is more or less synchronous, and laboratory experiments have confirmed that less developmentally advanced embryos 'catch up' with more advanced embryos, although the causative mechanisms are unknown (Spencer et al. 2001; Colbert et al. 2010). How can silent turtle embryos communicate with their clutchmates?

A recent body of literature has revealed that animal embryos can choose their own birth date. Environmentally cued hatching, whereby hatching-competent embryos alter their timing of hatching, may be widespread in animals (reviewed in Warkentin & Caldwell 2009). For example, some embryos delay hatching until environmental conditions are suitable for larvae, while others hatch prematurely in response to the immediate threat of egg

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predation (Martin 1999; Warkentin 1995, 2000). The relative costs of dynamic stage-specific threats can determine when an embryo should remain within the egg versus hatch into a new environment, and embryos hatch accordingly provided that they can perceive changing risks by sampling environmental variation (cues) from within the egg (Warkentin & Caldwell 2009).

Embryonic hatching responses are cued by a variety of stimuli, including chemical cues, vibrations, acoustic cues and hypoxia (reviewed in Warkentin & Caldwell 2009). Embryonic responses to these stimuli can influence survival, suggesting an adaptive function within some ecological contexts (Petranka et al. 1982; Warkentin & Caldwell 2009). In a complex example, the pig-nosed turtle, *Carettochelys insculpta*, delays its hatching until the wet season, then hatches 'explosively' under (hypoxic) flooding conditions (Fig. 1; Webb et al. 1986; Doody et al. 2001). Although the turtles hatch within minutes of immersion, anecdotal observations suggest that embryonic vibrations could further expedite and synchronize hatching: during field studies eggs often hatch while being handled, transported, or in response to thunder (Webb et al. 1986; Doody et al. 2001; J. S. Doody, personal observation). Collectively, these observations suggest a role for mechanical vibrations of sibling embryos as a hatching stimulus in nature.

Why would a vibration stimulus be beneficial, in addition to the hypoxia stimulus? When a nest of hatching-competent pig-nosed turtle embryos is inundated by river flooding, the ability of an embryo to hatch and emerge quickly for its first breath would be critical to its survival; for example, underdeveloped embryos drown after flooding (Doody et al. 2004). Embryos in flooded nests must hatch and move upwards through up to 25 cm of wet sand. Thus, any additional hatching stimulus that could expedite hatching (and emergence) beyond the effect of the hypoxia stimulus alone could increase survival.

We conducted laboratory experiments to address the broad hypothesis that sibling embryos can communicate with one another to increase survival. To our knowledge reports of embryo–embryo communication are restricted to birds and crocodilians, and fitness effects of embryo–embryo communication have rarely been demonstrated (Brua 2002; Vergne & Mathevon 2008). We tested the hypothesis that, relative to solitary embryos, groups of pig-nosed turtle embryos can reduce the latency to hatch from the egg and emerge from the nest, via embryo–embryo communication. Specifically, we used three laboratory experiments to test the hypothesis that groups of eggs would hatch and emerge faster than solitary eggs when exposed to hypoxia (perfusion with gaseous nitrogen or immersion in water). Because our laboratory experiments simplified the magnitude and direction of the flooding process that occurs in nature, we developed a simple mathematical model using parameters obtained from the hatching experiments and field inundation rates to reveal how sibling vibrations can decrease latency to hatching in nature, relative to using hypoxic cues alone. We discuss our findings within the broader context of embryo–embryo communication in animals.

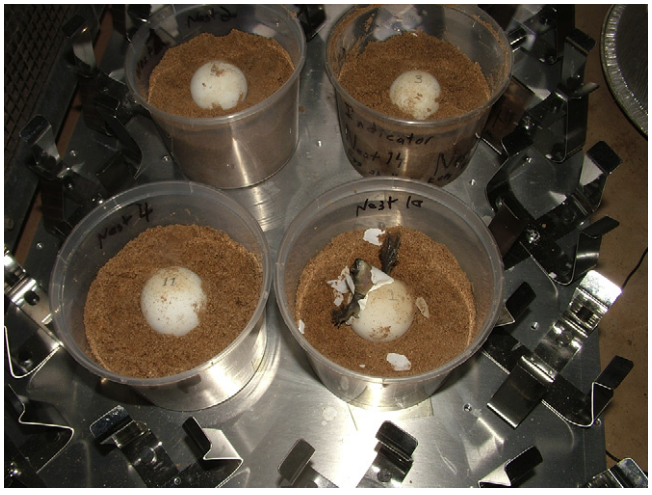

Figure 1. A pig-nosed turtle hatching in the vibration-induced hatching experiment, conducted on an electronic shaker in the laboratory. Note the position of eggshell fragments reflecting 'explosive' hatching.

METHODS

Study Species, Study Area and Egg Collection

The pig-nosed turtle is a riverine turtle inhabiting northern Australia and New Guinea (Georges et al. 2008). In Australia it lays two clutches of eggs in nests excavated and back-filled in sandy river banks during the dry season, July–October (Georges 1992; Doody et al. 2004). On the Daly River, Northern Territory, about 10 eggs are deposited between 12 cm and 22 cm below the surface (Doody et al. 2003). During incubation the eggs exposed to higher temperatures develop a 'desiccation patch', or a visible pocket of air between the outer shell membrane and the calcareous layer (J. S. Doody, unpublished data). Eggs do not hatch when development is complete, but rather embryos aestivate until the wet season. After a temperature-dependent incubation period of 65–109 days, eggs hatch with the onset of early wet season rainfall and flooding in November–January (Doody et al. 2001).

Postdevelopmental aestivation within the egg decouples the highly variable seasonal timing of nesting from timing of hatching, allowing turtles both to nest early and to hatch later under more favourable conditions (Doody et al. 2001, 2004). The importance of early nesting in this species is underscored by 20% flood mortality of nests with incomplete development during years when nesting is late (Doody et al. 2004), while the advantage of later hatching is probably reduced predation or increased food availability associated with wet season conditions (Webb et al. 1986; Doody et al. 2001). However, later hatching means that hatchlings must escape the subterranean nest during flooding to get their first breath, and embryos hatch 'explosively' within minutes, with laboratory experiments confirming hypoxia as a hatching cue (Webb et al. 1986). This explosive hatching behaviour is derived: latency to hatch in other turtle species is hours or days (Bustard 1972; Ewert 1985; S. Doody, unpublished data).

Fresh nests were located during daily surveys by boat along a 30 km stretch of the Daly River near Oolloo Crossing, Northern Territory, Australia (14°04′40″S, 131°15′00″E) in July and August 2008. Daily nest surveys allowed us to determine oviposition dates, which were important for predicting the completion of development. In some cases nests were marked and not disturbed for much of incubation, while in other cases nests were taken when discovered. Eggs were transported by car to an outdoor facility at Charles Darwin University, Darwin. Hatchlings were released near their natal beaches after the study.

The research was approved by the animal ethics committee of Charles Darwin University (1328) and the Northern Territory Parks and Wildlife Commission (27944).

Experimental Design, Set-up and Protocols

We conducted three laboratory experiments: (1) an experiment designed to investigate whether experimentally induced vibrations can stimulate hatching and to determine the hatching latency in response to vibrations; (2) an experiment testing the influence of the presence of siblings on hatching latency, using gaseous nitrogen as

a hypoxia stimulus; and (3) an experiment testing the influence of the presence of siblings on emergence latency, using immersion in water as a hypoxia stimulus. Hatching latency is broadly defined as the time elapsed between initiation of the hatching stimulus and hatching; emergence latency is the time elapsed between initiation of the hatching stimulus and emergence from the experimental nest.

Experiment 1: Hatching in Response to Vibrations

The aims of this experiment were to investigate vibrations as a hatching stimulus in pig-nosed turtle embryos and to determine the latency to hatching of eggs subjected to mechanical stimulation. Eggs were housed in an outdoor facility to avoid disturbance associated with experimental protocols that might cause vibration-induced hatching (e.g. by opening the door of an incubator). Eleven eggs from 11 clutches that had completed development in the laboratory were immediately individually placed half-buried in moist sand in 600 ml plastic containers on a moving electronic shaker (Fig. 1). One egg from each of these clutches served as a control, remaining undisturbed during the experiment. Time to pip the egg and time to hatching were recorded.

Experiment 2: Effect of Siblings on Hatching Latency

In this experiment developing eggs were housed in perfusion chambers in an outdoor facility to avoid disturbance associated with experimental protocols that might cause vibration-induced hatching. Individual clutches were split into two treatments: groups of eggs and solitary eggs. Clutch sizes (ca. 10 eggs per clutch but with one or two undeveloped eggs, Doody et al. 2003) were sufficiently large to divide across one group treatment and one to three solitary treatments. Each group treatment comprised six sibling eggs, while the solitary treatments comprised one sibling egg and five control eggs, which were ping pong balls filled with water. Ping pong balls were injected with water using a syringe, and approximated the size, weight and shape of the spherical pig-nosed turtle eggs (Doody et al. 2003). The position of the solitary egg within the ping pong balls was randomly selected. Eggs and ping pong balls were placed into small round plastic food containers (600 ml). In both groups the configuration was four eggs/balls touching one another forming a layer in the bottom of the container, one egg/ball located centrally on top of that layer, and another egg/ball touching the central top egg/ball and two eggs/balls below. Thus, in the group treatment each egg was touching at least three adjacent eggs, while in the solitary treatment each egg was touching at least three ping pong balls. Plastic containers were placed into nine perfusion chambers, which were either acrylic (30×30 cm and 12 cm high) or plastic (20×30 cm and 15 cm high). Each chamber was fitted with a hose (1.5 cm diameter) so that the nitrogen tank could be attached without inducing vibrations to eggs. Each chamber comprised all complements from each clutch (e.g. one group treatment and one to three solitary treatments) and a 600 ml plastic container of water to provide humidity to developing eggs. Eggs were placed into their treatments prior to completing development (before 55 days old; Doody et al. 2004) to avoid inducing hatching via incidental vibrations.

Nitrogen was perfused into each chamber rapidly but carefully to avoid inducing vibrations. Latency to pipping is defined as the time elapsed between perfusion in nitrogen and the first penetration of the outer eggshell. Latency to hatching was recorded as the time elapsed between perfusion and a hatchling's complete emergence from the egg. Hatchlings were not removed as they hatched because doing so would require removing the chamber lid, which could induce confounding vibrations.

Experiment 3: Effect of Siblings on Emergence Latency

This experiment was designed to test the influence of the presence of siblings on emergence from the nest (breaking the surface of the substrate). Although latency to emergence was confounded by hatching latency, the experiment was designed to mimic nest flooding in nature, and in combination with experiment 1 would provide a more comprehensive understanding of the importance of siblings for both hatching and emergence success.

Developing eggs were housed in moist sand in their experimental plastic containers in an outdoor facility to avoid disturbance that might cause vibrations and thus hatching upon completion of development. Egg temperatures approximated natural thermal regimes but with smaller daily fluctuations. As in experiment 2, clutches were split between two treatments: groups of eggs and solitary eggs. Each clutch spanned one group treatment and one to three solitary treatments. Each group treatment comprised six sibling eggs, while the solitary treatments comprised one sibling egg and five ping pong balls filled with water. In both groups eggs were placed in a cluster and were touching one another as in experiment 2. Both group and solitary treatments were buried in moist coarse sand at a depth roughly equivalent to the depth in field nests (10–18 cm below the surface).

Water was added rapidly but carefully via a 1 cm diameter hose buried in the sand. Eggs were inundated within 1 min. Latency to emergence was defined as the time elapsed between immersion in water and a hatchling breaking the surface of the water for its first breath.

RESULTS

Experiment 1: Hatching in Response to Vibrations

All 11 eggs from 11 clutches hatched successfully in response to vibrations on an electronic shaker (Fig. 1). The mean latency to pip the egg in response to vibrations $\pm$SD was 4.3 ± 2.93 min. The mean hatching latency in response to vibrations was 7.5 ± 5.55 min. One turtle that hatched in 45 min was excluded from calculation of the means. The 11 control eggs did not hatch for several days after the treatment eggs hatched.

Experiment 2: Effect of Siblings on Hatching Latency

We obtained successful hatching from nine replicates (clutches); however, in the first trial nitrogen introduction was more gradual than in the other replicates, and so this replicate was not included in the analysis. Hatching in another clutch was induced by a thunderstorm prior to the experiment. The presence of siblings did not significantly influence the latency to pip the egg (two-factor ANOVA, mixed model with treatment as a fixed effect and clutch as a random effect: $F_{1,6.6} = 4.95$, $P = 0.063$); however, the difference approached significance. The mean time to pipping in the group treatment averaged about 1 min shorter than that in the solitary treatment (Fig. 2).

There was a significant effect of the presence of siblings on latency to hatch (two-factor ANOVA, mixed model with treatment as a fixed effect and clutch as a random effect: $F_{1,50.0} = 9.69$, $P = 0.003$). Mean hatching latency in the group treatment averaged about 2 min shorter than that in the solitary treatment (Fig. 2). In one clutch a single egg from the group treatment failed to hatch; the opened egg revealed a deformed live hatchling. Time to hatch was not significantly influenced by mean desiccation patch size ($F_{1,7} = 2.63$, $P = 0.156$). However, eggs with smaller or no patches generally hatched sooner than those with larger patches.

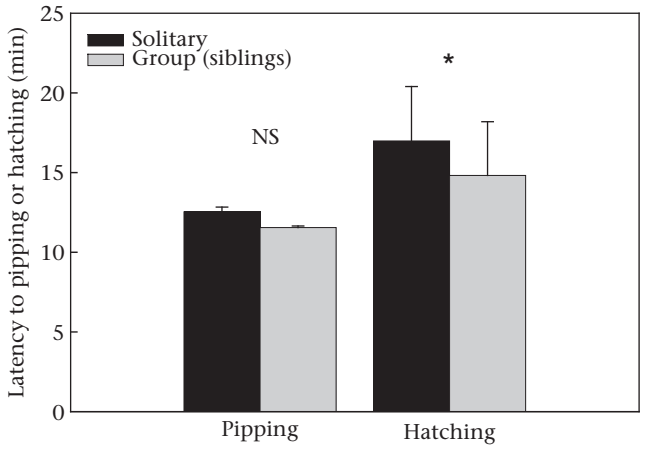

Figure 2. Influence of the presence of siblings on latency to pipping and hatching in pig-nosed turtle embryos, in response to hypoxic conditions (perfusion in gaseous nitrogen). Data are means + 1 SE. $^{*}P < 0.05$.

Experiment 3: Effect of Siblings on Emergence Latency

We obtained successful emergence from seven replicates (clutches). There was a significant effect of treatment on latency to emergence (two-factor ANOVA, mixed model with treatment as a fixed effect and clutch as a random effect: $F_{1,6.36} = 7.72$, $P = 0.030$). Mean latency to emergence of eggs in the group treatment averaged over 9 min shorter than that of eggs in the solitary treatment (Fig. 3). Three eggs from three separate solitary treatments failed to emerge: two hatched but failed to emerge and were found dead after 3 and 4 h, while the third hatchling was excavated alive after 80 min.

Modelling Effect of Hatching Stimuli on Hatching Latency

Our simplified experiments may not have captured the relative importance of multiple hatching cues in nature. Egg clutches were completely perfused/immersed in either nitrogen or water without delay. However, in nature, rising river levels reach and immerse the bottommost eggs first, rather slowly. If movements of these deeper embryos serve as a cue for hatching for upper egg layers, then predicting hatching latency of a clutch would require knowing the comparative speed at which the two cues operated

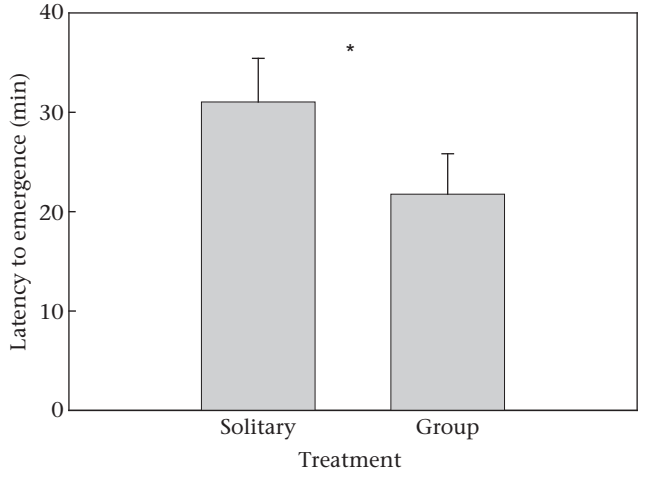

Figure 3. Influence of the presence of siblings on latency to emergence in pig-nosed turtle embryos in the laboratory, in response to hypoxic conditions (immersion in water). Data are means + 1 SE. $^{*}P < 0.05$.

across the different egg layers. For example, sibling vibrations (or some other means of communication such as vocalizations) caused by hypoxia in the bottommost eggs could essentially move instantaneously through the upper egg layers and thus faster than rising water levels, thereby causing earlier hatching in those layers than would be experienced by hypoxic conditions alone. In support of this, time to hatch in response to vibrations (7.5 min) was shorter than time to hatch in response to hypoxia in the present study (group = 11.6 min, single = 12.5) and in a previous study (single = 8.6 min, $N = 5$ eggs; Webb et al. 1986). Moreover, it is highly likely that vibrations confounded hatching time in the latter study, as one egg hatched immediately upon perfusion with nitrogen, and the values approximate those obtained in our vibration-induced hatching experiment.

Using hatching parameters obtained from our experiments and historical river level flooding data routinely collected at gauging stations, we can use very simple equations to predict hatching times for each egg, layer and thus an entire nest, taking both hatching stimuli into consideration. For simplicity, we assume three layers of three eggs, each layer touching the layer above and/or below (clutch size averages 10 at the Daly River site; Doody et al. 2003). A clutch of eggs occupies a spherical cavity with a diameter of approximately 7–12 cm (Georges 1992; Doody 2002). To be comprehensive we can use three rates of river level rises: fast, moderate and slow.

In the first scenario we consider hatching latency of embryos in response to hypoxia alone (without sibling vibrations/mechanical stimulation). Assuming that immersion time starts when the flood level reaches the bottom of the bottommost egg layer, and the hatching response is initiated upon complete immersion of the egg or layer, we can calculate the hatching latency for the bottommost layer (layer 1) as:

$$T_{\text{layer 1}} = t_{\text{i1}} + t_{\text{hhs1}}$$

where t_{i} = time to immerse the egg layer completely and t_{hhs} = latency to hatching after total immersion (hypoxia) for a solitary egg. Calculation of the hatching latency is similar for the other two layers, but includes immersion time for the previous layers:

$$T_{\text{layer 2}} = t_{\text{i1}} + t_{\text{i2}} + t_{\text{hhs2}} \text{ and}$$

$$T_{\text{layer 3}} = t_{\text{i1}} + t_{\text{i2}} + t_{\text{i3}} + t_{\text{hhs3}}$$

Assuming our three layers contain equal numbers of eggs we can then calculate the mean hatching latency for the clutch:

$$T_{\text{mean}} = \left(T_{\text{layer 1}} + T_{\text{layer 2}} + T_{\text{layer 3}}\right)/N$$

where N = the number of layers.

Now we consider the scenario in which embryos in the upper two layers have the ability to respond to sibling vibrations (or some other means of communication such as vocalizations) produced by embryos in the bottommost layer. For that layer (layer 1) we assume that the stimulus to hatch is initiated immediately upon complete immersion, but is also influenced by sibling movements both within the egg and possibly after hatching. We use:

$$T_{\text{layer 1}} = t_{\text{i1}} + t_{\text{hhg1}}$$

where t_{hhg} = latency to hatch after total immersion (hypoxia) in a group of eggs. For the next layer (layer 2) we assume that movements in layer 1, which begin at T_{layer1}, initiate the hatching stimulus in layer 2, independent of (and possibly prior to) the hypoxia stimulus. We use:

$$T_{\text{layer 2}} = t_{\text{i2}} + t_{\text{hv2}}$$

where t_{hv} = latency to hatch with sibling vibrations (only). We use the same principle for layer 3:

$$T_{\text{layer 3}} = t_{\text{i3}} + t_{\text{hv3}}$$

The mean hatching latency is calculated as in the first scenario:

$$T_{\text{mean}} = \left(T_{\text{layer 1}} + T_{\text{layer 2}} + T_{\text{layer 3}}\right)/N$$

We can then solve these equations using our experimental values and historical river level data obtained from a nearby gauging station. The slowest steady rises in the Daly River during wet season flooding are approximately 1–4 cm per 15 min, while the fastest steady rises are approximately 7–10 cm per 15 min (Fig. 4a; Australian Bureau of Meteorology). These values translate into 7.8 and 16.2 min to immerse one egg layer (4 cm), respectively (mean egg diameter was 39.6 ± 0.21 mm from Daly River turtles; Doody et al. 2003). Figure 4b shows the hatching latency predicted by the model, based on stimulus type (hypoxia, vibrations) for three rates of river rises: slow, fast and intermediate. Latency to hatching was more rapid with faster rates of river level rises, regardless of stimulus type. However, embryos responding to a vibration cue are predicted to hatch in about half the time, on average, than those responding to hypoxia alone (Fig. 4b).

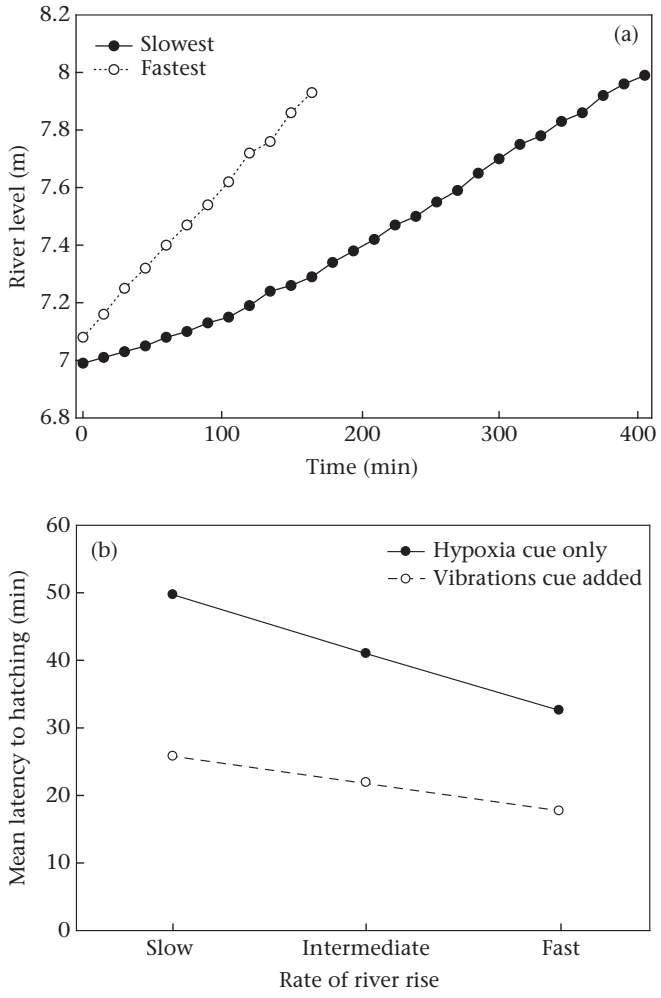

Figure 4. (a) Rates of river level rises during flooding gleaned from historical river stage data (Australian Bureau of Meteorology); (b) predicted time to hatch for pig-nosed turtle embryos generated by a model based on different rates of river rises in (a) and hatching stimulus type (anoxia, sibling vibrations).

A caveat of our analysis is that our predicted hatching times are longer than our experimental times because the former begin as the flood levels reach the bottom of the first egg layer, while the latter begin with immediate, complete inundation/perfusion of individual eggs. As such our predicted times do not represent the time an embryo would spend under water in nature. To calculate time spent underwater we would subtract t_{i} from latency to hatching for each egg layer and take the mean of the three layers.

Discussion

Our study suggests that embryos can communicate with one another to increase survival. Our experiments and model provide evidence that turtle embryos can expedite hatching and emergence during flood conditions through embryo–embryo communication. Latency to hatching and emergence from experimental nests was significantly reduced in groups of embryos relative to solitary embryos, under hypoxic conditions (Figs 2, 3). Although the mechanism in these experiments was not explicitly established, our complementary experiment confirmed vibrations as a hatching stimulus (Bustard 1972; Webb et al. 1986). Flood mortality of embryos may have selected for expedited hatching and emergence, leading to the evolution of an additional hatching cue, a supposition supported by the failed emergence and mortality of three solitary embryos in the present study, and by flood mortality of embryos in previous research (Doody et al. 2004). Although our first experiment suggests that the additional cue is embryo vibrations, we cannot rule out other cues such as vocalizations or heart rates (see below). Alongside other research our results suggest some generality in the ability of embryos to respond to risks, adding to a small but growing number of species with multiple hatching stimuli. Multiple hatching cues have also been demonstrated in frogs, fish and monogenean fish parasites (Gannicott & Tinsley 1997; Wedekind & Muller 2005; Gomez-Mestre et al. 2008). In the most complex example, embryos of the frog *Agalychnis callidryas* can hatch early in response to hypoxia, egg predation and pathogen invasion (Warkentin 1995, 2000, 2002; Warkentin et al. 2001). Our results are novel, however, in demonstrating multiple diverse cues within one environmental context (nest flooding).

Hatching Responses to Embryo Flooding

A recent review of environmentally cued hatching demonstrates a diversity of responses and associated cues in a wide variety of animal taxa (Warkentin & Caldwell 2009). The authors list 18 species that can hatch in response to flooding, dominated by frogs and fish, but including a dragonfly and a snail. The two species of reptiles listed include the pig-nosed turtle and the brown anole, *Anolis sagrei* (Losos et al. 2003). In addition, the yellow mud turtle, *Kinosternon flavescens*, can hatch after submersion in water (Ewert 1991), although a flooding stimulus in nature is unknown for this species. Although not experimentally demonstrated, the primary stimulus for hatching in the Indian flap-shelled turtle, *Lissemys punctata*, is also apparently flood-induced hypoxia. Like its relative the pig-nosed turtle, flapshell embryos complete development during the dry season but defer hatching until the onset of the wet season (Biswas & Acharjyo 1977; Vijaya 1983).

The adaptive significance of flood-induced hatching in animals can be either matching the seasonal timing of hatching with more favourable conditions or escaping stochastic flooding, and the selective mechanism is embryonic mortality via hypoxic stress (Warkentin & Caldwell 2009). In pig-nosed turtles and possibly flapshells, hatching during wet season conditions presumably either

decreases hatchling predation (owing to increased water volume and reduced water clarity) and/or increases hatchling food acquisition rates (Webb et al. 1986; Doody et al. 2001). As a consequence, however, embryos must be able to hatch in flooded nests, and pig-nosed turtle embryos pip the egg and hatch explosively with a physical vigour atypical of most newly hatched turtles. For example, most turtles have relatively large external yolk sacs at pipping and are not ready to leave the egg (Bustard 1972; Ewert 1985). The risk of drowning while attempting to hatch and emerge from the nest could select for a complementary hatching mechanism that would minimize hatching and emergence time. Our experiments support this notion, with groups of eggs pipping and hatching faster than solitary eggs, and this effect was also evident in our emergence experiment (Figs 2, 3). Moreover, our model indicates that sibling vibrations produced by the bottommost embryos during flooding could move up through adjacent embryos in upper egg levels more rapidly than the rising water level, providing a role for the production, detection and response to vibration cues. Pig-nosed turtle embryos can apparently also hatch in response to heavy rainfall, which can also induce hypoxic conditions for long enough to promote hatching (Doody et al. 2001). Further experiments are needed to determine the comparative hatching responses and sensitivity to potential vibrational and hypoxic stimuli such as rainfall, thunder and sibling movements within and outside the egg.

There were limitations to our interpretations. We could not remove hatchlings as they emerged in the hatching experiment because of the strong probability of our inducing vibrations by doing so. Thus, we could not disentangle embryo movements from movements of early hatchers as hatching stimuli for the other eggs in a group. However, latency to hatching of the first egg in the group treatments was significantly shorter than latency to hatch of solitary eggs (single-factor ANOVA: $F_{1,16} = 8.11$, $P = 0.012$), and the first group egg invariably hatched before the solitary egg from the same clutch. Thus, our treatment effect was not due to hatchlings climbing over the other eggs. Second, it is possible that vibrations could be felt by embryos between plastic containers within the chambers. However, this would theoretically cause single eggs to hatch faster, reducing the group effect. A final caveat for our results is that latency to hatching in pig-nosed turtles could be longer in desiccated eggs. Turtle eggs in hotter subterranean nests under dry conditions can dehydrate (especially the uppermost eggs), because the sand dries out from the surface downwards (Bustard 1972). For example, pig-nosed turtle eggs develop and aestivate during the northern Australian dry season during a period of little or no rainfall (Taylor & Tulloch 1985; Doody et al. 2001). Eggs at the shallowest depths can lose an average of 16% of their original wet mass, but will hatch successfully (J. S. Doody, unpublished data). Similarly, successful hatching also occurred after experimental reductions of 8% wet mass in eggs of the green sea turtle, *Chelonia mydas* (Bustard 1972). However, preliminary experiments indicated that latency to hatching is longer in dehydrated eggs than control eggs (J. S. Doody, unpublished data). Experiments manipulating the hydric environment around eggs to mimic natural incubation conditions are required to determine any increased risk imposed by desiccation.

Vibration-induced Hatching and Embryo–embryo Communication

Vibrational communication in animals is widespread but understudied (Hill 2008). Similarly, vibration-induced hatching may be more common than currently appreciated. In the best-studied example, vibrations of attacking snakes induce early hatching in the frog *A. callidryas* (Warkentin 1995; Warkentin et al. 2007). Embryos are able to distinguish between vibrations made by predators and those from benign sources such as rainfall and wind (Warkentin & Caldwell 2009). Predation can be high in pig-nosed turtle nests but a clear benefit for early hatching in response to predation is difficult to envision in response to their chief egg predators: the monitor lizards (Doody et al. 2003, 2006). Thus, the handling-induced hatching in the turtle embryos may reflect an evolved response to vibrations induced by siblings, rainfall or even thunder, rather than predators. Handling-induced hatching also occurs in the turtle *C. mydas* and in two lizard species, *Plica plica* and *Lampropholis delicata* (Vitt 1991; J. S. Doody, unpublished data). Further experiments are needed to determine whether these organisms have the ability to distinguish between different sources of vibrations.

Embryo–embryo interactions or communication have rarely been demonstrated in animals. Some late-stage bird embryos produce 'clicking noises' while others use vocalizations (reviewed in Brua 2002). In several cases hatching synchrony was reduced when eggs were not in contact with one another, suggesting a functional role for these interactions (e.g. Davies & Cooke 1983; Schwagmeyer et al. 1991). In reptiles, crocodile embryos hatched in response to playbacks of embryo vocalizations (Vergne & Mathevon 2008), and laboratory experiments demonstrating synchronous hatching suggested embryo–embryo interactions in two species of turtle (Spencer et al. 2001; Colbert et al. 2010). Our research with pig-nosed turtles supports a role for vibration-mediated embryo–embryo communication in turtles, but other mechanisms such as vocalizations or heart rates may exist (see below).

How pig-nosed turtle embryos receive sibling vibrations is not known. Vibrations are received by animals in two different ways: auditory–vestibular (hearing) and somatosensory (tactile) adaptations (Hill 2008). Spencer et al. (2001) suggested that auditory sounds from physically pipping the eggshell might explain synchronous hatching in turtle embryos. Auditory stimuli in late-stage bird embryos include clicking noises, vocalizations, bill tapping, breathing, heart beats, limb movements, head lifting and beak clapping (Vince 1969). Chemical communication occurs between larvae and embryos in certain molluscs (Voronezhskaya et al. 2004). It is conceivable that vibration-induced hatching in pig-nosed turtles may have evolved to promote synchronous hatching, rather than to expedite hatching in response to flooding, although it is difficult to disentangle these two phenomena within a flooding context. Synchronous hatching could theoretically reduce predation on hatchlings through dilution (Carr & Hirth 1961; Dial 1987; Spencer et al. 2001) or coordinate departure from the nest because chemical cues released during hatching could attract predators to the remaining eggs (Lack 1968; Vitt 1991).

Although we demonstrated that vibrations can induce hatching in pig-nosed turtles, it is possible that turtle embryos can produce and recognize vocalizations as in crocodilians and in (mainly) precocial bird embryos (Herzog & Burghardt 1977; Pooley 1977; Britton 2001; Brua 2002; Vergne & Mathevon 2008). Hatching in response to thunder in the pig-nosed turtle (present study) and in the turtle *L. punctata* (Vijaya 1983) is consistent with an auditory function, but does not rule out detection by tactile perception of vibrations of buildings during thunder. For example, researchers have recently documented vocalizations within the eggs of the turtle *Podocnemis expansa* (D. Vogt, unpublished data). The recent discovery of underwater vocalizations in aquatic turtles (Giles et al. 2009) perhaps increases the likelihood of vocalizations occurring between turtle embryos.

Modelling and Testing Comparative Hatching Responses

Our predictive model clarifies a pathway for future research. However, the model rests upon a few assumptions. First, it assumes that embryos utilize the vibration stimulus under flooding scenarios; mechanical stimulation induced hatching in the sea turtle *C. mydas*, which generally does not hatch in response to flooding (Bustard 1972). Second, it is feasible that the vibration-

induced hatching value we obtained in the hatching experiment and used in our predictive model is less than ideal because the mechanical stimulation in the laboratory (shaker) would produce much stronger vibrations (and probably at different frequencies) than those created and perceived by sibling embryos. Finally, the model assumes that the river floods a nest in a directional fashion without major changes in the integrity of the nest. For example, severe flooding can result in complete nest erosion with eggs spilling into the river, or conversely additional sand above the nest requiring more effort for escape (Doody et al. 2001). Nevertheless, our primary intention was not to predict the absolute time to hatch occurring in nature, but rather to predict the relative time to hatch based on the direct involvement of sibling embryos. A laboratory or field experiment with a more accurate rate and direction of flooding treatment would provide a test of the model.

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