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Repeatability of thermal reaction norms for spontaneous locomotor activity in juvenile newts

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ABSTRACT

Locomotor activity is a major attribute of animals. Although this trait determines important ecological processes, such as dispersal and species interactions, the sources of its variation are not fully understood. We examined the influence of body temperature (13, 18, 23, and 28 °C) and individual identity on spontaneous locomotor activity in juvenile alpine newts, *Ichthyosaura alpestris*, over three consecutive weeks. Locomotor activity was characterized by four parameters: distance covered, mean velocity, frequency of movements, and total activity rate (all directional and non-directional movements). Apart from total activity rate, thermal reaction norms for locomotor parameters had convex or concave curvilinear shapes. During the first trial series, i.e. across the four body temperatures that were tested, individual identity explained less variation in thermal reaction norms than during the second series. Individual means, i.e. the vertical positions of individual thermal reaction norms, were repeatable between trial series in all locomotor activity parameters but the frequency of movements. We conclude that spontaneous locomotor activity is a complex trait, which can be characterized by several parameters with varying individual repeatability and thermal dependency. This information should be considered for planning further locomotor activity experiments, conservation strategies, and modeling ectotherm responses to climate change.

1. Introduction

Locomotor activity is a typical attribute of non-sessile animals, which affects their ecology at different scales. At a population level, movement underlies major ecological processes, such as dispersal and migration (Nathan et al., 2008). Within a population, relationships among locomotor activity, energy acquisition, and predation risk may mediate the influence of environmental change on population dynamics (Frid and Dill, 2002). At an individual level, locomotor activity determines both the frequency of interactions with con- and heterospecifics (Dell et al., 2014) and the exposure to abiotic environmental factors (Williams et al., 2008). On the other hand, locomotor activity is a highly flexible trait, which is affected by diverse external and internal factors (Martin, 2003). However, the interactive influence of these factors on locomotor activity has received limited attention.

Among external factors, environmental temperature seems to be the most important determinant of locomotor activity, especially in ectotherms, because of its direct influence on body temperature. Indeed, after pioneering papers (Fry, 1947; Bennett, 1980; Putnam and Bennett, 1981), hundreds of studies have demonstrated the thermal dependence of various locomotor traits, such as maximum velocity, sustainable speed, jump length, or stamina. The thermal dependence of locomotor performance shows the typical pattern of linearly increasing values at body temperatures below thermal optimum, followed by a curved relationship around the thermal optimum, and a fast performance drop above this temperature (Huey and Stevenson, 1979). However, most of this information pertains to forced locomotion, which is largely determined by an ectotherm's morphological and physiological capacity. Spontaneous locomotion, which ectotherms mostly use for routine activities (Reilly et al., 2007), is affected by the individual's internal state and motivation rather than by maximum performance (Hertz et al., 1988). Accordingly, thermal dependence curves for maximum velocity differ between spontaneous activity and forced locomotion (Šamajová and Gvoždík, 2009).

Even when external factors are carefully controlled, locomotor activity varies considerably within a population because of consistent individual differences in behavior, i.e., personality. It is well established that some individuals within a population are consistently more active than others (Bell et al., 2009). However, it is unknown how personality (e.g., individual differences in relative activity) may affect thermal

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reaction norms for locomotor activity. Although many studies demonstrate thermal dependence of locomotor activity (Chiverton, 1988; Martin et al., 1999), they have often been performed at two experimental temperatures only, which provided insufficient information about the shape of the reaction norm across an ecologically relevant temperature range. If individual variation in thermal reaction norms for locomotor activity is, in fact, repeatable, it remains virtually unknown.

We examined the short-term repeatability of individual thermal reaction norms for spontaneous locomotor activity in juvenile alpine newts, *Ichthyosaura alpestris*. Newts are ectotherms so their body temperatures, and accordingly locomotor activity, are directly influenced by surrounding environmental (operative) temperatures that vary spatially and temporary in their natural habitat (Hadamová and Gvoždík, 2011). In newly metamorphosed newts, locomotor activity affects their dispersal from water bodies (Pittman et al., 2014) and the avoidance of costly heterospecific interactions (Janča and Gvoždík, 2017). Our two goals were to (1) evaluate individual variation in the magnitude and shape of thermal reaction norms for spontaneous locomotor activity and (2) examine the short-term repeatability of the reaction norms, which is an important assumption for their evolution by natural selection (Angilletta et al., 2002).

2. Materials and methods

2.1. Study species and maintenance

The alpine newt, *I. alpestris,* is a common European newt attaining a total length (TL) of 12 cm (Speybroeck et al., 2016). Its aquatic larvae usually metamorphose during August to September. Some larvae may overwinter and complete their metamorphosis the following spring. Metamorphosed individuals are strictly terrestrial with a crepuscular to nocturnal activity period. Newt dispersal from natal water bodies occurs largely during the juvenile stage (Kupfer and Kneitz, 2000). They feed on various invertebrates of appropriate size (Denoël, 2004).

Juvenile newts ($n = 21$; mean TL ± SD = 46.0 ± 5.6 mm; body mass = 0.741 ± 0.118 g) were haphazardly selected from a large stock of larvae ($n = 300$) reared in ten tanks (90 × 63 × 47 cm) under similar semi-natural conditions after completing metamorphosis (in September). Newts were placed in plastic tanks (40 × 26 × 18 cm) in small groups ($n = 4$–5). The bottom of each tank was covered with moistened filter paper. Two ceramic shelters were provided as refuges. Tanks were slightly inclined to maintain some free water on the lower side. They were placed in an air-conditioned room with daily fluctuating air temperatures (12–22 °C) and a natural light regime (12:12–14:10 [L:D]) for four weeks before the beginning of experiments. The air temperature range was chosen according to typical environmental temperatures from the field (Šamajová and Gvoždík, 2010). Newts were fed with live *Tubifex* worms and chironomid larvae twice weekly. Tanks were thoroughly washed with substrate replacement at three-day intervals.

All experimental procedures were approved by the Expert Committee for Animal Conservation of the Institute of Vertebrate Biology AS CR (research protocol no. 135/ 2016). The Agency for Nature Conservation and Landscape Protection of the Czech Republic issued permission to capture the newts (1154/ZV/2008). After experiments (November 2017), all animals were healthy and in good condition, and thus according to permit conditions, were released at the site of their parents.

2.2. Locomotor activity assays

We measured the spontaneous locomotor activity, i.e. steady state movement in a constant environment (Connolly, 1967), of newts in nine circular experimental glass arenas (140 × 10 mm) at 14, 20, 24.5, and 33 °C. The order of experimental temperatures was randomized for each trial series (see below). Chosen air temperatures correspond to newt operative temperatures of 13, 18, 23, and 28 °C, respectively, because of evaporative cooling from amphibian skin. Operative temperatures were measured inside agar models of similar size to the experimental animals, which provides reliable estimates of their body temperatures (Navas and Araujo, 2000). The temperature range was chosen according to information about typical environmental temperatures in newt terrestrial habitats (Šamajová and Gvoždík, 2010) and with respect to local climate change trends (Dvořák and Gvoždík, 2010). Two drops of water were added to each arena before newt placement in arenas. Arenas were then covered with a transparent acrylic lid to minimize water loss of experimental subjects during a trial. Arenas ($n = 9$) were separated by opaque walls that prevented newts from visually communicating during a trial. Activity trials were performed in an air-conditioned room without human presence between 19:00 and 23:00. Each newt was tested once daily and it was placed back to rearing tank immediately after a trial. Between trials, newts were exposed to the same thermal and light conditions prior to activity measurements (see above). After the first trial series (18, 13, 23, and 28 °C), newts rested for seven days and a second series of trials was performed at 28, 23, 18, and 13 °C, respectively. After 5 min of habituation to experimental settings, each trial lasted 30 min. Newt position was continuously recorded (3.75 fps) in darkness under infrared lighting using an automated tracking system (Ethovision XT, Noldus, Wageningen, Netherlands). Before each use, glass arenas were thoroughly washed in 95% ethanol to remove scent traces from previous trials.

We used four parameters to characterize newt locomotor activity: 1. Distance covered (mm) – distance traveled during a trial; 2. Mean velocity (mm s^{-1}) – average velocity during moving episodes within a trial; 3. Frequency of moving episodes – the number of time intervals with velocity $> 0.03\,\mathrm{mm\,s^{-1}}$. Marginal velocity was empirically determined as the minimum velocity when individual movement was not captured by the software; 4. Total activity rate – proportion of changed pixels relative to the total number of pixels within an arena. This measure includes all directional, i.e. shifts of body position in space, and non-directional movements, i.e. change in head, limb and tail position without spatial shifting of the animal's body. To examine the time course of locomotor activity during a trial, we also calculated distance covered at 5 min intervals. All calculations were performed using Ethovision software. Trackings were checked for missing values prior to calculations. Two trials with higher missing scores (> 5%) were discarded from further analyses.

2.3. Statistical analyses

We analyzed the effect of individual variation and body temperatures on locomotor activity traits using mixed effect models. The initial model contained two covariates, TL and body temperature (up to a second order polynomial), and individual identity as a random factor. The random factor in the full model contained both a random intercept and slope (second order polynomial). The repeatability of mean individual values between trial series was tested using the mixed effect model with week as a fixed factor and individual identity as a random factor.

We applied the model selection approach to obtain a minimum adequate mixed effect model using a likelihood ratio test. We assumed that the likelihood ratio followed χ^2 distribution. In the case of random intercept tests (testing on the boundary), the likelihood ratio follows $0.5 \times \chi^2$ distribution, and so the corresponding tabled *P*-values were divided by two (Zuur et al., 2009). The best fixed structure was selected according to the maximum likelihood, whereas we applied a reduced-maximum likelihood to find the optimal random structure, i.e. random slope and intercept, or random intercept only (Zuur et al., 2009). We visually checked the distribution of model residuals, and accordingly, all variables were log-transformed to improve their model residual distribution. This approach produced better data fits than using a

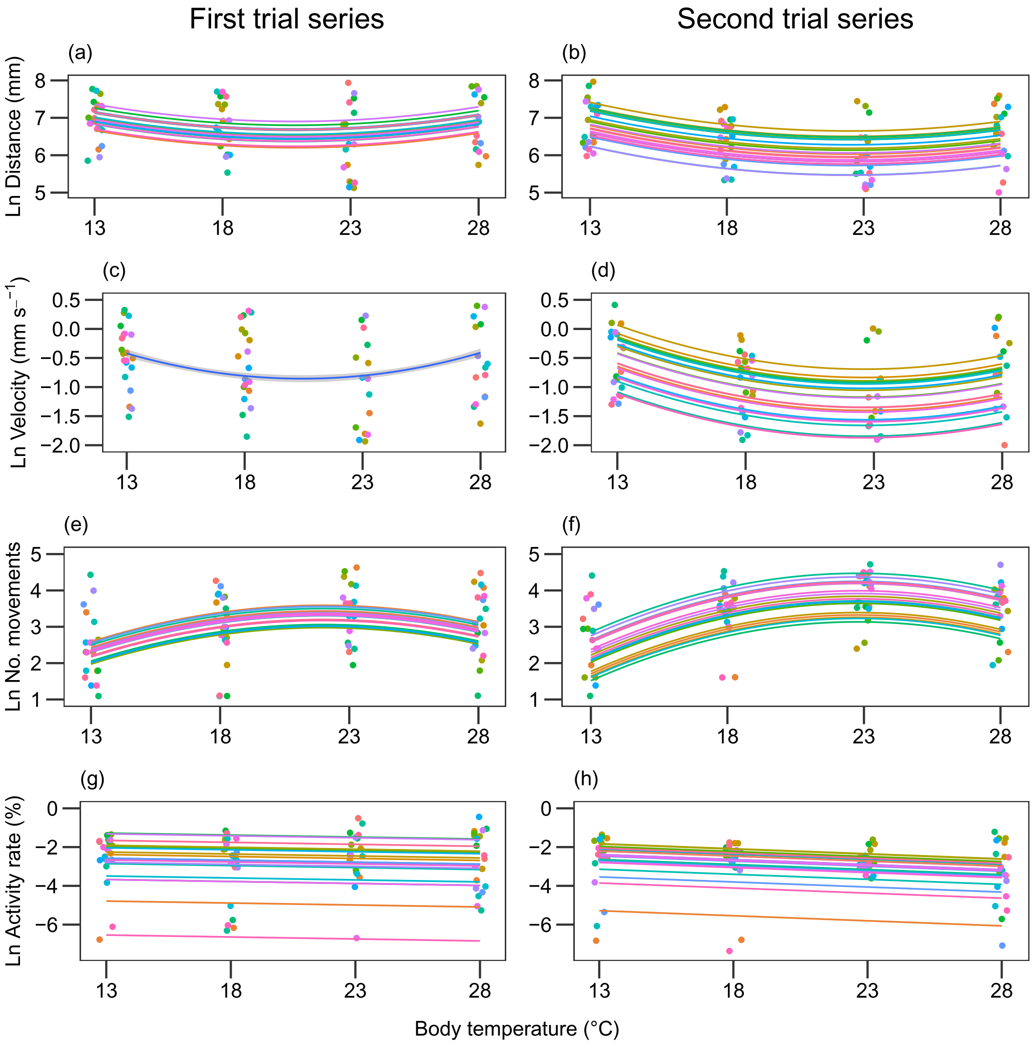

Fig. 1. Thermal dependence of (a, b) distance covered, (c, d) mean velocity, (e, f) the frequency of movements, and (g, h) activity rate in juvenile newts during a 30 min trial. The first trial series was repeated after seven days. Data from each individual were fitted using a linear or quadratic mixed effect model (see Table 1 for details). All fittings are parallel because of negligible individual variation in slopes of thermal reaction norms. Note that activity rate (g, h) was quantified as the proportion of changed pixels within an experimental arena. Data in (c) were fitted with a quadratic general linear model because of singularity in the mixed-effect model.

generalized model with non-normal errors. The proportion of total variance explained by a random factor was calculated as the difference between total variance explained by a model and variance explained by fixed factors (Nakagawa and Schielzeth, 2013). The repeatability of individual locomotor parameters both across temperatures and between trial series was estimated using intraclass correlation coefficients. All analyses were performed in the R environment using 'lme4' (Bates et al., 2014) and 'lmerTest' (Kuznetsova et al., 2017) packages.

3. Results

We obtained data from 21 individuals and 166 trials (Gvoždík and Baskiera, 2018). After the habituation period, there was no trend in distance covered during the trial across body temperatures (Fig. A1), and so observed behavior can be classified as spontaneous locomotor activity. All locomotor activity parameters were affected by body temperature, except total activity rate during the first trial series (Fig. 1; Table 1). The effect of body temperature on distance covered, mean velocity, and the number of movements was best modeled by quadratic polynomials. The shapes of reaction norms for the first two parameters were convex, and concave for the last one (Fig. 1a–f). Total activity rate showed none or a weak linear response to body temperature (Fig. 1g–h; Table 1).

For all locomotor activity parameters except mean velocity from the first trail series, the minimum adequate model contained random intercepts only (Table 2). The mixed effect model for mean velocity from the first trail series was singular, i.e. some elements of its variance–covariance matrix were close to zero. Accordingly, the model produced unreliable parameter estimates, and thus it was replaced with a general linear model without random factors. The magnitude of individual variation in thermal reaction norms varied between trial series (Table 1). During the first series, individual variation was non-significant and explained less variation in thermal reaction norms for locomotor activity parameters than during the second series. Individual thermal reactions norms varied in the magnitude (intercept), not in their linear or curvilinear shape (Fig. 1; Table 2). During the second series, individual identity explained 20–31% of total variation in locomotor activity parameters across body temperatures (Table 1).

For between-trial series comparisons we used individual means because intercepts from random models produce inaccurate estimates (Hadfield et al., 2010) and individual variation in the shape of thermal reaction norms was negligible (Table 2). Mean values of locomotor activity parameters calculated across body temperatures showed a marked decrease during the second trial series (Distance: $F_{1,20} = 16.94$, $P = 0.005$; Velocity: $F_{1,20} = 16.75$, $P = 0.006$; Movements: $F_{1,20} = 5.07$, $P = 0.04$; Activity: $F_{1,20} = 11.62$, $P = 0.003$). Apart from the frequency of movements, individual identity explained 45–53% of total variation in examined traits, which suggests good short-term repeatability of these parameters between weeks (Fig. 2).

4. Discussion

While individual repeatability and thermal dependence of forced locomotor performance have received ample attention (see Section 1),

Table 1
Parameters of the linear mixed effect model for the effect of body temperature and individual identity on distance covered, mean velocity, number of movements, and total activity rate in juvenile newts over two weeks of measurements. Note that fixed factor parameters for mean velocity from the first trial series were obtained from a general linear model because of singularity in the mixed effect model. Statistically significant values are in bold. Variance components are with 95%CIs. See Fig. 1 for graphs.

Activity metric	Factor	First trial series					Second trial series				
Distance	Fixed	Parameter	SE	*t*	df	*P*	Parameter	SE	*t*	df	*P*
	Intercept	9.806	1.498	6.54	61	**< 0.001**	10.340	1.183	8.74	62	**< 0.001**
	T_b	− 0.314	0.154	2.03	60	**0.047**	− 0.380	0.121	3.13	61	**0.003**
	T_b^2	0.008	0.004	2.02	60	**0.048**	0.008	0.003	2.87	61	**0.006**
	Random	Variance	ICC	χ^2	df	*P*	Variance	ICC	χ^2	df	*P*
	ID	0.008[0,0.15]	0.01	0.02	1	0.440	0.190[0.03, 0.44]	0.29	8.31	1	**0.002**
	Residual	0.716[0.48, 0.83]					0.455[0.30, 0.62]				
Velocity	Fixed	Parameter	SE	*t*	df	*P*	Parameter	SE	*t*	df	*P*
	Intercept	− 0.606	0.089	6.80	79	**< 0.001**	2.884	1.113	2.59	62	**0.012**
	T_b	0,063	0.807	0.08	79	0.938	− 0.369	0.114	3.23	61	**0.002**
	T_b^2	1.772	0.807	2.20	79	**0.031**	0.008	0.003	2.94	61	**0.005**
	Random	Variance	ICC	χ^2	df	*P*	Variance	ICC	χ^2	df	*P*
	ID	n/a	n/a	n/a	n/a	n/a	0.201[0.05, 0.44]	0.33	10.50	1	**< 0.001**
	Residual	0.807					0.403[0.28, 0.56]				
Movements	Fixed	Parameter	SE	*t*	df	*P*	Parameter	SE	*t*	df	*P*
	Intercept	− 2.712	1.753	1.55	60	0.127	− 4.970	1.548	3.21	62	**0.002**
	T_b	0.547	0.181	3.03	59	**0.004**	0.772	0.159	4.85	61	**< 0.001**
	T_b^2	− 0.012	0.004	2.85	59	**0.006**	− 0.017	0.004	4.40	61	**< 0.001**
	Random	Variance	ICC	χ^2	df	*P*	Variance	ICC	χ^2	df	*P*
	ID	0.103[0, 0.40]	0.09	0.91	1	0.170	0.274[0.12, 0.62]	0.26	6.56	1	**0.005**
	Residual	0.978[0.65, 1.28]					0.781[0.52, 1.08]				
Activity	Fixed	Parameter	SE	*t*	df	*P*	Parameter	SE	*t*	df	*P*
	Intercept	− 10.407	2.139	4.87	21	**< 0.001**	− 1.921	0.528	3.64	81	**< 0.001**
	TL	0.173	0.045	3.86	19	**0.001**					
	T_b	− 0.020	0.025	0.81	60	0.423	− 0.052	0.023	2.27	62	**0.026**
	Random	Variance	ICC	χ^2	df	*P*	Variance	ICC	χ^2	df	*P*
	ID	0.849[0.14, 1.85]	0.34	10.10	1	**< 0.001**	0.869[0.24, 1.72]	0.39	14.10	1	**< 0.001**
	Residual	1.651[1.08, 2.25]					1.382[0.96, 1.85]				

T_b, linear effect of body temperature; T_b^2, quadratic effect of body temperature; TL, total length; ID, individual identity; ICC, intraclass correlation coefficient, n/a, not available.

spontaneous locomotor activity has remained understudied from this view. We showed that the thermal sensitivity of spontaneous locomotor activity depends on the parameter that is used to characterize the activity of individuals. Individual variation in thermal reaction norms affected their magnitude (intercept), not shape. The vertical position of thermal reaction norms, characterized by individual means across body temperatures, of distance covered, mean velocity, and total activity was repeatable between weeks in juvenile newts.

Two parameters of locomotor activity, distance covered and average velocity, showed U-shaped thermal reaction norms with the lowest values between 18 and 23 °C (Fig. 1). This U-shaped thermal reaction norm markedly differs from the unimodal asymmetric shape of the thermal performance curve for maximum running velocity, which has a thermal optimum of 27 °C in alpine newts (Gvoždík and Van Damme, 2008). On the other hand, these findings concur with the thermal reaction norm for swimming velocity during diving, i.e. spontaneous locomotor activity (Šamajová and Gvoždík, 2009). This suggests that within an ecologically realistic temperature range, thermal reaction

Table 2
Comparison of models with random intercepts and with random intercepts and slopes in four metrics of locomotor activity during two trial series. Except for activity rates, random slopes contained both linear and curvilinear terms. All models contained total body length and body temperature as covariates (see Table 1). Note that results for mean velocity from the first trial series are unavailable (n/a) because of singularity in the mixed effect models.

Activity metric	Model random structure	First trial series				Second trial series			
		AIC	χ^2	df	*P*	AIC	χ^2	df	*P*
Distance	α	211.53				201.18			
	$\alpha + \beta$	215.46	0.07	2	0.97	204.37	0.81	2	0.67
	$\alpha + \beta + \beta^2$	218.85	2.61	3	0.46	206.38	4.00	3	0.26
Velocity	α	n/a	n/a	n/a	n/a	191.84			
	$\alpha + \beta$	n/a	n/a	n/a	n/a	193.30	2.54	2	0.28
	$\alpha + \beta + \beta^2$	n/a	n/a	n/a	n/a	195.41	3.89	3	0.27
Movements	α	246.82				244.44			
	$\alpha + \beta$	250.82	< 0.01	2	0.99	243.82	4.62	2	0.10
	$\alpha + \beta + \beta^2$	247.30	9.52	3	0.02	269.88	6.67	3	0.08
Activity	α	305.36				295.64			
	$\alpha + \beta$	306.35	3.00	2	0.22	298.89	0.75	2	0.69

Distance, distance covered; velocity, mean velocity; movements, the frequency of movements, activity, activity rate, i.e., all directional and non-directional movements; AIC, Akaike's Information Criterion; α, random intercept; $\alpha + \beta$, random intercept and slope; $\alpha + \beta + \beta^2$, random intercept and slope with linear and quadratic terms.

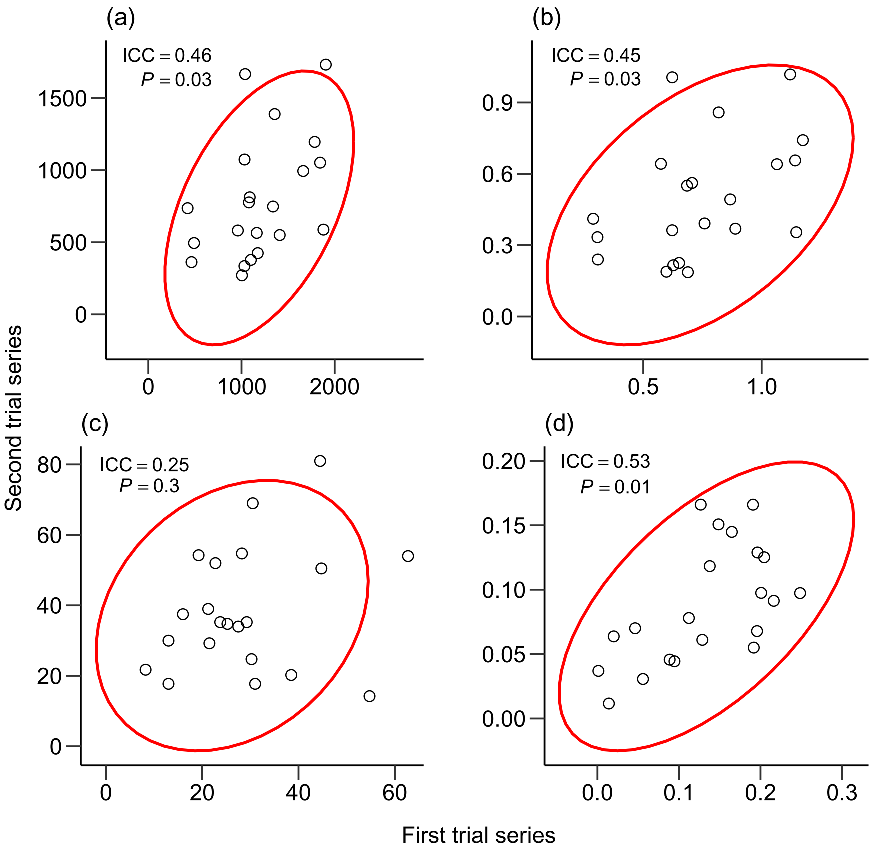

Fig. 2. Association between repeated measurements of locomotor activity parameters in juvenile newts after seven days. (a) distance covered (mm), (b) mean velocity (mm s^{-1}), (c) frequency of moving episodes, (d) total activity rate. Note that datapoints are mean values calculated across four body temperatures (13, 18, 23, and 28 °C). Association patterns are denoted by 95% confidence ellipses for illustrative purposes. ICC, intraclass correlation coefficient.

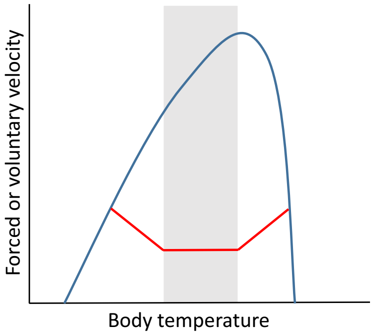

Fig. 3. Distinction between the thermal performance curve for maximum velocity during forced locomotion (blue) and the thermal reaction norm for mean velocity during voluntary locomotion (red). Grey area denotes the range of preferred body temperatures (T_{pref}). While an ectotherm attains the highest speed at an optimal temperature above the T_{pref} range, it increases voluntary mean velocity when its body temperatures are below or above the Tpref range. Compiled from empirical findings in alpine newts (Gvoždík and Van Damme, 2008; Šamajová and Gvoždík, 2009; Balogová and Gvoždík, 2015; present study).

norms for spontaneous locomotor activity markedly differ from thermal performance curves for maximum velocity during forced locomotion trials (Fig. 3).

Why were thermal reaction norms for distance covered and mean velocity U-shaped (Fig. 1)? Velocity during spontaneous locomotor activity is several times lower than the maximum performance during forced locomotion (Šamajová and Gvoždík, 2009). Accordingly, spontaneous locomotion is mainly determined by a motivation to move rather than by the physiological capacity for maximum performance, where thermal dependence follows a typical unimodal asymmetric shape (Martin and Huey, 2008; Fig. 3). In contrast, observations on ectotherms in thermal shuttle boxes (Withers and Campbell, 1985) suggest that motivation to move is affected by thermoregulatory behavior. If body temperatures are below or above a preferred body temperature (T_{pref}) range, they move to a site in which the environmental temperature allows them to attain their body temperature within the target range. The higher the departure of body temperature from the T_{pref} range, the more effort (higher velocity) should be invested for returning its body temperature to a target range. In the case of alpine newts, the lowest values of distance covered and mean velocity are indeed within the T_{pref} range for the studied population (Balogová and Gvoždík, 2015). Generally, thermal sensitivity of spontaneous velocity is weak, although it may vary substantially among taxa (Dell et al., 2011). Unfortunately, without additional information about ectotherm thermal requirements, additional data are needed to explain the contrasting shapes of the thermal performance curves for forced versus voluntary locomotion (Fig. 3).

Contrary to the distance covered and average velocity characteristics, the shape of thermal reaction norms for the frequency of movements was concave with the highest frequency of moving episodes between 18 and 23 °C. These temperatures are within the T_{pref} range for alpine newts (Balogová and Gvoždík, 2015). Because intermittent locomotion has been considered more costly than continuous locomotion (Gleeson and Hancock, 2001), one would expect that newts will move more continuously within their T_{pref} range, rather than outside of it. However, energy costs of increasingly intermittent locomotion may be partially offset by a lower average velocity at body temperatures within the T_{pref} range. In addition, intermittent locomotion increases endurance (Weinstein and Full, 1999), prey detection, and protection from predators (Kramer and McLaughlin, 2001). Hence, moving slowly with frequent stops appears the beneficial rather than detrimental mode of spontaneous locomotion in juvenile newts at their T_{pref}.

Total activity rate, involving all directional and non-directional movements, was thermally insensitive (first trial series) or weakly increased with body temperature (second trial series). To our knowledge, these strikingly disparate thermal dependencies of directional, i.e.

distance covered, and total movements have not been reported before. Total activity involves not only changes of body positions in space but also movement of all body parts, which may be less related to thermoregulatory motivation than distance covered or average velocity. Accordingly, variation in movement of body parts may mask the U-shaped thermal reaction norm for directional movements, resulting in its weak relationship with temperature.

Juvenile newts markedly differed in their individual variation in thermal reaction norms between the first and second trial series. The source of this variation is unknown. Perhaps the individual variation in reaction norms becomes visible only under reduced overall locomotor activity level, which happened during the second trial. The locomotor activity reduction during repeated measurements may be explained as increasing habituation to experimental conditions (Martin and Réale, 2008; Biro, 2012). However, if locomotor activity was affected by the trial order in our study, we should find (i) the highest locomotor activity level at the first measured temperature, i.e. 18 °C, and (ii) the shape of the reaction norm should vary between the first and second trial series. Because our results show exactly the opposite, it seems likely that the activity drop during the second trial series resulted from factors other than habituation, e.g. its interactive effect with individuality and the order of temperatures.

Mean values of all locomotor activity parameters, except frequency of movements, showed good short-term repeatability. Repeatability of flexible traits is an important prerequisite for their evolution by natural selection (Bennett, 1987). However, repeatability estimates of behavioral traits fade with time (Bell et al., 2009), and so our short term repeatability estimates have to be considered with caution. Despite this limitation, our results suggest that the vertical position of individual thermal reaction norms for locomotor activity may have some evolutionary potential. Further studies using larger sample sizes and sophisticated statistical tools (Dingemanse and Dochtermann, 2013) will show, whether this conclusion holds in other taxa and across longer time intervals.

In conclusion, our findings provide at least four important implications. 1. From a methodological view, parameters characterizing locomotor activity vary in their thermal dependency and individual repeatability. Hence, the activity parameters should be carefully selected according to the aims of a planned study. Specifically, it is important to decide whether only directional movements or all movements are important in quantifying spontaneous activity. For example, distance covered is relevant for studying spontaneous activity in relation to dispersal, while the total activity rate plays an important role in predator-prey interactions. 2. Mean moving velocity during spontaneous locomotion is several times lower than during forced locomotor trails. This provides potential for motivation-driven behavioral modifications, which buffer the effect of body temperature or even shape thermal reaction norms for locomotor activity in the opposite direction to thermal performance curves for locomotor capacity. Hence, we propose terminological discrimination between the thermal dependence of performance, i.e. thermal performance curves, and behavioral traits, i.e. behavioral thermal reaction norms. In addition, the lowest values of spontaneous locomotor activity were within the T_{pref} range, which seems key to understanding the shape of thermal reaction norms for locomotor activity and other aerobic activities (Gvoždík and Kristín, 2017). 3. The thermal dependence of locomotor velocity has recently received attention with respect to modeling the effect of climate change on predator-prey interactions (Dell et al., 2014). Our results showed that it is important to discriminate between body velocity during spontaneous and forced locomotion (Fig. 3), because a prey's probability of interaction with predators is likely influenced by body velocity during spontaneous locomotion, for which thermal dependence is disparate from the thermal performance curve for burst speed. 4. Finally, information about sources of variation in spontaneous locomotor activity may contribute to our understanding of movement patterns in juvenile amphibians, which is important for further conservation efforts and management of these threatened vertebrates (Pittman et al., 2014).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2019.01.010.

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