

The righting response as a fitness index in freshwater turtles

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Theoretical evolutionary ecology assumes the existence of fitness variability in natural populations. As realistic measures of fitness are usually difficult to perform directly, integrating fitness indices are proposed in all taxa. In sauropsids, locomotor performances have been proposed as a good integrating index of fitness in natural populations. Concerning aquatic turtles, a performance trait that may be important for the survival of juveniles is the righting response of individuals when they are placed on their carapace. In the present study, we examined the righting response in juveniles of the red-eared slider turtle, *Trachemys scripta elegans*. We tested two different measures of the righting response for 170 juveniles from constant incubation temperature and for 86 juveniles from three sinusoidal fluctuating incubation temperatures that are considered as more representative of natural conditions in the nest. We compared the effects of offspring sex, maternal identity, juvenile growth rate, and juvenile survival (i.e. individual characteristics), as well as the nutritional status of juveniles (i.e. experimental conditions), on the two different measures of righting response and for each thermal incubation treatments of the eggs (i.e. experimental treatments). We observed that the effects of the individual characteristics were markedly different between the two measures of the righting response and between experimental treatments. These results highlight the importance of the choice of the measure and of the experimental conditions and treatments in the study of a phenotypic trait. Results obtained for only one performance measure under constant laboratory conditions must therefore be extrapolated to the field with caution. Our results also show that the righting response presents individual variability that is probably heritable and is indirectly correlated with survival. These findings support the validity of the righting response as a good candidate for a fitness index in aquatic turtles. © 2007 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2007, 91, 99–109.

ADDITIONAL KEYWORDS: aquatic turtle – experimental conditions – locomotor performance.

INTRODUCTION

Most studies in evolutionary ecology assume the existence of fitness variability in natural populations. The estimation of fitness is possible through realistic measures of reproductive and survivorship traits. However, in most vertebrate species, these traits are difficult to assess directly. According to the assumption of a tight relationship between phenotype and fitness, many authors proposed that fitness in natural populations can be assessed by the study of phenotypic traits (Arnold, 1983; Garland & Losos, 1994). In ver-

tebrates, the relationships between locomotor performances, morphology, behaviour, and fitness have been extensively studied (Garland & Carter, 1994; Feder, Bennett & Huey, 2000). In particular, locomotor performance was proposed by Arnold (1983) as an integrating index of selective processes that influence fitness. These performance studies were frequently performed in vertebrates (Gaillard *et al.*, 2000; Ghalambor, Reznick & Walker, 2004; Thibodeaux & Hancock, 2004) and particularly in sauropsids. For lizards and snakes, the most commonly studied performances include running or swimming speed (snakes: Burger, 1989; lizards: Garland, Hankins & Huey, 1990; Huey *et al.*, 1990; Elphick & Shine, 1998, 1999; Irschick & Losos, 1998; Clobert *et al.*, 2000; Webb, Brown &

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Shine, 2001). Additionally, for turtles, the righting response (i.e. the return in a prone position after being placed upside down) has also been studied (Du & Ji, 2003; Janzen, 1993; Rhen & Lang, 1999; Freedberg, Ewert & Nelson, 2001; Steyermark & Spotila, 2001; Freedberg *et al.*, 2004). All of these phenotypic traits have been examined in relation with genetic, environmental (e.g. incubation conditions, maternal effect) and ontogenetic factors (Ashmore & Janzen, 2003; Miller, Packard & Packard, 1987; Janzen, 1995; Sorci & Clobert, 1995, 1997; Elphick & Shine, 1998; Finkler, 1999; Main & Bull, 2000; Webb *et al.*, 2001).

Within sauropsids, locomotor performances have been shown to be influenced by the hydric and thermal incubation conditions of the eggs (Burger, 1989; Miller *et al.*, 1987; Janzen, Ast & Paukstis, 1995; Shine & Harlow, 1997; Elphick & Shine, 1999; Freedberg *et al.*, 2001; Du & Ji, 2003), as well as by sex (Cullum, 1998; Elphick & Shine, 1999; Webb *et al.*, 2001; Lailvaux, Alexander & Whiting, 2003) and maternal identity (Ashmore & Janzen, 2003; Sorci, Massot & Clobert, 1994; Rhen & Lang, 1995; Sorci & Clobert, 1997; Steyermark & Spotila, 2001; Webb *et al.*, 2001; Warner & Andrews, 2002). Alternately, locomotor performances have been studied to investigate the existence and maintain of temperature-dependent sex determination (TSD) in some sauropsid species. In such species, the growth and the sex of the embryo are influenced by the temperature in the nest (Bull, 1980; Ewert, Jackson & Nelson, 1994). The most widespread hypothesis to explain the adaptative advantage of TSD, proposed by Charnov & Bull (1977), predicts that (1) incubation temperature influences both offspring sex and fitness and (2) the differential of fitness between males and females varies according to incubation temperature. To test this hypothesis, some authors have studied the influence of sex and incubation temperature on individual fitness through locomotor performance (Janzen, 1995; Rhen & Lang, 1995; Demuth, 2001; Webb *et al.*, 2001; Freedberg *et al.*, 2004).

In natural conditions, young aquatic turtles are frequently destabilized and find themselves upside down, particularly when running from their terrestrial nest to the closest pond (Burger, 1976). This frequently observed behaviour may considerably increase predation exposure or dehydration sensibility of juveniles (Finkler, 1999; Steyermark & Spotila, 2001; Kolbe & Janzen, 2002). The ability to right (i.e. the righting response) may thus be related to juvenile survival, and thus be a critical fitness component for juvenile turtles. Moreover, this performance trait is easy to measure in experimental conditions, partly because turtles are highly motivated to perform (Burger *et al.*, 1998; Freedberg *et al.*, 2001). Studies of several TSD species of turtles generally show that hydric and thermal incubation conditions (Finkler, 1999; Freedberg

et al., 2004), body size (Burger *et al.*, 1998), and maternal identity (Steyermark & Spotila, 2001; Ashmore & Janzen, 2003) affect righting response. However, the potential effect of offspring sex stays unclear because of the difficulty to separate sex and incubation temperature effects in TSD species (Rhen & Lang, 2004; Valenzuela, 2004).

It is generally assumed that locomotor performance somehow reflects individual fitness, even though the relationships between performance traits and survivorship remain somewhat elusive (Janzen, 1995). Because a perfect correlation between performance and fitness would never be expected, it is therefore hard to determine which performance trait is susceptible to influence fitness (Sorci *et al.*, 1994; Travis, McManus & Baer 1999). Moreover, a phenotypic response such as locomotor performance corresponds to a complex behavioural response that can be measured in many ways. Additionally, environmental conditions encountered at any given life-stage can potentially lead to trade-off between performance and other traits that would almost certainly weaken the correlation between performance and fitness (Le Gaillard, Clobert & Ferrière, 2004). Most locomotor performance studies are performed under laboratory conditions due to difficulties inherent to such studies in natural conditions. Furthermore, the experimental conditions are not always chosen to reflect biologically realistic conditions. In particular, in TSD species, the influence of incubation temperature on locomotor performance has usually been studied by using constant temperatures (Janzen, 1995; Freedberg *et al.*, 2001, 2004; Steyermark & Spotila, 2001) whereas, in natural nests, temperature fluctuates considerably on a daily and seasonal basis (Georges, Limpus & Stoutjesdijk, 1994; Packard & Packard, 1988). Freedberg *et al.* (2001: 960) argued that '(...) it is unlikely that [observed] strong pattern of male temperatures yielding lower fitness (...) would disappear or be reversed in natural nests'. However, several studies have found that thermal variance has a strong impact on offspring traits (Georges *et al.*, 1994; Doody, 1999; Demuth, 2001; Valenzuela, 2001). Results obtained under laboratory conditions can therefore be difficult to extrapolate to natural conditions (Ashmore & Janzen, 2003; Elphick & Shine, 1998, 1999; Webb *et al.*, 2001). Thus, it may appear necessary to use several different measures of locomotor performance and to consider precisely environmental conditions (i.e. ontogenic and experimental design) to yield reliable conclusions.

In the present study, we examined the righting response in a freshwater turtle with TSD, the red-eared slider turtle, *Trachemys scripta elegans*, using two different measures of this righting response. We examined the relationships between these two mea-

tures (i.e. the time elapsed until first move after being placed upside down and the duration of the active righting response) and individual characteristics (sex, maternal identity, growth rate, and survival), under two different experimental designs (fluctuating or constant incubation temperature of eggs and before or after feeding). We compared the results obtained for these two measures of the same locomotor behaviour and investigated the influence of experimental conditions on these results. Finally, we discuss the limits and perspectives of the righting response as fitness index in freshwater turtles. We also discuss the generalization and the extrapolation of this kind of study to the field.

MATERIAL AND METHODS

STUDY SPECIES

The red-eared slider turtle (*T. scripta elegans*) is native to USA. Between 1970 and 1997, millions of small young turtles (3–4 cm carapace length) were imported from USA and sold in France as pets, and most of them were released in natural ponds.

To date, in the field, production of viable hatchlings of this species was only observed in the South of France. Females lay their eggs from June to July and hatchlings emerge from their nests in September (D. Touzet, pers. comm.). Hatchlings do not overwinter in the nest as commonly observed in natural populations of *T. scripta elegans* in northern America (Tucker & Paukstis, 1999).

EGG COLLECTION

Sixty gravid females of *T. scripta elegans* were captured in artificial ponds in the South of France, in two zoological facilities located in the 'Ferme aux Crocodiles' (Pierrelatte, France) and in the 'Association Tortues Passion' (Vergèze, France). These two facilities are geographically very close to each other, and turtles present there come from everywhere in France; thus, we considered the origin of turtles as random. Moreover, densities of animals in these two artificial ponds are approximately the same and turtles are fed twice a week with the same food. We decided thus to consider all turtles as a single population.

Each captured female was isolated in a plastic box with some water. Oviposition was induced by injection of 1 mL of intramuscular oxytocin (Ewert & Legler, 1978). Each oviposited egg was identified by a code number on the eggshell for individual identification, weighed and placed on wet cotton in a cool box to prevent desiccation during transport to the laboratory. After egg deposition, adult females were released in their artificial ponds.

INCUBATION OF EGGS AT LABORATORY

Fertile eggs were two-thirds buried in moistened vermiculite under controlled and intermediate water potential (–398 kPa, i.e. 0.44 g of sterilized water·g⁻¹ vermiculite), and incubated in four programmable incubators (Memmert, IPP 200–400). A first set of 190 fertilized eggs was incubated at a constant temperature of 28.9 °C (regime A) that yields both sexes (Mrosovsky & Pieau, 1991; Godfrey, Delmas & Girondot, 2003). The other 112 eggs were shared and incubated at three different fluctuating temperatures (24-h fluctuating regimes, respectively, defined as 29 ± 3 °C, 28.5 ± 5 °C and 28.5 ± 1.5 °C). Eggs were placed in covered plastic boxes, with a maximum of ten eggs per box. Eggs from the same clutch were dispersed as completely as possible among boxes within each temperature treatments (constant vs. fluctuant) to prevent confounding box and clutch effects. Twice a week, boxes were rotated to prevent local variations of temperature in incubators and weighed to adjust water potential.

HUSBANDRY

After piping and before emerging, each hatchling turtle was placed on wet cotton in an individual plastic cup. After emergence, the turtles were maintained in the humidified cup until total absorption of yolk. Hatchlings were then weighed and individually marked by shell notching. Approximately 15 days after hatching, turtles were housed under standardized conditions in plastic tubs (60 cm long × 40 cm wide × 30 cm high) filled with 4 cm of water, in an air-conditioned room. Juveniles were randomly distributed among tubs, with a density of approximately 15 hatchlings per tub. Ambient temperature was maintained at 28 °C, whereas water temperature varied between 22 and 23 °C. Basking sites were placed in the tubs to allow thermoregulation. PowerSun ultraviolet (UV) lamps (Zoo Medical Laboratories Inc.) offered the appropriate intensity of UVB, UVA, heat and visible light with respect to photoperiod (10 : 14 h light/dark cycle). Turtles were fed twice a week *ad libitum* with Hatchling Aquatic Turtle Food (Zoo Medical Laboratories Inc.). Tubs were cleaned 2 h after each feeding. All juveniles were sacrificed when they were 16-months old for sexing.

PERFORMANCE MEASURES

Trials were performed on 1-month-old juveniles, in a wooden box containing nine open areas (9 cm long × 9 cm wide × 6 cm high) that were aligned in three rows of three. Each open area was considered as an independent unit. Trials were performed at an ambient temperature of approximately 28 °C. Turtles

were randomly distributed into groups of nine individuals. Each group of nine turtles was tested twice before and twice after feeding. The two replicate trials per nutritional status were performed alternatively during the morning and the afternoon, at least 2 days apart. Trials were videotaped with a digital camera, to prevent experimenter-linked inconvenience effect during trial. The videotaped record began when all turtles were placed upside down and was stopped after a maximal duration of 30 min. The data were collected a posteriori from the videotapes.

We studied two different and independent measures revealing different components in the righting response of the turtles: (1) the Latency time, defined as the time at the first movement of the turtle since it was placed upside down, and (2) the Time to right, defined as the time for a turtle to right itself since it started to move.

GROWTH, SURVIVAL, AND SEX IDENTIFICATION

The juvenile growth rate was defined as:

$$\text{Growth rate} = \frac{\ln(\text{weight}_t) - \ln(\text{weight}_{t_0})}{t - t_0}$$

where t is the time at experiment and t_0 is the time at birth. Juvenile survival was defined as the time (in days) that a given individual stayed alive (quantitative variable). Juvenile sex was determined a posteriori by dissection and microscopic observation of gonad morphology (Pieau, 1972; Yntema, 1976).

STATISTICAL ANALYSES

The dependent variables (Latency time and Time to right) were not normally distributed even after a variety of mathematical transformations. Therefore, we modelled both variables with a generalized linear mixed model, which included fixed and random effects and a gamma distribution with a log-link function as the error distribution. The associations between the measures of righting response and different variables were determined by using quasi-likelihood estimation of the parameter vector method through an iterative process. Statistical analyses were performed using SAS for Windows version 9.1 (SAS Institute Inc.). Computations were performed with SAS Macro program GLIMMIX, which iteratively runs SAS Procedure MIXED.

Separated analyses were conducted, depending on the dependent variables (Latency time and Time to right) and incubation treatment (constant and fluctuating temperatures regimes). The independent fixed effects included nutritional status (before or after feeding) and offspring sex (male 'M' or female 'F'). Juvenile growth rate and survival were added

as continuous individual covariables, and two-way interactions were also added to the model. We also implemented temperature regime (B, C or D) as fixed effect in fluctuating treatment analyses. Juvenile identity nested within clutch, clutch identity and all two-way interactions with clutch were defined as random effects. A backward stepwise procedure was used to select the final minimal model, according to the Akaike information criteria (AIC) for model comparison (Crawley, 1993; Burnham & Anderson, 1998).

RESULTS

Two hundred and ninety-two fertilized eggs produced 256 hatchlings (Table 1): 170 hatchlings from 25 clutches for the constant incubation temperature treatment and 86 hatchlings from 24 clutches for the fluctuating temperatures treatment (nonsignificant difference in hatching success for the two treatments: $\chi^2 = 0.11$, d.f. = 1, $P = 0.74$). The sex ratio yielded by each incubation temperature regime is presented in Table 1. All hatchlings survived until the performance tests.

CONSTANT INCUBATION TEMPERATURE

Latency time

According to the best minimal model ($AIC_C = 1892.4$ vs. general model: $AIC_C = 1899.4$), there was a significant individual effect on Latency time (Table 2). Clutch identity significantly influenced Latency time. We also detected significant effects of nutritional status and sex on Latency time (Table 2): juveniles stayed longer without any movement after feeding and females had a longer Latency time than males. Juvenile growth rate was significantly positively correlated with Latency time. Furthermore, we found a significant interaction between growth rate and sex (i.e. the interaction is positively related to Latency time and, for individuals with a higher growth rate, females have a longer Latency time than males whereas, for individuals with a lower growth rate, females have a shorter Latency time than males). No significant effect of juvenile survival or others two-way interactions were found.

Time to right

According to the best minimal model ($AIC_C = 1959.8$ vs. general model: $AIC_C = 1986.8$), there was a significant individual effect on Time to right (Table 2). We also detected a significant effect of nutritional status, with a longer Time to right after feeding. By contrast, clutch identity, sex, growth rate or juvenile survival did not have a significant main effect on this measure. Only the interaction between growth rate and nutritional status was significant (i.e. the interaction is

Table 1. Summary table of data collection for locomotor performance analyses realized separately for hatchlings from eggs incubated at constant temperature (regime A) and hatchlings from eggs incubated at fluctuating temperatures (regimes B, C or D)

Incubation regime	Constant	Fluctuating			Total
	A	B	C	D	
Number of eggs	190	35	33	34	102
Number of clutch	25	15	17	19	24†
Number of hatchlings	170	21	31	34	86
Males	123	0	0	22	22
Females	38	21	31	8	60
Undetermined	9	0	0	4	4
Sex ratio	0.23	1	1	0.25	–
Number of data used in the analyses*	574	79	115	109	303
Males	443	–	–	83	83
Females	131	79	115	26	220
Number of turtles dead after 1 year	67	5	9	10	24
Males	48	–	–	7	7
Females	19	5	9	3	17

*Only the turtles that have righted themselves during the 30-min trial to quantify Latency time and Time to right have been taken into account in our analyses.

†Twenty-four clutches were distributed among the three different fluctuating incubation regimes (B, C or D).

positively related to Time to right and individuals with a higher growth rate have a longer Time to right after than before feeding, whereas individuals with a lower growth rate have a longer Time to right before than after feeding).

FLUCTUATING INCUBATION TEMPERATURES

Latency time

According to the best minimal model ($AIC_C = 932.5$ vs. general model: $AIC_C = 982.1$), there was a significant individual variation in Latency time (Table 2). Nutritional status significantly influenced Latency time, with a longer time after feeding. No significant difference between incubation regimes or sexes was detected. However, difference between clutches was marginally significant (Table 2). Similarly, no main effect of juvenile survival or individual growth rate was detected, although the interaction between growth rate and incubation regime was significant (i.e. the interaction is negatively related to Latency time and individuals with a higher growth rate have a longer Latency time for regime D than regimes B and C, whereas individuals with a lower growth rate have a longer Latency time for regime C than regimes B and D).

Time to right

According to the best minimal model ($AIC_C = 1009.1$ vs. general model: $AIC_C = 1013.2$), there was a signifi-

cant individual effect on Time to right (Table 2). As in Latency time, the nutritional status also influenced this performance, with a longer Time to right after feeding. We also found a marginal significance of clutch identity and incubation regimes (Table 2). On the other hand, we detected a significant effect of juvenile survival with a positive correlation between Time to right and survival time. No other main effect was detected, whereas we found significant interactions between incubation regime and growth rate, similar to Latency time interaction; between sex and growth rate (i.e. the interaction is positively related to Time to right and for individuals with a higher growth rate, females have a longer Latency time than males whereas, for individuals with a lower growth rate, females have a shorter Latency time than males); between survival and growth rate, negatively correlated with Time to right; and between survival and incubation regime (i.e. the interaction is positively related to Time to right and for juveniles with a higher survival, juveniles from regime B have a longer Time to right than juveniles from regimes C and D whereas, for juveniles with a lower survival, juveniles from regime D have a longer Time to right than juveniles from regimes C and B).

DISCUSSION

Juvenile turtles righting response was analysed with two different experimental measures (Latency time

Table 2. Significant effects on *Trachemys scripta elegans* juveniles righting response obtained in the final models determined by a backward stepwise procedure

	Test statistic	<i>P</i>
'Latency time' model for constant incubation temperature		
Random effects		
Individual	$Z = 5.33$	< 0.0001***
Clutch	$Z = 2.15$	0.0314*
Fixed effects		
Growth rate	$F_{1,413} = 4.68$	0.0310*
Nutritional status	$F_{1,413} = 8.07$	0.0047***
Sex	$F_{1,413} = 12.23$	0.0005**
Survival	$F_{1,413} = 1.80$	0.1805
Sex × Growth rate	$F_{1,413} = 8.52$	0.0037**
'Time to right' model for constant incubation temperature		
Random effects		
Individual	$Z = 6.04$	< 0.0001***
Clutch	$Z = 1.36$	0.1786
Fixed effects		
Growth rate	$F_{1,412} = 1.35$	0.2453
Nutritional status	$F_{1,412} = 3.00$	0.0441*
Sex	$F_{1,412} = 2.05$	0.9815
Survival	$F_{1,412} = 0.58$	0.4456
Nutritional status × Growth rate	$F_{1,412} = 8.67$	0.0034**
'Latency time' model for fluctuating temperatures		
Random effects		
Individual	$Z = 4.48$	< 0.0001***
Clutch	$Z = 1.93$	0.0539
Fixed effects		
Growth rate	$F_{1,218} = 1.77$	0.1852
Nutritional status	$F_{1,218} = 8.48$	0.0040**
Regime	$F_{2,218} = 0.89$	0.4105
Sex	$F_{1,218} = 1.95$	0.1644
Survival	$F_{1,218} = 1.11$	0.2922
Regime × Growth rate	$F_{2,218} = 6.08$	0.0027**
'Time to right' model for fluctuating temperatures		
Random effects		
Individual	$Z = 2.58$	0.0098**
Clutch	$Z = 1.73$	0.0840
Fixed effects		
Growth rate	$F_{1,216} = 0.13$	0.7173
Nutritional status	$F_{1,216} = 6.53$	0.0113*
Regime	$F_{2,216} = 3.02$	0.0511
Sex	$F_{1,216} = 1.63$	0.2036
Survival	$F_{1,216} = 10.84$	0.0012**
Regime × Growth rate	$F_{2,216} = 13.00$	< 0.0001***
Sex × Growth rate	$F_{1,216} = 6.21$	0.0135*
Survival × Growth rate	$F_{1,216} = 5.64$	0.0184*
Survival × Regime	$F_{2,216} = 3.27$	0.0400*

Statistical tests are type III *F*-tests for fixed effects and *Z*-tests for random effects.

Results are presented for the four independent tests: the two dependent variables (Latency time and Time to right) tested for the two different incubation regimes (constant temperature vs. fluctuating temperatures).

****P* < 0.001, ***P* < 0.01, **P* < 0.05.

and Time to right) and two different incubation treatments of the eggs (constant and fluctuating temperatures). We investigated the same effects for all four analyses to test the generality of this kind of performance studies. Overall, significant effects were different for each analysis.

THE RIGHTING RESPONSE AS A FITNESS INDEX?

Despite the divergent results obtained in this study, some effects were consistent between analyses. For both incubation treatments (constant and fluctuating temperatures) and for both measures of the righting response, we found a large and significant individual variability, which potentially allows natural selection to operate. We also found an effect of maternal identity (i.e. clutch effect) on the Latency time at constant incubation temperature ($P = 0.031$). At fluctuating temperature, the effect of clutch identity on Latency time and Time to right was marginally significant ($P = 0.054$ and 0.084 , respectively). These results are partly in agreement with the results of two previous studies, which also found a contribution of clutch in the righting response (Ashmore & Janzen, 2003; Steyermark & Spotila, 2001), suggesting the existence of a composite effect of maternal and genetic contributions of parents to righting response. This, together with the significant individual variability, supports the hypothesis of heritable variability in this performance trait.

In addition to its heritability, a fitness index has to be correlated with at least one of the two principal components of fitness (i.e. survival until adulthood and fecundity). To test this second argument, we examined the relationship between the measures of the righting response vs. (1) the survival of the juveniles after 1 year in laboratory conditions and (2) juvenile growth rate, which has been suggested to be a good indicator of survival in aquatic turtles (Brooks *et al.*, 1991; Rhen & Lang, 1995; Congdon *et al.*, 1999). We found a significant effect of juvenile survival, as a main effect and in interaction, only on Time to right at fluctuating temperatures. By contrast, in both incubation treatments and for both measures of the righting response, juvenile growth rate significantly affects the righting response, either as a main effect or in interaction with another factor. The discrepancy between the effects of juvenile survival and growth rate may arise from the fact that our estimation of juvenile survival was performed under optimal conditions in the laboratory and may not well reflect survival rates in nature.

Finally, in our study, the conditions of operation of natural selection are met (Stearns, 1992), which further supports the validity of the righting response as a fitness index.

LIMITS AND PERSPECTIVES OF THE RIGHTING RESPONSE AS A FITNESS INDEX

To be a suitable fitness index, a performance measure must meet several requirements (Sorci *et al.*, 1994; Travis *et al.*, 1999). First, the performance must be relevant to the ecology of the species studied. In our case, the righting response is commonly observed in nature (Burger, 1976) and a delayed righting response may clearly increase the probability of predation, starvation or dehydration (Finkler, 1999; Finkler, 2001; Steyermark & Spotila, 2001). However, Tucker (2000) showed that the hatchlings of two aquatic turtle species use very different strategies to move overland: to reduce time spent migrating, *Chrysemys picta* hatchlings increase locomotion speed whereas *Trachemys scripta* hatchlings use stealth (i.e. slow locomotion speed) (see also Kolbe & Janzen, 2002). The same locomotor performance should therefore not be chosen to evaluate the efficiency of overland move for these two species. This underlines the importance of the choice of the locomotor performance measure to obtain reliable conclusions.

Second, because a behavioural response is generally complex, different measures of the same behaviour are possible, each capturing a particular aspect of that behaviour. In our experiment, we studied the composite righting response through two different measures that are independent: the Latency time, during which the turtle stays motionless, and the Time to right, which is the active return of the turtle in a prone position. Surprisingly, the significance and impact of most effects differs widely between the two measures (Table 3). For example, at constant incubation temperature, the effect of sex on Latency time is highly significant ($P = 0.0005$), whereas sex does not influence the Time to right ($P = 0.9815$). A third measure of righting response is the sum of Latency time and Time to right. Again, the results of significant effects detected were different of the results obtained for the two other measures (analysis not shown).

The strong discrepancies between our results for the two measures of the righting response show that the different aspects of a behaviour can be influenced by different factors. This may highlight the importance of using several measures of a locomotor performance to obtain valid conclusions.

Several parameters influence a priori the 'reaction norm' in locomotor performance performed in the laboratory: the environmental conditions during the incubation of the eggs (e.g. temperature and hydric potential), the husbandry environment and, finally, the conditions during measurement. All these environmental conditions may directly affect the expression of hatchling or performance traits; thus, these traits are not fixed but are phenotypically plastic

(Kingsolver & Huey, 2003). Behaviour, ontogeny, and environmental variation may complicate the relationship between performance and fitness (Arnold, 1983).

Many previous studies have found a significant effect of incubation temperature of the eggs on locomotor performance in sauropsids (Burger, 1989; Shine & Harlow, 1997; Downes & Shine, 1999; Elphick & Shine, 1999; Brana & Ji, 2000; Freedberg *et al.*, 2001; Webb *et al.*, 2001; Du & Ji, 2003). However, most of these studies have used extreme temperature regimes to test the effect of temperature. Extreme temperatures generate morphological and physiological constraints that do not exist in nature. Furthermore, most studies also used constant temperature regimes, which are not representative of the fluctuations observed in natural nests (Georges *et al.*, 1994; Valenzuela, 2001). In our study, we compared the 'reaction norm' of the righting response between juveniles from constant and fluctuating incubation temperatures of the eggs. We found that the significance of most effects (i.e. impact of explanatory variables on the righting response) differs widely between constant and fluctuating temperature treatments of the eggs, even though the means of the incubation temperature are approximately similar (Table 3). Our results also show that different measures of the same behaviour are liable to yield opposite results, depending on incubation treatment of the eggs (i.e. ontogenic factor). This confirms that the results obtained under constant temperature regimes cannot be extrapolated to the field without caution, and again stresses the need to use realistic (i.e. fluctuating) incubation regimes, which is

rarely done in studies examining Charnov and Bull's hypothesis (Valenzuela, 2004).

Furthermore, another factor that may influence performance measure is the rearing conditions of the animals. In the laboratory, husbandry conditions are usually optimal (e.g. food *ad libitum*, appropriate heat and UV intensity). The quasi-absence of environmental stress is likely to decrease the effect of natural selection. Under such conditions, the genetic and ontogenetic advantages of individuals may not express itself, and the observed phenotypic variability might therefore not be representative of individual fitness in nature (Le Gaillard *et al.*, 2004). In our study, the juvenile survival measured under standardized and optimal conditions probably only allowed the detection of low quality individuals and not distinction between medium and high quality individuals. We therefore encourage studies where laboratory studies are combined with field experiments to assess a real measure of the quality of individuals.

Finally, conditions during measurement itself will also influence the results. First, performance measures under laboratory conditions should reflect how organisms perform in nature (Hertz, Huey & Garland, 1988; Irschick & Losos, 1998). However, in many cases, the observed performance might reflect motivational factors in the laboratory more than physiological capacities (Le Gaillard *et al.*, 2004). For example running speed measured in the laboratory has often been linked to predation avoidance (Sorci *et al.*, 1994; Janzen, 1995; Irschick & Losos, 1998; Webb *et al.*, 2001) but the stimulation to perform is very different

Table 3. Summary table of significant effects obtained for the statistical analyses of the two different measures of righting response (Latency time vs. Time to right) performed under two different thermal incubation conditions (constant temperature vs. fluctuating temperatures)

	Constant temperature		Fluctuating temperatures	
	Latency time	Time to right	Latency time	Time to right
Individual	***	***	***	**
Clutch	*	NS	NS	NS
Growth rate	*	NS	NS	NS
Regime	–	–	NS	NS
Nutritional status	**	*	**	*
Sex	***	NS	NS	NS
Survival	NS	NS	NS	**
Regime × Growth rate	–	–	**	***
Sex × Growth rate	**	NS	NS	*
Survival × Growth rate	NS	NS	NS	*
Nutritional status × Growth rate	NS	**	NS	NS
Survival × Regime	–	–	NS	*

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

NS, nonsignificant; –, variable not tested for this analysis.

when there is no real predator. Second, all the factors than can influence the measure must be standardized and taken into account. In our study, we observed that the nutritional status of the turtles strongly influences their righting response (Table 3), with a longer response time after feeding. Feeding and digestion probably lead to an energetic cost in the righting response capacities of aquatic juvenile turtles, which indicates the possible intervention of a trade-off between physiological needs and performance capacities (Clobert *et al.*, 2000; Main & Bull, 2000; Ghalambor *et al.*, 2004). Similarly, several authors have shown a strong effect of the ambient temperature on locomotor performance (Elphick & Shine, 1998; Steyermark & Spotila, 2001; Freedberg *et al.*, 2004). Thus, the conditions under which measures are performed must be standardized and/or taken into account to permit comparison between performance studies and to avoid too many confounding effects.

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