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The influence of incubation temperature on post-hatching fitness characteristics of turtles

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Abstract. Turtle eggs collected immediately after oviposition were incubated in the laboratory at several different constant temperatures and fitness-related hatchling attributes measured at hatching, and in freshwater turtle species at 12 months after hatching. In green sea turtles, incubation temperature was found to influence sex, size, and amount of yolk material converted to hatchling tissue as well as swimming performance during the 24 h frenzy swimming period that occurs within 48 h of hatching. In freshwater turtles, incubation temperature influenced swimming performance, post-hatch survival, and post-hatch grow. These results clearly indicate that incubation temperature can have an important influence on hatchling fitness by influencing post-hatch mortality (sea turtles) and growth rates (freshwater turtles). © 2004 Elsevier B.V. All rights reserved.

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1. Introduction

Many studies have reported that incubation temperature influences hatchling attributes of reptiles including turtles [1]. The range of these effects is diverse and can include determining a hatchling's sex, body shape, colouring, size, amount of yolk converted to tissue during embryonic development, locomotor performance, and behaviour. Variation in these traits is most likely to have consequences for the hatchling's fitness [2], and although studies documenting this type of phenotypic variation are numerous, only a relative few have attempted to relate such variation to

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hatchling fitness in a quantitative way [2]. Direct measurements of fitness in hatchling turtles is made difficult because of their relatively long life spans (>20 years for most species). In this article, we summarize the results from two recent studies on how incubation temperature induced phenotypic variation in hatchling traits influence attributes (size, swimming performance and growth rates) that are correlates of fitness in hatchling turtles.

2. Methods

We studied two species of chelonian, the Green turtle *Chelonia mydas* that has temperature dependent sex determination, and Brisbane river turtle *Emydura signata* that has genetic sex determination.

2.1. Green turtles

Sixty eggs were collected immediately after oviposition from each of four nesting Green turtles at the Heron Island rookery in November 2000. These eggs were immediately chilled to 10–15 °C for transport to the laboratory in Brisbane. On arrival in the laboratory, 20 eggs from each clutch were incubated at 26, 28, and 30 °C in moist perlite® (water potential ~−100 kPa). At hatching, hatchlings were marked with white paint on their carapace for individual identification, and left with other hatchlings in a darkened container at their incubation temperature for 48 h to simulate the time hatchlings usually spend digging out of natural nests. After this period, hatchlings were weighed and had their carapace length and front flipper length and width measured with calipers. A front flipper area index was calculated from the product of flipper length and width. A sub-sample from each incubation temperature (10 from 26 °C, 9 from 28 °C, and 16 from 30 °C) was killed by cooling and then freezing, and once thawed the residual yolk was dissected free. Both the residual yolk and yolk-free carcass was dried to constant mass at 60 °C. Most of the remaining turtles underwent swimming trials. Hatchlings were swum individually in a glass aquarium filled with seawater at 28 °C (the average seawater temperature at Heron Island during the hatching season). A monofilament nylon line was glued to the carapace and the line attached to bracket suspended above the middle of the tank. A low intensity light was placed at one end of the tank, and the other sides of the tank covered with black plastic to encourage unidirectional swimming. The length of the tether was adjusted so that hatchlings could swim freely below the water or on the surface but could not touch the bottom or sides of the tank. Hatchlings were videotaped for 10 min at time intervals of 0.5, 2, 6, 12, 18 and 23 h after being introduced to the tank. Power stroke rate was later calculated for each time interval from the videotapes by manual counting with a stopwatch and hand-held counter. After swimming trials had concluded, a sample of hatchlings from 26 and 30 °C and all hatchlings from 28 °C were killed by a 1 ml injection of barbiturate (Nembutal) into the brain, and the kidney with gonad attached dissected free and preserved in Bouin's fixative. The fixed gonad was imbedded in wax and serially sectioned. Sections were stained with hematoxylin and eosin and examined using light microscopy. Males and females were identified by the different cellular structure of testis and ovaries according to the criteria of Miller and

Limpus [3]. Single factor ANOVA and ANCOVA in which initial egg mass was the covariate was used to analyze Green turtle morphological characteristics. Repeat measures ANCOVA in which initial egg mass was the covariate was used to analyze stroke rate data.

2.2. Brisbane river turtles

Fifteen gravid females were collected during September 1999 and oviposition induced via the administration of oxytocin [4]. A sample of eggs from each clutch was incubated in moist vermiculite (∼−150 kPa) at 24, 27, and 30 °C. On hatching, hatchlings were placed in individual plastic jars at their incubation temperature for a further 48 h to insure complete absorption of the yolk sac and allow the carapace to take on its natural shape. After 48 h, hatchlings were weighed and had their carapace length and width measured, and had their swim speed measured. Swim speed was measured by placing turtles in the middle of a tank containing 6 cm of 24 °C water with its bottom marked with concentric circles 2 cm apart. The turtle was videotaped while it swam away from the centre. This procedure was repeated 30 times for hatchling turtles and 10 times for yearling turtles. These trials were when analyzed for swimming over 12 cm and a mean swim speed calculated from the fastest 10 trial for hatchling turtles and all 10 trials for yearling turtles. Hatchling turtles were scute marked so individuals could be identified and released into a large outside tank and feed to satiation with nutra max™ turtle pellets every second day during the non-winter months. Turtles were not fed over winter because they were not active. Once per month turtles were weighed. After 12 months, swim speed was measured again by the previously described method. Two-factor mixed model (clutch random factor, incubation temperature fixed factor) ANOVA was used to analyze morphological and swim speed results.

3. Results

3.1. Green turtles

Of the 80 eggs set at each incubation temperature, 59, 60 and 56 eggs produced healthy hatchlings from 26, 28 and 30 °C, respectively. Samples of 20, 51, and 20 hatchlings from eggs incubated at 26, 28 and 30 °C had their gonads examined histologically. All hatchlings examined from 26 °C were males, all hatchlings examined from 30 °C were female, while 37% of hatchlings from 28 °C were female and 63% were male. Egg sizes did not differ between the incubation treatments (ANOVA, P=0.200) but sibling hatchings from 26 °C were larger in terms of mass, carapace length and front flipper size (Table 1). These differences are due to the effect of temperature and are independent of sex because males that hatched from eggs incubated at 28 °C had similar morphology to females hatched from eggs incubated at 28 and 30 °C (Table 1).

The proportion of yolk converted into hatchling tissue during incubation was greater in 26 °C males, resulting in larger yolk-free mass and smaller residual yolk compared to hatchlings from 28 and 30 °C (Table 1). All hatchlings fatigued during the swimming trials, with the largest drop-off in performance occurring within the first 2 h (repeat measures ANOVA with incubation treatment a fixed factor, time a repeated

Table 1
Incubation times and hatchling morphological parameters of Heron Island Green turtle (*C. mydas*) eggs incubated at different temperatures

Variable	26 °C, males *n*=59	28 °C, females *n*=19	28 °C, males *n*=32	30 °C, females *n*=56	Significant treatment effect?	Comparisons between temperature treatments
Incubation time (days)	80.6±0.2	62.5±0.1	62.8±0.1	52.6±0.1	yes *P*<0.001	26 ♂>28 ♂=28 ♀>30 ♀
Hatchling mass (g)	26.3±0.2	25.6±0.2	25.6±0.2	25.4±0.2	yes *P*<0.001	26 ♂>28 ♂=28 ♀=30 ♀
Carapace length (mm)	49.3±0.2	49.0±0.2	49.1±0.2	49.2±0.1	no *P*=0.824	26 ♂=28 ♂=28 ♀=30 ♀
Carapace width (mm)	39.2±0.1	38.0±0.2	38.1±0.2	38.0±0.2	yes *P*<0.001	26 ♂>28 ♂=28 ♀=30 ♀
Front flipper area index (mm^2)	757±7	722±15	713±12	707±6	yes *P*<0.001	26 ♂>28 ♂=28 ♀=30 ♀
Yolk-free carcass dry mass (g)	7.28±0.25 *n*=10	sex not known 5.43±0.52 *n*=9		5.10±0.54 *n*=16	yes *P*<0.001	26 ♂>28 ♂/♀=30 ♀
Residual yolk dry mass (g)	0.57±0.12 *n*=10	sex not known 1.48±0.08 *n*=9		1.61±0.12 *n*=16	yes *P*<0.001	26 ♂<28 ♂/♀=30 ♀

It was assumed that all hatchlings from 26 °C were male, and all hatchlings from 30 °C were female as all hatchlings examined histologically from these temperatures conformed with this assumption. Sex of hatchlings from 28 °C was determined by gonad histology. Statistical comparisons made between treatments with ANCOVA with initial egg mass as the covariate followed by a post hoc Tukey's honest significant difference test for unequal sample sizes. Data are presented as mean±standard error of the mean.

factor, and stroke rate as the dependent variable: $P<0.0001$; see Fig. 1). Males incubated at 26 °C were consistently poorer swimmers than males and females from the warmer incubation treatments in terms of power stroke rate. This finding was consistent across clutches. Once again this difference can be attributed to a temperature

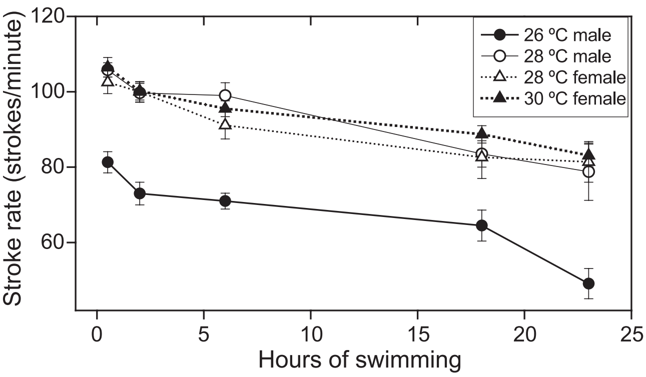

Fig. 1. Power stroke rate of Green turtle hatchlings incubated at different temperatures from Heron Island rookery during the frenzy swimming phase immediately after hatchlings were placed in water. Data are means±standard errors.

effect as males from 28 °C had a similar power stroke rate to females from 28 and 30 °C (P=0.506).

3.2. Brisbane river turtles

Incubation temperature influenced incubation length, while clutch of origin influenced all hatchling morphological characteristics, while incubation temperature influenced some hatchling morphological characteristics (Table 2). Incubation period was shortest at 30 °C, intermediate at 27 °C and longest at 24 °C. Hatchlings from eggs incubated at 30 °C were smaller in terms of mass, carapace width, and plastron width than those incubated at 27 and 24 °C. Swimming speed at hatching and also at 1 year was influenced by incubation temperature, but not by clutch (Table 2). Curiously, although turtles were larger at 1 year old, they did not swim any faster than when they were hatchlings. Relative swim performance was not repeatable between hatching and 1 year, i.e. within a temperature treatment, the fastest swimming turtles at hatching were not the fastest swimming at 1 year. Survival during the first year post-hatch was similar across all incubation temperature treatments and averaged 87% (Table 2). Growth patterns were similar across all incubation temperature treatments, with rapid growth during summer and autumn (November–May) little growth over the winter period (June–August) and rapid grow recommencing in Spring and Summer (September–January) (Fig. 2). Hatchlings from the 30 °C incubation treatment grew fast and were significantly larger after 12 months that hatchings from the

Table 2
Incubation times and hatchling morphological parameters of Brisbane river turtle (*E. signata*) eggs from 15 clutches incubated at different temperatures

Variable	24 °C, n=70	27 °C, n=74	30 °C, n=77	Significant temperature effect?	Significant clutch effect?	Comparisons between temperature treatments
Incubation time (days)	76±2	56±2	44±2	yes P<0.001	no P=0.824	24>27>30
Hatchling mass (g)	4.4±0.1	4.3±0.1	4.1±0.1	yes P<0.001	yes P<0.001	24=27>30
Carapace length (mm)	27.7±0.3	28.0±0.3	27.6±0.3	yes P=0.021	yes P<0.001	27>24=30
Carapace width (mm)	27.0±0.3	27.1±0.2	26.5±0.2	yes P<0.001	yes P<0.001	24=27>30
Plastron length (mm)	22.9±0.2	23.0±0.2	22.8±0.2	no P=0.383	yes P<0.001	24=27=30
Plastron width (mm)	13.0±0.1	13.1±0.1	12.8±0.1	yes P=0.002	yes P<0.001	24=27>30
Head width (mm)	9.2±0.1	9.2±0.1	9.2±0.1	no P=0.441	yes P<0.001	24=27=30
Hatchling swim speed (cm/s)	16.2±0.4	20.7±0.5	14.9±0.4	yes P=0.032	no P=0.13	27>24=30
Yearling swim speed (cm/s)	n=61 15.3±0.5	n=64 21.5± 0.6	n=67 15.5±0.4	yes P<0.001	no P=0.22	27>24=30
Yearling mass (g)	n=61 31.3±1.3	n=64 34.1±1.8	n=67 49.5±2.8	yes P<0.001	yes P=0.048	30>27=24
First year survival (%)	87	86	87			

Statistical comparisons made between treatments with a two-factor mixed model (temperature fixed factor, clutch random factor) ANOVA followed by a post hoc Tukey's honest significant difference test for unequal sample sizes. For all hatchling morphological attributes except plastron width there was a significant interaction between incubation temperature and clutch. For yearling attributes there was no significant interaction between incubation temperature and clutch. Data are presented as mean±standard error of the mean.

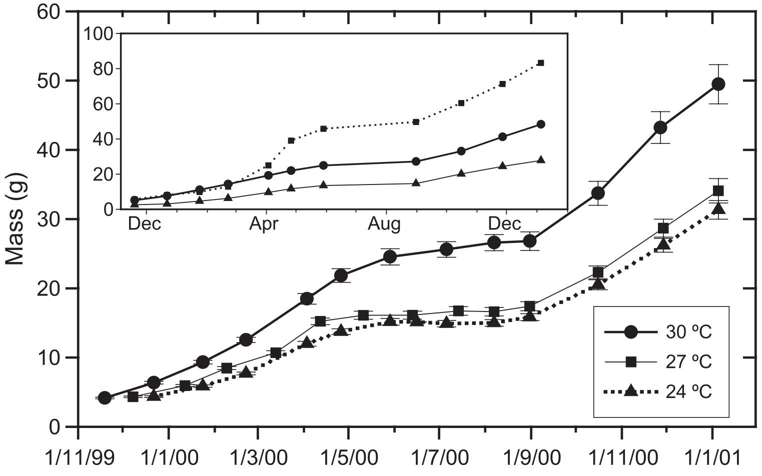

Fig. 2. Growth of Brisbane river turtles hatchlings from eggs incubated at different temperatures over the first 12 months post-hatching. Data are means±standard errors. Insert: growth of hatchlings from 3 of the 15 clutches from eggs incubated at 30 °C.

24 and 27 °C incubation treatment (Fig. 2; Table 2). Within a temperature treatment, hatchlings from different clutches grew at different rates (Fig. 2).

4. Discussion

Incubation temperature clearly has potential influence on hatchling fitness in both Green turtles and Brisbane river turtles, but the mechanism of by which fitness is influenced differ in the two species.

In Green turtles, incubation temperature influences sex determination, the amount of residual yolk converted to hatchling tissue, and swimming performance during the frenzy swimming period. The fitness consequence of temperature-sensitive sex determination has been extensively reviewed [5–8] and will not be discussed further. Conversion of more yolk to tissue during embryonic development at lower temperatures has been noted previously in the Green turtle [9], and in some other reptiles [10]. A larger hatchling with a smaller yolk reserve may be advantageous to hatchling entering an environment where food is easily obtained. The larger size may allow hatchlings to escape gape-limited predators, swim faster, and to successfully handle larger prey items hatchlings, i.e. the bigger is better hypothesis [11,12]. On the other hand, if the hatchling is entering an environment where food is scarce or difficult to locate, then a smaller body size and large residual yolk may be advantageous as the larger yolk could supply the hatchling with energy for a longer period of time than a smaller yolk. However, the difference in hatchling size/yolk reserve caused by differences in incubation temperature, although statistically significant are relatively small in Green turtles and is thus unlikely to play a major role in contributing differential fitness amongst hatchlings. By far the most important potential fitness outcome is the influence of incubation temperature on hatchling swimming performance during the frenzied swimming period when hatchlings are making their way from the natal beach to the open ocean.

In Green turtle hatchlings making their way from the nest to the open ocean, the highest rate of predation occurs in the water within the first 30–60 min of swimming as they pass through the gauntlet of predators found in the shallow water surrounding natal beaches [12–14]. Despite their larger body and front flipper size, 26 °C male hatchlings were consistently poorer swimmers than males and females from warmer incubation treatments, in terms of power stroke rate, and we anticipate that as a consequence predation of male hatchlings would be greater than females during this critical period. Curiously, nesting beach sand temperatures from Green turtle rookeries worldwide suggest a female-biased hatchling sex ratio [15–17]. Our results indicate that low temperature nest produce males of inferior swimming ability, and as a consequence the female-biased sex ratio of hatchling leaving nests may become even more skewed towards females in hatchlings that reach the open ocean.

The effect of incubation temperature on body size of hatchling Brisbane river turtles is small compared to interclutch differences in body size, so it probably has little or no ecological relevance. However, incubation temperature did influence swimming speed at both hatching and 1 year and also strongly influenced growth during the first year post-hatch, and both these traits could have significant effects of hatchling fitness. The faster burst swimming ability of hatchlings from 27 °C may enhance their fitness compared to hatchlings from 24 and 30 °C as these turtles may be more successful at avoiding predation [18]. Interestingly, clutch of origin did not influence swimming ability probably because the chief influence of clutch is on hatchling size, and there was no relationship between turtle size and swimming speed in our experiments. The absence of a correlation between swim speed at hatching and swim speed at 1 year also suggests that swim speed is not a good predictor of hatchling fitness in Brisbane river turtles.

The most significant influence of incubation temperature on Brisbane river turtles in terms of fitness outcomes is its effect on post-hatch growth rate. Hatchlings from 30 °C hatched earlier and grew faster than hatchlings from 24 and 27 °C. There are several potential advantages to having a faster growth rate. Firstly, predation rates on small turtles is thought to be greater than larger turtles [19,20], so the faster growing turtle will spend less time at a size that they are most vulnerable to predation. However, recent studies on wild populations of hatchling turtles indicate that size per se is not a good predictor of survival [10,21]. Secondly, sexual maturation in freshwater turtles is size-dependent and not age-dependent [19] and therefore the faster the growth, the sooner sexual maturity is achieved and this may increase the number of breeding seasons experienced and thus increase the life-time reproductive output.

In summary, incubation temperature has the potential to influence the fitness of hatchling turtles. In Green turtles this influence is manifested through sex determination and swimming ability, while in Brisbane river turtles it is manifested through post-hatch growth rate.

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