

Performance of Juvenile Tuatara Depends on Age, Clutch, and Incubation Regime

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ABSTRACT.—We investigated whether incubation treatment or size influence sprint speed of tuatara (*Sphenodon punctatus*) as artificial incubation is often used to produce founders for new populations of this endangered reptile. Sprint speed was affected by age, clutch and incubation regime (natural vs. artificial incubation). Older juveniles ran faster than younger juveniles. Maternal influence, represented by clutch, accounted for 18% of the variance in sprint speeds at one month of age. Naturally incubated juveniles were the smallest animals of any incubation regime, but at 10 months of age these juveniles were the fastest runners. Naturally incubated juvenile tuatara were more aggressive and faster for their size than artificially incubated juveniles, suggesting they may have higher fitness when released.

Incubation temperatures during embryonic development can affect phenotypic traits such as size and performance of hatchling reptiles, traits that potentially affect fitness (Van Damme et al., 1992; Elphick and Shine, 1999). Biological fitness is an organism's relative lifetime reproductive success (Ridley, 1997). For phenotype at hatching to affect fitness, phenotypic differences must affect either probability of survival to reproductive age or success in reproduction (Ferguson and Fox, 1984; Stearns and Koella, 1986). Running speed is an example of a performance measure used to assess behavioral responses with respect to embryonic incubation regimes in reptiles (Elphick and Shine, 1998). Sprint speed potentially affects survival and growth through access to food resources. We investigated whether incubation treatment has consequences for performance differences that might affect fitness of juvenile tuatara (*Sphenodon punctatus*).

Tuatara are medium-sized, cryptically colored, sexually dimorphic reptiles with temperature-dependent sex determination (TSD; Dawbin, 1982; Cree et al., 1995). Now restricted to offshore islands of New Zealand, they are of high conservation importance (Cree and Butler, 1993) and are biologically significant as the sole living representatives of the reptilian order Sphenodontia (Benton, 1990). Eggs have been collected and artificially incubated to produce founders for new captive and wild populations and to augment existing rare populations (Daugherty, 1998). Incubation regime (natural vs. artificial) and temperature during embryonic development do not affect size of tuatara at hatching (Nelson et al., 2004a). By 10 months of age, however, juveniles from warmer artificial incubation treatments are larger (snout-vent length = SVL, and mass) than those from cooler treatments and juveniles incubated in natural nests. Juveniles incubated in natural nests have a greater incidence of tail loss than those from artificially incubated eggs (Nelson et al., 2004a).

We addressed the following questions with juvenile tuatara: (1) Is performance heritable? (2) Does performance vary with size? (3) Is performance related to incubation treatment? (4) Do performance patterns change by 10 months posthatching? To assess these questions we examined sprint speed, a performance measure that could be closely connected to survival and growth of tuatara in natural habitats. Tuatara are capable of short sprints but tend to move in a stop-start manner, avoiding predators by retreating quickly into burrows, and often detecting prey using sit-and-wait tactics (Daugherty and Cree, 1990). We assessed sprint speed over a short distance and make the assumption that faster animals will be more likely to avoid predation and possibly obtain food.

MATERIALS AND METHODS

Source Animals, Test Times, and Conditions.—The source of tuatara (*S. punctatus*) eggs was Stephens Island, New Zealand. Details of captive incubation and husbandry procedures are presented in Nelson et al. (2004a,b), but briefly, this study involved 398 juvenile tuatara that were incubated and head-started as part of an ecological restoration program. The eggs were incubated either artificially at one of three constant temperatures (18, 21, and 22°C) in a split clutch design or naturally in undisturbed nests where they experienced variable temperatures throughout incubation. The resulting hatchlings were held at Victoria University of Wellington in similar rearing conditions (Nelson et al., 2004a).

Juveniles (aged 0–1 yr) were housed in groups of 16 animals, based on hatching date, in enclosures (size: 600 × 700 × 350 mm) furnished with a leaf litter base, tree bark for shelter, and two water dishes. There was no attempt to keep siblings together, or to isolate them. Juveniles were fed three times per week with similar amounts of crickets, house flies, or blow flies per enclosure regardless of juvenile size or hatching date. Enclosures were exposed to daily temperature variations of approximately 18–22°C and a 12:12 light:dark cycle beginning at 0600 h throughout the year (Duro-test® True-lite® power twist fluorescent tubes, placed 750 mm above the enclosure). At the

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end of the study, when juveniles were approximately one year old, all juveniles were released into an outside head-start facility. They were released into the wild, as founders for new populations, in October 2004.

Performance of juvenile tuatara from controlled artificial incubation conditions and natural nests was measured at one and 10 months posthatching, in the same room in which animals were housed. Before testing commenced, SVL (mm) and total length (TL) were measured. Performance testing was conducted at room temperature (which was recorded and used in analyses, range 18–22°C), and centered around mid-day. Although tuatara are reportedly nocturnal, they are also active during the day. Experimental group sizes varied because every juvenile was tested within three days of their one- and 10-month posthatch dates, hence a universal start and finish time for experimentation was not feasible. The same person conducted all tests and measurements (NJN).

Performance Measures.—Sprint speed was measured on a 1.5 m straight, flat racetrack using an electronic stopwatch. A stopwatch was wired to five pairs of infrared transmitters, and receivers were placed 0.25 m apart along, and 4 mm above the floor of the track. When the tuatara crossed the first set of lights, the stopwatch began timing on four panels. Each timer stopped when its respective beam was crossed, providing four measurements for the time taken (in seconds) to run each 0.25 m stretch (or the total time taken to run 1 m). Approximately 0.2 m was available at one end of the track for positioning the tuatara prior to testing, and 0.3 m at the other end of the track to receive the animal. Tuatara were encouraged to run along the racetrack using a paintbrush to flick their tails. Three tests were conducted on each animal, consecutively, without a rest period.

Statistical Analyses.—The full dataset was analyzed with a repeated measures analysis of covariance (ANCOVA) using the computer program R (Gentleman et al., 2003). The dependent variables were the logs of the fastest sprint speed (ms^{-1}) of each tuatara over 0.25 m at one and 10 months of age. Incubation treatment (i.e., artificial vs. natural incubation regime), sex and age were fixed effects, and clutch was a random effect nested in incubation treatment. Covariates used were size (SVL and TL) and test temperature. A subset of the data including log (sprint speed) of artificially incubated juveniles was analyzed using incubation temperature as a fixed categorical factor. Sex was not included in these analyses as incubation temperatures covaried with sex of hatchlings. Similarly, a subset of the data including only log (sprint speed) of naturally incubated juveniles was analyzed. Constant temperature equivalent (CTE) in February was the variable used to represent the incubation temperature of each nest (Nelson et al., 2004a), modelled as a random effect. Mean CTE accounts for the variability of a nest as well as its mean temperature. The CTE is defined as the temperature above which half of embryonic development occurs (Georges, 1989; Georges et al., 1994).

Akaike's information criterion (AIC; Akaike, 1973; Burnham and Anderson, 1998) was used to screen models. *P*-values are reported (sequential ANOVA; Type I sums of squares) for models that provide the

TABLE 1. Fastest sprint speeds (ms^{-1}) of juvenile tuatara over a 0.25 m distance.

Data category	<i>N</i>	Mean (\pm SE)	Range
<u>At 1 month posthatching</u>			
Artificial incubation	278	0.49 (0.01)	0.22–1.11
Natural incubation	124	0.47 (0.01)	0.20–0.98
Male ^a	186	0.48 (0.01)	0.20–1.02
Female	215	0.49 (0.01)	0.24–1.11
Artificial incubation			
18°C	99	0.51 (0.02)	0.30–1.11
21°C	74	0.49 (0.02)	0.35–0.91
22°C	105	0.48 (0.01)	0.22–1.02
Natural incubation			
Male	78	0.49 (0.02)	0.20–0.98
Female	46	0.44 (0.02)	0.24–0.77
<u>At 10 months posthatching^b</u>			
Artificial incubation	277	0.67 (0.02)	0.24–1.56
Natural incubation	123	0.84 (0.03)	0.25–3.16
Male	183	0.77 (0.03)	0.24–3.16
Female	216	0.69 (0.02)	0.25–2.46
Artificial incubation			
18°C	99	0.64 (0.02)	0.33–1.13
21°C	74	0.67 (0.03)	0.25–1.07
22°C	104	0.72 (0.03)	0.24–1.56
Natural incubation			
Male	76	0.85 (0.05)	0.25–3.16
Female	46	0.83 (0.05)	0.37–2.46

^a We were unable to ascertain the sex of one juvenile; this animal was not included in analyses involving sex as a factor.

^b Differences in the number of individuals between one- and 10-month tests were caused by equipment failure. If equipment failed, individuals were not retested.

best explanation of variance, that is, those with the lowest AIC values. All datasets satisfied the assumptions of normality and homogeneity of variances.

RESULTS

Sprint speeds of juveniles ranged from 0.20–1.11 m s^{-1} at one month of age and 0.24–3.16 m s^{-1} at 10 months posthatching (Table 1). The best model representing the whole dataset included age ($F_{(1,396)} = 243.59$; $P < 0.0001$), incubation treatment ($F_{(1,69)} = 5.87$; $P = 0.018$), and an interaction between these two factors ($F_{(1,396)} = 23.60$; $P < 0.0001$; AIC lowest by 15.8, $df = 7$). Sprint speeds increased with age of tuatara, and there was no significant correlation ($r = 0.0199$; $P = 0.692$) between the first and second observation on individuals. Age accounted for 21.8%, incubation treatment for 1.1%, their interaction for 2.1% and clutch for 4.3% of the variation in sprint speeds.

Total length ($t_{(1,325)} = 3.78$; $P = 0.0002$) and incubation temperature ($t_{(1,325)} = -2.38$; $P = 0.0181$) significantly influenced sprint speed of all juveniles at one month of age. Longer juveniles were faster, but size accounted for only 2.6% of the variance (Fig. 1), compared to 18% of the variance explained by clutch, representing the maternal influence. Incubation temperature explained only 1.4% of the variance in sprint

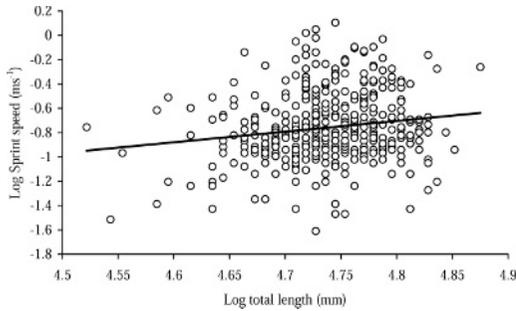


FIG. 1. Sprint speeds of juvenile tuatara at one month of age as a function of total length. Longer juveniles ran faster.

speeds. When sprint speeds of one-month-old juveniles from the two incubation treatments were analyzed separately, clutch remained the only common factor affecting both groups ($F_{(1,224)} = 1476.49$; $P < 0.0001$ for artificially incubated; $F_{(1,101)} = 253.97$; $P < 0.0001$ for naturally incubated). Incubation temperature was no longer a significant factor and SVL was a significant covariate only for those juveniles incubated from natural incubation conditions ($F_{(1,101)} = 9.65$; $P = 0.0025$).

At 10 months of age, the best model for explaining variance in sprint speeds of all juveniles included incubation treatment (Fig. 2; $t_{(1,69)} = 4.29$; $P = 0.0001$), experimental temperature ($t_{(1,326)} = 2.64$; $P = 0.0087$), and clutch ($t_{(1,326)} = -3.46$; $P = 0.0006$). Although incubation treatment accounted for only 6.2% of the variance, it accounted for much of the among-clutch variation (for example, if incubation treatment was omitted, the clutch component increased from 0.08–6.1%). Naturally incubated juveniles ran faster than artificially incubated juveniles. Experimental temperature accounted for 1.6% of the variation. Sprint speed of artificially incubated juveniles was weakly, but significantly, affected by experimental temperature ($F_1 = 6.24$; $P = 0.0132$). Speed increased with increasing test temperature. Sprint speed of naturally incubated juveniles was only weakly related to SVL ($F_1 = 6.20$, $P = 0.0144$), and not correlated with TL.

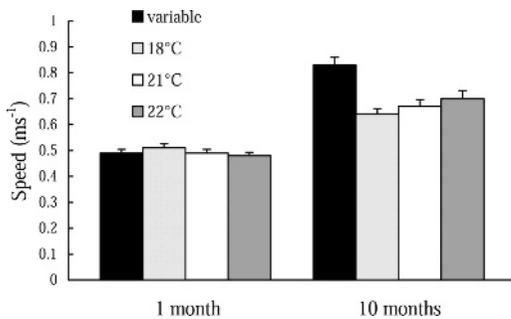


FIG. 2. Mean (\pm SE) of fastest speeds of juvenile tuatara posthatching over 0.25-m distance. Juveniles are grouped by artificial (18, 21 and 22°C) and natural incubation regime (variable). At 10 months of age, naturally incubated juveniles ran faster than artificially incubated juveniles.

DISCUSSION

We investigated factors that affect performance and potentially fitness of tuatara by asking whether juvenile tuatara performance varies with clutch, size, and/or incubation treatment and whether performance patterns change by 10 months posthatching. Burst speeds of juvenile tuatara are similar to those of lizards; for example, they are faster than smaller sized *Urosaurus* (Miles, 2004) but not as fast as smaller *Sceloporus* (Sinervo, 1990) or larger *Anolis* (Macrini and Irschick, 1998). Clutch origin accounts for a small proportion of the variation in sprint speeds at one month of age, suggesting that performance is in some part heritable. However, by 10 months of age, most of the among clutch variability is accounted for by incubation treatment.

We found that performance of juvenile tuatara varies with size. Longer juveniles are faster than shorter juveniles at one month of age. Increasing speed with increasing size is a common performance pattern for lizards (e.g., Sinervo and Adolph, 1989; Van Damme et al., 1992). Larger juvenile reptiles that sprint faster are likely to be more successful at hunting prey (Avery et al., 1982; Warner, 2001), obtaining access to limited resources and a wider size range of prey items (Ferguson and Fox, 1984; Ferguson et al., 1982), avoiding predation (Christian and Tracy, 1981; Webb, 1986), and retaining high social ranks (Garland et al., 1990).

Performance is affected by incubation regime, that is, natural versus artificial incubation. Naturally incubated juveniles are the shortest animals from any incubation regime (Nelson et al., 2004a), but at 10 months of age, these juveniles are the fastest runners. Long-term consequences of these effects on fitness are yet to be determined. Performance is not, however, directly affected by incubation temperature. A potential beneficial finding of this study is that within the laboratory setting, choice of incubation temperatures can be made with respect to desired sex ratios or hatching times, within constraints, without the concern over physiological effects on locomotor performance.

Factors other than speed may also be important determinants of fitness. Juveniles from the natural incubation treatment are significantly shorter than those artificially incubated, in part because of substantial tail loss by 10 months of age (Nelson et al., 2004a). High rates and large amounts of tail loss in the naturally incubated group indicate that artificially incubated juveniles are comparatively less aggressive in the colonial rearing conditions (Nelson et al., 2004a), and this could help explain the pattern of size differences among incubation treatments. Differences in aggression are associated with incubation temperature in geckos also (Flores et al., 1994). Hence, although artificially incubated juveniles do well in captivity and grow faster than those incubated naturally (Nelson et al. 2004a), they may have lower success in nature if cryptic coloration is insufficient protection for motionless juveniles.

Older juveniles run faster than younger juveniles. The leap from performance at 10 months to interpretations for fitness of tuatara, however, is a substantial one. Tuatara have an indeterminate growth pattern (Nelson et al. 2002), may live 100 yr (Dawbin,

1982), and performance may change with time (Jayne and Bennett, 1990). For example, subadult and juvenile tuatara run faster than adults (Pepperell, 1982). Reptiles may have different performance requirements with age (Greene, 1988). For example, juvenile tuatara may need to be faster than adults to avoid being eaten by them. In addition, sprint performance of individuals at one month of age was not repeatable at 10 months. This finding has important consequences for interpretations of speed on fitness. For example, fitness consequences may be missed or misinterpreted if only one test period is used, or depending on the test period/age chosen for any particular study.

Size and performance can affect growth and survival, that is, life-history traits that have the potential to have strong effects on lifetime reproductive success. Larger size may mean female reptiles reach maturity earlier and hence have greater relative fitness because they have opportunities to reproduce sooner and/or a longer reproductive life. Larger size may also mean female reptiles are capable of producing larger clutches once they reach maturity (Schwarzkopf and Brooks, 1986; Cree, 1994). Reproductive success in males of species with sexual dimorphism, where males are larger than females, may also be affected by size (Berry and Shine, 1980).

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