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Plasticity of the duration of metamorphosis in the African clawed toad

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Abstract

In organisms with complex life cycles, such as amphibians, selection is thought to have minimized the duration of metamorphosis, because this is the stage at which predation risk is presumed to be highest. Consequently, metamorphic duration is often assumed to show little if any environmentally induced plasticity, because the elevation in the extrinsic mortality risk associated with prolonging metamorphosis is presumed to have selected for a duration as short as is compatible with normal development. We examined the extent to which metamorphic duration in the anuran amphibian *Xenopus laevis* was sensitive to environmental temperature. Metamorphic duration was influenced by body size, but independent of this effect, it was strongly influenced by environmental temperature: the duration at 18 °C was more than double that at 24 and 30 °C. We also compared the vulnerability of larval, metamorphosing and post metamorphic *Xenopus* to predators by measuring their burst swimming speeds. Burst swim speed increased through development and while we found no evidence that it was reduced during metamorphosis, it did increase sharply on completion of metamorphosis. We therefore found no evidence of a substantial increase in vulnerability to predators during metamorphosis compared with larval stages, and hence the slowing of metamorphosis in response to temperature may not be as costly as has been assumed.

Introduction

Organisms with complex life cycles often have a number of distinct life-history stages, each with substantially different requirements and risks (Wilbur, 1980). The durations of different stages have been shown to vary with environmental circumstances. Variation in the duration of the larval period, for example, has been demonstrated in several studies covering a wide range of taxa (e.g. marine invertebrates: Twombly, 1996; insects: Blakley, 1981; fish: Policansky, 1983; amphibians: Werner, 1986; Harris, 1999). The duration of the transition between stages, often involving a metamorphosis, has received considerable attention in terms of developmental mechanisms, but much less in terms of the effects of environmental circumstances. The duration of metamorphosis is presumed to be minimized by selection because of the high vulnerability to predators during this period (Williams, 1966), due to reduced locomotor ability and hence to be insensitive to environmental circumstances (Rose, 2005). Contrary to Rose's (2005) statement that metamorphosis is a developmental phase of fixed duration, with no intraspecific variation, a recent analysis of metamorphic duration has shown that it varies considerably among and within anuran species. This analysis also suggested that minimizing predation risk is not the sole factor in determining the duration of metamorphosis, but that it is related to local growth conditions, as indicated by body condition and size, and to environmental temperature (Downie, Bryce & Smith, 2004).

Anuran amphibians are widely used in studies of the effect of environmental factors on life cycles because they go through clear and distinct stages. Following hatching from the egg, there is a larval period characterized by rapid growth. Metamorphosis then occurs, during which time individuals do not feed and change from a tail-driven tadpole to a four-legged froglet; this is then normally followed by further growth and then by sexual maturation. It has been suggested that metamorphosis is a particularly vulnerable period in this group. For example, Arnold & Wassersug (1978) found that chorus frogs *Pseudacris triseriata* were more frequently captured by garter snakes during metamorphosis than as pre-metamorphic larvae or post-metamorphic juveniles. Additionally, it was established that the impaired locomotor performance (measured as swimming endurance) of metamorphosing individuals compared with tadpoles observed in this species was responsible for the increased vulnerability (Wassersug & Sperry, 1977).

The locomotor impairment thought to be typical of metamorphosis is of critical importance to the hypothesis that the duration of anuran metamorphosis has been minimized by selection. While there have been several studies evaluating the effects of conditions during the larval period on the speed of larval and juvenile movement (Van Buskirk & Saxer, 2001; Alvarez & Nicieza, 2002; Altwegg & Reyer,

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2003), and differences in locomotion between pre- and post-metamorphic urodeles (Shaffer, Austin & Huey, 1991; Azizi & Landberg, 2002; Wilson, 2005), the locomotor performance of metamorphosing individuals has only been examined in two other anuran species since Wassersug & Sperry's (1977) original study. Watkins (1997) did not find a significant difference in maximum burst swim speed between premetamorphic individuals (Gosner stage 37; Gosner, 1960) and individuals at the start of metamorphic climax (Gosner stage 42) in *Hyla regilla*. However, Huey (1980) demonstrated that burst speed in *Bufo boreas* decreased during metamorphosis, from a peak just before the onset, in tandem with a decrease in tail length.

In this study, we examined the plasticity of metamorphic duration in the fully aquatic *Xenopus laevis* in relation to conditions during metamorphosis. Our aims were twofold. Firstly, we examined the effect of experimentally imposed temperatures during metamorphosis, taking into account variation in body size due to differential growth during the larval period. Secondly, we examined the predator avoidance capability of individuals at different stages in their development to determine whether this is impaired during metamorphosis compared with the pre-metamorphic larval and post-metamorphic juvenile stages.

There are several methods for assessing the predator avoidance capability in anurans, such as turning speed, endurance swimming, maximum attainable swim speed or burst swim speed (Wassersug & Sperry, 1977; Huey, 1980; Wassersug, 1989). Burst swim speed, the starting velocity from a stationary position, has been shown to be important for avoiding predation across all developmental stages (Miller, 1982; Azizi & Landberg, 2002; Wilson, Kraft & Van Damme, 2005). Therefore, we measured predator avoidance capability using burst swim speed.

Methods and materials

Animals and rearing conditions

Approximately 250 wild-type eggs of *X. laevis* Daudin were obtained from St Andrews University (St Andrews, Fife, Scotland, UK) in 2005. Tadpoles were kept in a single 40 L holding tank for 20 days before being transferred to 10 smaller holding tanks, by which time they had reached approximately Nieuwkoop and Faber (NF) stage 42 (Nieuwkoop & Faber, 1994).

Each of the smaller holding tanks ($30 \times 20 \times 20$ cm) contained 25 tadpoles and was filled with *c.* 11 L of aerated, de-chlorinated, copper-free water. The temperature in the tanks was controlled by heaters, which were switched off during the night, and maintained the temperature at 30 °C during daytime and 18 °C at night. This range is representative of the temperatures that *X. laevis* would experience under natural conditions (Tinsley & Kobel, 1996). Thus, all tadpoles were exposed to the same range of experimental temperatures before metamorphosis; that this range encompassed the full range of temperatures to be used in the experiments during metamorphosis avoided any acclimatization problems. Perspex sheets were placed over the tanks to reduce water loss from evaporation, and a constant water level was maintained in all tanks. Tadpoles were exposed to a 12 h:12 h light/dark cycle throughout the larval period. They were fed daily on *c.* 0.2 g of dried algal pellets per tank, ground and hydrated before being suspended in water.

Experimental protocol

Checks were made three times a day for individuals showing emergence of one or both forelimbs, indicative of the commencement of metamorphosis (stage 60). In total, 139 tadpoles were transferred to separate, individual experimental tanks ($15 \times 20 \times 20$ cm) at the onset of metamorphosis. The remainder were not included due to natural mortality before the onset of metamorphosis or because they did not commence metamorphosis within the time span of the study. Individuals were checked three times a day for completion of metamorphosis (stage 66), identified by complete tail absorption (tail length < 0.5 mm). Experimental tanks were maintained at constant water temperatures of 18, 24 and 30 °C, and tadpoles were allocated sequentially to the three temperature treatments at the onset of metamorphosis. The 18 and 24 °C treatments each had 46 individuals, while the 30 °C treatment had 47 individuals.

Wet mass, after removal of surface water (± 0.001 g), snout–vent length (SVL), head width, tail length and maximum tail depth measurements (± 0.1 mm, using callipers) were taken at the commencement of metamorphosis. There were no significant differences in mass, SVL, head width, tail length, tail depth (Table 1) or body condition (mean: 0.106 ± 0.001 SEM; $F_{2,135} = 1.573$, $P = 0.211$) at the start of metamorphosis between the three temperature treatments. Tail length and depth (± 0.1 mm) measurements were taken every 3 days during metamorphosis, to determine whether metamorphosis progressed with the same pattern in all three temperature treatments. At the completion of metamorphosis, mass, SVL and head width were measured. Body condition at the start and end of metamorphosis was calculated from body measurements using the formula provided in Veith (1987), defined as condition = $(\text{mass}/\text{SVL}^3) \times 1000$.

Newly metamorphosed juvenile individuals to be tested for locomotor performance were maintained at their metamorphic temperature until testing; after testing, they were transferred to a stock tank maintained at 24 °C.

Table 1 Starting measurements of different temperature treatment groups (ANOVA results are displayed; NS indicates non-significant difference)

	18 °C	24 °C	30 °C
Mass^{NS} (g)	0.65 ± 0.03	0.69 ± 0.02	0.67 ± 0.02
SVL^{NS} (mm)	18.1 ± 0.2	18.5 ± 0.2	18.4 ± 0.2
Head width^{NS} (mm)	8.6 ± 0.1	8.8 ± 0.1	8.8 ± 0.1
Tail length^{NS} (mm)	32.1 ± 0.6	31.7 ± 0.6	31.9 ± 0.7
Tail depth^{NS} (mm)	5.8 ± 0.1	5.8 ± 0.1	5.6 ± 0.2

SVL, snout–vent length.

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Swimming speed data collection

Measurements of swimming speed were taken from NF stages 48–66, comprising the larval stages (stages 48–59), the middle of metamorphosis (stage 63, when 50% of the tail was reabsorbed) and soon after metamorphosis was complete (stage 66+). Pre-metamorphic tadpoles were grouped as follows: stages 48–51 (small hind-limb buds); stages 52–55 (differentiation and flattening of hind foot); and stages 56–59 (separation of toes and development of hind legs). Mid-metamorphosis (stage 63) was selected because it is likely to represent the most critical period during which metamorphosis would be impaired, because the tail is being rapidly absorbed and the hind limbs are still developing.

Owing to the sensitivity of the equipment being used, filming of swimming had to be performed in one room (air temperature 20 °C) for all temperature treatments. Individuals to be filmed were brought into the room in a shallow dish 1 h before being filmed to acclimatize to the temperature (the water temperature ranged between 19 and 27 °C, due to heat from the lighting equipment required for filming). The 1 h time period was selected as an acceptable period for acclimatization, as a longer period might have affected the developmental processes being investigated. Water temperature at the time of trials was recorded and included in the analysis as a covariate. This did not significantly affect locomotor performance at any of the three developmental stages (tadpoles: $F_{1,28} = 1.784$, $P = 0.194$; metamorphs: $F_{1,52} = 1.330$, $P = 0.255$; and juveniles: $F_{1,78} = 1.911$, $P = 0.171$).

A Photron FASTCAM-PCI high-speed camera (Photron USA Inc., San Diego, CA, USA) was used to capture video footage. The camera was placed facing directly down into a tank 20 cm long, with laminated grid-paper along the bottom; there was a 4 cm wide corridor through the middle of the grid, filled with *c*. 1 cm depth of water. The camera was mounted sufficiently far above the water surface (0.8 m) relative to water depth (1 cm) to minimize parallax errors in determining position. Each individual was placed at one end of the tank and allowed to settle, and then gently prodded at the base of the tail with a glass stirring rod wrapped in red electrical tape to elicit an escape response. These procedures limited the tadpoles to swimming forward in response to the stimulus.

Filming was carried out at 250 frames per second (fps) and the camera recorded up to 5 s of swimming; the swim speed of each individual was measured five times with *c*. 1 min break between each. There was no evidence of habituation through the series of trials in any of the three developmental groupings (pre-metamorphic: $r^2 = 0.029$, $P = 0.06$; metamorphic: $r^2 = 0.006$, $P = 0.23$; juvenile: $r^2 = 0.0$, $P = 0.67$). As in other studies (e.g. Wilson *et al*., 2005), the first ~300 ms (37 frames) following initial movement were used representing burst speed, a critical variable in fleeing from a predator (Watkins, 1996; Dayton *et al*., 2005).

Data analysis

Data are presented as mean ± SEM. *F*-tests were used to determine whether variation in metamorphic duration differed between temperature treatments. Principal components analysis was carried out on the four body measurements (SVL, head width, tail length and depth) for use in analysis on metamorphic duration. A single factor was extracted (PC1), which explained 62.4% of the variation, representing predominately body rather than tail measurements. χ^2 was used to determine differences in metamorphic mortality between temperature treatments. Tail loss during metamorphosis was assessed using 3-parameter, sigmoid regression (SigmaPlot 10, Systat Software Inc.). The remaining analyses were performed using analysis of variance with *post hoc* Tukey tests (SPSS 14, SPSS Inc.).

For film analysis, Photron Motion Tools software was used, which allowed digital analysis, giving the distance per frame (velocity) and the distance travelled. The maximum measurement achieved from the five trials was used for data analysis.

Before stage 60, individual tadpoles were taken at random from the small holding tanks for swim testing; they were then returned to the tank, and so it was not possible to track them as individuals. Therefore, the data on locomotor performance between larval and metamorphic stages are treated as independent samples. Comparisons were made between four developmental groups: three pre-metamorphic stages (all experiencing the fluctuating temperature environment) and one at mid-metamorphosis (stage 63). Three such analyses were performed to allow comparisons between larval and metamorph swim speeds at the three different temperature treatments experienced only after metamorphosis commenced. Comparative analysis of locomotor performance between metamorphic and juvenile stages (stages 63 and 66) was performed on known individuals using a linear mixed model with repeated measures.

Results

Metamorphic duration

Metamorphic duration differed significantly between the three experimental treatments. It was the slowest at the lowest temperature, taking twice as long at 18 °C than at 30 °C (Fig. 1). The difference between the two higher temperatures, while significant, was relatively small (*c*. 1 day). The variability in metamorphic duration differed significantly among the temperature treatments, with the 24 °C treatment being the least and the 30 °C treatment the most variable ($F = 15.113$, $P = 0.000$; 18 °C: range 14.04–20.02 days, CV: 9.32%; 24 °C: 7.19–10.00 days, CV: 6.96%; 30 °C: 6.00–10.19 days, CV: 13.76%).

As the body size (PC1) increased, the duration of metamorphosis increased ($F_{1,117} = 7.646$, $P = 0.007$). Metamorphic duration was not influenced by starting body condition ($F_{1,117} = 2.803$, $P = 0.097$; Fig. 2). The effects of temperature were significant ($F_{2,117} = 3.812$, $P = 0.024$) and there was no interaction between temperature and body size ($F_{2,117} = 0.721$, $P = 0.488$) or temperature and condition ($F_{2,117} = 0.089$, $P = 0.915$).

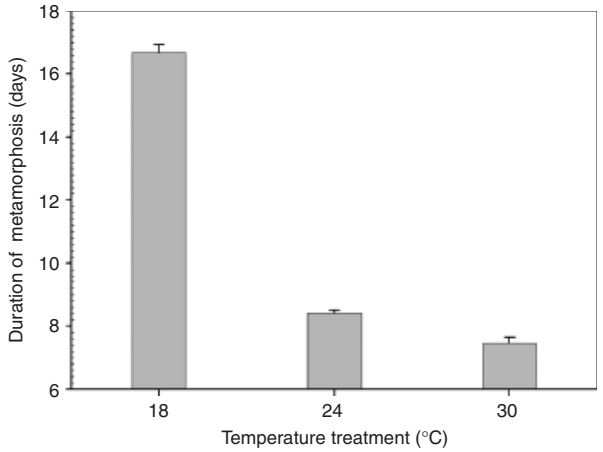

Figure 1 Duration of metamorphosis in days (±SEM) for the three temperature treatments.

Mortality levels were significantly different among treatments. No mortality occurred in the 24 °C treatment. The 30 °C treatment had the highest mortality rate (11 mortalities, 23.4%), while the 18 °C treatment had less (six mortalities, 13%) ($\chi^2 = 11.905$, d.f. = 2, $P = 0.003$).

Tail loss during metamorphosis

Tail re-absorption followed a sigmoid pattern, with a rapid decrease in tail length occurring between days 3 and 6 in the two higher temperatures (24 and 30 °C) and between day 6 and 12 in the 18 °C treatment (Fig. 3). Inflection points (x_0) and steepness of curves (b) were significantly different between the three treatments (x_0: $F_{2,124} = 210.986$, $P = 0.000$; b: $F_{2,124} = 60.279$, $P = 0.000$); *post hoc* analysis showed that this difference was between 18 °C and the two higher temperature treatments.

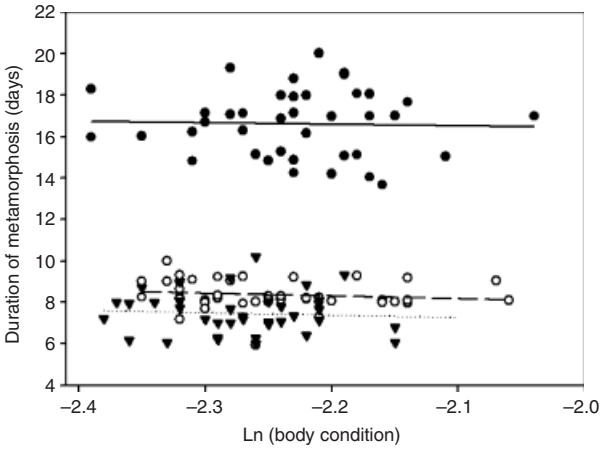

Figure 2 Duration of metamorphosis in days in relation to body condition at the onset of metamorphosis (18 °C: •, solid line; 24 °C: ○, dashed line, 30 °C: ▼, dotted line).

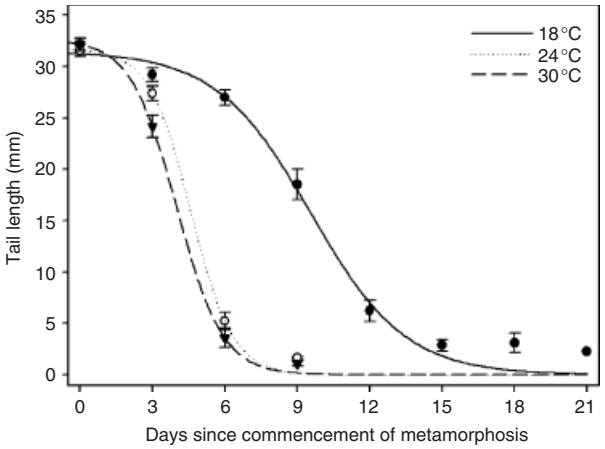

Figure 3 Decrease in mean tail length (±SEM) through metamorphosis (18 °C: $y = 31.437/[1 + e^{((x-4.188)/0.653)}]$ $r^2 = 0.768$, $P = 0.000$; 24 °C: $y = 31.692/[1 + e^{((x-2.533)/0.289)}]$ $r^2 = 0.857$, $P = 0.000$; 30 °C: $y = 32.627/[1 + e^{((x-2.335)/0.319)}]$ $r^2 = 0.801$, $P = 0.000$).

Locomotor performance

SVL had a significant positive effect on burst swim speed during the larval ($F_{1,28} = 9.223$, $P = 0.005$) and juvenile stages ($F_{1,78} = 17.154$, $P = 0.000$), but only marginally during metamorphosis ($F_{1,52} = 4.000$, $P = 0.051$). The temperature treatment experienced during metamorphosis had a significant effect on metamorph burst swim speed ($F_{2,52} = 5.586$, $P = 0.006$), but this was not found in juveniles ($F_{2,78} = 0.245$, $P = 0.783$). During metamorphosis, individuals from the 24 °C treatment had a significantly faster burst swim speed than the higher and lower temperature treatment groups, which were not significantly different from one another (Fig. 4).

To assess whether locomotion is impaired during metamorphosis, comparisons were made between larvae and metamorphs and between metamorphs and juveniles.

The comparisons between the three larval stage groups and metamorphic individuals at the three temperature treatments all showed a significant relationship between maximum swimming speed and stage (18 °C: $F_{3,44} = 6.807$, $P = 0.0007$; 24 °C: $F_{3,45} = 11.989$, $P = 0.0001$; 30 °C: $F_{3,47} = 6.378$, $P = 0.001$; Fig. 4). We found no evidence of locomotor impairment during metamorphosis: at 18 and 30 °C, metamorphic burst swim speeds did not differ from burst speeds attained by pre-metamorphic larvae. In fact, metamorphs at 24 °C actually swam significantly faster than the late-stage larvae. The maximum swimming speed increased as individuals progressed through larval development and continued to increase up to mid-metamorphosis (NF stage 63). Therefore, swimming speed at metamorphosis was not reduced compared with late-stage, pre-metamorphic larvae of similar sizes and was significantly faster than smaller, early-stage larvae.

Correcting for metamorphic temperature treatment ($F_{2,80.506} = 0.299$, $P = 0.742$) and SVL ($F_{1,80.782} = 6.807$, $P = 0.011$), at the end of metamorphosis individuals swam

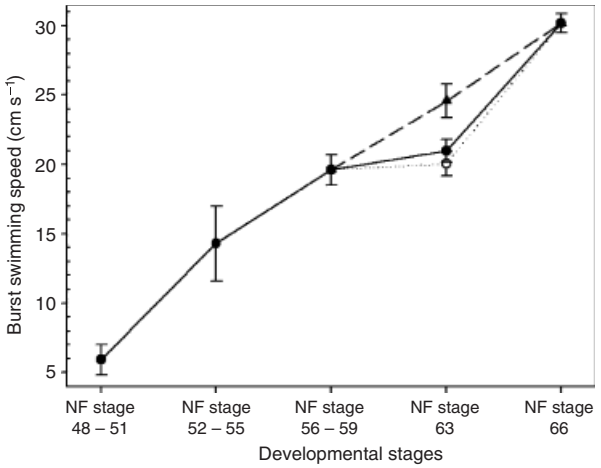

Figure 4 Burst swimming speed (±SEM) at different developmental stages. At mid-metamorphosis, the three temperature treatments are represented individually (•: 18 °C, ▲: 24 °C, ○: 30 °C).

significantly faster than they did during metamorphosis ($F_{1,80.125} = 57.741$, $P = 0.000$).

Discussion

Metamorphic duration

Our results show that metamorphic duration is sensitive to environmental temperature. At temperatures commonly experienced by *X. laevis* (*c.* 21 °C), metamorphosis takes *c.* 8 days (Huang *et al.*, 2001). Metamorphosing in cold temperatures resulted in more than double the standard length of time to complete metamorphosis. Metamorphosis at the two higher temperature treatments (24 and 30 °C) was much closer in duration, with the duration at 30 °C taking about a day less than at 24 °C. This result indicates that above a certain temperature threshold, the speed of metamorphosis is optimized, as is suggested by Downie *et al.* (2004) for other species.

There was also substantial variability in metamorphic duration within the temperature treatment groups. Body size was the main component explaining this variation. Contrary to the prediction of Downie *et al.* (2004), metamorphic duration was not related to the index of body condition. However, Downie *et al.* (2004) did not use the term body condition in the precise manner defined by Veith (1987). The lack of relation with body condition might be a result of the rearing conditions. All individuals were reared at relatively low densities, with an abundance of food on a daily basis. Therefore, the condition at the start of metamorphosis was relatively high and less variable than would be expected in the wild. Rearing individuals at different densities or lower food availabilities may allow the effects of body condition on metamorphic duration to be studied in more detail.

Within-group variability in metamorphic duration differed between the three temperatures. At 30 °C, metamorphosis was completed in the fastest time, but the degree of variation was the highest. Additionally, the mortality rate was the highest in this group. The higher mortality indicates that there may be costs associated with such a rapid rate of metamorphosis, as has been found in rapid growth rates (Arendt, 1997). At 24 °C, the temperature treatment closest to what would be experienced by *X. laevis* in the field (Tinsley & Kobel, 1996), the variation was the smallest. This indicates that at the temperature a species is adapted for, a thermal developmental optimum is established (Stahlberg, Olsson & Uller, 2001).

Locomotor performance

The temperature experienced during metamorphosis had different effects on swimming speed during and after metamorphosis. At stage 63, the 24 °C temperature treatment resulted in individuals having a faster maximum swim speed, with either extreme having slower speeds. Alvarez & Nicieza (2002) demonstrated a similar result in juvenile jumping performance in the Iberian painted frog *Discoglossus galganoi*, with performance peaking at an optimal temperature and decreasing as the temperature increased or decreased. Miller (1982) found a similar trend in adult *Xenopus*, with the highest locomotor performance occurring at 27 °C and performance decreasing with elevated or lowered temperatures. Additionally, in our study, size was found to have only a marginally significant effect on swimming speed during metamorphosis, possibly due to other factors, such as the progression of hind limb development, being more critical. This result suggests that the temperature experienced during metamorphosis may have some effect on musculoskeletal development, such as the type or capacity of hind limb musculature being developed during metamorphosis, which influences swimming performance.

On completion of metamorphosis, temperature no longer had an effect on swim speed, but size, specifically SVL, did have an effect. This result contrasts with the report of a temperature effect on juvenile locomotion reported by Alvarez & Nicieza (2002). However, in their study, the temperature treatments were experienced throughout the larval period, while in this study individuals were only subjected to different temperatures from the start of metamorphosis. Thus, the temperature effects found by Alvarez & Nicieza (2002) might be a consequence of effects operating during larval development.

Comparisons between larval and juvenile locomotor performance may be complicated by differences in thermal capacity between the musculature that the two stages use for locomotion. Sherman (1980) showed that, in *Bufo woodhousii fowleri*, the developing hind limbs were less able to cope with thermal stress than the tail, suggesting that extremely high temperatures would favour tadpole speed over juvenile speed. However, this is not likely to be the case in this study, because the thermal range used was well within the species tolerance and well below the thermal maxima of *Xenopus* (Sherman & Levitis, 2003). Additionally, differences in thermal preference between larval and adult amphibians

have not been shown in all species (e.g. *Triturus cristatus*: Wilson, 2005), and it has even been suggested that the thermal environment of adult and larval *Xenopus* is likely to be the same, as they occur in the same ponds (Sherman & Levitis, 2003), but this will be dependent on pond depth.

Comparison of metamorph predator avoidance

In this study, even when corrected for size, individuals did not experience the decrease in locomotor performance predicted and observed by Wassersug & Sperry (1977), using chorus frogs. In *X. laevis*, metamorphic swimming speed was slightly, but not significantly, faster than speeds displayed by larval individuals of a similar size. There was a dramatic increase in swimming performance on completion of metamorphosis. There could be several explanations for this: (1) this species is fully aquatic, and so the transition costs between terrestrial and aquatic locomotion are not present. (2) Research on *Xenopus* development has shown that during metamorphic climax, individuals are able to use both tail and hind limb-based locomotion (Combes *et al*., 2004), which may be different from other species (e.g. species with rapid tail loss; see Downie *et al*., 2004), and could improve metamorphs' locomotion performance. (3) Hind limb development, which has been shown during the larval stage to aid swimming (Park *et al*., 2003), may confer a greater advantage to metamorphosing *Xenopus* because of the large, well-developed webbed feet found in this species. It has been shown that drag forces are considerably increased during metamorphic climax due to forelimb emergence (Dudley, King & Wassersug, 1991), but the advantage of the thrust generated by the developing hind limbs could offset this cost. However, Dudley *et al*. (1991) used *Rana catesbeiana* tadpoles, which have a narrower, more streamlined anterior end compared with *X. laevis* tadpoles. (4) Finally, this study examined locomotion performance as burst speed, whereas Wassersug & Sperry (1977) examined swimming endurance. However, burst speed is more accurate than endurance as an indicator of predator evasion (see Dayton *et al*., 2005).

In two studies on locomotor performance, where burst speed was examined, conflicting results were observed. Huey (1980) did show a decrease in burst speed in *B. boreas* during metamorphosis, with individuals with longer tails at stage 43 (*c*. NF stage 63) having faster swim speeds. Watkins (1997), working on the Pacific tree frog, found that the maximum burst speed was not significantly different between premetamorphic and stage 42 individuals, but argued that using the mean of some of the speed trials, there was a significant decrease in locomotor performance in metamorphic individuals. Watkins (1997) did not investigate post-metamorphic locomotor performance in the Pacific tree frog. In the present study, using either mean or maximum, burst speed did not demonstrate a decrease in performance during metamorphosis in any of the three temperature treatments.

In conclusion, our data show that metamorphic duration varies with both environmental temperature and body size, and that locomotion is not impaired during metamorphosis compared with the pre-metamorphic stage. These findings present some fundamental complications for the idea that selection fuelled by high predation risk has minimized the duration of metamorphosis. It is possible that variation in natural predation risk results in different strengths of selective pressure for minimizing metamorphic duration, and it would be interesting to compare populations from high and low predation risk habitats. It would also be interesting to know more about the costs associated with rapid metamorphosis.

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