Plasticity of the duration of metamorphosis in the African clawed toad

Citation for published version:

Digital Object Identifier (DOI):
10.1111/j.1469-7998.2007.00367.x

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Early version, also known as pre-print

Published In:
Journal of Zoology

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Plasticity of the duration of metamorphosis in the African clawed toad

P. T. Walsh, J. R. Downie & P. Monaghan

Division of Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK

Keywords
metamorphic climax; metamorphic duration; *Xenopus laevis*; locomotor performance; life-history variation.

Correspondence
Patrick T Walsh, Division of Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow, G12 8QG, UK.
Email: P.Walsh.1@research.gla.ac.uk

Received 3 May 2007; accepted 22 June 2007
doi:10.1111/j.1469-7998.2007.00367.x

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 by 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 & Reyner...
2003), and differences in locomotion between pre- and post-
metamorphic urodeles (Shaffer, Austin & Huey, 1991; Azizi & Landberg, 2002; Wilson, 2005), the locomotor perfor-
mance of metamorphosing individuals has only been exam-
inied in two other anuran species since Wassersug & Sperry’s
(1977) original study. Watkins (1997) did not find a signifi-
cant 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 avoid-
ance 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
holding tank for 20 days before being transferred to 10
Scotland, UK) in 2005. Tadpoles were kept in a single 40 L
obtained from St Andrews University (St Andrews, Fife,
Van Damme, 2005). Therefore, we measured predator
(1977) original study. Watkins (1997) did not find a signifi-
cant 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

**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 × 20 × 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; $F_{2,125} = 1.573, P = 0.211$) 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 = (mass/SVL$^3$) × 1000.

Newly metamorphosed juvenile individuals to be tested for locomotor performance were maintained at their meta-
morphic 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)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Mass$^{NS}$ (g)</th>
<th>SVL$^{NS}$ (mm)</th>
<th>Head width$^{NS}$ (mm)</th>
<th>Tail length$^{NS}$ (mm)</th>
<th>Tail depth$^{NS}$ (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 °C</td>
<td>0.65 ± 0.03</td>
<td>18.1 ± 0.2</td>
<td>8.6 ± 0.1</td>
<td>32.1 ± 0.6</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>24 °C</td>
<td>0.69 ± 0.02</td>
<td>18.5 ± 0.2</td>
<td>8.8 ± 0.1</td>
<td>31.7 ± 0.6</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>30 °C</td>
<td>0.67 ± 0.02</td>
<td>18.4 ± 0.2</td>
<td>8.8 ± 0.1</td>
<td>31.9 ± 0.7</td>
<td>5.6 ± 0.2</td>
</tr>
</tbody>
</table>

SVL, snout-vent length.
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 may 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 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 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. Performance at any of the three developmental stages (tadpoles: $F_{1,28} = 24.607$, $P = 0.000$; metamorphs: $F_{1,52} = 1.300$, $P = 0.255$; and juveniles: $F_{1,78} = 0.059$, $P = 0.446$). Pre-metamorphic tadpoles were grouped representing predominately body rather than tail measures. $r^2$ 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’s 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 ($F_{1,117} = 6.96$, $P = 0.007$). 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 ($F_{2,117} = 0.089$, $P = 0.915$).

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 measures. $r^2$ 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’s 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.
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.

**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: $F_{3,43} = 11.989$, $P = 0.0001$; 30°C: $F_{3,42} = 6.378$, $P = 0.001$; 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 swim 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 ($F_{2,80.306} = 0.299$, $P = 0.742$) and SVL ($F_{1,80.782} = 6.807$, $P = 0.011$), at the end of metamorphosis individuals swim...
Our results show that metamorphic duration is sensitive to environmental experienced by X. laevis (e. 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 a 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).

**Discussion**

**Metamorphic duration**

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) may 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 woodhoussi 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 accurately related 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 pre-metamorphic 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.

Acknowledgements

We would like to thank Isobel Maynard for providing the eggs used in this experiment and two anonymous referees for helpful comments. P.T.W. would like to thank the Carnegie Trust for the Universities of Scotland for providing the PhD studentship, during which this research was conducted; the Louise Hiom Award provided funds for purchasing equipment.

References


Dear Author,

During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers clearly on the query sheet if there is insufficient space on the page proofs. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication.

<table>
<thead>
<tr>
<th>Query No.</th>
<th>Description</th>
<th>Author Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>AQ: Please provide manufacturer information for Photron FASTCAM-PCI high-speed camera: company name, town, state (if USA) and country.</td>
<td></td>
</tr>
</tbody>
</table>
Please correct and return this set

Please use the proof correction marks shown below for all alterations and corrections. If you wish to return your proof by fax you should ensure that all amendments are written clearly in dark ink and are made well within the page margins.

<table>
<thead>
<tr>
<th>Instruction to printer</th>
<th>Textual mark</th>
<th>Marginal mark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leave unchanged</td>
<td>--- under matter to remain</td>
<td></td>
</tr>
<tr>
<td>Insert in text the matter indicated in the margin</td>
<td>/ through single character, rule or underline or --- through all characters to be deleted</td>
<td></td>
</tr>
<tr>
<td>Delete</td>
<td>/ through letter or --- through characters</td>
<td></td>
</tr>
<tr>
<td>Substitute character or substitute part of one or more word(s)</td>
<td>/ through character or / where required</td>
<td></td>
</tr>
<tr>
<td>Change to italics</td>
<td>--- under matter to be changed</td>
<td></td>
</tr>
<tr>
<td>Change to capitals</td>
<td>--- under matter to be changed</td>
<td></td>
</tr>
<tr>
<td>Change to small capitals</td>
<td>--- under matter to be changed</td>
<td></td>
</tr>
<tr>
<td>Change to bold type</td>
<td>~ under matter to be changed</td>
<td></td>
</tr>
<tr>
<td>Change to bold italic</td>
<td>~ under matter to be changed</td>
<td></td>
</tr>
<tr>
<td>Change to lower case</td>
<td>Encircle matter to be changed</td>
<td></td>
</tr>
<tr>
<td>Change italic to upright type</td>
<td>(As above)</td>
<td></td>
</tr>
<tr>
<td>Change bold to non-bold type</td>
<td>(As above)</td>
<td></td>
</tr>
<tr>
<td>Insert ‘superior’ character</td>
<td>/ through character or / where required</td>
<td></td>
</tr>
<tr>
<td>Insert ‘inferior’ character</td>
<td>(As above)</td>
<td></td>
</tr>
<tr>
<td>Insert full stop</td>
<td>(As above)</td>
<td></td>
</tr>
<tr>
<td>Insert comma</td>
<td>(As above)</td>
<td></td>
</tr>
<tr>
<td>Insert single quotation marks</td>
<td>(As above)</td>
<td></td>
</tr>
<tr>
<td>Insert double quotation marks</td>
<td>(As above)</td>
<td></td>
</tr>
<tr>
<td>Insert hyphen</td>
<td>(As above)</td>
<td></td>
</tr>
<tr>
<td>Start new paragraph</td>
<td>(As above)</td>
<td></td>
</tr>
<tr>
<td>No new paragraph</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transpose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Close up</td>
<td>linking characters</td>
<td></td>
</tr>
<tr>
<td>Insert or substitute space between characters or words</td>
<td>/ through character or / where required</td>
<td></td>
</tr>
<tr>
<td>Reduce space between characters or words</td>
<td>between characters or words affected</td>
<td></td>
</tr>
</tbody>
</table>