



# Behavioral syndrome persists over metamorphosis in a pond-breeding amphibian

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## Abstract

In recent years, behavioral ecology has shifted from assuming animal behavior is infinitely plastic and situation specific to recognizing that behavior can be limited in its plasticity and correlated across different ecological situations. At the center of this new framework are behavioral syndromes or consistent individual differences in behavioral tendencies. Over the past decade, numerous studies have identified the evolutionary mechanisms and ecological implications of behavioral syndromes. However, the persistence of behavioral syndromes over ontogeny remains an open question. Species with complex life cycles present an interesting system in which to test the persistence of behavioral syndromes, because such life histories are thought to evolve when correlations between life stages are costly. In this study, we tested the hypothesis that behavioral tendencies of species with complex life histories are consistent within a life stage (before or after metamorphosis) but not between life stages. We experimentally assayed the activity, boldness, and exploration of spotted salamanders (*Ambystoma maculatum*) before and after metamorphosis. We found most behaviors to be at least moderately repeatable. Additionally, there was support for a behavioral syndrome within the larval stage as well as between larval behaviors and juvenile boldness. Our results reject the adaptive decoupling hypothesis and instead suggest that behavioral syndromes in species with complex life cycles can be maintained over metamorphosis.

## Significance statement

A central prediction of behavioral syndromes is that individual behavioral consistency should be maintained over the life of an organism. However, in species with complex life cycles, evolution is thought to act independently on each stage, leading to the prediction that behavioral syndromes should not persist over metamorphosis. We tested for behavioral correlations over metamorphosis by assaying salamander activity, boldness, and exploration in larval and juvenile salamanders. We found support for behavioral syndromes within and between life stages. These findings contradict the predictions of complex life cycle evolution and instead suggest that behavioral syndromes may span metamorphosis. However, because support for the persistence of syndromes over metamorphosis varies between taxa, we caution researchers against extrapolating inferences from the larval stage to the juvenile stage.

**Keywords** *Ambystoma maculatum* · Boldness · Complex life history · Personality · Temperament

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## Introduction

For much of the history of behavioral ecology, the assumption has been made that animal behavior is infinitely plastic, situation specific, and different ecological situations can be studied in isolation (Sih et al. 2004a). These assumptions have led behavioral ecologists to focus on possibly adaptive population means and to regard inter-individual variation around this mean as non- or maladaptive (Dall et al. 2004). However, in recent years, it has become apparent that these three assumptions are frequently violated. Indeed, there is a growing body

of literature identifying limited behavioral plasticity and behavioral correlations across different ecological situations (Dall et al. 2004; Sih et al. 2004a; Réale et al. 2007; Wolf and Weissing 2012). For example, individuals that are more active while foraging may also be more active in the presence of predators (Stamps 2007). Many terms have been used to describe consistent individual differences in behavioral tendencies, including behavioral syndromes (Sih et al. 2004a), temperaments (Réale et al. 2007), and personalities (Stamps 2007; Wolf and Weissing 2012). While these terms vary in their nuances, they all describe when an individual behaves consistently over time and across contexts, and there is variation among individuals in a population. Hereafter, we will use behavioral syndrome to refer to covariance of behaviors between contexts and personality to refer to repeatable among-individual variation. Studies of behavioral syndromes have established the evolutionary mechanisms that lead to the maintenance of behavioral variation within populations (Dall et al. 2004; Dingemans and Réale 2005; Sih et al. 2015). Over the past decade, it has also become apparent that behavioral syndromes affect many areas of ecology (Sih et al. 2012; Wolf and Weissing 2012), including physiology (Watkins 1997; Careau et al. 2014; Gifford et al. 2014), growth-predation trade-offs (Nannini et al. 2012), parasitism (Grim et al. 2014), dispersal (Verbeek et al. 1994; Ducatez et al. 2012; van Overveld et al. 2014), and the spatial distribution of individuals (Duckworth 2006; Alcalay et al. 2014). Behavioral syndromes have also facilitated an understanding of applied issues such as invasions (Cote et al. 2010; Phillips et al. 2010; Carere and Gherardi 2013), functional connectivity (Baguette and Van Dyck 2007), and fisheries management (Conrad et al. 2011).

A central prediction of behavioral syndromes is that behavior will be correlated across different situations (Sih et al. 2004a), with “situations” most frequently being interpreted as ecological contexts. For example, female fishing spiders that are more aggressive foragers are also aggressive during mating and display precopulatory sexual cannibalism (Johnson and Sih 2005). Likewise, animals that have activity syndromes will be more active while foraging but also in the presence of predators (Nannini et al. 2012). While there are many examples of behaviors being correlated across ecological contexts, tests of the repeatability of behavioral syndromes over time are much rarer, with most studies taking a snapshot approach and measuring behavior types over hours to days (Bell et al. 2009). The degree to which behavioral syndromes persist over ontogeny remains a topic of debate (Sih et al. 2004b; Stamps and Groothuis 2010; Stamps 2016), and there are few empirical tests (Bell and Stamps 2004; Bell and Sih 2007; Brodin 2009; Niemelä et al. 2012a; Brodin et al. 2013; Wuerz and Krüger 2015).

Complex life cycles present an interesting evolutionary context in which to consider behavioral syndromes. Complex life histories apply to animals which undergo an abrupt

developmental change in morphology, physiology, and behavior by undergoing metamorphosis (Wilbur 1980; Ebenman 1992; Wilson and Krause 2012a). Metamorphosis is expected to evolve when life stages are under different selection pressures (e.g., rapid growth versus dispersal), making correlations between life stages more costly than evolution acting independently on each stage (Wilbur 1980; Ebenman 1992). Under the adaptive decoupling hypothesis (Moran 1994), behaviors that are beneficial before metamorphosis may very well be maladaptive after. This leads to the prediction that behavioral syndromes and personality should not persist over metamorphosis. However, results from quantitative genetics studies are mixed, with some supporting adaptive decoupling while others indicate developmental constraints such that correlations persist over metamorphosis (Blouin 1992; Phillips 1998; Watkins 2001; Aguirre et al. 2014). This mixed bag is recapitulated by studies testing for the consistency of behavioral syndromes across metamorphosis: 30% of behaviors tested were consistent over metamorphosis, 55% of behaviors were inconsistent, and 15% had mixed support (Table 1).

We assessed whether behavioral syndromes persist over metamorphosis by conducting a series of experiments with spotted salamanders (*Ambystoma maculatum*) to assay activity, boldness, and exploration (Réale et al. 2007). Pond-breeding amphibians such as the spotted salamander have complex life cycles, inhabiting ponds as larvae before metamorphosing and moving into terrestrial habitat as juveniles. Previous studies of amphibians have found correlations among multiple behaviors within a single life stage (Sih et al. 2003; Koprivnikar et al. 2012; Brodin et al. 2013; González-Bernal et al. 2014; Videliér et al. 2014), and these correlations appear to be stronger in older larvae (Urszán et al. 2015). However, there is less support for a relationship between larval and juvenile behavior or performance (Shaffer et al. 1991; Watkins 1997; Austin and Shaffer 2010; Brodin et al. 2013). To our knowledge, only two studies in anurans have tested for behavioral syndromes persisting over metamorphosis (Wilson and Krause 2012b; Brodin et al. 2013), and multi-stage repeatability of behavior has not been assessed in salamanders. Salamanders undergo a smoother transition at metamorphosis than anurans, maintaining the same body plan and eating similar food items as late-stage larvae and juveniles. As such, salamanders may be more likely to have similar behaviors before and after metamorphosis.

We tested the hypotheses that (i) behavior varies among life stages and contexts, (ii) among-individual differences in specific behaviors are consistent (i.e., repeatable) within and between life stages, and (iii) behaviors are correlated between ecological contexts within and between life stages. We predicted that behavioral correlations would persist within a life stage. However, because larvae and juveniles are under selection for different niches, we predicted that specific behaviors would not be repeatable between life stages.

**Table 1** Review of empirical studies that tested for the persistence of behavioral syndromes over ontogeny in species with complex life histories

Taxon	Species	Behavior	<i>R</i>	Citation	
Insect	<i>Holometabolous</i>	<i>Drosophila melanogaster</i>	Activity	–	Anderson et al. (2015)
		<i>Drosophila melanogaster</i>	Exploration	+	Edelspanne et al. (2014)
		<i>Drosophila melanogaster</i>	Social	–	Anderson et al. (2015)
		<i>Eriopsis connexa</i>	Activity	+	Rodrigues et al. (2016)
		<i>Eriopsis connexa</i>	Aggression	–	Rodrigues et al. (2016)
		<i>Eriopsis connexa</i>	Boldness	–	Rodrigues et al. (2016)
		<i>Eriopsis connexa</i>	Exploration	+	Rodrigues et al. (2016)
		<i>Eriopsis connexa</i>	Social	–	Rodrigues et al. (2016)
		<i>Phaedon cochleariae</i>	Activity	–	Müller and Müller (2015)
		<i>Tribolium castaneum</i>	Activity	–	Wexler et al. (2016)
		<i>Tribolium castaneum</i>	Boldness	–	Wexler et al. (2016)
	<i>Hemimetabolous</i>	<i>Gryllus integer</i>	Boldness	±	Hedrick and Kortet (2012)
		<i>Lestes congener</i>	Activity	–	Brodin and Drotz (2011)
		<i>Lestes congener</i>	Activity	+	Brodin (2009)
		<i>Lestes congener</i>	Boldness	+	Brodin (2009)
	Amphibian	<i>Rana ridibunda</i>	Activity	±	Wilson and Krause (2012b)
		<i>Rana ridibunda</i>	Boldness	–	Wilson and Krause (2012b)
		<i>Rana ridibunda</i>	Exploration	±	Wilson and Krause (2012b)
		<i>Rana temporaria</i>	Boldness	–	Brodin et al. (2013)
<i>Rana temporaria</i>		Exploration	–	Brodin et al. (2013)	

Insects were tested as larvae and adults and amphibians were tested as larvae and juveniles. Behavior was scored as repeatable over ontogeny (+), not repeatable (–), or repeatable in some assays but not others (±)

*R* repeatability

## Materials and methods

**Approach** To test our hypotheses, we conducted a series of experiments to determine the repeatability of specific behaviors and correlations between behavior types within and between life stages. We were specifically interested in three categories of behavior, each representing a different ecological context: activity, boldness, and exploration. Activity is a baseline measure of how much an animal moves in a familiar habitat in the absence of risk (Réale et al. 2007). Boldness is an individual's reaction to a risky situation but not a novel situation (Réale et al. 2007). This can include exposure to predators or handling by humans. Exploration is an individual's response to a new place (Réale et al. 2007). For both the larval and juvenile life stage, we assayed activity and boldness in an individual's home container on two consecutive nights (Beckmann and Biro 2013). On the following two nights, we assayed exploration in a novel arena. We assayed each behavior twice, because estimates of repeatability do not typically change with additional observations per individual (Bell et al. 2009). We measured behaviors using scan sampling and minimized the intervals between scans to minimize error (Wirth et al. 2014). All assays were conducted in this fixed order, with

exploration last, because we expected any handling and a lack of a refuge to be more stressful for salamanders than our simulated predation attempt in the boldness assay when a refuge was present (Bell 2013). We measured all behaviors at night (21:30–23:20 h), when larvae and juveniles have peak activity (Brodman and Jaskula 2002; BHO personal observation), by using a dim red headlight (Buchanan 1993). To minimize bias, observers were blind to identity of individuals.

**Subjects** Spotted salamanders are a common pond-breeding amphibian and can be found in the eastern USA and Canada (Petranka 1998). Adult spotted salamanders migrate to forested, fishless ponds (Peterman et al. 2014) in the early spring (Hocking et al. 2008) where they lay eggs in submerged vegetation (Petranka 1998). Larvae metamorphose into terrestrial juveniles June–August (Hocking et al. 2008), which then disperse into the terrestrial landscape. Both larvae and juveniles are sit-and-wait predators (Wells 2007).

In this experiment, we opportunistically used salamanders that had been reared in mesocosms at either low larval density ( $N = 10$  hatchlings/mesocosm) or high density ( $N = 20$  hatchlings/mesocosms). These densities are within the range of those observed in natural populations (Ousterhout et al.

2015). Several spotted salamander egg masses were collected from natural ponds in Fort Leonard Wood, MO, USA and hatched in captivity. On 17 April 2015, we randomly assigned free-swimming hatchlings of a similar age to mesocosms. Mesocosms ( $N=9$ , 1000 L volume, 0.5 m depth, 1.52 m diameter polyethylene cattle tanks) were established on 13 March 2015 with the addition of dechlorinated tap water, 1 kg of dried leaf litter (primarily *Quercus* spp. and *Acer* spp.), and a 1.3-L concentrated aliquot of plankton to establish plankton and periphyton communities.

On 26 and 27 June 2015, we collected 40 late-stage larvae from mesocosms (2.5–8.5 days from metamorphosis; 31 from high density mesocosms, 9 from low density) and randomly assigned each larva to an individual plastic container (hereafter, larval home container;  $31 \times 18 \times 11$  cm). Each container was filled with 3 L of conditioned tap water and had a  $25 \times 5$  cm refuge cut from fiberglass window screen. Containers were separated by an opaque material to prevent salamanders from seeing conspecifics in neighboring containers. After each trial, we fed each salamander four mosquito larvae. As larvae metamorphosed (gills reduced to less than 2 mm; 29 June – 5 July 2015), we placed them into individual plastic containers ( $15 \times 10 \times 7$  cm) partially filled with moist sphagnum moss. On 9 July 2015, after all larvae had metamorphosed, we transferred juveniles to larger individual plastic containers in which juvenile activity and boldness trials would occur (hereafter, juvenile home container;  $31 \times 18 \times 11$  cm). Juvenile home containers had a 1-cm layer of moist sphagnum moss and a refuge burrow (4.5 cm long  $\times$  2 cm diameter PVC pipe). Each burrow contained a small amount of moss substrate. We fed juveniles approximately 80 mg of mealworms (*Tenebrio molitor*) twice a week. All animals were housed in a temperature-controlled room (air, 26 °C; water, 21–23 °C; 15:9 h light/dark schedule). To minimize handling, we measured individual mass and snout-vent length (SVL) after the conclusion of the experiment (mass, 17 July; SVL, 21 July).

**Larval behavior assays** We measured larval behavior in home containers on 28 and 29 June 2015 (activity, 22:00–22:40 h; boldness, 22:40–23:20 h). After allowing animals 2 min to acclimate to the presence of observers, we used a scanning method to score movement (walking or swimming) as a measure of activity every 2 min for 40 min ( $N_{\text{Activity}} = 20$  observations per trial). We also recorded whether an animal was using its refuge as a measure of boldness. We considered an individual to be in a refuge if more than 50% of its body was under the refuge.

Immediately after this first assay, we quantified boldness by scoring behavior in a situation with perceived risk. We simulated the presence of a predator by introducing an aqueous cue from a predatory sunfish (*Lepomis* sp.) (Kats et al. 1988; Sih et al. 2003). To collect cues, we housed three sunfish (12 cm total length) in an aquarium for 4 days (50 mL of

water per 1 mL of fish; DeSantis et al. 2013). We fed the sunfish red wigglers (*Eisenia foetida*) and mosquito larvae. On 26 June, we removed the fish, stirred the tank water, and collected and froze 65 mL aliquots. On trial dates, we thawed aliquots to room temperature before use (Hickman et al. 2004). We poured one aliquot into the center of each enclosure, and recorded movement and refuge use as before, scoring behavior every 2 min for 40 min.

We measured larval exploration behavior on 30 June and 1 July 2015 (22:00–22:40 h) by quantifying movement in a novel environment (Réale et al. 2007). We removed larvae from home containers and placed each in an unfamiliar, circular plastic container filled with 2 L of conditioned tap water (30 cm diameter, 2.5 cm deep). After a 2-min acclimation period, we recorded whether salamanders were moving using a scanning method at 1-min intervals for 40 min ( $N_{\text{Exploration}} = 40$  observations).

**Juvenile behavior assays** After a 5-day acclimation period to their juvenile home container, we re-tested salamanders post-metamorphosis in their home containers on 14 and 15 July 2015 (activity, 21:30–22:10 h; boldness, 22:30–23:10 h). We quantified juvenile behavior in the same manner as for larvae, by recording movement (any amount of walking or climbing) and refuge use (at least 50% of body in refuge) in the home container every 2 min over a 40-min trial ( $N_{\text{Activity}} = 20$  observations). After this initial trial, we then measured juvenile behavior in a situation of a perceived predation risk. Because juvenile spotted salamanders do not respond to chemical cues from terrestrial predators (M. Osbourn and S. Pittman personal communication), we simulated a terrestrial predation attempt by handling each animal and firmly pinching the base of the tail using forceps. We then recorded movement and refuge use every 2 min over a 40-min trial ( $N_{\text{Boldness}} = 20$  observations).

We measured juvenile exploration on 16 and 17 July 2015 (22:00–22:40 h) by scoring movement in a novel environment. We placed juveniles in 30-cm-diameter plastic containers lined with moist paper towels as a substrate. Containers had opaque walls so that salamanders could not see each other. After a 2-min acclimation period, we scored whether animals were moving every minute over a 40-min trial ( $N_{\text{Exploration}} = 40$  observations).

## Statistical analyses

**H1: Behavior varies among life stages and contexts** We first tested whether behaviors differed between life stages, context, or their interaction with generalized linear mixed effects models (GLMM). We assessed three responses: count of moves, time spent in refuge (activity and boldness assays only), and latency calculated as the number of intervals that elapsed before an individual moved. In these models, we included the covariates mesocosm density, date of



metamorphosis, and SVL to control for developmental differences and a random intercept of individual identity. Because these models revealed that behavior differed between life stages and contexts, we then constructed life stage and context-specific GLMMs from which we extracted individual variance ( $V_{ind0_y}$ ) as a measure of inter-individual differences in behavior (Dingemanse and Dochtermann 2013). As before, these models contained the covariates larval density, date of metamorphosis, and SVL, as well as a random effect of individual identity. All GLMMs were univariate and built with a zero-inflated Poisson error distribution. Additionally, we calculated the repeatability of among-individual variation for each behavior using the full data set. We conducted all analyses in R version 3.3.2 (R Core Team 2017). We conducted the analysis with packages “glmmADMB” (Fournier et al. 2012), “car” (Fox and Weisberg 2011), and “rptR” (Schielzeth et al. 2016).

**H2: Among-individual differences are consistent (i.e., repeatable) within a life stage** To assess intra-individual repeatability of behavior, we calculated Intraclass Correlation Coefficients ( $R$ ), the variance accounted for by individual divided by the total variance (Nakagawa and Schielzeth 2010). Unsurprisingly, latency to move and movements were negatively correlated, so we reduced behavioral responses within a context and life stage using a principal component analysis (PCA) (Table 2). For example, in the larval boldness PCA, we included larval latency to move, movements, and refuge use in the boldness assay. We also included SVL, mass, and date of metamorphosis in the PCA. We calculated  $R$  of each principal component that behavior loaded onto using univariate GLMMs with a normal error distribution (Nakagawa and Schielzeth 2010). We included PC1 as a covariate to account for differences in size and development as well as a random effect of individual identity. Following the recommendations of Bell et al. (2009), we calculated both the unadjusted  $R$  as the null linear mixed effects model with an intercept term and random effect of individual, and the adjusted  $R$ , upon which we focus in our results, which also included the covariates larval density and PC1. We estimated principal components using “prcomp” in base R (R Core Team 2017) and univariate repeatability using package “rptR” (Schielzeth et al. 2016).

**H3: Correlations between behaviors within and among life stages** We estimated the strength of behavioral syndromes by extracting variance components from multivariate GLMMs and calculating the correlation between behaviors (Dingemanse and Dochtermann 2013)

$$r_{ind0_y, ind0_z} = \frac{Cov_{ind0_y, ind0_z}}{\sqrt{V_{ind0_y} \times V_{ind0_z}}}$$

where  $Cov_{ind0_y, ind0_z}$  is the individual covariance between traits  $y$  and  $z$  and  $V_{ind0_y}$  is the individual variance in phenotype for

attribute  $y$ . As response variables, we included all behavior principal components from the previous hypothesis test that were at least marginally repeatable (adjusted  $R > 0.2$ ,  $p < 0.10$ ) (Dingemanse and Dochtermann 2013). We determined the statistical support for behavioral syndromes within and between life stages by comparing the deviation information criteria (DIC) of each model to one where the covariance was constrained to  $Cov_{ind0_y, ind0_z} = 0$ . Models with lower DIC values better fit the data. We conducted separate comparisons for each of the following data sets: larval data, juvenile data, and data from both life stages. Using the variance components estimated in these models, we also calculated multivariate repeatability as a measure of the repeatability of behavioral syndromes (Dingemanse and Dochtermann 2013). We calculated multivariate behavioral syndromes with package “MCMCglmm” (Hadfield 2010).

## Results

**H1: Behavior varies among life stages and contexts** After controlling for any effects of developmental differences, individuals differed in their behaviors within a life stage and context (Fig. 1). The mean behavioral response of salamanders also differed between life stages and contexts (life stage  $\times$  context: movement:  $F_{2, 407} = 53.82$ ,  $p < 0.001$ ; latency to move:  $F_{2, 407} = 46.71$ ,  $p < 0.001$ ; time in refuge:  $F_{1, 287} = 4.58$ ,  $p = 0.033$ ; latency to enter refuge:  $F_{1, 287} = 125.44$ ,  $p < 0.001$ ). Larvae moved more and earlier in trials, whereas juveniles used refuges more than larvae. Behavior was (or tended to be) affected by date of metamorphosis (movement:  $F_{1, 407} = 14.06$ ,  $p < 0.001$ ; latency to move:  $F_{1, 407} = 3.18$ ,  $p = 0.075$ ; time in refuge:  $F_{1, 287} = 7.42$ ,  $p = 0.007$ ; latency to enter refuge:  $F_{1, 287} = 3.02$ ,  $p = 0.083$ ) but not larval density ( $0.40 < p < 0.50$ ) or SVL ( $0.10 < p < 0.56$ ).

**H2: Among-individual differences are consistent (i.e., repeatable) within and between life stages** In the principal component analysis, the covariates SVL, mass, and date of metamorphosis loaded strongly onto PC1, latency to move and moves onto PC2, and, when applicable, refuge use onto PC3 (Table 2). The magnitude of variances was similar across ontogenetic stages (Table 3). However, the variance accounted for by individual identity was two orders of magnitude greater for movement/latency during the larval boldness assay and juvenile activity assay than for any other assays (Table 3). These two behaviors also had very high repeatabilities (Table 3), which were likely driven by the strong effects of fixed effects (density, body size, and development) rather than stronger among-individual differences. The repeatability of behaviors differed between contexts and, with the exception of juvenile boldness, was very low for refuge use (Table 3 and

**Table 2** Loadings of principal components (PC) used to test repeatability of behavior. Developmental covariates loaded onto PC1, movements and latency to move on PC2, and refuge use on PC3. The proportion of variance explained by principal components for each life stage and context are presented as footnotes

Stage	Context	Coefficient	PC1	PC2	PC3
Larvae	Activity <sup>a</sup>	SVL	-0.608	0.158	-0.019
		Mass	-0.614	0.202	-0.095
		Date of metamorphosis	0.446	0.153	-0.021
		Movements	0.233	0.619	-0.201
		Refuge use	0.010	-0.331	-0.942
	Boldness <sup>b</sup>	SVL	-0.017	-0.647	0.250
		Mass	-0.496	0.418	0.000
		Date of metamorphosis	-0.509	0.425	0.004
		Movements	0.494	0.103	0.358
		Refuge use	0.389	0.498	-0.271
	Exploration <sup>c</sup>	SVL	-0.050	-0.342	-0.820
		Mass	-0.313	-0.519	0.355
		Date of metamorphosis	-0.548	0.302	
		Movements	-0.551	0.297	
		Latency to move	0.539	0.067	
Juvenile	Activity <sup>d</sup>	SVL	0.234	0.661	
		Mass	-0.225	-0.616	
		Date of metamorphosis	-0.505	0.298	0.319
		Movements	-0.492	0.361	0.313
		Refuge use	0.290	-0.374	0.319
	Boldness <sup>e</sup>	SVL	0.384	0.585	0.007
		Mass	-0.331	0.025	-0.826
		Date of metamorphosis	-0.403	-0.546	0.132
		Movements	-0.633	0.022	0.192
		Refuge use	-0.649	0.007	0.121
	Exploration <sup>f</sup>	SVL	0.419	0.169	0.431
		Mass	-0.048	0.683	-0.152
		Date of metamorphosis	-0.004	-0.277	-0.806
		Movements	0.031	-0.654	0.302
		Latency to move	-0.628	0.083	
	SVL	-0.637	0.118		
	Mass	0.404	-0.149		
	Date of metamorphosis	-0.141	-0.693		
	Movements	0.130	0.691		
	Latency to move				

Stage = life stage at time of assay. Context = context of behavioral assay

<sup>a</sup> PC1, 36%; PC2, 30%; PC3, 15%

<sup>b</sup> PC1, 37%; PC2, 31%; PC3, 16%

<sup>c</sup> PC1, 48%; PC2, 34%

<sup>d</sup> PC1, 43%; PC2, 25%; PC3, 14%

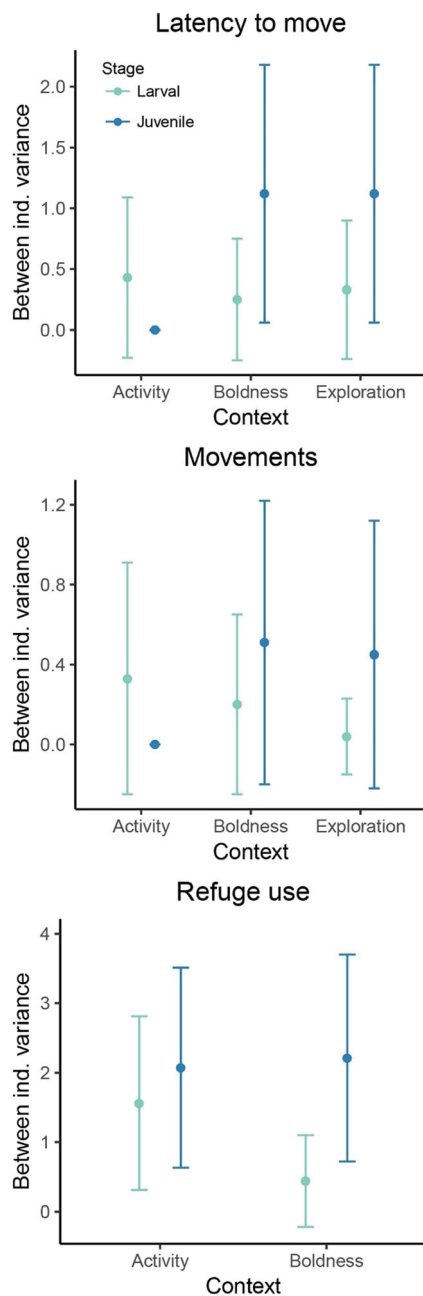
<sup>e</sup> PC1, 35%; PC2, 28%; PC3, 18%

<sup>f</sup> PC1, 42%; PC2, 35%

Fig. 2). Although the confidence intervals of moderately low estimates of repeatability included zero, these should be interpreted with caution as confidence intervals tend to be overestimated when repeatability < 0.3 (Wolak et al. 2012; Dingemans and Dochtermann 2013). It should be noted that repeatability is a property of the population and that

significant repeatability does not indicate that all individuals within the population are repeatable (Bell et al. 2009).

**H3: Correlations between behaviors within and between life stages** The larval and full models with behavioral correlations received substantially more support than the respective



**Fig. 1** Individuals differed in their behavior. Points and error bars are mean  $\pm$  1 SD of among-individual variance in latency to move, movements, and refuge use by context and life stage

constrained models without behavioral correlations ( $\Delta\text{DIC}_{\text{Larv}} = 21.2$ ,  $\Delta\text{DIC}_{\text{Full}} = 26.9$ ), indicating support for behavioral syndromes within the larval stage and between the larval and juvenile life stages. Within the larval stage, all three behaviors were positively correlated, with the strongest correlation between activity and boldness (Fig. 3). Additionally, larval behavior was positively correlated with juvenile boldness (Fig. 3). Individuals that were more active, bolder, and explored more as larvae became bolder juveniles (i.e., used their refuge less). We did not find support for correlations between juvenile behaviors ( $\Delta\text{DIC}_{\text{Juv}} = 0.4$ , Fig. S1).

## Discussion

In recent years, there has been increased interest among behavioral ecologists in inter-individual differences in behavior and intra-individual behavioral consistency. The evolutionary and ecological significance of such behavioral syndromes (e.g., parasitism, growth-predation trade-offs, speciation) has received considerable attention (Réale et al. 2007; Dall et al. 2012; Sih et al. 2012; Wolf and Weissing 2012), and empirical studies have identified behavioral syndromes in many taxa (Johnson and Sih 2005; Bell and Sih 2007; Brodin 2009; Carlson and Langkilde 2013; Favati et al. 2016). Although theoretical studies predict that behavioral syndromes should persist over ontogeny (Sih et al. 2004b; Stamps and Groothuis 2010), few empirical investigations have tested this hypothesis (van Overveld et al. 2014; Wuerz and Krüger 2015 and sources therein). This is particularly true for species with complex life histories (Table 1), which present a paradox to behavioral syndromes. Whereas behavioral syndrome theory predicts the persistence of behavioral consistency over ontogeny (Sih et al. 2004b), the evolutionary theory of complex life histories predicts that factors which govern behavior will not be correlated between life stages and specifically will not persist over metamorphosis (Wilbur 1980; Ebenman 1992).

Here we examined if spotted salamanders demonstrate individual behavioral consistency within a life stage and if behavioral consistencies persist over metamorphosis. We found evidence for a relationship between larval behaviors and juvenile boldness. This is congruent with other studies of species with complex life histories (Table 1). For example, fruit flies (*Drosophila melanogaster*) that explore more as larvae also explore more as adults, and this behavior has been linked to a specific polymorphism in the *for* gene (Edelsparre et al. 2014). Likewise, damselflies (*Lestes congener*) that were more active and bolder as larvae remained so as adults (Brodin 2009). These results as well as those from other behavioral studies (Table 1) and quantitative genetics (Watkins 2001; Aguirre et al. 2014) suggest that correlations between life stages may persist over metamorphosis in some species with complex life cycles. However, it is important to note that support for ontogenetic stability of behavioral syndromes is not universal among species that undergo metamorphosis. Among other studies that have tested for a relationship between larval and juvenile amphibian behavior (Wilson and Krause 2012b; Brodin et al. 2013; this study) or performance (Shaffer et al. 1991; Watkins 1997; Austin and Shaffer 2010; Johansson et al. 2010), two have supported the adaptive decoupling hypothesis (Wilson and Krause 2012b; this study). Likewise, some quantitative genetic studies fail to find significant correlation between pre- and post-metamorphic traits (Blouin 1992; Phillips 1998). This species specific support for adaptive decoupling is not limited to amphibians. A variety of invertebrates, including ladybird beetles

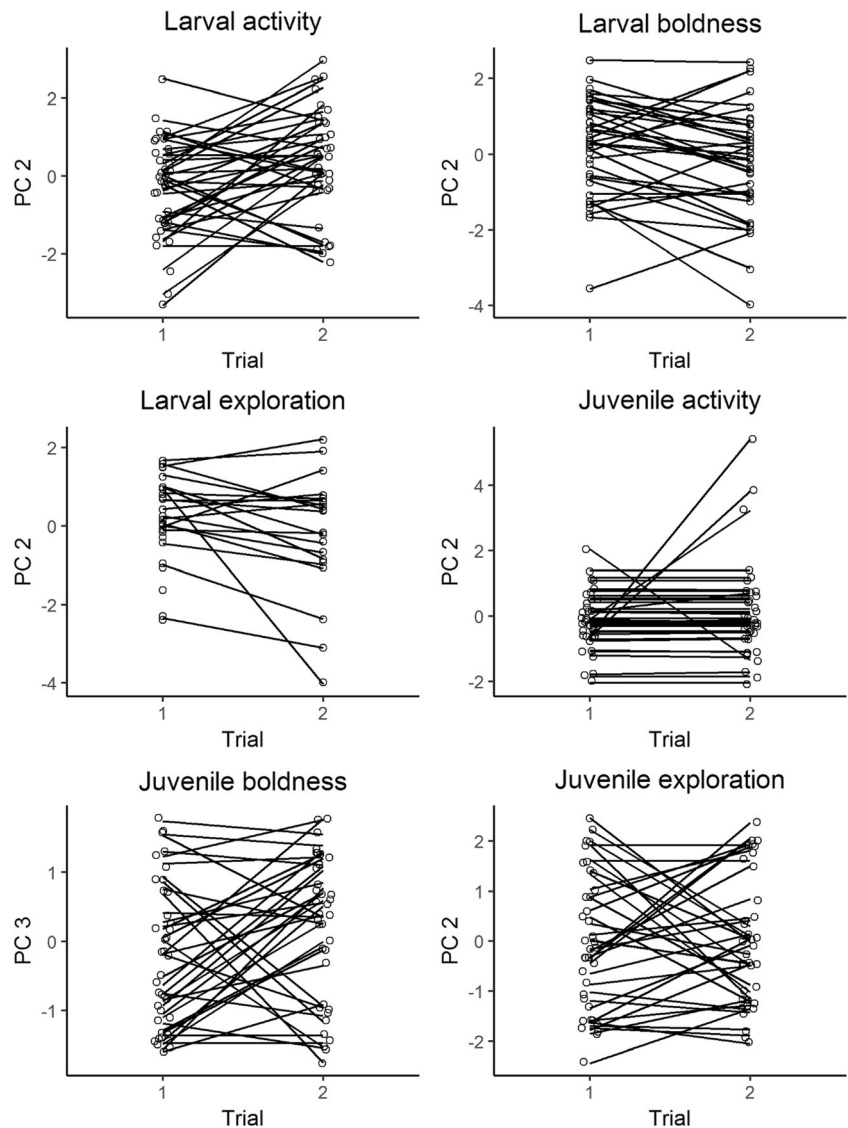
**Table 3** Variance estimates ( $\pm 1$  SE) and intraclass correlation coefficients (ICC) of behaviors within life stages. Bolded values have 95% confidence intervals (CI) of R that do not include 0. An R of 1 equals perfect repeatability. It should be noted that  $R < 0.3$  have inherently low power. Adjusted estimates are calculated after controlling for the

variance of covariates and do not include variance explained by the fixed effects in the denominator, whereas unadjusted estimates do not control for the effects of covariates and include fixed effect variance in the denominator

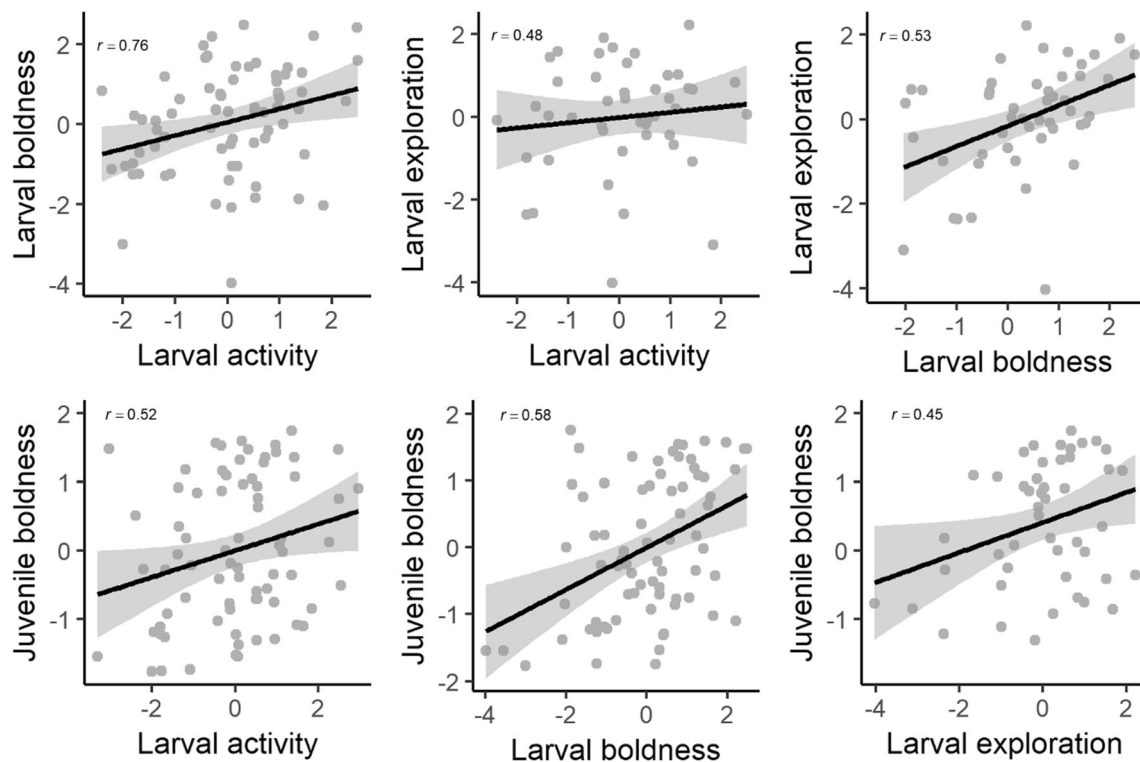
Stage	Behavior	Measure	Variance estimates			Adjusted <i>R</i>	Unadjusted <i>R</i>
			Random	Fixed	Residual		
Larva	Activity	Moves/Latency	0.44 $\pm$ 0.30	0.08 $\pm$ 0.12	1.36 $\pm$ 0.31	0.25 (0, 0.52)	0.24 (0, 0.50)
		Refuge use	0.04 $\pm$ 0.10	0 $\pm$ 0.03	0.89 $\pm$ 0.17	0.05 (0, 0.36)	0.05 (0, 0.34)
	<b>Boldness</b>	<b>Moves/Latency</b>	<b>5.00 <math>\pm</math> 1.19</b>	<b>3.63 <math>\pm</math> 0.55</b>	<b>0.08 <math>\pm</math> 0.02</b>	<b>0.98 (0.97, 0.99)</b>	<b>0.57 (0.42, 0.68)</b>
Juvenile	<b>Exploration</b>	Refuge use	0 $\pm$ 0.10	0.05 $\pm$ 0.06	0.96 $\pm$ 0.18	0 (0, 0.31)	0 (0, 0.3)
		<b>Moves/Latency</b>	<b>0.95 <math>\pm</math> 0.44</b>	<b>0.06 <math>\pm</math> 0.15</b>	<b>0.89 <math>\pm</math> 0.28</b>	<b>0.52 (0.12, 0.78)</b>	<b>0.50 (0.10, 0.73)</b>
	<b>Activity</b>	<b>Moves/Latency</b>	<b>3.00 <math>\pm</math> 0.75</b>	<b>1.99 <math>\pm</math> 0.39</b>	<b>0.20 <math>\pm</math> 0.05</b>	<b>0.94 (0.88, 0.97)</b>	<b>0.58 (0.41, 0.69)</b>
		Refuge use	0.12 $\pm$ 0.12	0 $\pm$ 0.03	0.74 $\pm$ 0.16	0.13 (0, 0.44)	0.13 (0, 0.42)
	Boldness	Moves/Latency	0.08 $\pm$ 0.19	0.06 $\pm$ 0.09	1.60 $\pm$ 0.31	0.04 (0, 0.37)	0.04 (0, 0.34)
		Refuge use	0.34 $\pm$ 0.20	0 $\pm$ 0.04	0.82 $\pm$ 0.19	0.29 (0, 0.55)	0.29 (0, 0.55)
<b>Exploration</b>	<b>Moves/Latency</b>	<b>0.75 <math>\pm</math> 0.32</b>	<b>0.12 <math>\pm</math> 0.14</b>	<b>0.99 <math>\pm</math> 0.23</b>	<b>0.43 (0.13, 0.66)</b>	<b>0.41 (0.11, 0.62)</b>	

*R* repeatability

**Fig. 2** Repeatability of behaviors within a life stage. A repeatability of 1 would be represented by a horizontal line. Open circles are individual observations







**Fig. 3** Larval salamander activity, boldness, and exploration were correlated suggesting a within stage behavior syndrome. Larval behaviors were also correlated with boldness during the juvenile life

stage. Points represent individual observations, and shaded area represents  $\pm 1$  SE

(*Eriopis connexa*), flour beetles (*Tribolium castaneum*), and leaf beetles (*Phaedon cochleariae*), also lack correlations between larval and adult behaviors (Müller and Müller 2015; Rodrigues et al. 2016; Wexler et al. 2016).

These conflicting findings may indicate that the effects of correlations between life stages are more subtle in some systems or traits than others, such that traits before and after metamorphosis are correlated, but the strength of these correlations is variable and frequently less than one (Aguirre et al. 2014). They may also indicate that gene by environment interactions have a larger effect on the persistence of behavioral syndromes over ontogeny than previously thought. The effect of environmental factors or gene by environment interactions on the persistence of behavioral syndromes over ontogeny remains an open question. Whereas we deliberately limited any environmental differences experienced by salamanders by rearing larvae in a common environment, Stamps and Groothuis (2010) suggest that, in addition to genes influencing natural selection on behavioral syndromes, biologically relevant experiences (i.e., environment) can also affect the strength of behavioral correlations. For example, a boldness-aggression syndrome in three-spined sticklebacks (*Gasterosteus aculeatus*) only emerged after exposure to predators (Bell and Sih 2007). Likewise, field crickets (*Gryllus integer*) exposed to predation had stronger behavioral responses in ensuing tests (Niemelä et al. 2012b). Future investigations of the persistence of behavioral syndromes over

metamorphosis should consider manipulating biologically relevant experiences of individuals (Barbasch and Benard 2011).

We found support for a behavioral syndrome within the larval stage, with more active larvae consistently being bolder and exploring more. Stamps (2007) suggests that such behavioral correlations are most likely to emerge in behaviors that contribute to growth-mortality trade-offs. This prediction is consistent with our findings and may be generalizable to species with complex life cycles that rely on ephemeral resources as larvae. Amphibian larvae are under strong selection for rapid growth to avoid predation by gape-limited predators (Urban 2007a, b) and to metamorphose from vernal pools before they dry out (Altwegg and Reyer 2003). However, they are also subject to growth-mortality trade-offs. To grow more rapidly, larvae must forage more, perhaps in unfamiliar habitat, and this additional movement exposes larvae to greater mortality risks (Werner and Gilliam 1984; Werner 1986; Urban 2007a, b). This hypothesized relationship between behavioral correlations and growth-mortality trade-offs may explain the maintenance of different growth rates within a population (Stamps 2007). Further work that measures individual behavior and growth rates under varying strengths of predation is required to test these hypotheses.

Our results should be interpreted as conservative, because confidence intervals for intraclass correlation coefficients tend to be overestimated when sample size ( $N < 60$ ) or repeatability

( $R < 0.3$ ) is low (Wolak et al. 2012; Dingemans and Dochtermann 2013). However, it should be noted that the persistence of behavioral syndromes over metamorphosis has been found by testing as few as 16 individuals (Brodin 2009) and studies with much larger sample sizes have found no relationship ( $N = 80$ ; Brodin et al. 2013). The amount of time that passed between larval and juvenile assays may have also affected our ability to detect behavioral syndromes. There is likely an element of temporal autocorrelation such that behaviors measured consecutively will be more similar than those separated by 2 weeks. Despite this, we were able to detect correlations between life stages. Likewise, behavioral correlations that spanned metamorphosis were detected in frogs (*Rana ridibunda*) after 8 days (Wilson and Krause 2012b) and in damselflies (*L. congener*) after 21 days (Brodin 2009). However, the effects of temporal autocorrelation may overestimate weaker behavioral correlations.

An implication of this study is that carryover effects of the larval period to later life stages in amphibians may not be limited to morphological traits (i.e., body size or body condition) (Scott 1994; Barbasch and Benard 2011). If larval behaviors are also carrying-over to the juvenile stage, as suggested by our findings, it is possible that selection in the larval habitat may affect the ecology of later life stages. For example, if larvae are under selection for reduced activity by visual predators, they may mature into juveniles with reduced activity despite being released from their larval predator. In this manner, behavioral syndromes in general, and especially in species with complex life cycles that undergo an abrupt change in habitat over ontogeny, may result in maladaptation in later life stages. Alternatively, in systems where the constraints experienced by larvae are very different from those faced by life stages following metamorphosis, we might expect greater plasticity rather than behavioral syndromes. These trade-offs may in part account for the seemingly discordant findings of studies testing for behavioral syndromes in species with complex life cycles (Table 1).

In conclusion, our results provide the first test of a behavioral syndrome persisting across metamorphosis in a salamander. Moreover, our data suggest that larvae and juveniles both have consistent among-individual variation in behavior. These findings reject the adaptive decoupling hypothesis and instead suggest that behavioral syndromes can persist over ontogeny in species with complex life cycles. We encourage future researchers to investigate the relationship between growth-mortality trade-offs, growth, and the persistence of behavioral syndromes over ontogeny.

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**Data availability** The data generated and analyzed during the current study are available in the figshare repository, <https://doi.org/10.6084/m9.figshare.7255868>.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in this study were in accordance with the ethical standards of the University of Missouri Animal Care and Use Committee (8402) and collected under Missouri Department of Conservation permit no. 16463.

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