

Ontogenetic changes in activity, locomotion and behavioural complexity in tadpoles

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Metamorphosis is a widespread developmental process that involves considerable changes in morphology, habitat use, ecology and behaviour between early developmental (larval) stages and adult forms. Among amphibians, anuran larvae (tadpoles) undergo massive morphological and ecological changes during their development, with early stages characterized by somatic growth, whereas more conspicuous changes (i.e. metamorphosis) occur later during development. In this study, we examined how locomotor and behavioural traits covary with morphology (body size) and metamorphosis (hindlimb and forelimb development) across developmental stages in spined toad (*Bufo spinosus*) tadpoles. As expected, we found that locomotion and behaviour undergo significant changes during tadpole development. These changes are curvilinear across developmental stages, with a phase of increasing activity and locomotion followed by a phase of stasis and/or reduction in locomotion and behavioural complexity. All the metrics we investigated indicate that the peak of activity and associated behaviour is situated at a pivotal stage when somatic growth decreases and significant morphological changes occur (i.e. hindlimb growth). Future studies that aim to investigate determinants of locomotion should include developmental stages as covariates in order to assess whether the sensitivity of locomotion to environmental variables changes across developmental stages.

ADDITIONAL KEYWORDS: activity – amphibian – behaviour – fractal analysis – metamorphosis – swimming speed.

INTRODUCTION

Metamorphosis is a key developmental process that is widespread across animal phylogeny (insects, fish, amphibians, molluscs, crustaceans, cnidarians, echinoderms and tunicates; Heyland & Moroz, 2006). This biological process involves massive organismal modifications after birth between early developmental (larval) stages and adult forms. Metamorphosis is not limited to changes in the body architecture, but often involves considerable shifts in other life-history traits, such as habitat use, trophic ecology and behaviour (Mueller *et al.*, 2015; Truman & Riddiford, 2019).

Among vertebrates, amphibians are well-known examples of taxa that undergo metamorphosis. Many amphibian species are biphasic: they live on land but

reproduce in freshwater environments, where eggs and larvae develop (Dodd, 2010). In most urodeles (salamanders), larvae broadly resemble adults, and metamorphosis mainly involves a shift from aquatic to terrestrial life and a consequent modification from branchial aquatic respiration to pulmonary aerial respiration (Vitt & Caldwell, 2014). Anuran (frog and toad) larvae undergo comparatively more massive morphological and ecological changes during their development (Vitt & Caldwell, 2014). In these species, eggs hatch as tadpoles, characterized by a globose body and an elongated tail used for locomotion, which are very different from adults both morphologically and ecologically. In tadpoles, early developmental stages are mainly marked by significant somatic growth, whereas more conspicuous changes occur later during development (i.e. hindlimb and forelimb development), ending with the atrophy of the tail and gills and the

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acquisition of aerial respiration and a feeding system that allows foraging on mobile terrestrial prey (Vitt & Caldwell, 2014).

These two different phases of tadpole development are expected to influence several parameters of their day-to-day life, including activity levels, locomotor performance (i.e. speed; Arnold, 1983) and behaviour (Kurali *et al.*, 2018). During early developmental stages, tadpoles are expected to increase activity levels and locomotion with increasing body size, because tadpole locomotion involves the entire anteroposterior axis, from the snout to the tail (Hoff & Wassersug, 2000). Conversely, at later stages of development, hindlimb and forelimb development is expected to influence mobility negatively (increased drag) and thus to decrease locomotor performance (but see Liu *et al.*, 1996). Finally, during the ultimate steps of the metamorphosis, tail atrophy is expected to decrease locomotor performance, because tail undulations are used as the main propulsive force in tadpoles (Liu *et al.*, 1996, 1997; Fabrezi *et al.*, 2014).

An important body of literature has investigated how environmental parameters (i.e. water temperature, predation, contamination or a combination thereof) can influence tadpole activity, performance or behaviour at specific developmental stages (Capellán & Nicieza, 2007; Touchon *et al.*, 2013; Johnson *et al.*, 2015; Kurali *et al.*, 2018; Lee-Jenkins & Robinson, 2018; Moreira *et al.*, 2019; Gallo *et al.*, 2020; Zhang *et al.*, 2020). Yet, to our knowledge, no study has investigated comprehensively the ontogenetic trajectories in activity and behaviour across the entirety of tadpole development and the relationships between body size, morphological changes linked to metamorphosis and locomotor and behavioural traits across the entirety of tadpole development. Thus, in this study, we examined how locomotor and behavioural traits covary with morphology (body size) and metamorphosis (hindlimb and forelimb development) across developmental stages in spined toad (*Bufo spinosus*) tadpoles.

Bufo spinosus tadpoles are benthic tadpoles, usually found foraging on the substrate, but also less frequently crossing the water column for 'bobbing' (Hoff *et al.*, 1999). Tadpoles swim continually and have remarkable endurance, even against slow currents (Wassersug & Feder, 1983). Swimming abilities fall into two categories: rapid bursts to evade predators and slow, continuous swimming while foraging or 'bobbing' (Hoff *et al.*, 1999). We investigated various metrics of locomotion and behaviour, including activity, distance travelled, swimming speeds and number of bouts, in undisturbed tadpoles across five discrete ontogenetic stages spanning the entire tadpole development, from hatching to forelimb appearance and tail atrophy. We also implemented an analysis of long-range correlations in locomotion sequences as an index of behavioural complexity using detrended fluctuation

analysis (DFA; Peng *et al.*, 1994; MacIntosh *et al.*, 2013). These analyses used the same developmental stages as those listed above to assess whether changes in body size and locomotion were associated with changes in behavioural complexity throughout development.

We predicted that all studied parameters (i.e. swimming speed, distance travelled, activity, number of activity bouts, and behavioural complexity) should increase with body and tail length during somatic growth and decrease in later developmental stages during metamorphosis.

MATERIAL AND METHODS

TADPOLE SAMPLING AND MAINTENANCE

Bufo spinosus egg strings ($N = 10$) were collected immediately after laying from a pond near the study site (Centre d'Etudes Biologiques de Chizé, France) in February 2019. For each string, 30 eggs were randomly selected and kept for the experiment. The remaining eggs (i.e. 3000–5000) were released at the site of capture. For each string, the 30 eggs were placed in 2 L glass tanks that contained dechlorinated tap water until hatching. Upon hatching, two tadpoles from each clutch were selected randomly and kept for the experiment (all other tadpoles were released in the pond of capture). The selected tadpoles were individually placed in individual 2 L tanks ($N = 20$), and their development was monitored until the end of the metamorphosis. Such husbandry methods allow for the development of each tadpole to be monitored individually and for the locomotion and behaviour of each individual to be assessed repeatedly throughout larval development. Importantly, maintaining the tadpoles individually in aquaria should not generate stressful conditions (e.g. Bókony *et al.*, 2021). All the experiments took place in a thermally controlled room with the temperature set at 17 °C (both air and water). The photoperiod was controlled (12 h dark–12 h light) using light-emitting diodes that were placed evenly across the room. Water was changed once a week. Tadpoles were fed *ad libitum* with organic spinach.

DEVELOPMENTAL STAGES AND MORPHOLOGICAL MEASUREMENTS

We used morphological features to classify and select specific stages according to Gosner (1960). We selected Gosner stages 25, 30, 37, 41 and 42 (hereafter, GS 25, GS 30, GS 37, GS 41 and GS 42, respectively) because those stages correspond to specific changes in the morphology of tadpoles (Table 1).

For each of the stages, we measured total length and tail length, following Watters *et al.* (2016), in order to

Table 1. Gosner stages (Gosner, 1960) selected to investigate morphological, locomotor and behavioural changes over time in developing *Bufo spinosus* tadpoles and corresponding pictures

Stages	Features	Pictures
25	Free-swimming tadpoles and feeding behaviour	
30	Hindlimb bud fully shaped	
37	Beginning of toe differentiation and development	
41	Cloacal tail pieces lost; fully grown hindlimbs	
42	Forelimb emergence; tail atrophy	

assess the overall growth of tadpoles. Each tadpole was put into a Petri dish with the water from its own tank, and photographs were taken using a fixed camera (Panasonic Lumix DC-TZ55) placed above the Petri dish. Morphological measurements were performed with the software IMAGEJ (Schneider *et al.*, 2012).

ACTIVITY AND LOCOMOTOR PERFORMANCE

At each developmental stage, tadpoles were removed from their tank and placed in an individual arena (Petri dish, diameter 13.5 cm, water level 1.5 cm) placed above a white background. After 15 min of acclimation (Dayton *et al.*, 2005), tadpoles were filmed for 30 min using a video camera [GoPro HERO (2018); GoPro, San Mateo, CA, USA] placed above the arena. Camera settings were as follows: 1080 pixel resolution, 60 frames s⁻¹ and narrow field of view. Codec for video was H.264 to produce MP4 files, and image stabilization was built into the GoPro (electronic image stabilization). A narrow field of view was used to limit the fish-eye effect, which was corrected further using *ToxTrac* during calibration. Operators left the room during experiments. Videos were analysed with the software *ToxTrac* (Rodriguez *et al.*, 2018). The contrast between the white arena and the dark tadpole allowed *ToxTrac* to detect and follow the tadpoles during the whole video. The successive positions of a tadpole (defined from the *x*- and *y*-calibrated coordinates within the Petri dish) were then extracted per frame, and the distance moved between two consecutive frames was calculated. We defined the tadpole as inactive when the distance moved between two consecutive frames was < 0.25 mm (Touchon & Wojdak, 2014; Hänzi & Straka, 2017). Initially, we calibrated the cameras to correct for lens distortion. We tested different calibration models for lens distortion, resulting in selection of ‘radial 3 + tangential 2’ distortion parameters. The calibration image was recorded in the same conditions

as the experiment and was a black-and-white chessboard pattern made using editing tools (GIMP, 2019). Calibrations were performed before each video. Definition of the arena was performed manually for each video to ensure that the entire arena was captured during the process. The animal detection threshold and minimal and maximal object size were adapted to the size of the tadpole for each video. We used the tutorial explained by Rodriguez *et al.* (2018). Using ‘Tracking RealSpace’ data and RSTUDIO scripts, we determined the following variables: number of activity bouts; total duration of activity; and total distance travelled. We measured total distance travelled as both the absolute distance (in millimetres) and relative to body size (per total length). We also extracted locomotion as the mean and maximal swimming speeds, calculated both as absolute speeds (in millimetres per second) and relative to total length (total lengths per second) (Wassersug, 1989). To minimize observer bias, blinded methods were used when all behavioural data were recorded and analysed.

BEHAVIOURAL COMPLEXITY

Detrended fluctuation analysis was conducted to measure long-range dependence as an index of temporal complexity in tadpole activity sequences (Peng *et al.*, 1994, 1995). By measuring fractal properties, animal time-series data can be defined as ranging from stochastic (uncorrelated) behaviour to deterministic (autocorrelated) behaviour that persists across measurement scales (see MacIntosh, 2014). The degree of complexity depends on both the internal conditions (e.g. stress, disease; Alados *et al.*, 1996; MacIntosh *et al.*, 2011; Cottin *et al.*, 2014) and external conditions (e.g. environment; MacIntosh *et al.*, 2011; Meyer *et al.*, 2017) of an individual.

To prepare the locomotion data for DFA, we followed the methodology as reported by Touchon & Wojdak (2014) and Hänzi & Straka (2017) to extract bouts of activity and inactivity. The process of DFA is described in detail elsewhere (Peng *et al.*, 1994, 1995). Briefly, the derived binary activity sequences were transformed into a time series, in which minus one corresponded to inactivity and one to activity (see previous subsection, ‘Activity and locomotor performance’). Time series were then integrated (through cumulative summation) to create an ‘activity walk’ sequence $[y(k)]$. Next, equally sized windows (n) were aligned along the sequence, and a least squares line was fitted within each to reflect the trend $[y_n(k)]$. Local trends in each box were then subtracted from the sequence, so that the average fluctuation as a function of window size $[F(n)]$ could be calculated. The process was repeated across a range of increasing window sizes (from 2^{10} to $2^{28/2}$), described by the following formula:

$$F(n) = \sqrt{\frac{1}{N} \sum_{k=1}^N [y(k) - y_n(k)]^2}$$

This analysis produces a sequence of values that produce a positive linear relationship on a double logarithmic plot of $F(n)$ against n , the slope of which is used to calculate the scaling exponent of the sequence (α DFA). We calculated α DFA using the package ‘fractal’ (Constantine & Percival, 2014) in R statistical software v.4.0.0 (R Core Team, 2020). Values of α DFA range between {0,1} for fractional Gaussian noise (fGn) and between {1,2} for fractional Brownian motion (fBm) (Eke et al., 2000; Seuront, 2009). Crucial to our study aims, α DFA of 0.5 for fGn and 1.5 for fBm reflect noise in time series data (random uncorrelated sequences), whereas values between {0.5,1} and {1.5,2} reflect persistence and those between {0,0.5} and {1,1.5} reflect antipersistence. Thus, values of α DFA approaching the extremes reflect higher long-range dependence and thus greater determinism in behavioural sequences, whereas values approaching the midpoints (0.5, 1.5) reflect increased stochasticity (MacIntosh, 2014). Thorough details of the analytical approach used, including DFA calculation, validation of scaling and its relationship to other fractal dimension estimates and illustrations, can be found in the studies by MacIntosh et al. (2013) and Burgunder et al. (2018).

STATISTICAL ANALYSES

We retained in our analyses only the individuals for which the entire duration of the behavioural experiments (30 min) was recorded and successfully extracted using *ToxTrac*. Given that *ToxTrac* did not detect some individuals satisfactorily during the whole duration of the behavioural experiments and because some mortality occurred at later stages of development ($N = 4$), we were able to analyse data on 13 individuals from GS 25, 18 individuals from GS 30, 17 individuals from GS 37, 14 individuals from GS 41 and 12 individuals from GS 42.

All statistical analyses were conducted with R statistical software v.4.0.0 (R Core Team, 2020) and RSTUDIO v.1.2.5042 (RStudio, 2020, Inc.). All residuals were tested for homogeneity of variance and normality with Bartlett’s test and the Shapiro–Wilks test, respectively. We also checked the normality of the residuals using diagnostic plots. Overall, clutch identity always influenced measured parameters significantly (all $P < 0.001$), hence we kept clutch identity as a random factor in all our analyses. Given that we assessed locomotion and behaviour in the same individuals throughout development, we nested the identity of the tadpole in their respective clutch. We tested a random intercept model and a random slope

model and kept the models with the lowest Akaike information criterion corrected for small sample size (AICc; Supporting Information, Table S1).

We fitted linear mixed-effect models (LMERs; package ‘lme4’; Bates et al., 2015) to analyse differences in total activity time, total distance, number of activity bouts, swimming speed (maximum, mean and relative) and α DFA across developmental stages (Supporting Information, Table S2). We fitted LMERs to analyse the relationship between total activity time, total distance, number of activity bouts, swimming speed (maximum and mean) and α DFA with total length and another round of models for tail length (Supporting Information, Tables S3 and S4). In order to extract random effects variances, model estimates and R^2 , we used the ‘parameter’ package with 1000 bootstrap iterations (Lüdecke et al., 2020) and the ‘lme4’ package (Supporting Information, Tables S5–S7). Tukey’s *post hoc* (‘multcomp’ package) tests were carried out to analyse the differences. Finally, to investigate whether behavioural traits were correlated together, we calculated the Pearson correlation coefficient using the *corr.test* function in the *corr.plot* package.

ETHICAL APPROVAL

All applicable institutional and/or national guidelines for the care and use of animals were followed. This work was approved by the French authorities (COMETHEA ethical committee and Ministère de l’Enseignement Supérieur, de la Recherche et de l’Innovation).

DATA AVAILABILITY

Data will be made available upon reasonable request.

RESULTS

All behavioural traits at each stage are summarized in Table 2.

TADPOLE SIZE

Total length ($F_{4,69} = 160.70$, $P < 0.0001$; Fig. 1) and tail length ($F_{4,69} = 97.62$, $P < 0.0001$; Fig. 1) differed among all stages. Both variables increased across the first three stages before reaching a plateau at GS 41 (Fig. 1). A slight but significant ($P < 0.001$) decrease was visible at GS 42, when tail atrophy occurs (Fig. 1).

ACTIVITY AND DISTANCE

Total activity time varied across developmental stages ($F_{4,51} = 8.69$, $P < 0.0001$; Fig. 2). It increased by 72% from GS 30 [190 s, 95% confidence interval

Table 2. Summary statistics (mean ± SE) of behavioural and morphological traits

Trait	Developmental stage				
	GS 25 (n = 13)	GS 30 (n = 18)	GS 37 (n = 17)	GS 41 (n = 14)	GS 42 (n = 12)
Behavioural trait					
Total activity time (s)	132.15 ± 29.46	319.27 ± 17.62	459.27 ± 38.52	398.24 ± 30.40	341.67 ± 40.52
Number of activity bouts	4899.62 ± 844.55	3126.78 ± 234.71	11 175.47 ± 1244.28	13 715.21 ± 781.76	12 085.00 ± 1213.61
Total distance (mm)	3314.59 ± 976.64	14 510.81 ± 1021.44	14 904.87 ± 1269.47	10 155.46 ± 1170.18	8360.61 ± 1220.93
Absolute mean swimming speed (mm s ⁻¹)	22.14 ± 1.24	44.10 ± 1.37	31.87 ± 1.32	23.77 ± 1.15	22.80 ± 0.80
Absolute maximal swimming speed (mm s ⁻¹)	65.16 ± 4.45	248.78 ± 13.17	254.94 ± 18.99	130.42 ± 12.70	144.20 ± 15.31
Relative mean swimming speed (total lengths s ⁻¹)	-8.96 ± 1.24	13.75 ± 1.36	2.12 ± 1.31	-5.86 ± 1.14	-7.08 ± 0.81
Relative maximal swimming speed (total lengths s ⁻¹)	-62.46 ± 4.41	81.84 ± 13.36	56.30 ± 19.41	-74.70 ± 13.29	-47.70 ± 14.35
αDFA	0.97 ± 0.00	0.91 ± 0.01	0.90 ± 0.01	0.97 ± 0.00	0.98 ± 0.00
Morphology					
Total length (cm)	1.04 ± 0.02	1.90 ± 0.03	2.59 ± 0.04	2.73 ± 0.06	2.45 ± 0.11
Tail length (cm)	0.63 ± 0.01	1.15 ± 0.02	1.56 ± 0.03	1.63 ± 0.04	1.43 ± 0.09

Abbreviation: αDFA, scaling exponent of the sequence.

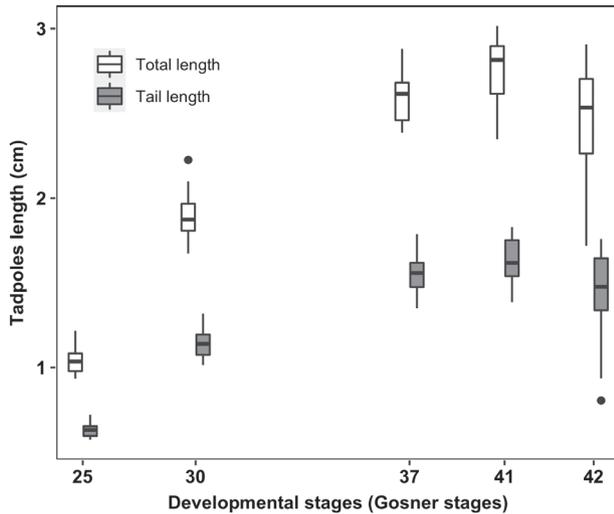


Figure 1. Total length (white boxes) and tail length (grey boxes; both in centimetres) relative to Gosner developmental stages in *Bufo spinosus* tadpoles. The top and bottom of the boxes represent the first and last quartiles, the horizontal line within the box represents the median, the whiskers represent the fifth and 95th percentiles, and the circles represent outliers. Dots represent the distribution of all individuals. Intervals between Gosner developmental stages were calculated using the mean number of days between two Gosner stages for all individuals.

(CI) = 77–310] to reach maximal values at GS 37 (328 s, 95% CI = 213–440) and subsequently decreased by 19% at GS 41 (266 s, 95% CI = 138–400) and again by 19% upon metamorphosis (216 s, 95% CI = 86–345), although all confidence intervals overlapped.

The number of activity bouts differed significantly between developmental stages ($F_{4,51} = 14.35$, $P < 0.0001$), with tadpoles at GS 25 and GS 30 being significantly less active than in all other stages (all $P < 0.001$, Fig S1). The total distance travelled was also significantly different between stages $F_{4,51} = 10.62$, $P < 0.0001$; Fig. 2). Early developmental stages (GS 25) moved more than three times less than GS 30 (11.2 cm, 95% CI = 7.1–15.5) and GS 37 (11.7 cm, 95% CI = 7.2–15.8), when maximal values were obtained; and total distance travelled gradually decreased at GS 41 (6.8 cm, 95% CI = 2.0–11.4) and at GS 42 (5.3 cm, 95% CI = 0.3–9.9), although all confidence intervals overlapped.

MEAN AND MAXIMAL SWIMMING SPEED

Both swimming speed variables differed between developmental stages (all $P < 0.001$, Fig S1). GS 25 displayed lower swimming speeds than GS 30 (all $P < 0.04$). After GS 37, maximal and mean swimming speeds decreased along development stages (all $P < 0.02$). Absolute maximal swimming speed increased

by almost 300% for GS 30 (183 mm s⁻¹, 95% CI = 130–237) to reach maximal values at GS 37 (190 mm s⁻¹, 95% CI = 135–248) and returned to the same value as GS 25 for GS 41 (66 mm s⁻¹, 95% CI = 7–125) and finally increased by 17% upon metamorphosis (216 s, 95% CI = 86–345), although all confidence intervals overlapped.

BEHAVIOURAL COMPLEXITY

Scaling exponent differed significantly between developmental stages ($F_{4,51} = 10.34$, $P < 0.0001$; Fig. 2), with GS 30 and GS 37 displaying higher complexity (lower α DFA, all $P < 0.006$) than other stages.

RELATIONSHIPS BETWEEN LOCOMOTION AND MORPHOLOGY

Total activity time was positively correlated with both total length and tail length across stages (total length, $F_{1,72} = 23.44$, $r^2 = 0.23$, $P < 0.0001$; tail length, $F_{1,72} = 24.85$, $r^2 = 0.24$, $P < 0.0001$), but not within stages (all $P > 0.14$). The number of activity bouts was positively correlated with total length and tail length across stages (total length, $F_{1,72} = 28.01$, $r^2 = 0.27$, $P < 0.0001$; tail length, $F_{1,72} = 26.65$, $r^2 = 0.26$, $P < 0.0001$), but not within stages (all $P > 0.19$), except for a positive correlation with tail length at GS 41 ($F_{1,12} = 6.85$, $r^2 = 0.31$, $P = 0.02$). Total distance travelled was positively correlated with total length and tail length across stages (total length, $F_{1,72} = 7.60$, $r^2 = 0.08$, $P = 0.007$; tail length, $F_{1,72} = 8.73$, $r^2 = 0.09$, $P = 0.004$), but not within stages (all $P > 0.2$).

Absolute mean swimming speed was not correlated with total length or with tail length across (all $P > 0.67$) or within stages (all $P > 0.09$), whereas absolute maximal swimming speed was positively correlated with total length and tail length across stages (total length, $F_{1,72} = 5.59$, $r^2 = 0.06$, $P = 0.02$; tail length, $F_{1,72} = 6.39$, $r^2 = 0.07$, $P = 0.01$), but not within stages (all $P > 0.1$).

Scaling exponent was not correlated with total length either across ($P = 0.07$) or within stages (all $P > 0.2$), except for a positive relationship at GS 37 ($F_{1,15} = 4.59$, $r^2 = 0.18$, $P = 0.04$). However, α DFA was negatively correlated with tail length across stages ($F_{1,72} = 4.29$, $r^2 = 0.04$, $P = 0.04$), but not within stages (all $P > 0.07$).

RELATIONSHIPS BETWEEN BEHAVIOURAL TRAITS

Most behavioural traits were correlated with one another (all $P < 0.001$; Table 3). The α DFA was negatively correlated with all locomotion traits except for the number of activity bouts ($P > 0.05$; Table 3), indicating that as locomotion increased, behavioural sequences exhibited greater complexity.

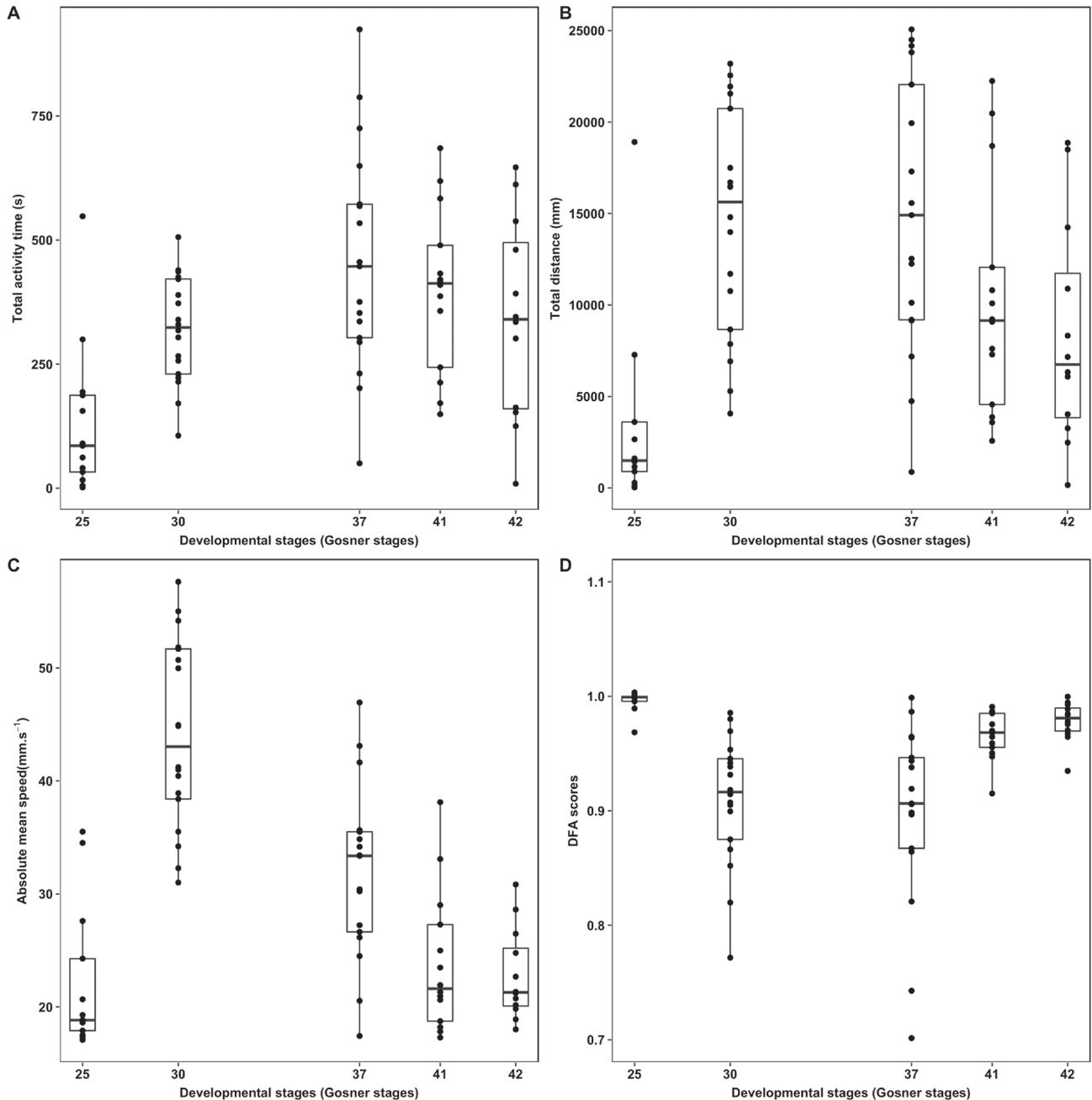


Figure 2. Total activity duration (in seconds; A), total distance travelled (in millimetres; B), absolute maximal swimming speed (in millimetres per second; C) and α DFA (unitless scaling index inversely related to behavioural complexity; D) relative to Gosner developmental stages in *Bufo spinosus* tadpoles. The top and bottom of the boxes represent the first and last quartiles, the horizontal line within the box represents the median, the whiskers represent the fifth and 95th percentiles, and the circles represent outliers. Dots represent the distribution of all individuals. Intervals between Gosner developmental stages were calculated using the mean number of days between two Gosner stages for all individuals.

DISCUSSION

Overall, we showed that activity levels, locomotion and behaviour undergo massive changes during tadpole development. These changes are not linear, but rather curvilinear across developmental stages, with

a phase of increasing activity followed by a plateau and/or reduction in the values of these parameters. These two different phases dovetail relatively well with the morphological changes associated with metamorphosis (i.e. a phase of intense growth followed

Table 3. Correlations (r) among all behavioural traits in *Bufo spinosus* tadpoles

	Total activity duration (s)	Number of activity bouts	Total distance (mm)	Absolute mean swimming speed (mm s ⁻¹)	Absolute maximal swimming speed (mm s ⁻¹)	Relative mean swimming speed (total lengths s ⁻¹)	Relative maximal swimming speed (total lengths s ⁻¹)	α DFA
Total activity duration (s)	/	0.73	0.87	0.3	0.48	0.32	0.36	-0.63
Number of activity bouts		–	0.33	-0.34	0.01	-0.31	-0.03	-0.06
Total distance (mm)			–	-0.69	0.72	0.71	0.66	-0.8
Absolute mean swimming speed (mm s ⁻¹)				–	0.76	1	0.81	-0.61
Absolute maximal swimming speed (mm s ⁻¹)					–	0.78	0.96	-0.58
Relative mean swimming speed (total lengths s ⁻¹)						–	0.81	-0.62
Relative maximal swimming speed (total lengths s ⁻¹)							–	-0.54
α DFA								–

Numbers in bold indicate significant average intra-individual correlations at $P < 0.05$. Abbreviation: α DFA, scaling exponent of the sequence.

by morphological changes linked to metamorphosis; [Brown & Cai, 2007](#)).

As predicted, the two observed phases of tadpole development strongly influenced all the metrics we studied. The highest values of activity levels, distance travelled, swimming speeds and behavioural complexity (i.e. lowest value of α DFA) were attained between GS 30 and GS 37, which represent the later developmental stages at the onset of the most intensive changes linked to metamorphosis. Our results suggest that, in order to sustain the intensive growth occurring between GS 25 and GS 37, tadpoles increased their activity levels and mobility, presumably to optimize foraging while evading predation ([Van Buskirk & Saxer, 2001](#)). In turn, greater body and tail lengths are likely to improve locomotion ([Wilson & Franklin, 2000](#); [Wilson et al., 2005](#); [Johnson et al., 2015](#); [Lindgren et al., 2018](#)).

During later stages of development (GS 41 and GS 42), the total length of tadpoles remained roughly constant, while locomotion-related traits decreased significantly. These stages are characterized by strong morphological changes linked to metamorphosis, with the end of growth of the hindlimbs (GS 41), the emergence of forelimbs from the body cavity and the onset of tail atrophy (GS 42). Interestingly, a previous theoretical study has suggested that the shape of tadpoles, along with large lateral oscillations at the snout, compensates for the reduced hydrodynamism (increased drag) linked to presence of the hindlimbs ([Liu et al., 1996](#)). Accordingly, it has been suggested that hindlimbs remain bound to the side of the body to improve streamlining ([Stehouwer & Farel, 1984](#)).

In contrast, our results showed that locomotion was strongly reduced at GS 41, when fully developed hindlimbs are present but probably not yet useful and/or used for propulsion ([Wilson & Franklin, 2000](#)). These appendages might have a negative influence on hydrodynamism and/or impede efficient undulatory body and tail movements (e.g. presence of hindlimbs on both sides of the base of the tail might impede tail undulations). Accordingly, hindlimb presence has been shown to influence postural and directional changes and predation avoidance ([Stehouwer & Farel, 1984](#); [Brown & Taylor, 1995](#)). GS 41 is also characterized by the growth of forelimbs that occur in the atrial chambers where gills are located ([Wells, 2007](#)). This process might also limit the drag imposed by limb development at this stage. Yet, comparatively lower activity was also observed at GS 42, when both hindlimbs and forelimbs are present and when tail atrophy begins, all of which seem to constrain aquatic locomotion of tadpoles further ([Calsbeek & Kuchta, 2011](#)). Similar changes in the index of behavioural complexity confirm these hypotheses: α DFA was markedly lower at stages GS 30 and GS 37, indicating greater levels of behavioural complexity (stochasticity) at these stages ([MacIntosh, 2014](#)).

Alternatively, but not mutually exclusively, we can hypothesize that after GS 37 activity is no longer dedicated to acute growth (as suggested by the stabilization of body size), but rather towards organismal maintenance and metamorphosis. In this respect, by decreasing the costs of locomotion (lower activity levels and swimming speeds) tadpoles dedicate energy to metamorphosis ([Beck & Congdon, 2003](#);

Wright *et al.*, 2011; Kirschman *et al.*, 2017; Ruthsatz *et al.*, 2020). Additionally, other major changes linked to metamorphosis are also expected to influence locomotion negatively; for instance, the shift from herbivory and detritivory to carnivory, characterized by significant anatomical modifications in the digestive system and mouth (Vitt & Caldwell, 2014). The feeding apparatus and oral disc disappear, and alterations of the intestinal epithelium preclude efficient nutrient absorption (Hourdry *et al.*, 1996). This might prevent tadpoles at later stages of development from foraging efficiently (e.g. inability to acquire and/or digest food), thereby limiting the necessity of displaying well-developed locomotor performance. Accordingly, it has been suggested that during later stages of development, tadpoles become aphagic and rely, in part, on tail resorption to fuel energetic demands (Gonçalves *et al.*, 2015; Bouchard *et al.*, 2016; Ruthsatz *et al.*, 2018).

To conclude, our study shows that tadpole ontogeny is characterized by strong variations in locomotion and behaviour according to two different phases of development (i.e. somatic growth vs. morphological changes linked to metamorphosis). All the metrics we investigated indicate that the peak of activity and associated behaviour is situated at a pivotal stage when somatic growth decreases and significant morphological changes occur (i.e. hindlimb growth). Future studies that aim to investigate determinants of activity should include developmental stages as covariates in order to assess whether the sensitivity of locomotion to environmental variables (e.g. temperature and predation) changes across developmental stages. Additionally, future studies should investigate the influence of tadpole development on their movements throughout the water column.

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during data curation. L.R., X.M. and A.K. arranged the dataset for fractal analysis. A.K., L.R. and M.C. conducted all fractal analyses. F.B., M.C., L.R., A.K. and Y.R.-C. interpreted the data. F.B., M.C. and L.R., designed the figures and wrote the initial draft. All authors contributed to manuscript revision. All authors read and approved the final manuscript. The authors declare that they have no competing interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Table S1. Model comparison between random intercept model and random slope (individual identification) model using the Akaike information criterion corrected for small sample size (AICc). We retained the model with the smallest AICc value.

Table S2. Linear mixed model of the difference in several behavioural traits of developmental stages (Gosner stages). We kept individual identification nested in clutches in all our models. σ represents variance of random effect. When possible, marginal and conditional R^2 were used.

Table S3. Linear mixed model of the relationship between several behavioural traits and total length of the tadpoles. We kept individual identification nested in clutches in all our models. σ represents variance of random effect. When possible, marginal and conditional R^2 were used.

Table S4. Linear mixed model of the relationship between several behavioural traits and tail length of the tadpoles. We kept individual identification nested in clutches in all our models. σ represents variance of random effect. When possible, marginal and conditional R^2 were used.

Table S5. Linear mixed model of several behavioural traits and developmental stages. We kept individual identification nested in clutches in all our models. The confidence interval (CI) and P -value were estimated from 1000 bootstrap iterations (‘parameter’ package; Lüdecke *et al.*, 2020).

Table S6. Linear mixed model of several behavioural traits and total length. We kept individual identification nested in clutches in all our models. The confidence interval (CI) and P -value were estimated from 1000 bootstrap iterations (‘parameter’ package; Lüdecke *et al.*, 2020).

Table S7. Linear mixed model of several behavioural traits and tail length. We kept individual identification nested in clutches in all our models. The confidence interval (CI) and *P*-value were estimated from 1000 bootstrap iterations ('parameter' package; Lüdecke *et al.*, 2020).

Figure S1. A, number of activity bouts. B, absolute mean swimming speed (in millimetres per second). C, D, relative mean swimming speed (per total length; C) and relative maximal swimming speed (per total length; D) relative to Gosner developmental stages in *Bufo spinosus* tadpoles. The top and bottom of the boxes represent the first and last quartiles. The line across the box represents the median. The whiskers represent the fifth and 95th percentiles, and the dots represent the distribution of the individuals.