

Behavioural consistency and life history of *Rana dalmatina* tadpoles

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Abstract The focus of evolutionary behavioural ecologists has recently turned towards understanding the causes and consequences of behavioural consistency, manifesting either as animal personality (consistency in a single behaviour) or behavioural syndrome (consistency across more behaviours). Behavioural type (mean individual behaviour) has been linked to life-history strategies, leading to the emergence of the integrated pace-of-life syndrome (POLS) theory. Using *Rana dalmatina* tadpoles as models, we tested if behavioural consistency and POLS could be detected during the early ontogenesis of this amphibian. We targeted two ontogenetic stages and measured activity, exploration and risk-taking in a common garden experiment, assessing both individual behavioural type and intra-individual behavioural variation. We observed that activity

was consistent in all tadpoles, exploration only became consistent with advancing age and risk-taking only became consistent in tadpoles that had been tested, and thus disturbed, earlier. Only previously tested tadpoles showed trends indicative of behavioural syndromes. We found an activity—age at metamorphosis POLS in the previously untested tadpoles irrespective of age. Relative growth rate correlated positively with the intra-individual variation of activity of the previously untested older tadpoles. In previously tested older tadpoles, intra-individual variation of exploration correlated negatively and intra-individual variation of risk-taking correlated positively with relative growth rate. We provide evidence for behavioural consistency and POLS in predator- and conspecific-naive tadpoles. Intra-individual behavioural variation was also correlated to life history, suggesting its relevance for the POLS theory. The strong effect of moderate disturbance related to standard behavioural testing on later behaviour draws attention to the pitfalls embedded in repeated testing.

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Introduction

One of the more recent goals of evolutionary behavioural ecology is to understand the proximate and ultimate mechanisms resulting in individual behavioural consistency. The term “behavioural consistency” commonly refers to individual consistency that results in systematic differences between individuals in terms of their mean behaviour (Sih et al. 2004, 2012; Bell 2007; Kortet et al. 2010; Wolf and Weissing 2012). Animal personality, behavioural syndrome,

Box 1 Definitions of the key terms used in the current study

Term	Definition
Animal personality	Consistent between-individual differences in a single behaviour
Behavioural syndrome	Consistent between-individual differences across functionally different behaviours
Behavioural type	The mean behaviour of an individual
Individual behavioural plasticity	Individual behavioural variation induced by environmental change
Intra-individual behavioural variation	Individual behavioural variation unrelated to the environment, i.e. the precision of the expression of the behavioural type
Pace-of-life syndrome (POLS)	Consistent individual differences across behavioural, physiological and life-history traits

temperament, among others, are often used interchangeably as synonyms in the behavioural consistency literature. However, it has been suggested that animal personality and behavioural syndromes should refer to different patterns in order to achieve consistency in experimental design and analysis (Garamszegi and Herczeg 2012; Jandt et al. 2014), and we use this separation of terms in the study reported here. The terminology used in the behavioural consistency field can be confusing. Thus, for clarity, we provide definitions of the key terms used in this study in Box 1.

Researchers usually test first for the presence of personality (repeatability of single behaviours) and behavioural syndromes (correlations between repeatable behaviours) in a group of individuals. Upon proving the presence of personality or a syndrome, they then focus on the analysis of the individual behavioural types (mean behaviour) observed in the studied populations or species. However, this approach is problematic as it totally ignores intra-individual variation in behaviour; consequently, it does not take into account an important component of individual behaviour. Recently developed approaches to quantify intra-individual behavioural variation in both animal personality and behavioural syndromes allow this issue to be circumvented (Herczeg and Garamszegi 2012; Stamps et al. 2012; Dingemans and Dochtermann 2013). By adopting these approaches, it is possible to characterize an individual simultaneously by its behavioural type and behavioural variation, thereby incorporating two potentially independent aspects of its behaviour. Whenever environmentally induced behavioural shifts (individual behavioural plasticity; Dingemans et al. 2010) are controlled for, and measurement error is distributed evenly among the studied individuals, the remaining differences in behavioural variation should represent the precision how individuals express their behavioural type. Hereafter, we use the term “intra-individual behavioural variation” to describe this behavioural component, following Stamps et al. (2012).

Studies on animal personalities and behavioural syndromes are being published at a fast pace (e.g. Smith and Blumstein 2008; Bell et al. 2009; Garamszegi et al. 2012, 2013). Réale et al. (2010) integrated behaviour in

pace-of-life syndromes (POLS) describing life-history strategies along a fast–slow lifestyle continuum, with the aim to explain the adaptive nature of behavioural consistency. According to the POLS hypothesis, behavioural types may associate with life-history characteristics—as certain combinations of life history and behaviour can be more adaptive in specific situations than others—but various combinations may eventually yield equal expected life-time fitness in a heterogeneous environment (Biro et al. 2006; Réale et al. 2010; Wolf and Weissing 2010). In this view, behavioural consistency arises from a variation of life-history strategies between individuals in a population (Stamps 2007; Wolf et al. 2007; Careau and Garland 2012). Individuals following the fast POLS strategy are expected to be more active, more prone to taking risks, more aggressive and more likely to explore more superficially. They are also expected to mature earlier and have a faster metabolism and a weaker immune system (i.e. “live fast, die young”). In comparison, slow POLS individuals are expected to avoid risks, be less active, thoroughly explore situations and be less aggressive, while having a longer life span, longer developmental time and more efficient immune responses (Réale et al. 2010).

Even though the POLS hypothesis seems logical, only a few studies have actually investigated it, with mixed results. For example, Careau et al. (2011) provided support for the hypothesis by observing a link between exploration and metabolic rate in deer mice (*Peromyscus maniculatus*). In field crickets (*Gryllus integer*), Niemelä et al. (2012a) reported a boldness–immune response correlation which supported the POLS hypothesis, but these authors found no link between boldness and timing of maturation. David et al. (2012) found a connection between feeding motivation and the degree of proactivity in zebra finches (*Taeniopygia guttata*), supporting POLS. In their study on brown trout (*Salmo trutta*), Adriaenssens and Johnsson (2013) found a positive aggression–mortality correlation which supported POLS and a negative activity–mortality correlation which contradicted it. It should also be noted here that many of the predicted POLS associations have not been detected even in the most supportive studies. Further, it

is possible that not only behavioural types, but also intra-individual behavioural variation is also included in POLS. Considering that the fast POLS relies on fast growth and early reproduction, and that a fixed behavioural strategy is less energy demanding (no need for costly cognitive abilities; Coppens et al. 2010; Niemelä et al. 2012b), we hypothesize that fast-paced individuals that perform better in predictable, stable environments are characterized by low intra-individual behavioural variation.

Environmental effects are complicating factors when the aim is to draw evolutionary conclusions from phenotypic data collected in the wild (e.g. Kuparinen and Merilä 2007; Gienapp et al. 2008; Teplitsky et al. 2008; Merilä 2009). Accordingly, several studies have emphasized the importance of experience during early ontogeny on personality expressed later in life (Dingemanse et al. 2009; Rodel and Monclus 2011; Butler et al. 2012). Further, if the influence of early experience is manifested in multiple traits, it cannot only affect the mean expression of these traits, but also their correlations. Therefore, exposure to different environmental factors during the early phase of life can have consequences for behavioural syndromes and POLS measured at a later phase. Such environmental effects can stem from experimental manipulations. For example, when behaviour is tested multiple times throughout ontogeny, tests and handling, including novel stimuli or stress, can directly alter the later behaviour of the same individual. This potential confounding effect has rarely been addressed experimentally (but see Ruiz-Gomez et al. 2008; Stamps and Groothuis 2010).

The primary goal of our study was to test for behavioural consistency and POLS at different ontogenetic stages using agile frog (*Rana dalmatina*) tadpoles as a model. Amphibian larvae in general are excellent candidates for studies on behavioural consistency (Sih et al. 2003; Wilson and Krause 2012). A secondary aim was to test whether the disturbance connected to standard behavioural testing affected behaviour later during ontogenesis. To ensure a complete coverage of individual behavioural variation, we focused not only on behavioural type, but also on intra-individual behavioural variation. We reared *R. dalmatina* tadpoles individually in a standardized common garden experiment, providing food ad libitum. This approach excluded the effects of previous experience with predators or conspecifics, as well as energetic constraints on the behaviour and life-history characteristics of the focal tadpoles. Therefore, as environmental variation was negligible and no systematic variation in measurement error could be expected, the behavioural variation expressed by an individual would represent intra-individual behavioural variation. Half of the tadpoles were tested at two ontogenetic stages, while the other half only at the later stage. In particular, we tested for (1) presence of animal personality

and behavioural syndromes at different ontogenetic stages of *R. dalmatina*, (2) correlations of individual behavioural type or intra-individual behavioural variation with age and size at metamorphosis and (3) an effect of experimental manipulations, including stress, on later behavioural consistency and POLS.

Materials and methods

Field sampling and rearing

We collected *R. dalmatina* eggs from a pond on the Island of Szentendre, near Szigetmonostor (47°40'40.77"N, 19°5'31.47"E) where both invertebrate and vertebrate aquatic predators are present (e.g. Aeshnid dragonfly larvae, Dytiscid water beetle larvae, different fishes). We sampled 80 freshly laid clutches between 17 and 20 March 2011, collecting 30 randomly selected eggs from each clutch and placing them in separate plastic containers (volume 8 l; dimensions 34 × 23 × 16 cm) holding 2 l of reconstituted soft water (RSW; American Public Health Association 1985) at 19 °C and a 12:12 light:dark photoperiod. Another ten eggs were randomly collected from each clutch and photographed (model s7000 digital camera; Fujitsu Ltd., Tokyo, Japan; pictures taken from a standard distance and angle using a size standard for each image). The mean egg diameter per clutch was determined (egg diameter was measured using the free imaging and processing software program UTHSCSA ImageTool v. 3.0; <http://compdent.uthscsa.edu/dig/itdesc.html>) as a proxy for maternal investment (Laugen et al. 2002).

After hatching, one randomly selected healthy tadpole was left in each rearing container. Hence, the experimental setup consisted of 80 containers, each containing a single tadpole from a different clutch. In this way, we could maximize the genetic variation between the studied individuals, achieving a good representation of the original population. We should note that the analysis of full-sib families collected in the wild would have not been useful in terms of drawing quantitative genetic inferences, so we chose to maximize the number of families included by not including within-family replicates in the experiment. The remaining tadpoles were released at the original collection site. Experimental tadpoles were fed chopped and slightly boiled spinach ad libitum, with food provided again 2 h before the end of the daily light period. Water was changed every 4 days. Everything that came in contact with the tadpoles was thoroughly rinsed beforehand to ensure that all individuals remained naïve regarding the presence of conspecifics. Each of the containers was placed inside white polystyrene cells to facilitate the recording of movements and to prevent visual contact between adjacent cells.

The development of each individual tadpole was observed and recorded on daily basis. We were particularly interested in stage 32–36 (Gosner 1960: early stages of toe development) when we performed the second round of behavioural assays (the first assays were conducted based on tadpole age; see text below) and in stage 42 (emergence of forelimbs) when we evaluated age and mass at metamorphosis. When a tadpole approached stage 42, monitoring was changed to 2-h intervals in order to record age and mass at metamorphosis with a high accuracy. We randomly assigned tadpoles to two groups. The first group's behaviour was assessed on two occasions, first at the age of 11 days (after the onset of the free swimming stage; hereafter "11-day-old") and second at stage 32–36 (hereafter "pre-tested stage 32–36"). The second group's behaviour was only assessed at stage 32–36 (hereafter "naïve stage 32–36"). In this way, we were able to evaluate the effect of behavioural measurements performed 11 days after hatching on the behaviour at stage 32–36—i.e. we could evaluate tadpole behaviour at stage 32–36 independently of the potential effects of previous behavioural tests. Taken together, we measured behaviour at two ontogenetic stages and recorded age and mass at metamorphosis in a third ontogenetic stage.

Behavioural assays

We assessed three different behaviours (following Réale et al. 2007; Garamszegi et al. 2013): activity, novel area exploration and risk-taking. One measurement period lasted for 3 days, during which time all three behaviours were assessed for each individual separately on a daily basis. Our first step was to measure activity (movement rate in a familiar environment) as this behaviour could be estimated without disturbance; this was followed by measuring exploration and risk-taking in random order, as the latter two behaviours are invasive processes that include handling and novel stimuli (see following test, for details). Between the two invasive behavioural tests, we allowed the tadpoles to rest in their respective rearing container (familiar environment) for at least 2 h. We recorded the tadpoles' behaviour with webcams using the open source Dorgem software (Frank Fesevur: <http://dorgem.sourceforge.net/>). Upon the completion of all tests, the tadpoles were released back to their pond of origin.

Activity Activity in a familiar environment was measured in the rearing container without disturbing the tadpole, 2 h after the beginning of the light period. Activity recordings lasted for 30 min, resulting in approximately 1,800 images (sampling time 1 frame/s). Activity (movement frequency) was measured by dividing the number of images showing changes in the position of the sampled individual compared to the previous image by the total number of images.

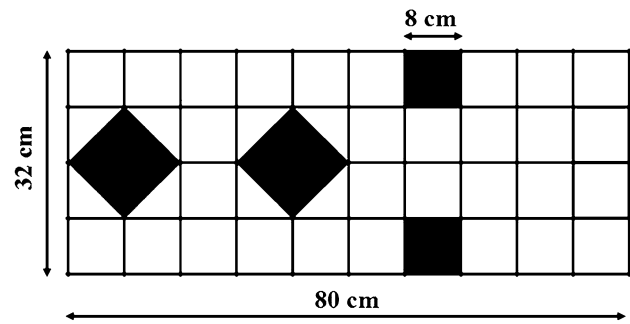


Fig. 1 Experimental setup to study novel area exploration. The arena dimensions were: 80 × 32 × 18 cm (length × width × height). Grey squares represent the fenced starting area, black areas represent the obstacles (boxes filled with gravel), while the grid was used to quantify movements

Exploration Exploration in a novel environment was recorded in four 36-l plastic containers (for details, see Fig. 1). These containers had opaque grey walls and their bottom was divided into 40 equal-sized rectangles. In each container, there were four smaller containers filled with gravel, which functioned as obstacles, placed in a way to prevent an overview of the whole area. For each trial, we filled the arenas with 4 l of RSW and placed the sampled tadpole behind a three-sided veil. After 5 min of acclimation, we lifted the veil and recorded the individual's movements for 25 min. Exploration was quantified as the number of rectangles visited at least once divided by the number of available rectangles. We deemed a rectangle to be visited if an individual crossed the line separating two adjacent rectangles at least with its full body without the tail. Containers were thoroughly washed between trials.

Risk-taking Risk-taking was measured in the rearing containers by using a threat stimulus. In this experiment, we used a 55-cm-long plastic tube with handles mounted on the sides, inside of which a metallic rod was suspended (length 11 cm, width 6 mm). The metallic rod could be released so that it fell through the plastic tube but come to a halt 11 cm below the lower opening of the tube. We provided a threat stimulus by placing this device over the container of the tadpole and letting the rod fall next to the focal tadpole. This activity always carried out by the same investigator (TJU). Aiming the device was done by eye. We could not fully exclude variation in the distance between the tadpole and the threat stimulus but are confident that any bias introduced by this variation was minor and randomly distributed among all test animals. Tadpoles responded to the stimulus by quickly swimming away and freezing (immobility). Their behaviour was recorded for 15 min after the threat stimulus. To quantify risk-taking,

we measured the latency to restart activity, which included the time spent swimming away and the time spent freezing, with the former typically lasting only for a few seconds. If an individual remained inactive for >15 min, we stopped the observation and assigned the maximum score (900 s) to the individual.

Statistical analyses

We only included individuals in the analyses that had reached Gosner stage 42 and for which we had complete behavioural data. Consequently, 13 individuals were excluded from the analysis because behavioural data were lost due to camera malfunction, 12 individuals were excluded due to abnormal development, 5 individuals became stressed/injured during handling (by, for example, jumping out of the holding net) and ten individuals died from unknown reasons. From the remaining individuals, 19 were in the group that was assessed twice during ontogeny, providing the 11-day-old and pre-tested stage 32–36 data, and 21 individuals were in the group that was assessed only once at the later developmental stage, providing the naïve stage 32–36 data. In our analyses, we treated the three data batches separately because of the imbalanced design and the different patterns regarding the presence/absence of personalities and syndromes between the batches (see “Results”).

To assess if tadpoles exhibited personality, we estimated the repeatability of the different behaviours by comparing the between-individual component of variation to the total variation based on the three measurements of every individual. We used an analysis of variance-based approach following Becker (1985), which generally gives a reliable estimate (Nakagawa and Schielzeth 2010). To calculate repeatabilities, we also ran general linear mixed models (GLMMs) with individual as the random factor and the behavioural variable of interest as the dependent variable, obtained almost identical repeatability estimates (data not shown). To test directly whether behavioural consistency changed throughout ontogeny after the disturbance involved with behavioural testing at the early stage, we ran GLMMs with the given behaviour as the dependent variable, developmental stage (11 days old vs. Gosner stage 32–36) as a fixed effect and individual as a random effect. The main interest here was in the individual \times developmental stage interaction entered into the model as a random effect, which would indicate that the individual effect differs between ontogenetic stages when testing for behavioural consistency.

We tested for behavioural syndromes using Spearman rank correlations between behavioural types using repeatable behaviours only. We also included the intra-individual

variation of the different behaviours in the correlations, irrespective of whether the behaviour was repeatable or not, as this variable might be informative even in the absence of significant individual variation in behavioural types. This step was necessary since complex behavioural strategies, such as different behavioural types being expressed at different levels of variation or intra-individual variation of functionally different behaviours being non-independent, could also be present. The calculation of behavioural type and variation is described in detail in the following text. In the case of the group whose behaviour was assessed at two ontogenetic stages, we also tested for correlations between behavioural types across these stages.

To control for the statistical problems arising from the large number of non-independent tests, we applied the False Discovery Rate (FDR) correction (Benjamini and Hochberg 1995), which is thought to perform best among Bonferroni-type corrections (as suggested by García 2004; Verhoeven et al. 2005). In the variable that describes risk-taking, individuals that remained immobile during the whole observation period of 15 min received the maximum score of 900 s. As individuals receiving this score more than once would falsely increase repeatability, they were excluded from repeatability calculations ($N = 0$ in 11-day-old tadpoles; $N = 1$ in pre-tested stage 32–26 tadpoles; $N = 3$ in naïve stage 32–36 tadpoles). This was a typical “right censoring” effect which is often observed in latency variables, as most researchers are not able to sample individuals beyond a given threshold (Stamps et al. 2012), and there is no unbiased method of repeatability calculations avoiding right censoring without using a much longer observational period or using different tests to record the same behaviour (Carter et al. 2013). In a follow-up experiment, we doubled the length of the observation period, and we still could not avoid this effect.

In the subsequent analyses, individual behaviour was characterized by two variables: (1) behavioural type and (2) intra-individual behavioural variation. When significant repeatability indicated the presence of personality in terms of the given behaviour, behavioural type was represented by the mean behaviour. We calculated intra-individual behavioural variation as the standard deviation of the subsequent three behavioural measures. Those individuals (see above) that received a 900 s score more than once in the risk-taking trials were not used in analyses using intra-individual behavioural variation variables. As we found no strong support for behavioural syndromes (see “Results”), we did not quantify complex behavioural types.

We applied general linear models (GLMs) to test for relationships between variables describing individual behaviours and age and mass at metamorphosis in the different groups to test for the presence of POLS. Age and mass at metamorphosis were not independent ($r = 0.414$,

$N = 40$, $P = 0.008$). Therefore, we analysed (1) age at metamorphosis and (2) mass at metamorphosis corrected for age by including age at metamorphosis as a covariate in the latter models. In this way, we were able to analyse relative growth rate irrespective of the timing of metamorphosis. We also added mean egg size to our models as a proxy for maternal effects. The starting models were built with all explanatory variables, and then we applied backward stepwise model simplification based on the P values (only effects with $P < 0.05$ were kept in the final models; Grafen and Hails 2002) to avoid potential problems due to the inclusion of non-significant terms (Engqvist 2005). This method is generally considered to be a conservative approach (Murtaugh 2009; Hegyi and Garamszegi 2011). We found no significant deviations from normality in model residuals applying Kolmogorov–Smirnov tests with the Lilliefors correction.

Pre-tested stage 32–36 tadpoles showed correlations between behavioural types and intra-individual behavioural variation (see “Results”). In this case, the original variables should not be put into the same GLM as explanatory variables to avoid multicollinearity. Therefore, we first ran a principal component analysis (PCA) on the behavioural

Table 1 Results of the principal component analysis ran on the behavioural variables of the pre-tested developmental stage 32–36 *Rana dalmatina* tadpoles

Variable	PC1	PC2
Mean activity	0.698	−0.501
SD activity	−0.706	0.029
Mean exploration	−0.234	0.711
SD exploration	0.584	0.488
Mean risk-taking	0.827	0.035
SD risk-taking	0.921	0.243
Proportion of variation explained (%)	48.6	17.6
Eigenvalue	2.92	1.06

Behavioural type is presented as the mean and behavioural variation as the standard deviation

PC Principal component

variables of this group, and then after entering the individual variables separately, we also ran our models with the new, by definition independent, unrotated PCs. The PCA resulted in two PCs with eigenvalues of >1 (Table 1). The first PC explained 48.6 % of the total variation and described relationships with all variables but exploration (Table 1); the second PC explained 17.6 % of the total variation and described mainly variation in exploration (Table 1). In addition to significance, we also report effect sizes (partial eta squared, η^2) in our GLM results. All analyses were performed with PASW Statistics 18 (PASW Inc., Chicago, IL).

Results

Personality and behavioural syndromes

Only activity was repeatable in all three experimental groups. Exploration was repeatable in both the older tadpole groups, and risk-taking was only repeatable in the pre-tested stage 32–36 tadpole group (Table 2). Hence, these behaviours can be considered as those which describe personality in the different tadpole groups. The GLMMs indicated that behavioural consistency changed throughout ontogeny following disturbance (individual \times developmental stage interaction; activity: $\chi^2 = 3.49$, $P = 0.031$; exploration: $\chi^2 = 2.45$, $P = 0.059$; risk-taking: $\chi^2 = 6.29$, $P = 0.006$). Consistency of activity became weaker, while consistency of exploration and risk-taking emerged only at the later stage (see Table 2). The mean behavioural type changed only in terms of exploration (activity: $F_{1,18} = 0.11$, $P = 0.92$; exploration: $F_{1,18} = 17.13$, $P < 0.001$; risk-taking: $F_{1,18} = 0.24$, $P = 0.63$), with older tadpoles exploring larger areas (data not shown). We note that here we were unable to separate age effect from size effect because older tadpoles were also larger and exploration was tested in similarly sized arenas. However, this should not influence estimates of behavioural consistency or any of the following results.

Table 2 Repeatability of the different behaviours of *R. dalmatina* tadpoles

Experimental group ^a	Assessed behaviours		
	Activity	Exploration	Risk-taking
11-day-old ($N = 19$)	$0.4 \pm 0.15^*$ ($P = 0.0021$)	0.01 ± 0.15 ($P = 0.22$)	0.005 ± 0.13 ($P = 0.5$)
Naïve stage 32–36 ($N = 18$)	$0.55 \pm 0.12^*$ ($P = 0.00013$)	$0.24 \pm 0.14^*$ ($P = 0.032$)	0.087 ± 0.15 ($P = 0.27$)
Pre-tested stage 32–36 ($N = 18$)	$0.3 \pm 0.15^*$ ($P = 0.015$)	$0.39 \pm 0.15^*$ ($P = 0.002$)	$0.24 \pm 0.16^*$ ($P = 0.048$)

* Repeatability is significant at $P < 0.05$. The P value is that of the general linear model (GLM)

^a Tadpoles were assigned to one of two groups. The first group’s behaviour was assessed on two occasions, first at the age of 11 days (after the onset of the free swimming stage; 11-day-old) and second at stage 32–36 (pre-tested stage 32–36). The second group’s behaviour was only assessed at stage 32–36 (naïve stage 32–36)

Table 3 Significant multiple behavioural type–intra-individual behavioural variation correlations^a after Bonferroni correction in pre-tested stage 32–36 tadpole group

Spearman correlations of pre-tested stage 32–36	r_s	N	P
Activity–variation of activity	−0.63	18	0.005
Risk-taking–variation of risk-taking	0.81	18	<0.0001
Variation exploration–variation of risk-taking	0.72	18	0.001

^a Namely, more active individuals were less variable in their activity; more risk-taking individuals were also less variable in their risk-taking; individuals less variable in exploration were also less variable in risk-taking. Note that in the case of risk-taking, high numbers represent shy individuals

We found no correlations between behavioural types and intra-individual behavioural variation in 11-day-old or naïve stage 32–36 tadpoles, but various correlations emerged in the pre-tested stage 32–36 group tadpoles (for details, see Table 3). There were strong trends for an activity–risk-taking correlation in the pre-tested stage 32–36 tadpoles ($r_s = 0.53$, $N = 18$, $P = 0.023$) and for a correlation between the activity of 11-day-old tadpoles and exploration of naïve stage 32–36 tadpoles ($r_s = -0.59$, $N = 18$, $P = 0.009$), but these correlations disappeared after the FDR correction. The significance of these, otherwise strong, effects (the mean effect size of behavioural correlations was found to be approximately 0.2 in a meta-analysis; Garamszegi et al. 2012) might have been higher with a larger sample size. Hence, it is possible that a behavioural syndrome emerged in the pre-tested stage 32–36 group and that there was also an ontogenetic syndrome between different behaviours, but that the significance of these correlations was sensitive to the large number of tests we ran. For all correlations, see Electronic Supplementary Material (ESM) Table 1. The PC1 from our PCA contained both activity and risk-taking with high and positive loadings, further emphasizing the possibility for a valid activity–risk-taking syndrome in this group (Table 1).

Pace-of-life syndrome

The activity of both 11-day-old and naïve stage 32–36 tadpoles showed a negative relationship with age at metamorphosis (11-day-old: $F_{1,17} = 19.23$, $P < 0.001$, $\eta^2 = 0.53$; naïve stage 32–36: $F_{1,16} = 7.16$, $P = 0.017$, $\eta^2 = 0.31$; Fig. 2). The intra-individual variation of activity in naïve stage 32–36 tadpoles showed a negative correlation with relative mass at metamorphosis ($F_{1,15} = 6.69$, $P = 0.021$, $\eta^2 = 0.31$; Fig. 3a). These results imply that more active individuals metamorphosed earlier and less variable individuals reached a higher mass relative to their age.

In the pre-tested stage 32–36 group, the intra-individual variation of exploration showed a negative relationship

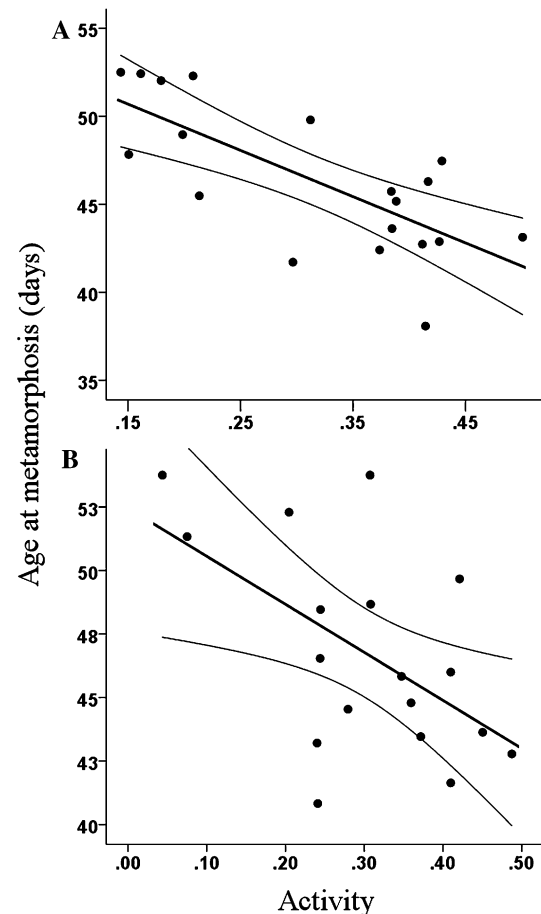


Fig. 2 An activity–age at metamorphosis pace-of-life syndrome (POLS) observed in 11-day-old ($N = 19$; **a**) and naïve stage 32–36 ($N = 18$; **b**) *Rana dalmatina* tadpoles. More active individuals started metamorphosis earlier

with relative mass at metamorphosis ($F_{1,14} = 5.98$, $P = 0.028$, $\eta^2 = 0.3$; Fig. 3b), while the intra-individual variation of risk-taking showed a positive relationship with relative mass at metamorphosis ($F_{1,14} = 5.14$, $P = 0.04$, $\eta^2 = 0.27$; Fig. 3c). In other words, individuals expressing low variation in exploration or high variation in risk-taking gained more mass during the tadpole stage. The effect sizes obtained from the above tests can be seen as strong (Cohen 1988). No other explanatory variable had significant effect on age or mass at metamorphosis (all $P > 0.07$; for more details, see ESM Table 2).

Discussion

We found strong support for personalities and POLS, but only a marginally significant trend for behavioural syndromes in naïve *R. dalmatina* tadpoles and in addition to behavioural type, intra-individual behavioural variation

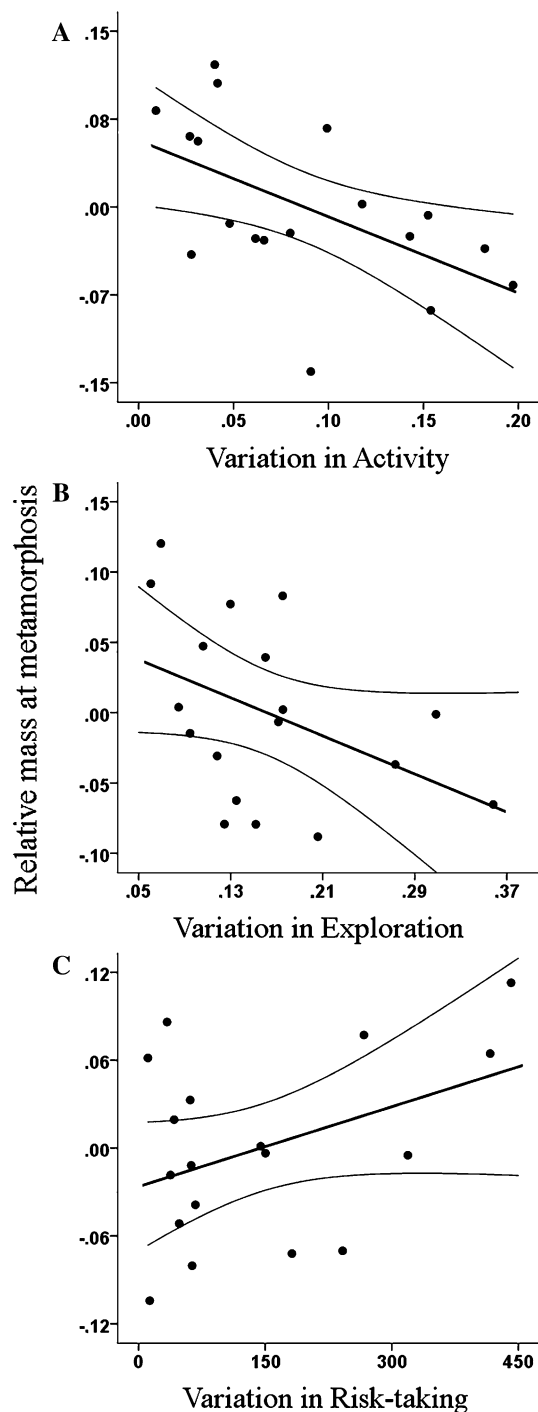


Fig. 3 The relationship between intra-individual behavioural variation and relative mass at metamorphosis (mass corrected for age) in *R. dalmatina* tadpoles. **a** Negative correlation between variation of activity and relative mass in naïve stage 32–36 tadpoles ($N = 18$), **b** negative correlation between variation of exploration and relative mass in pre-tested stage 32–36 tadpoles ($N = 18$), **c** positive correlation between variation of risk-taking and relative mass in pre-tested stage 32–36 tadpoles ($N = 18$)

was also linked to life history. Strong effects of the minor manipulation associated with our behavioural assays were found on all levels of behavioural patterns recorded approximately 30 days after the first manipulation.

Personality and behavioural syndromes

Behavioural traits typically show low-to-moderate repeatability (0.37–0.47), with wild-caught individuals showing higher consistency than laboratory-reared ones (Bell et al. 2009). Our results generally concur with this trend. We found that while certain behaviours were consistent throughout ontogeny (activity), others only became consistent during development (exploration) or as a response to an environmental stimulus (risk-taking). Hence, the developmental component in emerging personalities is an important one (e.g. Grootuis and Trillmich 2011; Trillmich and Hudson 2011). Wilson and Krause (2012) showed that in the marsh frog (*Pelophylax ridibundus*), activity and exploration are consistent even during metamorphosis. However, in their study, these authors used wild-caught tadpoles, and thus the circumstances resulting in the emergence of behavioural consistency were not controlled for. In our study on *R. dalmatina*, ontogenetic consistency appears to be weak at best within the tadpole stage. This implies strong time-scale dependency in both the presence/absence of personality and in the actual behavioural type in predator- and conspecific-naïve tadpoles. In addition to aging, individual experience during development also seems to be crucial in emerging personalities, as in our case risk-taking became consistent only in individuals that were previously subjected to potentially stressful situations. This draws attention to the fact that the interpretation of patterns observed in wild-caught individuals is not straightforward (see, for example, Merilä 2009) and also adds to the growing body of literature emphasizing that individual experience during ontogeny is an important component to consider in behavioural studies (Stamps and Grootuis 2010).

Correlations among behavioural traits occur in many taxa in the wild (Garamszegi et al. 2012, 2013). However, studies based on laboratory-reared predator- and conspecific-naïve individuals are scarce and typically report on the lack of behavioural syndromes (Herczeg et al. 2009; present study; but see Riechert and Hedrick 1993). After we applied the FDR method, we could only find a fairly strong but non-significant trend for an activity–risk-taking syndrome in the pre-tested group which had experienced some disturbance during an earlier stage. Sweeney et al. (2013) compared wild-reared spiders with laboratory-reared ones through multiple ontogenetic stages and found

syndromes only in the older wild-reared spiders, suggesting that behavioural syndromes are manifestations of environmentally induced phenotypic plasticity. The same inference was also supported by an elegant experiment of Bell and Sih (2007), where behavioural syndromes in three-spined sticklebacks (*Gasterosteus aculeatus*) emerged as a plastic response to predation risk. In contrast, some studies have shown behavioural correlations on the genetic level (e.g. van der Waaij et al. 2008; Dingemanse et al. 2009; Dochtermann and Dingemanse 2013; Rigterink et al. 2014). Studies which focus on separating environmental and genetic contributions to the emergence of behavioural syndromes are necessary to resolve this issue.

Studies focusing on behavioural consistency in general should not only consider individual behavioural type, but also the behavioural variation expressed by an individual (Herczeg and Garamszegi 2012; Stamps et al. 2012). In theory, this variation can have three main components: (1) behavioural plasticity, which is an environmentally induced, potentially adaptive shift; (2) intra-individual behavioural variation, which is an environment-independent estimate of how precisely an individual expresses its behavioural type; (3) measurement error. Here, we analysed variables measured in standardized behavioural assays of common garden-reared tadpoles; consequently, behavioural plasticity should be close to zero, and measurement error should be low and even across individuals. Thus, individual divergence in behavioural variation reflects divergence in intra-individual behavioural variation in this case. An interesting pattern emerged in terms of intra-individual behavioural variation: several correlations were present between intra-individual behavioural variation of different behaviours or between intra-individual behavioural variation and behavioural type—but only in the previously tested stage 32–36 group. This leads to two conclusions. First, these results provide evidence for complex behavioural strategies in which different behavioural types are expressed with different variation. More risk-taking individuals were less variable in their risk-taking, more active individuals were also less variable in their activity and individuals less variable in exploration were also less variable in risk-taking. We are aware that there are non-biological explanations for a positive correlation between the mean and variation of any variable. However, in the present study, high risk-taking, represented by low values, and high activity, represented by high values, were both coupled to low variation. Hence, it is unlikely that the correlations are mere statistical artefacts. Second, the emergence of this pattern in the late stage was a result of the disturbance related to the behavioural trials at the early stage and, therefore, can be considered to be environmentally induced. This notion is discussed in detail in the following section.

Pace-of-life syndromes

The integrative POLS hypothesis predicts complex relationships between behaviour, life history, immune defence and physiology (Réale et al. 2010). As the POLS framework is relatively recent, there are as yet only a few published studies which have focussed on finding evidence pro or contra the POLS hypothesis—with mixed results (Carreau et al. 2011; David et al. 2012; Niemelä et al. 2012a; Adriaenssens and Johnsson 2013; Le Galliard et al. 2013; Sweeney et al. 2013). Our results add to the body of literature in support of the POLS hypothesis: in our study, tadpoles with high activity developed faster, showing support for the integration of behaviour to POLS at both ontogenetic stages. In fact, in our survey activity was the only trait repeatable at both ontogenetic stages in predator- and conspecific-naïve tadpoles, further suggesting its importance in tadpole life-history strategies. The minor disturbance which occurred when testing the tadpoles at the early ontogenetic stage uncoupled the POLS in this group at the later stage.

We found no POLS for exploration, risk-taking or mass at metamorphosis, suggesting that even in studies supporting POLS, only a subset of the expected correlations can ever be found. However, we did find strong links between intra-individual behavioural variation and life-history: among the naïve stage 32–36 tadpoles, individuals with relatively lower variation in their activity grew faster than their more variable conspecifics, while in pre-tested stage 32–36 tadpoles, individuals with a relatively lower variation in exploration and higher variation in risk-taking gained more mass. These results imply that intra-individual variation in behaviour can be linked to fitness and possibly fit into the POLS framework. In our case, low intra-individual behavioural variation in activity was coupled with higher growth rates in tadpoles kept in a predictable environment (ad libitum food, predator and conspecific free)—i.e. low intra-individual behavioural variation seems to be an attribute of high pace-of-life individuals. This makes sense if we consider that the proactive (fast-pace-of-life) strategy is beneficial in stable environments (Sih et al. 2004) where low behavioural variation is also expected (Coppens et al. 2010; Niemelä et al. 2012b). In contrast, in the disturbed environment, we found low intra-individual variation in risk-taking to be associated with a relatively low growth rate, while low intra-individual variation in exploration was associated with a higher growth rates. Hence, the relationships between intra-individual behavioural variation and life-history are not always straightforward to explain. Further, it appears that both thorough and superficial explorers could have a high relative mass gain if they had exhibited low intra-individual behavioural variation, which would be against the general predictions of the POLS hypothesis. Integration of behavioural variation into the POLS theory is

indeed a fascinating possibility, but further studies are necessary to establish a general pattern.

The importance of maternal effects on larval phenotypes has been recognized in amphibians. Larvae hatching from larger eggs have higher growth rates and increased survival with possible carry-over effects into juvenile frog stage and even adulthood (Kaplan 1998). Laugen et al. (2002) found that egg size positively affected the size of offspring and their growth rate—but only under ad libitum food availability in *R. temporaria*. Egg size may also influence offspring personality (Andersson and Höglund 2012). In our study, egg size did not influence any of the measured fitness traits directly during ontogeny.

The effects of prior manipulation

We found considerable differences in behaviour between the pre-tested and naïve tadpoles at stage 32–36. Only the pre-tested group showed (1) repeatability in risk-taking, (2) a strong, near-significant behavioural correlation, (3) correlation of individual behavioural type and intra-individual behavioural variation, (4) correlation between intra-individual behavioural variation and relative body mass and (5) the absence of the activity–age at metamorphosis POLS. Mean behavioural type and mean behavioural variation did not differ between the naïve and pre-tested groups (data not shown). The only difference between these groups' development was the disturbance caused by the behavioural assays (carrying tadpoles to the exploration-arena and exposing them to the simulated predator-strike three times during a 3-day period) approximately 1 month before the differences between the pre-tested and naïve stage 32–36 groups were documented. We emphasize that (1) apart from the tests performed at 11 days age, the rearing procedure was totally standardized and (2) the behavioural testing-related disturbance can be seen as nothing extraordinary in the behavioural literature. These results draw attention to two facts. First, behaviour seen in the wild must have a strong environmentally induced component and cannot be used for evolutionary inference *sensu stricto*. Second, it will be challenging to study ontogenetic shifts in individual behaviour when the given behavioural assay includes novelty stimuli or stress. These finding coupled with the important role of individual experience in the emergence of complex behavioural strategies draws attention to a serious methodological problem regarding the study of behavioural consistency and suggests that current methodologies based on repeated and invasive testing of individuals will have to be reconsidered.

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References

- Adriaenssens B, Johnsson JI (2013) Natural selection, plasticity and the emergence of a behavioural syndrome in the wild. *Ecol Lett* 16:47–55
- Andersson MÅ, Höglund E (2012) Linking personality to larval energy reserves in rainbow trout (*Oncorhynchus mykiss*). *PLoS One* 7(11):e49247
- American Public Health Association (APHA) (1985) Standard methods for the examination of water and wastewater, 16th edn. APHA, Washington DC
- Becker WA (1985) Manual of quantitative genetics. Academic Enterprises, Washington State University, Pullman
- Bell AM (2007) Future directions in behavioural syndromes research. *Proc R Soc B* 274:755–761
- Bell AM, Sih A (2007) Exposure to predation generates personality in three-spined sticklebacks (*Gasterosteus aculeatus*). *Ecol Lett* 10:828–834
- Bell AM, Hankison SJ, Laskowski KL (2009) The repeatability of behaviour: a meta-analysis. *Anim Behav* 77:771–783
- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B* 57:289–300
- Biro PA, Abrahams MV, Post JR, Parkinson EA (2006) Behavioural trade-offs between growth and mortality explain evolution of sub-maximal growth rates. *J Anim Ecol* 75:1165–1171
- Butler MW, Toomey MB, McGraw KJ, Rowe M (2012) Ontogenetic immune challenges shape adult personality in mallard ducks. *Proc R Soc B* 279:326–333
- Careau V, Garland T Jr (2012) Performance, personality, and energetics: correlation, causation, and mechanism. *Physiol Biochem Zool* 85:543
- Careau V, Thomas D, Pelletier F, Turki L, Landry F, Garant D, Réale D (2011) Genetic correlation between resting metabolic rate and exploratory behaviour in deer mice (*Peromyscus maniculatus*). *J Evol Biol* 24:2153–2163
- Carter AJ, Feeney WE, Marshall HH, Cowlishaw G, Heinsohn R (2013) Animal personality: what are behavioural ecologists measuring? *Biol Rev* 88:465–475
- Cohen J (1988) Statistical power analysis for the behavioral sciences 2nd edn. Academic Press, San Diego Press
- Coppens CM, de Boer SF, Koolhaas JM (2010) Coping styles and behavioural flexibility: towards underlying mechanisms. *Philos Trans R Soc B Biol Sci* 365:4021–4028
- David M, Auclair Y, Giraldeau LA, Cezilly F (2012) Personality and body condition have additive effects on motivation to feed in Zebra Finches *Taeniopygia guttata*. *Ibis* 154:372–378

- Dingemanse NJ, Kazem AJN, Reale D, Wright J (2010) Behavioural reaction norms: animal personality meets individual plasticity. *Trends Ecol Evol* 25:81–89
- Dingemanse NJ, Dochtermann NA (2013) Quantifying individual variation in behaviour: mixed-effect modelling approaches. *J Anim Ecol* 82:39–54
- Dingemanse NJ, Van der Plas F, Wright J, Réale D, Schrama M, Roff DA, Van der Zee E, Barber I (2009) Individual experience and evolutionary history of predation affects expression of heritable variation in fish personality and morphology. *Proc R Soc B* 276:1285–1293
- Dochtermann NA, Dingemanse NJ (2013) Behavioural syndromes as evolutionary constraints. *Behav Ecol* 24:806–811
- Engqvist L (2005) The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Anim Behav* 70:967–971
- Le Galliard JF, Paquet M, Cisel M, Montes-Poloni L (2013) Personality and the pace-of-life syndrome: variation and selection on exploration, metabolism and locomotor performances. *Funct Ecol* 27:136–144
- Garamszegi LZ, Herczeg G (2012) Behavioural syndromes, syndrome deviation and the within-and between-individual components of phenotypic correlations: when reality does not meet statistics. *Behav Ecol Sociobiol* 66:1651–1658
- Garamszegi LZ, Markó G, Herczeg G (2012) A meta-analysis of correlated behaviours with implications for behavioural syndromes: mean effect size, publication bias, phylogenetic effects and the role of mediator variables. *Evol Ecol* 26:1213–1235
- Garamszegi LZ, Markó G, Herczeg G (2013) A meta-analysis of correlated behaviors with implications for behavioral syndromes: relationships between particular behavioral traits. *Behav Ecol* 24:1068–1080
- García LV (2004) Escaping the Bonferroni iron claw in ecological studies. *Oikos* 105:657–663
- Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J (2008) Climate change and evolution: disentangling environmental and genetic responses. *Mol Ecol* 17:167–178
- Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190
- Groothuis TGG, Trillmich F (2011) Unfolding personalities: the importance of studying ontogeny. *Dev Psychobiol* 53:641–655
- Grafen A, Hails R (2002) *Modern statistics for the life sciences*. Oxford University Press, Oxford
- Hegyí G, Garamszegi LZ (2011) Using information theory as a substitute for stepwise regression in ecology and behavior. *Behav Ecol Sociobiol* 65:69–76
- Herczeg G, Garamszegi LZ (2012) Individual deviation from behavioural correlations: a simple approach to study the evolution of behavioural syndromes. *Behav Ecol Sociobiol* 66:161–169
- Herczeg G, Gonda A, Merilä J (2009) Predation mediated population divergence in complex behaviour of nine-spined stickleback (*Pungitius pungitius*). *J Evol Biol* 22(544):552
- Jandt JM, Bengston S, Pinter-Wollman N, Pruitt JN, Raine NE, Dornhaus A, Sih A (2014) Behavioural syndromes and social insects: personality at multiple levels. *Biol Rev* 89:48–67
- Kaplan RH (1998) Maternal effect, developmental plasticity, and life history evolution: an amphibian model. In: Mousseau TA, Fox CW (eds) *Maternal effects as adaptations*. Oxford University Press, New York, pp 244–260
- Kortet R, Hedrick AV, Vainikka A (2010) Parasitism, predation and the evolution of animal personalities. *Ecol Lett* 13(12):1449–1458
- Kuparinen A, Merilä J (2007) Detecting and managing fisheries-induced evolution. *Trends Ecol Evol* 22:652–659
- Laugen AT, Laurila A, Merilä J (2002) Maternal and genetic contributions to geographical variation in *Rana temporaria* larval life-history traits. *Biol J Linn Soc* 76:61–70
- Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol Rev* 85:935–956
- Niemelä PT, Vainikka A, Hedrick AV, Kortet R (2012a) Integrating behaviour with life history: boldness of the field cricket, *Gryllus integer*, during ontogeny. *Funct Ecol* 26:450–456
- Niemelä PT, Vainikka A, Forsman JT, Loukola OJ, Kortet R (2012b) How does variation in the environment and individual cognition explain the existence of consistent behavioral differences? *Ecol Evol* 3:457–464
- Merilä J (2009) Genetic constraints on adaptation? *Science* 325:1212–1213
- Murtaugh PA (2009) Performance of several variable-selection methods applied to real ecological data. *Ecol Lett* 12:1061–1068
- Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. *Biol Rev* 82:291–318
- Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio PO (2010) Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philos Trans R Soc B: Biol Sci* 365:4051–4063
- Riechert SE, Hedrick AV (1993) A test for correlations among fitness-linked behavioural traits in the spider *Agelenopsis aperta* (Araneae, Agelenidae). *Anim Behav* 46:669–675
- Rigterink A, Houpt K, Cho M, Eze O, Xu R, Horii T, Hatada I (2014) Genetics of canine behavior: a review. *World J Med Genet* 4:46–57
- Rodel HG, Monclus R (2011) Long-term consequences of early development on personality traits: a study in European rabbits. *Behav Ecol* 22:1123–1130
- Ruiz-Gomez MDL, Kittilsen S, Höglund E, Huntingford FA, Sørensen C, Pottinger TG, Bakken M, Winberg S, Korzan WJ, Øverli Ø (2008) Behavioral plasticity in rainbow trout (*Oncorhynchus mykiss*) with divergent coping styles: when doves become hawks. *Horm Behav* 54:534–538
- Sih A, Kats LB, Maurer EF (2003) Behavioural correlations across situations and the evolution of antipredator behaviour in a sunfish–salamander system. *Anim Behav* 65:29–44
- Sih A, Bell A, Johnson JC (2004) Behavioural syndromes: an ecological and evolutionary overview. *Trends Ecol Evol* 19:372–378
- Sih A, Cote J, Evans M, Fogarty S, Pruitt J (2012) Ecological implications of behavioural syndromes. *Ecol Lett* 15:278–289
- Smith BR, Blumstein DT (2008) Fitness consequences of personality: a meta-analysis. *Behav Ecol* 19:448–455
- Stamps JA (2007) Growth-mortality tradeoffs and ‘personality traits’ in animals. *Ecol Lett* 10:355–363
- Stamps JA, Groothuis TG (2010) Developmental perspectives on personality: implications for ecological and evolutionary studies of individual differences. *Philos Trans R Soc B: Biol Sci* 365:4029–4041
- Stamps JA, Briffa M, Biro PA (2012) Unpredictable animals: individual differences in intraindividual variability (IIV). *Anim Behav* 83:1325–1334
- Sweeney K, Gadd RD, Hess ZL, McDermott DR, MacDonald L, Cotter P, Armagost F, Chen JZ, Berning AW, DiRienzo N, Pruitt JN (2013) Assessing the effects of rearing environment, natural selection, and developmental stage on the emergence of a behavioural syndrome. *Ethology* 119:436–447
- Teplitsky C, Mills JA, Alho JS, Yarrall JW, Merilä J (2008) Bergmann’s rule and climate change revisited: disentangling environmental and genetic responses in a wild bird population. *Proc Natl Acad Sci USA* 105:13492–13496
- Trillmich F, Hudson R (2011) The emergence of personality in animals: the need for a developmental approach. *Dev Psychobiol* 53:505–509

- van der Waaij EH, Wilsson E, Strandberg E (2008) Genetic analysis of results of a Swedish behaviour test on German shepherd dogs and labrador retrievers. *J Anim Sci* 86:2853–2861
- Verhoeven KJ, Simonsen KL, McIntyre LM (2005) Implementing false discovery rate control: increasing your power. *Oikos* 108:643–647
- Wilson AD, Krause MJ (2012) Personality and metamorphosis: is behavioral variation consistent across ontogenetic niche shifts? *Behav Ecol* 23:1316–1323
- Wolf M, Weissing FJ (2010) An explanatory framework for adaptive personality differences. *Philos Trans R Soc B Biol Sci* 365:3959–3968
- Wolf M, Weissing FJ (2012) Animal personalities: consequences for ecology and evolution. *Trends Ecol Evol* 27(8):452–461
- Wolf M, van Doorn GS, Leimar O, Weissing FJ (2007) Life-history trade-offs favour the evolution of animal personalities. *Nature* 447:581–584