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Compensation for inundation-induced embryonic diapause in a freshwater turtle: achieving predictability in the face of environmental stochasticity

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Summary

1. We investigated the influence of duration of inundation-induced diapause, incubation temperature and clutch of origin on incubation duration and survivorship of eggs of the Snake-Necked Turtle, *Chelodina rugosa*, from the wet–dry tropics of northern Australia.

2. Eggs of *C. rugosa* survive at least 25 weeks' inundation, almost 6 months, with a clear optimal inundation duration of 6 weeks. Eggs not held under water suffered the same mortality as eggs inundated for 25 weeks. Underwater nesting is not a facultative capacity but, rather, inundation is essential for optimal survivorship of embryos.

3. Inundation duration had a profound effect on incubation period, reducing it by up to 9 weeks over what would be expected for a given temperature. Eggs inundated for up to 7 weeks complete incubation faster than had they been laid at the same time in dry ground.

4. There was remarkable variability in incubation period remaining after correcting for the effect of incubation temperature, inundation duration and clutch.

5. We interpret these traits as adaptations that match the timing and duration of the period available for nesting to the timing and duration of the period available for successful hatching, emergence from the nest and hatchling survival. Our interpretation is placed in the context of considerable environmental stochasticity in the factors driving these variables.

Key-words: Chelidae, *Chelodina rugosa*, Chelonia, developmental compensation, nesting, nest inundation, reproductive strategy

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Introduction

Studies of a wide range of oviparous organisms have demonstrated that the incubation period is often greater than the minimum time required to develop from egg to offspring. Perhaps the most extreme cases are in the insect world, where extended periods of diapause, broken in response to a specific environmental cue, effectively uncouple the timing of egg-laying from the timing of emergence of the larvae (Powell 1989). In other cases, insects have extended periods of larval life in order to match timing of egg-laying and larval emergence to conditions suitable for adult dispersal phase (Jewel Beetle, *Buprestis aurulenta*: Linsley 1943). Among vertebrates, many annual fish occupying ephemeral waters cope with unpredictability in the duration of dry seasons by extending the period of development via a series of diapause (Wourms 1972a,b,c), the last terminated by conditions suitable for the survival of the larvae. In amphibians, the duration of the larval period is highly variable within and among species, and is a central characteristic of their life history (Denver 1997). Among amniotes, costs of gestation in mammals and incubation in birds limit the scope for adjustment of developmental times in response to variability in conditions suitable for the offspring, though some birds can adjust development times to ensure synchronous hatching despite staggered laying times (Vinuela 1997; Wiebe, Wiehn & Korpimaki 1998; Both & Visser 2005). Oviparous reptiles are not so constrained since, with notable exceptions (Shine 1988), they deposit their eggs in a nest that they subsequently abandon and have little further direct influence on the fate of their offspring. Morphogenesis can be normal over a range of thermal and hydric environments despite varying rates of development (Ewert 1985; Gutzke & Packard 1987) and an increase in temperature, within

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bounds, accelerates development (Packard *et al.* 1987; Shine & Harlow 1996; Shine *et al.* 1997b; Georges *et al.* 2005), as does a dry nesting environment (Janzen, Ast & Paukstis 1995). The capacity to vary rates of development through seasonal timing of egg-laying (Ewert 1979; Olsson & Shine 1997) and choice of nest site (Cagle *et al.* 1993; Shine, Elphick & Harlow 1997a; Wilson 1998), coupled with embryonic diapause and embryonic aestivation (Webb, Choquenot & Whitehead 1986; Ewert 1991; Ewert & Wilson 1996; Booth 2000) provides reptiles with great scope for adjusting developmental period. Reptiles therefore provide good models for exploring the trade-offs between the potentially competing interests of mother and offspring and the role of control over developmental period in moderating costs of such trade-offs.

From the perspective of the mother, eggs need to be laid at a time dictated by the constraints on accumulation of resources and when seasonal temperatures are conducive to mobilization of those resources for vitellogenesis, ovulation and the deposition of oviducal contributions to the egg (Ewert 1985). The eggs must be laid at a time when conditions allow nesting activity and when those conditions least compromise survivorship of the mother (Spencer 2002). From the perspective of the offspring, the eggs must be in the nest when conditions are conducive to successful incubation and appropriate developmental outcomes, and they must be laid at a time that allows hatching and emergence from the nest when conditions are suitable for offspring survival and growth (Webb *et al.* 1986; Madsen & Shine 1998). Timing of egg-laying and subsequent timing of hatching and emergence are linked by the developmental period. Adjustment of the timing and location of nesting by the mother, in response to natural selection acting upon her survivorship, will potentially result in a shift in the timing of emergence of her offspring. This may in turn compromise the survivorship of the offspring and so her overall fitness. Meeting the needs of these two influences on the mother's overall fitness will require coincident adjustment of the developmental period. So the period during which embryonic development occurs serves not only the purpose of providing sufficient time for the embryo to develop, but also provides coordination between the timing of egg-laying and the timing of hatchling emergence in order to maximize overall parental fitness. The developmental period can therefore be expected often to exceed the minimum physiological and developmental requirements for embryonic development.

In this paper, we investigate the influence of duration of inundation-induced diapause (Kennett, Georges & Palmer-Allen 1993b) and incubation temperature on the incubation period of a tropical turtle, *Chelodina rugosa.* This species occupies ephemeral swamps and wetlands of the wet–dry tropics of northern Australia (Kennett 1994). Turtle activity is punctuated each year by the dry season, when the wetlands draw down and often dry completely. The turtles survive this time, which typically ranges from August/September to December/January, buried beneath the ground. The aestivation period spans the season in which most temperate turtle species and many tropical species nest. Female *C. rugosa* nest under water or in highly saturated soils (Kennett, Christian & Bedford 1998) in the late wet season and early dry season, a strategy that allows them to annually lay multiple clutches of eggs prior to aestivation (Kennett 1999). Modification of the vitelline membrane allows oviducal arrest to continue in the form of embryonic diapause while the eggs are inundated (Seymour, Kennett & Christian 1997), and development proceeds only when the soil dries and the hypoxic conditions are removed (Kennett *et al.* 1993b).

The wet–dry tropics of Northern Australia experience extremes of high rainfall during the monsoonal wet seasons and the near absence of rainfall in the intervening dry seasons, coupled with great unpredictability in the quantity that falls and its timing (Taylor & Tulloch 1985; Georges *et al.* 2003).We interpret the influence of inundation-induced diapause and temperature on incubation period in the context of environmental unpredictability in both the period available to turtles for nesting and the period in which conditions are suitable for hatching. For *C. rugosa*, matching optimal timing of egg-laying to optimal timing of hatching and emergence, through incubation period as an intermediary, presents a considerable challenge.

Materials and methods

Female *C. rugosa* were collected between May and June 2004 using baited hoop traps set in ephemeral billabongs on the Blyth-Cadel floodplain of Arnhem Land, Northern Territory. Turtles found to be gravid by palpation were X-rayed to confirm reproductive status and to determine clutch size (after Gibbons & Greene 1979). Turtles carrying 10 or more eggs ($n = 25$) were shipped by air to the University of Canberra; all other turtles were released at their site of capture. Ten days after capture, each turtle was induced to lay her eggs with an intramuscular injection of synthetic hormone Oxytocin® (Troy Laboratories, Smithfield, NSW, Australia) at a dosage rate of 1 unit/100 g body mass (Ewert & Legler 1978). The turtles were returned to their site of capture and released.

Each egg was labelled with a unique number, weighed (±0·1 g) and measured (length and width ±0·1 mm). Ten eggs from each of the 25 clutches were selected at random for allocation to treatments. Pairs of eggs selected at random from each of five of these clutches were systematically allocated to each of five constant temperature treatments (26, 28, 29, 30, 32 °C) and each of five inundation treatments (0, 2, 6, 10, 25 weeks) in a Latin square design. This basic experiment was replicated four more times using eggs from the remaining 20 clutches. Each replicate was thus based on a total of 50 eggs, and the full experiment was based on 250 eggs. The inundation treatments involved totally immersing the clutches of eggs under water in 250-ml plastic containers fitted with lids that minimized evaporation but did not exclude oxygen exchange between the water and the atmosphere. Inundated eggs were stored in complete darkness at room temperature. Container effects were considered to be negligible, and this effect was incorporated in the clutch effect (eggs were stored as clutches) in subsequent analyses.

Eggs in the 0 weeks treatment were covered immediately after laying with moist vermiculite (four parts water to three parts vermiculite by mass) in 18-compartment plastic boxes fitted with clip-down lids. Each box, with its eggs and vermiculite, was weighed and placed within a Ziploc® (SC Johnson, Lane Cove, NSW, Australia) plastic bag with a small quantity of water (5 ml). A corner of each bag was snipped to allow air transfer to the eggs. The bags were monitored daily and water was added as required. This ensured a constant 100% humidity level throughout incubation. Each box of eggs was placed in a incubator (Refrigerated Incubator Model RI 170, Thermoline Scientific, Smithfield, NSW, Australia) set at the appropriate temperature. Incubators were not replicated, and incubator effects were kept to a minimum by the use of identical equipment in each treatment. These effects were assumed to be negligible, and differences between incubators are assumed to be in temperature only. Incubation temperatures were monitored daily using thermometers calibrated against a reference thermometer certified as accurate (±0·1 °C) by the National Authority of Testing Agencies (NATA). The incubators maintain temperature using opposing heating and cooling apparatus on a short cycle and are fan forced. Thermal inertia within the chamber was increased by the addition of a 5-l water container. The incubators maintain constant temperature within an empty chamber of +0·2 °C. Egg containers were rotated within the incubator each time they were inspected. As the remaining eggs completed the inundation phase of their treatment, they were transferred to free compartments in the boxes as outlined above at the appropriate temperature dictated by the experimental design.

Eggs that failed to begin development, that is, eggs that failed to develop a white patch indicating embryonic activity (Thompson 1985), were regarded to have died under inundation. Note that our estimates of survivorship under the inundation treatments (including the control) will include a random element derived from infertile eggs that would have failed to develop whether inundated or not. After an incubation period of 70 days, eggs were checked daily for signs of pipping. Date of pipping was recorded for each egg and taken as the point at which incubation was terminated. Pipped eggs were left in place until hatching was completed. Eggs that developed a white patch but that failed to hatch were counted as having died during incubation. Once eggs had hatched, the compartment into which they were placed was cleaned of vermiculite and fluid, and then partially filled with fresh incubation medium and the hatchling left in place. Hatchlings were checked daily until egg yolk sac had been fully internalized. They were then removed from the incubator, their scutes were notched according to a binary number system, and they were transferred to husbandry experiments.

Data from the Latin square experiment were analysed to determine the impact of inundation duration, temperature and clutch on incubation period using PROC MIXED in SAS Version 8 (SAS Institute 2001). Replication of the clutches (five arbitrary sets, as opposed to blocks) allowed a test of the interaction between inundation duration and temperature. Temperature and period of inundation were treated as fixed factors and clutch was treated as a random factor. The response surface for incubation period as a function of inundation duration and incubation temperature was obtained using a general linear model (PROC GLM) following appropriate transformation (see Results) and examination of residuals. Survivorship during inundation was related to the three factors of inundation duration, temperature and clutch using generalized linear models (PROC GENMOD) with a logistic link function and binomial error structure.

Results

SURVIVORSHIP

Rates of egg survivorship while under water varied significantly with duration of inundation ($\chi^2 = 17{\cdot}5$; df = 4; $P < 0{\cdot}002$) but not with clutch ($\chi^2 = 21{\cdot}1$; df = 24; $P = 0{\cdot}63$). Survivorship was high at 96–98% for 0, 2 and 6 weeks' inundation, declining to 92% at 10 weeks, declining sharply to 78% at 25 weeks.

Rates of survivorship during incubation varied significantly with temperature ($\chi^2 = 65{\cdot}26$; df = 4; $P < 0{\cdot}0001$) and duration of inundation ($\chi^2 = 20{\cdot}3$; df = 4; $P < 0{\cdot}0005$) but not among clutches ($\chi^2 = 33{\cdot}4$; df = 24; $P = 0{\cdot}09$). There was a steady decline in embryo survivorship with increasing temperature, from 80% at 26 °C (pooled over clutches and inundation treatments) to 10% at 32 °C (Fig. 1). Survivorship under different inundation treatments (pooled over clutches and temperature treatments) showed a unimodal distribution with mode of 72% at 6 weeks' duration dropping to 35% at the extremes of zero inundation and 25 weeks' inundation (Fig. 1).

INCUBATION PERIOD

Incubation period was profoundly influenced by both incubation temperature ($F = 7{\cdot}85$; df = 4,73; $P < 0{\cdot}0001$) and duration of inundation ($F = 13{\cdot}27$; df = 4,73; $P < 0{\cdot}0001$). There was no interaction between the effects of temperature or inundation ($F = 0{\cdot}99$; df = 14,73; $P = 0{\cdot}48$). The combined effect of temperature and inundation was strong – the expected incubation period of 180 days at 26 °C without inundation was 100 days longer than at 32 °C and 25 weeks' inundation (Fig. 2). An increase in temperature from 26 °C to 32 °C

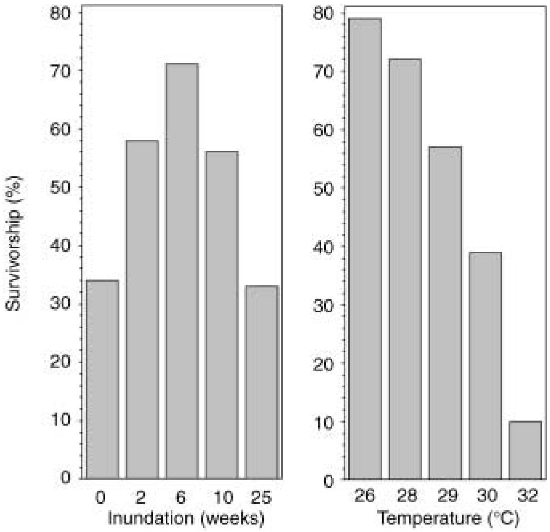

Fig. 1. Survivorship of embryos of *Chelodina rugosa* to prior egg inundation and incubation temperature. Note that survivorship values for inundation are pooled across temperatures and clutches, and survivorship values for temperature are pooled across inundation treatments and clutches in a Latin square design (see Materials and methods).

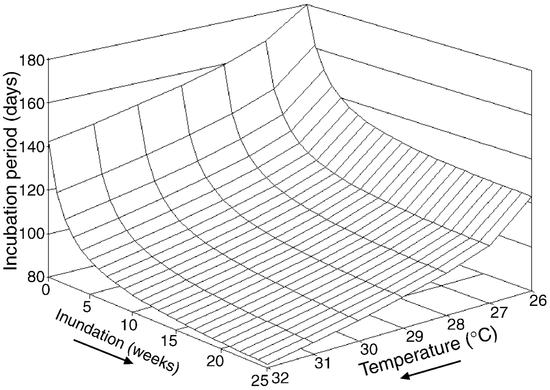

Fig. 2. Response of incubation period of *Chelodina rugosa* to variation in incubation temperature and inundation duration. Note that incubation period declines with increasing temperature and increasing inundation duration.

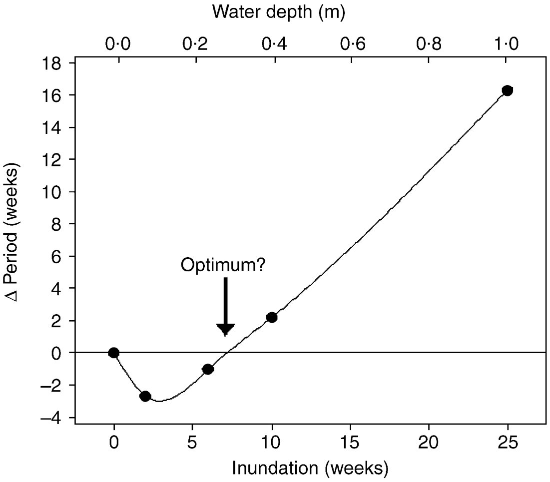

Fig. 3. Relationship between the change in incubation period (including the period of inundation-induced diapause) and period of inundation for *Chelodina rugosa*. Inundation period is rescaled to water depth based on a relationship describing reduction in water depth with time (2·04 m per annum) for Gid-da-della Billabong (May to September, 2005). Note that nests that undergo 7 weeks' inundation are ready to hatch at the same time as those that are not inundated. This corresponds to the inundation that yields maximum survivorship, and suggests an optimum period of inundation and corresponding laying depth (27·5 cm). If so, and mothers are laying at this depth, nests falling to the right of the optimum are those that have been laid early and subjected to water level rise postlaying.

decreased incubation period across all inundation treatments, by approximately 40 days, though almost half of this variation could be attributed to change in incubation period in the range 26–27 °C. Inundation had a stronger effect. An increase in duration of inundation from 0 to 25 weeks resulted in a decrease in incubation period of approximately 60 days, though most of this could be attributed to the first 10 weeks of inundation (Fig. 2). The equation for the least-squares surface of best fit relating incubation period (*IP*) to inundation duration (*ID*) and incubation temperature (*T*) was:

$$IP = 118{\cdot}0 + \frac{130{\cdot}90}{ID + 2} - 16{\cdot}85\sqrt{T - 26} \qquad \text{eqn 1}$$

($26 \leq T \leq 32$ °C; $0 \leq ID \leq 25$ weeks; *IP* in days) was established by first transforming *ID* and *T* to linear form with an inverse and square root transformation, respectively, as indicated in the equation. The proportion of variation in incubation period explained by temperature and inundation duration was 52·8%, without accounting for clutch effects.

There was considerable residual variation in incubation period after the effects of temperature, inundation duration and clutch were removed (variation explained 77·1%), well beyond the few days' variation of the synchronous hatching expected of most species of turtle (Booth 1998; Hewavisenthi & Parmenter 2001; Spencer, Thompson & Banks 2001; Ashmore & Janzen 2003). Incubation duration for embryos of *C. rugosa*, after correcting for temperature, period of inundation and clutch, varied about the predictive relationship by up to 50 days, with a standard deviation of 17·2 days. We lacked an appropriate error term to test the statistical significance of this residual variation, but its magnitude suggests that there are other factors, independent of temperature, inundation duration and clutch, that influence incubation period in this species.

The relationship between incubation and inundation can be expressed in terms of difference in the total time in the ground (inundation-induced diapause plus development time) between inundated and noninundated eggs to more clearly the impact of inundation on hatching date (Fig. 3). Adjustment of development rates in response to inundation compensates entirely for the period of inundation for eggs inundated for up to 7 weeks. Indeed, eggs inundated for 7 weeks or less

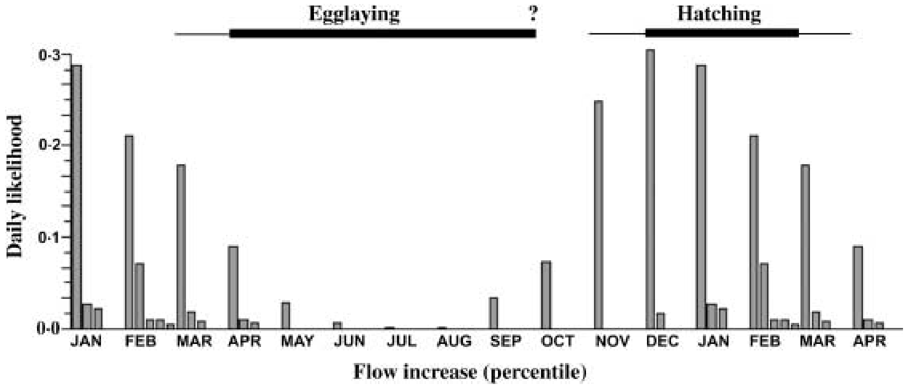

Fig. 4. Likelihood of daily stage height rises by month calculated from data for Dorisvale gauging station, Daly River, Northern Territory (1960–98, Station G8140067, data from Georges *et al.* 2003) as an indication of rainfall of sufficient quantity to influence surface water runoff. The horizontal axis is scaled to percentile flow increases by month (columns for each month represent the 10th, 30th, 50th, 70th and 90th percentiles, respectively). These data can be used as an index to probable water depth increases across months for areas affected by the monsoonal troughs. Substantial surface flow increases (50th percentile or greater) occur in all months from January to April, with decreasing frequency. Steady water level decline can be anticipated from May to August. Egg-laying occurs from early April (rarely March) until late September or until waters recede (indicated by the ?), whichever is the earliest. Conditions for hatching occur commonly in the months of December to February, rarely in November or March.

will be ready to hatch at the same time or before those laid in dry ground. Figure 3 also shows a rescaling of the *x*-axis to units of water depth. This was achieved using the relationship between water depth and time established for Gid-da-della Billabong (May to September, 2005). These billabongs have a simple pan shaped structure, and water depth decreases linearly through time, as a result of evaporation, evapotranspiration and seepage, at a rate of 2·04 m per annum ($R^2 = 0{\cdot}9975$). Using this relationship, 7 weeks corresponds to 27·5 cm water depth which, when taking into account core depth of a nest chamber, corresponds to nests laid close to the waters edge and is in close agreement with the depth at which the nests are constructed in the wild (Kennett, Christian & Pritchard 1993a).

Discussion

Our experiments show a number of remarkable results. First, the eggs of *Chelodina rugosa* can survive 25 weeks' inundation, almost 6 months, albeit with increased mortality. This is a considerable extension over the open-ended tolerance of 10–12 weeks demonstrated in earlier studies (Kennett *et al.* 1993b; 1998) and is more than adequate to cater for any period of inundation likely to be experienced in the wild. Second, there is a clear optimal inundation duration of 6 weeks, with eggs not held under water suffering the same mortality as eggs inundated for 25 weeks. Previous studies have shown that eggs of *C. rugosa* can survive inundation and can be laid underwater (Kennett *et al.* 1993a). Our result suggests that underwater nesting is not a facultative capacity but rather that inundation is essential for optimal survivorship of embryos in this species. Third, inundation duration has a profound effect on incubation period, reducing it by up to 9 weeks over what would be expected for a given temperature. Eggs that are inundated and undergo diapause for up to 7 weeks complete incubation faster than had they been laid at the same time in dry ground. Finally, there is remarkable variability in incubation period remaining after correcting for the effect of incubation temperature, inundation duration and clutch, confirming the results of previous studies on this and closely related species (Beynon 1991).

The challenge is to interpret these new results in the context of the reproductive strategy of *C. rugosa*. To do this we need to characterize the period suitable for egg-laying and the period suitable for hatching and emergence, and view the demonstrated influences on developmental time in this context. By nesting underwater, *C. rugosa* is released from the constraint of finding suitable dry land to nest, allowing nesting to begin as early as March, but more typically in April, regardless of the timing and or intensity of the previous wet season, except in exceptionally dry years when reproduction fails entirely (D. Fordham, unpublished data). Nesting is terminated by habitat loss when billabongs draw down and turtles prepare to aestivate (Grigg *et al.* 1986; Kennett & Christian 1994), an event that varies considerably in timing from year to year, or in late September, whichever comes first. Thus the timing of egg-laying can be viewed as a broad period set intrinsically to fall between April and September, but commonly abbreviated when waters recede and the turtles are forced into aestivation (Fig. 4). The period suitable for hatching and emergence is the onset of the wet season, when torrential rains soften the soil, allowing hatchling emergence, and fill the billabongs providing hatchlings with habitat necessary for survival. In the wet–dry tropics of northern Australia, the onset of the wet season is a narrow, highly variable window in time. The onset of the rains occurs commonly as early as December and as late as February (Taylor & Tulloch

1985; Georges *et al.* 2002). However, in extreme years turtles may need to be ready to hatch as early as November (D. Fordham, unpublished data). Turtle eggs that fail to complete development prior to the onset of the rains will perish as a result of inundation (Plummer 1976; Webb *et al.* 1983; Kennett *et al.* 1993b). Thus the time suitable for hatching and emergence can be viewed as a narrow target moving unpredictably in a 3-month window, December to February (Fig. 4). We believe that inundation-induced diapause, coupled with the compensatory effects of inundation on development period demonstrated in our study, serves to match the 6–7 month (or less) period of egg-laying to the 3-month period within which conditions suitable for hatching and emergence are likely to occur.

Some hypothetical examples serve to illustrate this point. A nest is laid on 1 April in 22 cm of water with a shallow chamber depth of 10 cm (Kennett *et al.* 1993a). A rainfall event, likely at this time of year (Fig. 4), increases the standing water depth above the nest to 50 cm by 1 May, after which water depth decreases in time through evaporation, evapotranspiration and seepage at a rate estimated to be 2·04 m per annum, as judged from the linear decline in water depth for Gid-da-della Billabong (May to September, 2005). The inundation-induced diapause thus adds 19·6 weeks to the incubation period of eggs laid on 1 April. Taking incubation compensation into account (equation 1) the subsequent incubation period at 26 °C will be 124 days. Thus, developmental compensation for inundation shifts hatching date back in time by 59 days bringing the eggs to the point of hatching on 18 December. A second nest is laid on 1 June, at a water and chamber depth identical to the first nest (a depth of 22 cm and 10 cm, respectively). In the absence of rainfall in June (Fig. 4), water level drops progressively at a rate of 2·04 m per annum (3·9 cm per week), ending the inundation-induced diapause after 8·2 weeks. Subsequent incubation will take 131 days, bringing the eggs to the point of hatching on 6 December. Developmental compensation for inundation shifts the hatch date back in time by 53 days. Note that the order of hatching is reversed in comparison with the order of egg-laying, so that eggs laid early in the season may well hatch later than eggs laid late in the season. This is a direct consequence of the inundation-induced diapause. Note also that the time interval between the laying dates of these two clutches is 8·7 weeks, but the interval between hatching dates is only 1·7 weeks. A substantial portion of this contraction of hatching period compared with laying period can be attributed to compensation in the incubation period for inundation, in this case 6 days or 12·2%.

Our examples are hypothetical in that we know very little of the incubation temperatures in natural nests (we have set it at an average of 26 °C, the optimum for hatching success in our experiment). Variation in incubation temperatures with season, mid-term diapause, delayed hatching after maturation, the unexplained variation in incubation period among eggs, or delay in emergence after hatching may each also contribute to setting the emergence dates of natural nests, and we know little of these aspects of the species biology. However, the examples serve to show that, all other factors held constant, inundation-induced diapause and developmental compensation for inundation can serve to (a) potentially reduce the variation in hatching date compared with the variation in laying date, and (b) shift the mean hatching date back in time – these provide a mechanism for exerting some control over egg-laying, development and hatching in the face of environmental stochasticity in the timing, intensity and duration of the monsoonal wet. This reproductive strategy provides potential for complete 'folding-over' of hatching times, whereby nests laid early in the season are ready to hatch at the same time as the latest nests of the season. This would commonly occur when clutches laid in March/April experience localized flooding postlaying in an average breeding season. We have demonstrated that *C. rugosa* has sufficient flexibility in its nesting strategy to match the timing of hatching and emergence to the probability distribution for the timing of conditions suitable for hatching and emergence (3 months, December to February), while at the same time, extending its nesting period to the maximum extent (6–7 months, March/April–September).

Chelodina rugosa is not the only freshwater turtle of the wet–dry tropics, and so not the only turtle that must contend with unpredictability in the conditions suitable for egg-laying, development and hatching. Rather than nesting underwater, other species occupying ephemeral swamps with seasonally unpredictable water level either seek out the limited opportunities to nest in higher ground (e.g. *Elseya branderhorsti* in the Suki-Aramba swamps of Papua New Guinea) or nest on floating mats that rise and fall with the water level (e.g. *Emydura subglobosa*) (Georges, Guarino & Bito 2006). The Pig-Nosed Turtle (*Carettochelys insculpta*), a riverine species, lays relatively few clutches, the embryos develop rapidly to maturity before entering embryonic aestivation and hatching is stimulated by hypoxia brought about by torrential rain or flooding (Webb *et al.* 1986; Georges 1992; Doody, Georges & Young 2003). Delayed hatching, in response to an appropriate cue, overcomes variability in the timing of conditions suitable to hatchling emergence and subsequent survival.

Notwithstanding the adaptive responses of other turtle species of the wet–dry tropics, *C. rugosa* shows a diversity of developmental responses to environmental stochasticity usually regarded as the domain of non-amniotes. For example, annual fish of several genera that occupy seasonally ephemeral habitat respond to environmental stochasticity in the timing and duration of dry periods through early anoxic-induced diapause, mid-term diapause and late term embryonic aestivation, the latter terminated by the refilling of their wetland habitat (Wourms 1972a,b,c). This, coupled with high fecundity, ensures their persistence. *C. rugosa* are highly fecund, embryos undergo anoxic-induced diapause, and there are strong indications of a mid-term diapause (Beynon 1991), probably terminated by a cool mid-year chill as in the congener, *C. expansa* (Booth 2002). Hatchling survivorship depends upon emergence that is coincident with resource availability (Van Noordwijk, McCleery & Perrins 1995), so emergence is likely to be delayed until an appropriate cue such as early wet season rains, though this has not been demonstrated. In addition to these developmental responses, *C. rugosa* appear to have a scattergun strategy for coping with unpredictability in the onset of the period suitable for hatchling emergence and survival. A protracted nesting season, during which many clutches are laid by individual females, and embryonic diapause, coupled with high variability in developmental rates among clutches and among eggs within clutches (Beynon 1991), ensures that some eggs will be available to hatch at the onset of the wet season, whether it be early or late. These diverse reproductive traits, comparable to those of some lower vertebrates facing similar environmental challenges (Wourms 1972a,b,c), present *C. rugosa* as possibly a unique amniote model for the study of evolutionary responses to environmental stochasticity in the variables that govern timing of reproduction, duration of development and timing of hatching and emergence.

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References

Ashmore, G.M. & Janzen, F.J. (2003) Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. *Oecologia* **134**, 182–188.

Beynon, F. (1991) *Comparative rates of oxygen consumption and development in two species of turtle from the genus Chelodina*. Honours Thesis, University of Canberra, Canberra.

Booth, D.T. (1998) Effects of incubation temperature on the energetics of embryonic development and hatchling morphology in the Brisbane river turtle *Emydura signata*. *Journal of Comparative Physiology B* **168**, 399–404.

Booth, D.T. (2000) Incubation of eggs of the Australian broad-shelled turtle, *Chelodina expansa* (Testudinata: Chelidae), at different temperatures: effects on pattern of oxygen consumption and hatchling morphology. *Australian Journal of Zoology* **48**, 369–378.

Booth, D.T. (2002) The breaking of diapause in embryonic broad-shell river turtles (*Chelodina expansa*). *Journal of Herpetology* **36**, 304–307.

Both, C. & Visser, M.E. (2005) The effect of climate change on the correlation between avian life-history traits. *Global Change Biology* **11**, 1606–1613.

Cagle, K.D., Packard, G.C., Miller, K. & Packard, M.J. (1993) Effects of the microclimate in natural nests on development of embryonic Painted turtles, *Chrysemys picta*. *Functional Ecology* **7**, 653–660.

Denver, R.J. (1997) Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. *American Zoologist* **37**, 172–184.

Doody, J.S., Georges, A. & Young, J.E. (2003) Twice every second year: reproduction in the pig-nosed turtle, *Carettochelys insculpta*, in the wet-dry tropics of Australia. *Journal of Zoology, London* **259**, 179–188.

Ewert, M.A. (1979) The embryo and its egg: development and natural history. *Turtles: Perspectives and Research* (eds M. Harless & H. Morlock), pp. 333–416. John Wiley, New York.

Ewert, M.A. (1985) Embryology of turtles. *Biology of the Reptilia* (eds C. Gans, F. Billett & P.F.A. Maderson), pp. 75–267. John Wiley, New York.

Ewert, M.A. (1991) Cold torpor, diapause, delayed hatching and estivation in reptiles and birds. *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles* (eds D.C. Deeming & M.J.W. Ferguson), pp. 173–191. Cambridge University Press, Cambridge.

Ewert, M.A. & Legler, J.M. (1978) Hormonal induction of oviposition in turtles. *Herpetologica* **34**, 314–318.

Ewert, M.A. & Wilson, D.S. (1996) Seasonal variation of embryonic diapause in the striped mud turtle (*Kinosternon baurii*) and general considerations for conservation planning. *Chelonian Conservation and Biology* **2**, 43–54.

Georges, A. (1992) Thermal characteristics and sex determination in field nests of the pig-nosed turtle, *Carettochelys insculpta* (Chelonia: Carettochelydidae), from northern Australia. *Australian Journal of Zoology* **40**, 511–521.

Georges, A., Webster, I., Guarino, E., Thoms, M., Jolley, P. & Doody, J.S. (2003) *Modelling Dry Season Flows and Predicting the Impact of Water Extraction on a Flagship Species*. Report No. ID23045 in Environmental Water Requirements of the Daly River, Northern Territory. Northern Territory Government, Department of Infrastructure, Planning and Environment, Daruin. March 2003.

Georges, A., Beggs, K., Young, J.E. & Doody, J.S. (2005) Modelling reptilian development under fluctuating temperature regimes. *Physiological and Biochemical Zoology* **78**, 18–30.

Georges, A., Guarino, F. & Bito, B. (2006) Freshwater turtles of the TransFly Region of Papua New Guinea – notes on diversity, distribution, reproduction, harvest and trade. *Wildlife Research* **33**(5), in press.

Gibbons, J.W. & Greene, J.L. (1979) X-ray photography: a technique to determine reproductive patterns of freshwater turtles. *Herpetologica* **35**, 86–89.

Grigg, G.C., Johansen, K., Harlow, P., Beard, L.A. & Taplin, L.E. (1986) Facultative aestivation in a tropical freshwater turtle *Chelodina rugosa*. *Comparative Biochemistry and Physiology Part A* **82**, 321–323.

Gutzke, W.H.N. & Packard, G.C. (1987) Influence of the hydric and thermal environments on eggs and hatchlings of bull snakes *Pituophis melanoleucus*. *Physiological Zoology* **60**, 9–17.

Hewavisenthi, H.S. & Parmenter, C.J. (2001) Influence of incubation environment on the development of the flatback turtle (*Natator depressus*). *Copeia* **2001**, 668–682.

Janzen, F.J., Ast, J.C. & Paukstis, G.L. (1995) Influence of the hydric environment and clutch on eggs and embryos of two sympatric map turtles. *Functional Ecology* **9**, 913–922.

Kennett, R. (1994) *Ecology of two species of freshwater turtle,* Chelodina rugosa *and* Elseya dentata, *from the wet–dry* *tropics of northern Australia*. PhD Thesis, University of Queensland, Brisbane.

Kennett, R. (1999) Reproduction of two species of freshwater turtle, *Chelodina rugosa* and *Elseya dentata*, from the wet–dry tropics of northern Australia. *Journal of Zoology, London* **247**, 457–473.

Kennett, R. & Christian, K. (1994) Metabolic depression in estivating long-necked turtles (*Chelodina rugosa*). *Physiological Zoology* **67**, 1087–1102.

Kennett, R., Christian, K. & Pritchard, D. (1993a) Underwater nesting by the freshwater turtle *Chelodina rugosa* from tropical northern Australia. *Australian Journal of Zoology* **41**, 47–52.

Kennett, R., Georges, A. & Palmer-Allen, M. (1993b) Early developmental arrest during immersion of eggs of a tropical freshwater turtle, *Chelodina rugosa* (Testudinata: Chelidae), from northern Australia. *Australian Journal of Zoology* **41**, 37–45.

Kennett, R., Christian, K. & Bedford, G. (1998) Underwater nesting by the Australian freshwater turtle *Chelodina rugosa*: effect of prolonged immersion and eggshell thickness on incubation period, egg survivorship, and hatchling size. *Canadian Journal of Zoology* **76**, 1019–1023.

Linsley, E.G. (1943) Delayed emergence of *Buprestis aurulenta* from structural timbers. *Journal of Economic Entomology* **35**, 348–349.

Madsen, T. & Shine, R. (1998) Quantity or quality? Determinants of maternal reproductive success in tropical pythons (*Liasis fuscus*). *Proceedings of the Royal Society of London B* **265**, 1521–1525.

Olsson, M. & Shine, R. (1997) The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why early clutches are better. *Journal of Evolutionary Biology* **10**, 369–381.

Packard, G.C., Packard, M.J., Miller, K. & Boardman, T.J. (1987) Influence of moisture, temperature and substrate on snapping turtle eggs and embryos. *Ecology* **68**, 983–993.

Plummer, M.V. (1976) Some aspects of nesting success in the turtle, *Trionyx muticus*. *Herpetologica* **32**, 353–359.

Powell, J.A. (1989) Synchronized, mass-emergences of a yucca moth, *Prodoxus y-inversus* (Lepidoptera: Prodoxidae), after 16 and 17 years in diapause. *Oecologia* **81**, 490–493.

SAS Institute (2001) *SAS*, Version 8·2. SAS Institute Inc., Cary, NC.

Seymour, R.S., Kennett, R. & Christian, K. (1997) Osmotic balance in the eggs of the turtle *Chelodina rugosa* during developmental arrest under water. *Physiological Zoology* **70**, 301–306.

Shine, R. (1988) Parental care in reptiles. *Biology of the Reptilia* (eds C. Gans & R.B. Huey), pp. 275–329. Alan Royal Liss, New York.

Shine, R. & Harlow, P.S. (1996) Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* **77**, 1808–1817.

Shine, R., Elphick, M.J. & Harlow, P.S. (1997a) The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* **78**, 2559–2568.

Shine, R., Madsen, T.R.L., Elphick, M.J. & Harlow, P.S. (1997b) The influence of nest temperatures and maternal brooding on hatchling phenotypes in water pythons. *Ecology* **78**, 1713–1721.

Spencer, R.J. (2002) Experimentally testing nest site selection: fitness trade-offs and predation risk in turtles. *Ecology* **83**, 2136–2144.

Spencer, R.J., Thompson, M.B. & Banks, P.B. (2001) Hatch or wait? A dilemma in reptilian incubation. *Oikos* **93**, 401–406.

Taylor, J. & Tulloch, D. (1985) Rainfall in the wet-dry tropics: extreme events at Darwin and similarities between years during the period 1870–1973. *Journal of Ecology* **10**, 281–295.

Thompson, M.B. (1985) Functional significance of the opaque white patch in eggs of *Emydura macquarii*. *Biology of Australasian Frogs and Reptiles* (eds G. Grigg, R. Shine & H.E. Ehmann), pp. 387–395. Australian Zoological Society of New South Wales, Sydney.

Van Noordwijk, A.J., McCleery, R.H. & Perrins, C.M. (1995) Selection for the timing of great tit breeding in relation to caterpillar growth and temperature. *Journal of Animal Ecology* **64**, 451–458.

Vinuela, J. (1997) Laying order affects incubation duration in the black kite (*Milvus migrans*): counteracting hatching asynchrony? *Auk* **114**, 192–199.

Webb, G.J.W., Sack, G.C., Buckworth, R. & Manolis, S.C. (1983) An examination of *C. porosus* nests in two northern Australian freshwater swamps, with analysis of embryo mortality. *Australian Wildlife Research* **10**, 571–605.

Webb, G.J.W., Choquenot, D. & Whitehead, P. (1986) Nests, eggs and embryonic development of *Carettochelys insculpta* (Chelonia: Carettochelidae) from northern Australia. *Journal of Zoology, London* **1B**, 512–550.

Wiebe, K.L., Wiehn, J. & Korpimaki, E. (1998) The onset of incubation in birds: can females control hatching patterns? *Animal Behaviour* **55**, 1043–1052.

Wilson, D.S. (1998) Nest-site selection: microhabitat variation and its effect on the survival of turtle embryos. *Ecology* **79**, 1884–1892.

Wourms, J.P. (1972a) Developmental biology of annual fishes. I. Stages in the normal development of *Austrofundulus myersi* Dahl. *Journal of Experimental Zoology* **182**, 143–168.

Wourms, J.P. (1972b) The developmental biology of annual fishes. II. Naturally occurring dispersion and reaggregation of lastomeres during the development of annual fish eggs. *Journal of Experimental Zoology* **182**, 169–200.

Wourms, J.P. (1972c) The developmental biology of annual fishes. III. Pre-embryonic and embryonic diapause of variable duration in the eggs of annual fishes. *Journal of Experimental Zoology* **182**, 389–414.

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