Individual consistency in exploration and shyness but not activity in smooth newts (Lissotriton vulgaris): the effect of habituation?

P. Chajma[1] (iD), O. Kopecký[2] & J. Vojar[1]

1Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Praha, Czech Republic
2Department of Zoology and Fish Farming, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Praha, Czech Republic

Abstract

Behavioural consistency (i.e. personality) is an important aspect of behavioural ecology that has yet to be thoroughly examined in amphibians. Currently published studies often address only one or two aspects of personality, using many different methods for evaluating behavioural consistency. This is the first study focusing on all relevant behavioural traits and their relationships in urodele amphibians. Based on three trials within the experiment, we examined the consistency of activity (time spent moving), the boldness (latency of the first movement and time spent escaping) and the exploration (number of visited segments of the test arena) of 42 smooth newts (Lissotriton vulgaris). Individual consistency, calculated through the intraclass correlation coefficient (ICC), was low in newt activity (ICC = 0.192) and was moderate in boldness (0.476) and in exploration (0.403). Activity was moderately consistent for each trial (0.425), indicating possible habituation, supported by a decrease in mean activity throughout the trials. Newt activity, shyness and exploration were correlated throughout the experiment, suggesting the possible existence of a common selective pressure. With a summary of existing studies and their effect sizes, we aim to highlight the inconsistencies in the methods and in the results of these studies, to emphasize the need for a complex approach to the study of amphibian personality and the need for a standardized methodology, which would solve the current difficulties in comparing published results.

Introduction

Behavioural consistency (i.e. personality) is a well-known phenomenon that was studied in many taxa (e.g. Gosling, 2001; Sih *et al.*, 2004; Réale *et al.*, 2007; Garamszegi, Marko & Herczeg, 2013) including amphibians (e.g. Aragón, 2011; Koprivnikar, Gibson & Redfern, 2011; Maes, Van Damme & Matthysen, 2012; Wilson & Krause, 2012; Brodin *et al.*, 2013; Carlson & Langkilde, 2013, 2014a). Consistency in the expression of behavioural traits over time and in different situations, as well as the correlation of these traits, that is behavioural syndrome (Sih *et al.*, 2004), is often linked to survival in predator–prey situations (Dingemanse & de Goede, 2004; Carlson & Langkilde, 2014b) (but see Carlson & Langkilde, 2014a), reproductive success (Dingemanse & Réale, 2005; Cole & Quinn, 2014), disease risk (Koprivnikar *et al.*, 2011) and dispersal tendencies (Cote *et al.*, 2010, 2013; Gruber *et al.*, 2017a, 2017b; reviewed in Cayuela *et al.*, 2018). Consistent behaviour leads to consistent (dis)advantages in certain situations and, if heritable (if genetically determined), is subjected to selective pressure. Behaviour correlations can imply trade-offs that can result in maladaptive behaviour in

some contexts and can potentially maintain individual variation in behaviour in a variable environment (Sih *et al.*, 2004). Thus, animal personality plays an important part in individual life histories and should be inspected and carefully considered when dealing with most aspects of animal ecology.

Amphibian personality research, focused mostly on anurans (for a recent review, see Kelleher, Silla & Byrne, 2018), has various approaches to behavioural consistency. Consistency of the same behaviour across time (e.g. Maes *et al.*, 2012; Wilson & Krause, 2012; Brodin *et al.*, 2013; Carlson & Langkilde, 2013) is measured in a time frame ranging from four hours to nine weeks. Different contexts in which the consistency of the behaviour is being measured can mean many different things, starting from the introduction of different odours to the introduction of a novel object or parasite infestation (e.g. Sih *et al.*, 2003; Aragón, 2011; Koprivnikar *et al.*, 2011). Even the behavioural traits representing certain types of behaviour (axes of personality) and their level of consistency (effect sizes) vary considerably.

The aim of our study was to measure the temporal consistency of the most commonly examined behavioural traits—activity, exploration and boldness—in the smooth newt, a

common but surprisingly understudied urodele species. In addition, we wanted to assess the correlations between these behaviour traits and to see which behaviour traits, if any, might be shaped together. In particular, we were interested to find whether active individuals explored more, and how boldness/shyness interacted with these two traits. Additionally, to clearly show how the level of behavioural consistency in our study compares to existing amphibian personality research, without neglecting the differences in experimental approach mentioned above, we have summarized the most important findings in the Supplementary information section (Tables S1).

Materials and methods

Experimental design

The experiment was carried out in laboratory conditions at the Czech University of Life Sciences in Prague. We chose the urodele that was most abundant locally, the smooth newt (*Lissotriton vulgaris*). At the start of the reproductive season at the beginning of May 2017, 21 males and 21 females were captured using nets in a single pond in the village of Stará Lysá in the Central Bohemia region. The net catching was carried out manually, using a rapid, torrent-creating movement, and a sudden change of direction, to capture individuals that were swept by this torrent. This way of capturing, in our opinion, minimizes the advantages of certain personality types and reduces the risk of bias.

The newts were housed separately in plastic containers with dimensions of $18 \times 12 \times 14$ cm that were filled with aged tap water, and the newts were fed *Daphnia* and Chironomidae larvae *ad libitum*. The air temperature in the laboratory was constant and was set to 17°C. Sufficient light intensity in a diurnal cycle was provided by the translucent roof of the laboratory.

The experiment itself was conducted between May 13th and 27th in two experimental arenas made of non-transparent round green water barrels with bottom diameters of 80 cm. Using a non-toxic waterproof marker, a square grid of 7-cm segments was drawn at the bottom to better assess the position of each newt. The arena was filled with 5 cm of cold tap water (10.8–11.2°C). After each recording, the water was changed, and the arena was thoroughly cleaned with a clean sponge and pressurized water, and was then left to dry to eliminate any potential chemical cues that remained from the previous tested individual.

Each trial within the experiment was 12 min long. Behaviour was recorded at 25 frames per second with a full HD camera, positioned approximately 150 cm above the water level. Newts were separately inserted under the transparent glass dome (10 cm diameter) into the centre of the arena and were left to acclimatize for the first two minutes. Then, the dome was carefully removed in a motion perpendicular to the ground, and the recording was initiated. To measure the temporal repeatability of the behaviour, each individual was recorded three times with a six-day gap between each recording. This was the longest gap possible before the newts started to shift to the terrestrial phase of the season,

substantially changing their behaviour, and becoming unwilling to stay in the water for long periods of time. In total, we tested 39 newts in the behavioural assay three times and 3 newts twice (due to a loss of data as a result of technical difficulties).

Three types of behaviour (personality traits) were tracked: activity, exploration and boldness. Activity was measured as the amount of time [s] during which the individual moved. In addition, the movement activity was divided into walking and swimming, in order to distinguish the role of each in the total activity and the consistency of each activity, and also to determine the consistency of the choice of locomotion (i.e. the proportion of the activity that consisted of walking). Exploration was recorded as the number of grid blocks that an individual entered, not including blocks that had already been visited. For the sake of better comparison with other studies, boldness/shyness was measured as the latency of the first movement [s] (the most common but imprecise measure of boldness, see Discussion) and also the time [s] spent at the outermost edge of the arena (our preferred measure). Staying in the vicinity of the edge of the arena (thigmotaxis) can be interpreted as an escape response and can therefore be a valuable measure of shyness (Burns, 2008; Harris, D'Eath & Healy, 2009; Carlson & Langkilde, 2013). Behaviour was scored manually by the same person, using Observer XT v. 10 software (Noldus, 2010). The study was carried out in accordance with permit SZ-092744/2012KUSK/3, issued by the Regional Office of the Central Bohemian Region of the Czech Republic and approved by an institutional committee based on institutional accreditation No. 63479/2016-MZE-17214 of the Ministry of Agriculture of the Czech Republic.

Data analysis

To test the differences in activity, time spent walking, swimming, number of visited squares (exploration), latency of the first movement (boldness) and time spent near the outermost edge of the arena (shyness) between trials and sexes (independent variables), we created separate linear mixed effects models (LMM) for each of the characteristics (dependent variable) fitted by the restricted maximum likelihood (REML), with the individual (1–42) as a random intercept. Apart from the variables mentioned above, we also tested the dependency of the proportion of walking activity (i.e. time spent walking divided by time spent active) of each newt on the same fixed (trial and sex) and random effects (individual). This was done to assess whether the preferred type of locomotion differed between sexes and between trials. Although proportions were being modelled, this model reasonably met the assumptions for LMM.

Each model was also tested for the effect of the time of day at which the experimental trial took place. Because the dependency on time is rarely linear, we decomposed this variable to the sine and cosine of the time in radians, in order to take into account the periodic nature of the variable. When all underlying assumptions had been considered, the models were evaluated using Type III F-tests. Neither the time of day nor the sex of the newts affected any of the tested variables (see

Supplementary information Tables S1-S9 for details), and they were therefore not included in the repeatability analyses.

Individual consistency (repeatability) in the measured traits (dependent variables from previous models) was calculated using the intraclass correlation coefficient (ICC), computed from the variance components (available in Supplementary information section Tables S1-S9) of models like those discussed above, but with no fixed effects and with the trial number as a second random variable. Note that adding the trial number as a random intercept allowed us to estimate its consistency, that is the between-subject similarity in the expression of measured traits during each trial within the experiment and to estimate the individual repeatability while accounting for the effect of trial order.

Confidence intervals (CI) for the ICC were estimated by parametric bootstrapping with 1000 iterations (for details, see Nakagawa & Schielzeth, 2010). The confidence interval for the proportion of walking activity was estimated from the fixed intercept of the linear mixed effects model, with the individual and the trial as random intercepts, using the profile likelihood method.

The correlation of behavioural traits was tested using Kendall's coefficient of concordance, because unlike ICC, it relies on the number of concordant groups of measurements and is not influenced by the differences in the means of the selected behavioural responses. In order to reduce the potential dependence caused by the simultaneous scoring of all behavioural responses in the same assay, each trial was split into three equally long sections, and one of the traits (activity, shyness, exploration) was randomly assigned to each section. This was performed 500 times, and much like in non-parametric bootstrapping, the 95% confidence interval of the coefficient of concordance was constructed from the 2.5% and 97.5% quantile of its empirical distribution. To avoid pseudoreplication, repeated measurements of individuals were averaged. Pairwise similarities were analysed using Pearson's correlation coefficient. All statistical analyses were performed in R 3.5.1 (R Core Team, 2018) using *lme4* (Bates *et al.*, 2015), *car* (Fox & Weisberg, 2011) and *rptR* (Stoffel, Nakagawa & Schielzeth, 2017) packages at the level of significance $\alpha = 0.05$.

Results

Activity

The individual repeatability of activity was significant, but lower than the individual repeatability of both of its parts – walking and swimming (Table 1A). Walking was the most frequent type of locomotion, taking up 71% of the time spent moving (CI = [0.583, 0.840]). The individual choice of locomotion (i.e. the proportion of activity classified as walking) was also moderately consistent (Table 1A).

The mean activity differed significantly between trials (for details, see Supplementary information Tables S1-S9). The initial mean activity of 309.9 s decreased by 32% in the second trial and by 20% in the third trial, and was consistent throughout each trial (Table 1B), meaning that the change in activity was similar for each newt (see Figure 1).

Both the amount of walking ($P < 0.01$) and the amount of swimming ($P < 0.001$) fell significantly between the trials within the experiment. The proportion of activity classified as walking, however, increased only slightly during the second and third trials ($P = 0.05$). Unlike general activity, however, walking, swimming and also the proportion of walking activity were not repeatable for each trial (i.e. the trial number did not contribute to the differences in these measurements, for details, see Table 1B).

Exploration

We observed two different exploration patterns. Most of the newts started the trial with a quick escape response and then began by exploring the outer ring of the arena, rarely exploring the inner parts. The squares were usually visited only briefly and in rapid succession. A smaller group was startled at first and then explored the inner parts of the arena, eventually reaching the outer ring, and they stayed in the same square for a longer period of time. Individual exploration was significantly repeatable with a moderate ICC (0.403). As in the case of activity, there was a significant difference in mean exploration between each of the trials within the experiment ($P = 0.01$, see Supplementary information Tables S1-S9). The initial mean of 27.8 explored squares decreased by 2.5% in the second trial and by 18% in the third trial. Unlike in the case of activity, the general decrease in exploration was not consistent among individuals from the same trial (see Table 1B).

Boldness

Boldness was measured as the latency to move and the time spent with an escape response. These two measures differed in individual repeatability (latency to move: ICC = 0.121; time spent escaping: ICC = 0.476), which seems to be a relatively common finding for other studies as well (see Tables S1). There was no difference in the boldness values between the trials within the experiment. However, it should be noted that the time spent escaping was only marginally insignificant ($P = 0.07$, see Supplementary information Tables S1-S9). The initial mean time spent with an escape response of 226.9 s decreased by 5.5% in the second trial and then rose by 30% in the third trial. The repeatability of movement latency and time spent escaping was not significant for the trials within the experiment (see Table 1B).

Correlated behaviour

The similarity in mean activity, exploration and time spent escaping (shyness) of individuals was relatively high (*Kendall's* $W = 0.647$, CI = [0.55, 0.72]). Pairwise correlations showed a moderate positive relationship between activity and time spent escaping ($r = 0.542$, CI = [0.39, 0.69]), and no correlation between activity and exploration ($r = 0.259$, CI = [−0.03, 0.50]) and between time spent escaping and exploration ($r = 0.240$, CI = [−0.02, 0.48]).

Table 1 Intraclass correlation coefficients (ICC) and confidence intervals (CI) for repeatability of behavioural traits in the smooth newt

A. Repeatability of individual behaviour between the trials of experiment			B. Repeatability of behaviour demonstrated by different individuals in the same trial of the experiment		
Variable	ICC	CI	Variable	ICC	CI
Activity	0.192[a]	[0.06, 0.42]	Activity	0.416[a]	[0.01, 0.73]
Walking	0.254[a]	[0.05, 0.45]	Walking	0.058	[0, 0.22]
Swimming	0.385[a]	[0.18, 0.57]	Swimming	0.156	[0, 0.42]
Walking proportion	0.373[a]	[0.17, 0.55]	Walking proportion	0.046	[0, 0.18]
Squares explored	0.403[a]	[0.2, 0.59]	Squares explored	0.051	[0, 0.18]
Latency of the first movement	0.121	[0, 0.32]	Latency of the first movement	0	[0, 0.05]
Time spent escaping	0.476[a]	[0.27, 0.64]	Time spent escaping	0.022	[0, 0.12]

Activity – time spent moving.
Walking – time spent walking.
Swimming – time spent swimming.
Walking proportion – proportion of activity spent walking.
Squares explored – number of visited squares.
Latency of the first movement – time spent idle, before the first movement.
Time spent escaping – time spent in the outermost part of the test arena.
ICC – intraclass correlation coefficient.
CI – 95% confidence intervals.
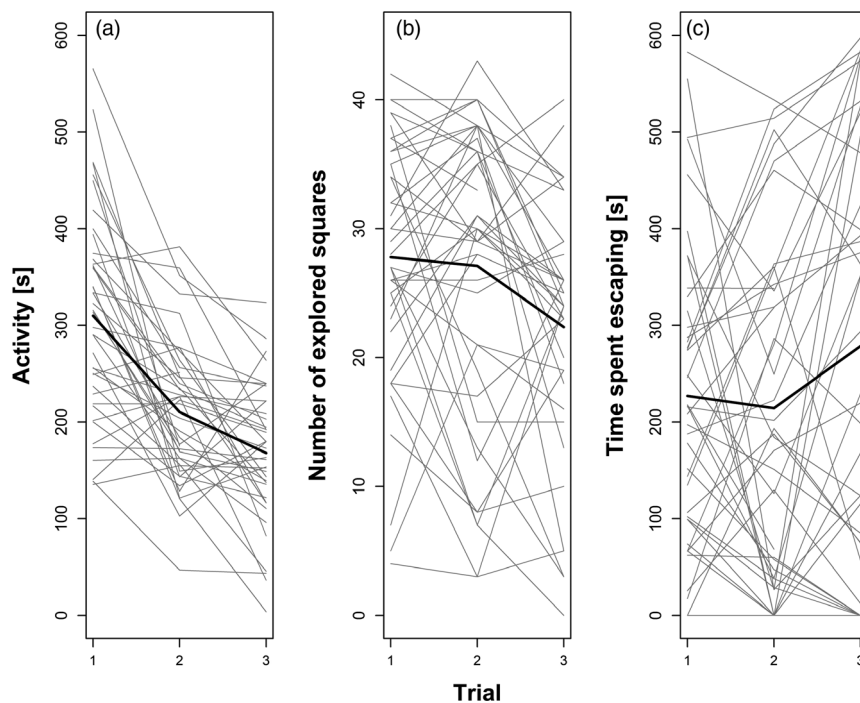[a]The estimate is significantly different from 0.

Figure 1 Individual (grey lines) and mean (bold line) responses of activity (a), exploration (b) and shyness (c) of smooth newts during three consecutive trials of the experiment. (a) – activity between trials, (b) – exploration between trials, (c) – escape response between trials.

Discussion

The observed behavioural traits of the newts in our study were moderately individually consistent for swimming activity, proportion of walking activity, exploration and escape response (thigmotaxis). They were weakly consistent for general activity and walking activity, and inconsistent for the latency of the first movement. Activity was moderately consistent for each trial, meaning that it decreased for all newts similarly between each of the trials within the experiment. The behaviour responses also did not differ between the sexes and were unaffected by the time of day when the experiment started.

Individual consistency in activity

The measures of activity presented in our study were all individually consistent. Slight differences in the repeatability estimates of walking and swimming might be more prominent with a larger sample, but in our case, they were well within the estimated confidence intervals. The division of activity into walking and swimming might be important for species where swimming is closely related to the escape response. Thus, the substitution of general activity with walking activity might separate it from a possible inherent correlation with shyness. The choice of the type of locomotion was also individually consistent, as shown by the individual consistency of the proportion of activity spent walking (walking proportion). Therefore, the consistency of the choice of locomotion might be a valuable indicator of individuality in smooth newts.

Consistency of activity did not depend on the sex of the newts, as opposed to the only other study that has dealt with the same question in urodele amphibians. Aragón (2011) reported stronger consistency for male newts, but weaker consistency for females. He tested this consistency in the context of different odours, so his results may reflect a sex-specific response to odours (his treatments) that does not translate into the temporal consistency of the behaviour when no odours are presented.

Taking into account all of the reviewed studies for both anuran amphibians and urodele amphibians, the repeatability of the activity was affected by odour treatments (Urszán *et al.*, 2015a), arena structure (Smith & Doupnik, 2005), by breeding origin – wild or captive (Maes *et al.*, 2012), by sex (Aragón, 2011, but not in our study), by previous experience (Urszán *et al.*, 2015b), by methods of measurement (Videlier *et al.*, 2014; Kelleher *et al.*, 2017) and slightly by age (Urszán *et al.*, 2015b) (see Tables S1). These are all factors that should be kept constant or should be taken into account when comparing multiple studies, populations or species. A less important factor (i.e. one that did not seem to affect the repeatability of the activity) was the time gap between repeated measurements (Maes *et al.*, 2012). In addition, the overall repeatability of the activity of larval and post-metamorphic amphibians did not differ much (Tables S1). However, none of the studies compared multiple life stages of the same individuals, probably due to the vast differences in the locomotor abilities of larval and post-metamorphic anurans.

Individual consistency in exploration

The number of squares explored by newts in our study was individually consistent. Consistency in exploration is often linked to all three stages of animal dispersal (departure, transience and settlements; for details see Cote *et al.*, 2010 or Cayuela *et al.*, 2018). For example, natal dispersal distance was found to be positively related to exploratory behaviour (Dingemanse *et al.*, 2003). Dispersal distance in natural streams was found to be positively related to the exploration score in an unfamiliar habitat (Fraser *et al.*, 2001). Immigrants were found to be faster explorers than locally born individuals

(Dingemanse *et al.*, 2003), and individuals from island populations were found to have higher exploration than individuals from mainland populations (Brodin *et al.*, 2013). Consistent explorative behaviour in parents was also found to positively affect the explorative behaviour in offspring (Dingemanse *et al.*, 2003). While all these findings suggest that consistency of exploration might play an important role in smooth newt's ecology, they have yet to be tested on this species.

The only other study that describes consistency of exploration (but not other personality traits) in urodele amphibians shows a similar level of correlation (Gifford *et al.*, 2014). The consistency, however, decreased with the time between repeated measurements (see Tables S1). Other factors that can potentially affect the repeatability of exploration are age and experience (Urszán *et al.*, 2015b). However, the differences in consistency between the stages of development are not conclusive (Table S1).

The greatest drawback to a comparison of the published results is the difference in the definition of exploration. Exploration has been defined as a buffer around the trajectory of the individual (Brodin *et al.*, 2013), the number of visited squares (our study; Carlson & Langkilde, 2013; Gifford *et al.*, 2014) or the percentage of visited squares (Urszán *et al.*, 2015b). Kelleher *et al.* (2017) even used three definitions – distance travelled, time spent mobile and number of mobile events – each of which showed different repeatability (Tables S1). The strength of the correlation (repeatability) is usually used as the main indicator of the suitability of a certain measure as the best representative of exploration behaviour. However, the research would benefit above all from standardized approaches that would enable the studies to be compared effectively (Roche, Careau & Binning, 2016).

Individual consistency in boldness

In our study, repeatability of boldness was observed only for the escape response, which is a similar measure to that of Carlson & Langkilde (2013), who counted the number of square sides on the inner portion of the test arena crossed by an individual. This is also a measure of thigmotaxis. The accuracy of the latency of the first movement as a measure of boldness was impaired for our study by the inability to raise the glass dome in the centre of the arena consistently at the start of the experiment. We suspect that this has also been an issue in other studies, which makes the method impractical. This problem can be mitigated by changing the first movement to a movement longer than one body length (Wilson & Krause, 2012). However, in our opinion, it is better to choose a completely different option, for example shelter use, escape initiation distance or thigmotaxis.

Boldness has often been linked to predator-induced selection. Contrary to the common belief that more exposed (bolder) individuals have higher probability of being depredated (e.g. Brydges *et al.*, 2008; Smee & Weissburg, 2008), Carlson & Langkilde (2014a) found no dependence of tadpole survival on boldness, and Sih *et al.* (2003) even found a negative relationship between boldness and predator pressure for

urodele amphibians. They attribute their results to the existence of behavioural syndromes that dilute the significance of predator pressure by other selective pressures.

Sih *et al.*, (2003) reported consistency of boldness for *Ambystoma barbouri* and *A. texanum* similar to our study, using shelter use as the preferred measure. Our investigation of other studies has shown that the consistency of boldness varied greatly (Tables S1). The variation was caused by previous experience (Urszán *et al.*, 2015a, 2015b) and by the breeding origin (Maes *et al.*, 2012) of the study subjects, and probably by differences in the definition of boldness (supported by Kelleher *et al.*, 2017). There does not seem to be any difference between larval and post-metamorphic amphibians, or between different time gaps, in the repeatability measurements (see Tables S1).

Trial repeatability

Activity and exploration decreased significantly with each trial in the experiment, which suggests that habituation may have taken place (but see Carlson & Langkilde, 2013). Habituation involves learning to disregard stimuli that are without significance to an animal, that is neutral stimuli that are not associated either with punishment or with reward (Cloninger, 1994). That being said, the difference in the mean, by itself, does not necessarily indicate that habituation took place. For this reason, it is important to show that the difference happened overall, that is to report the consistency of the expression of behavioural traits for each trial within the experiment (Roche *et al.*, 2016).

The decrease in the expression of behaviour traits could have been caused by insufficient time between the trials within the experiment, allowing the newts to remember the previous trial. The multisensory orientation system (see Sinsch, 2006) could then have allowed the newts to recognize the experimental arena and to adjust their behaviour across the trials. The speed of habituation and the habituation recovery time are unknown for the studied species. For the common toad, however, Ewert & Kehl (1978) stated that 6–24 h is long enough for recovery from habituation to an artificial rectangular-shaped prey dummy. Unfortunately, it was not possible to allow more time between the trials, because we feared that the newts might switch to the terrestrial phase and change their behaviour. The repeatability of behaviour traits does not seem to have changed with the amount of time between the trials in other studies (Tables S1), but it would be beneficial to test this relationship specifically through a single manipulative experiment.

With the exception of activity, no behavioural trait was repeatable for each trial within the experiment. This might mean that the extent of habituation varied individually, that is individuality was stronger than habituation (see Fig. 1), or that habituation was not present for the behavioural traits in question.

Correlated behaviour

Activity, shyness and exploration were positively correlated for our study. Unfortunately, the interpretation of the correlation of behavioural traits that were measured in the same behavioural assay is complicated, because the same movement can be classified at the same time both as general activity and as exploration or boldness. This causes an inherent correlation of unknown magnitude that we weakened by using randomly chosen non-overlapping parts of the assay.

A positive correlation of some of the measured traits has, however, already been found in a couple of previously published studies. Activity has been found to correlate with exploration (Koprivnikar *et al.*, 2011; Maes *et al.*, 2012; Wilson & Krause, 2012) and with boldness (Maes *et al.*, 2012; Wilson & Krause, 2012; Urszán *et al.*, 2015a). Boldness has also been found to correlate with exploration (Maes *et al.*, 2012) (but see Brodin *et al.*, 2013), and also with sociability (González-Bernal, Brown & Shine, 2014). No positive correlation of activity, exploration and boldness has, however, ever been found to occur at the same time, mostly because it has not been thoroughly tested (but see Maes *et al.*, 2012).

Active individuals from our study tended to explore more and were less bold, spending more time escaping. Although counter-intuitive at first sight, the negative correlation of boldness and exploration can be explained by differentiation of the exploratory behaviour. Verbeek, Drent & Wiepkema (1994) described two consistent types of explorers in juvenile great tits – slow explorers, which tended to explore slowly and to stay longer in each visited place, and fast explorers, which explored faster and did not spend much time in one place. In total, fast explorers were able to make more visits within the 10-min trial time. In our case, we discovered a similar pattern in exploring, too. As most of the tested newts could be classified as fast explorers, the positive correlation between time spent with an escape response (shyness) and exploration may be an artefact of this situation.

More time spent near the edge of the arena could also be caused by a size constraint of the arena, that is by the tendency of fast explorers to explore more, but to be restricted by the wall in front of them. This would also render the measure unsuitable for representing boldness, as it would automatically also contain a lot of information about exploration. Another cause of the correlation between shyness and exploration might be a common selective pressure that favours fast explorers, caused by increased predation or reduced mate availability – an increase in locomotor activity has been found to provide a benefit in mate searching (Martin, Joly & Bovet, 1989). Furthermore, an increase in mate searching ability might also result in a positive correlation between exploration and sociability, which was unfortunately not measured in the study. The absence of behavioural differences between the sexes might indicate that this pressure is beneficial for both males and females, or is at least not harmful for either. Whatever the cause, correlated behaviours should not be studied in isolation, because they develop as a group (Sih *et al.*, 2003, 2004). Additionally, they should be tested in a different study design, to see whether the correlations persist in different ecological contexts.

In conclusion, amphibian (especially urodele) personality research is still sparse, and the findings differ considerably both in approach and in results. Behavioural consistency is often studied for one or two types of behaviour only, in study-specific conditions, and behavioural correlations are sometimes neglected. The differences in the means of the expressed traits

are never accompanied by consistency of the behaviour for each experimental trial and are therefore unsuitable as indicators of the habituation process. We believe that there is a need for a more complex approach (measuring more types of behaviour) and a standardized methodology (i.e. definition of behaviour types, correlation in time and different contexts, a standard time gap between repeated measurements, number of repeated measurements, duration of the experiment and sampling effort, and the shape and size of the test arena). It would only then be possible to make general assumptions on the global nature and consequences of the phenomena investigated here.

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Conflict of interest

The author(s) declare no competing interests.

Author contributions

O. Kopecký captured and cared for the newts, and together with P. Chajma participated in carrying out the experiment. P. Chajma analysed the data, prepared all figures and tables and, together with J. Vojar, wrote the main manuscript. All authors have reviewed the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Summary of amphibian personality studies' effect sizes.

Table S2. The influence of sex, time and trial order on general newt activity.

Table S3. The influence of sex, time and trial order on newt walking activity.

Table S4. The influence of sex, time and trial order on newt swimming activity.

Table S5. The influence of sex, time and trial order on the proportion of walking.The influence of sex, time and trial order on the proportion of walking.

Table S6. The influence of sex, time and trial order on newt exploration.

Table S7. The influence of sex, time and trial order on the latency of the first movement.

Table S8. The influence of sex, time and trial order on thigmotaxis.

Table S9. Variance components of repeatability estimates.