Original Article

Personality and metamorphosis: is behavioral variation consistent across ontogenetic niche shifts?

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Understanding the manner by which individual differences in personality arise and are maintained in animal populations is currently a topic of considerable research interest. This is particularly the case when it comes to developmental processes and understanding how behaviors change over ontogeny. Such developmental perspectives are essential given that the vast majority of animal species possess complex life cycles or undergo some form of metamorphosis. Yet, in spite of the broad taxonomic relevance and the obvious potential importance of metamorphosis for understanding the basis of consistency in personality over ontogeny, almost no research has been done on this topic. Using the lake frog (*Rana ridibunda*) as a study organism, we tested whether individual-level differences in personality (activity, exploration and boldness) were consistent within both larval and juvenile frog life-history stages and across metamorphosis. We found that most behaviors of interest were highly consistent within a given life-history stage and at least some traits were consistent across metamorphosis (e.g., activity and exploration). Generally, more active, exploratory individuals in novel experimental arenas were also bolder and more likely to spend time in more risky open areas of a familiar environment. To our knowledge, our study is the first to both characterize personality traits across anuran development and provide evidence of consistency in behavior across metamorphosis in a vertebrate species. *Key words:* activity, amphibian, anuran, boldness, frog, temperament. *[Behav Ecol]*

INTRODUCTION

Behavioral biologists have long been interested in the mechanisms by which consistent individual-level differences in behavior may evolve and be maintained over time or across contexts. In recent years, this consistency in behavior has been documented in a wide range of taxa and has generally been characterized as representing behavioral syndromes (Sih et al. 2004) or animal personalities (Dall et al. 2004; Réale et al. 2007). However, in spite of numerous conceptual and empirical advances in the animal personality literature (Krause et al. 2010; Schuett et al. 2010; Wilson and Godin 2010), certain areas of research remain largely unexplored (Stamps and Groothuis 2010a), for example, the ways in which life-history and developmental processes are integrated with personality.

Developmental perspectives have been posited to be essential for properly understanding the function and evolution of animal personality as until relatively recently, individual consistency in behavior has most commonly been assessed by observations over short time periods or within a given life-history stage (Stamps and Groothuis 2010b). This approach, however, tends to both underestimate the potential consequences of long-term consistency in behavior and often fails to recognize the potential importance of seemingly maladaptive behaviors later in life. Additionally, personality traits may also couple with life-history characteristics over ontogeny, with some general combinations of behavioral and developmental traits being superior to others in terms of fitness (Wolf et al. 2007; Réale et al. 2010). This notion, however, is complicated in animals that have complex life cycles or undergo abrupt ontogenetic transformations (i.e., metamorphosis).

Metamorphosis, in the broadest sense, refers to an abrupt series of changes in an individual's morphology, physiology, and behavior during postembryonic development (Wilbur 1980). The importance of metamorphosis for ecological research is that this phenomenon is frequently associated with a discrete shift in an individual's habitat or ecological niche (e.g. aquatic to terrestrial environments), and thus affects a range of important behaviors, including predator avoidance, foraging, mating and dispersal. Thus, these dramatic niche shifts during development may result in selection acting to decouple syndromes between life-history stages (i.e. larval and adult forms) that differ dramatically in terms of predation pressure and environmental factors in each niche (Sih et al. 2004). Furthermore, although metamorphosis is unique to amphibians among tetrapods, greater than 80% of all animal species have complex multistage life cycles involving abrupt ontogenetic transformations (Wilbur 1980; Werner 1988). In spite of this abundance and although the value of such studies have been mentioned (Sih et al. 2004; Stamps and Groothuis 2010b), almost no research has been conducted on the consistency of individual differences in personality traits across metamorphosis (but see Brodin 2009; Hedrick and Kortet 2012).

In this study, we used a wild population of the lake frog (*Rana ridibunda*) to ascertain whether individual-level differences in personality (i.e. activity, exploration, and boldness) were consistent both within life-history stage (larval tadpole

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and frog ecomorphs) and across metamorphosis. Although few studies have attempted to characterize individual differences in personality traits among amphibians in general (but see Nicieza 2000; Sih et al. 2003), anurans specifically provide an ideal, yet heretofore largely unstudied vertebrate taxonomic group by which to study the impact of metamorphosis on individual-level differences in personality. Because larval and adult life-history stages in anurans tend to inhabit extremely different habitats together with different foraging behaviors and predation pressures, anurans also provide an ideal system by which to compare the mechanistic bases for the coupling and perhaps uncoupling of behavioral syndromes and life-history characteristics during ontogeny. Larval tadpoles typically inhabit still water pools or low flow streams, are primarily herbivorous, and their ecology is primarily dedicated to rapid growth to reach metamorphosis prior to a change in environmental conditions (i.e. pool drying). Post-metamorphosis, the adult stage frog or toad is carnivorous, inhabiting a range of terrestrial or semiaquatic environments, and its ecology is primarily dedicated to reproduction and dispersal (Rowe and Ludwig 1991).

To quantitatively assess the relationship between personality and metamorphosis, we captured larval tadpoles from a single population consisting of a series of pools that were colonized naturally by *R. ridibunda*. Individual tadpole larvae were collected and tested twice for repeatability under standardized conditions for individual differences and repeatability of activity, exploration, and risk-taking behaviors. After initial behavioral trials, larvae were held and observed in the laboratory until 1 week post-metamorphosis whereupon they were tested repeatedly to examine individual behavioral consistency across metamorphosis. Because selection should uncouple behavioral syndromes across ontogeny when environmental conditions differ significantly between those experienced as juveniles and those experienced as adults (Bell and Stamps 2004; Sih et al. 2004), we predicted individuals would be consistent and repeatable when tested multiple times as either larvae or as frogs, but not across metamorphosis due to contrasting behavioral, morphological, and environmental needs that differ between each life-history stage/niche.

MATERIALS AND METHODS

Subjects

Larval tadpoles (total length: 22–68 mm, wet weight 82–243 mg) were collected from several small pools (~5–6 m² surface area) on the edge of a large temperate lake (52º26´57 N, 13º38´58 E) in Berlin-Brandenburg region, Germany. Fifteen individuals were collected on 5 sampling trips over a 9-day period to insure an adequate range of developmental stages (see Gosner 1960) in captured larvae. The acclimation period and behavioral tests for test subjects were staggered according to a standardized protocol, which insured that each larval cohort spent a similar amount of time in the laboratory before behavioral testing (see below).

Early experience gained through rearing in a natural or semi-natural environment has recently been posited to be particularly important in order to allow valid comparisons to be made between studies of wild and laboratory test populations (Stamps and Groothuis 2010b). As such, we collected active larvae that had already begun exogenous feeding (stages 25–43; Gosner 1960) as opposed to collecting egg clutches. This was meant to allow for greater genetic heterogeneity in the collected individuals and for individual differences in experience. Given that we were primarily interested in the carryover of personality traits from larval to adult morphs, irrespective of the potential causal mechanisms of the expression of such traits (e.g. genetic,

hormonal, and experience), this design was appropriate for our study. Additionally, because we were studying larval and juvenile frogs, it was not possible to ascertain the sex of tested individuals as they had not yet reached sexual maturity.

Experimental holding conditions

On arrival, all larvae were housed communally, but separate from those captured on different days in 1 of 2 large fiberglass holding tanks (216×41×14 cm, water depth: 7 cm). Partitions consisting of perforated, transparent plastic were used to separate groups before testing and during the acclimation period (see below). Each holding tank contained aerated and filtered dechlorinated tap water maintained at 18±1 °C, and illuminated by overhead fluorescent lights on a 14:10-h light cycle. In addition, each holding tank contained abundant natural sediment, detritus, and plant matter collected from pools similar to that of initial capture. This material was meant to provide substratum and food to the developing larvae. Foraging and refuge material was replenished/ changed every 72 h or when necessary with similar natural material from local sites. Holding tanks were equipped with a large recirculating pump to remove waste and oxygenate the water. Larvae were also provided with aquarium algae wafers (Tetra) for supplemental feeding throughout the study. All larvae were held for approximately 1 week before the onset of behavioral testing to allow for acclimation to laboratory conditions and insure feeding in all individuals to be tested.

During testing, 10 individuals from each collection period were selected haphazardly and tested individually using a standardized protocol (Experiments 1–3). After initial larval phase testing (pre-metamorphosis), all larvae were placed individually in holding aquaria. Holding aquaria consisted of small transparent plastic containers (23×14×13 cm, water depth: 7 cm) that contained ample detritus and plant matter for food and refuge. These aquaria were placed within the same initial larger holding tanks and were also equipped with a mesh (3×3-mm square pattern) bottom to change water and allow waste removal as well as allow olfactory cues to be shared between individuals. After initial tests (approximately 72 h) and later during development, any individuals of approximate developmental Stage 43 (Gosner 1960) were then transferred to a second stage of larger holding aquaria to allow further growth and eventual metamorphosis.

Second stage holding aquaria consisted of 24 rectangular clear Plexiglas compartments (29×24×29 cm, water depth: 16 cm) connected in series and to a large external pump to allow recirculation and oxygenation of water between compartments. As with the first stage holding containers, larvae were provided detritus and plant matter ad libitum as well as supplemented with algae wafers. These aquaria also contained a perforated metal partition that extended out of the water that allowed larvae to rest and exit the water in the final stages of metamorphosis.

On completing metamorphosis, frogs were collected and placed in a third and final housing experimental setup. This housing setup consisted of identical plastic containers as those used in Stage 1. However, these containers consisted of a continuous base to allow a water depth of 1.5 cm instead of a mesh screen base. In addition, frogs were provided a translucent white PVC tube (13 cm long and 2 cm diameter) for rest and refuge. During the first week after metamorphosis, frogs were provided ad libitum access to live fruit flies (*Drosophila* spp.) and first to third instar cricket nymphs (*Acheta domestica* and *Gryllus integer*) for foraging. All insects used for feeding test subjects post-metamorphosis were obtained from a commercial laboratory supplier to insure uniform size, quality and quantity in provided food items for

all tested individuals. On the eighth day post-metamorphosis, all focal individuals were tested again in the identical test conditions (Experiments 1 and 2) as those used on tadpole larvae to test the repeatability of personality across metamorphosis. Because individual animals were in different stages of development on capture and also likely had different developmental rates (see Nicieza 2000), individuals were in holding for slightly different time periods. That said, all individuals were held for identical time periods post-capture and post-metamorphosis before testing to attempt to mediate any potential effects this might have on behavior.

After final behavioral testing, all frogs, in addition to any larvae that not been used as experimental subjects, were released near their point of capture. In some instances wherein a focal individual escaped their holding container such that positive identification could not be made, or where individuals were not observed to be feeding in the laboratory (as evidenced by visual confirmation of feeding events or via fecal evidence), animals ($N = 5$) were removed before final testing and excluded from further analyses.

Behavioral experiments

Quantifying activity, exploration, risk-taking, and antipredator behavior

We characterized personality traits in individual larvae and frogs using 6 different behavioral measures in 3 experimental components with each component (other than Experiment 3) being performed twice during each life-history stage (larval tadpoles and juvenile frogs) to quantify repeatability of behavior within each developmental stage and across metamorphosis. All focal individuals were tested in 2 consecutive days to allow testing within a given developmental stage and not across stages, although order of testing within each day was randomized. Experiment 3 was only conducted once in the larval stage as it occurred after the behavioral trials in Experiments 1 and 2 and involved multiple observations over multiple days and thus, may have occurred over more than 1 development stage (between Experiments 1 and 3). As such it would not be possible to replicate all potential factors in a second observation session. All behavioral variables were quantified by a single observer.

Experiment 1: open arena test

The experimental arena used for this test consisted of a rectangular glass aquarium ($79 \times 59 \times 20$ cm, water depth: 5 cm) and was covered in gray chloroplast (sides and bottom) to prevent outside stimulus from disturbing the focal individual during observation. The aquarium was placed behind a large observation blind positioned so as to allow remote observations of behavior and the release of focal individuals at the onset of behavioral trials (see below). The experimental aquarium contained a mix of aged water and water from the appropriate holding tank, which was refreshed between trials. Located within the aquarium was a small number of mixed natural color aquarium gravel pieces ($n = \sim 25$), which were haphazardly placed throughout the arena so as to provide additional environmental stimuli. The gravel was kept consistent through each experimental day, but changed between days for novelty purposes.

Just before the beginning of a behavioral trial, a focal individual was haphazardly dipnetted from one of the appropriate holding containers, placed in a small vial (500 mL) and promptly transported to the experimental arena. The individual was slowly poured into a tall (20 cm) translucent plastic tube (12 cm diameter) that acted as a temporary refuge to allow for acclimation to the test arena. After a 5-min acclimation period, the refuge tube was then raised and individual

differences in latency to become active, general activity, and flight initiation distance (FID) from a threat stimulus were recorded. Latency to become active was recorded as the first instance of movement of greater than 1 body length (approximately 3 cm) from the focal individual's initial position. To account for the possibility that initial movement may have been a startle response to the tube being raised, no measurements were recorded for the first 10 s after tube removal. General activity was quantified as the total time spent active after initial movement more than a 10-min period. To quantify individual differences in FID, an opaque glass rod was placed into the aquarium approximately 15 cm from the focal individual and moved slowly through the water column and toward the larvae/frog (1–2 cm/s) until the individual moved. FID was recorded after the 10-min activity measure and only while the individual was in a sedentary position.

All focal individuals were tested in 2 consecutive days to allow testing within a given developmental stage and not across stages, although order of testing within each day was randomized.

Experiment 2: response to predator cues

Immediately after the conclusion of Experiment 1, each focal individual was placed singly into a second novel experimental arena, given 20 min to acclimatize, and then tested for their response to simulated predation threat. These experimental aquaria were made of the same material and dimensions as those used during holding except for the fact that they had a continuous plastic bottom instead of a mesh screen. Each aquarium contained approximately 500 mL of aged tap water. A 2-mL pipette was used to present each focal individual with 1 mL of water from a holding tank containing European perch (*Perca fluviatilis*). Presentation was accomplished by releasing the stimulus water directly above the dorsal section of the focal individual (while the individual was active) from approximately 20 cm above. This stimulus was meant to be both a mechanical and chemical stimuli as anuran larvae and adults can detect both forms of stimuli via sensory receptors in their skin. The use of fish sensory cues represented a novel stimulus as there were no fish predators present in the ponds when captured. An individual response to simulated predation threat was quantified as individual latency to resume normal activity level more than a 5-min observation period. Any individual that did not resume activity within 5 min was arbitrarily assigned the 5 min maximum ($n = 4$ larvae and $n = 0$ frogs).

Experiment 3: refuge use and activity during holding

Immediately after Experiment 2, focal individuals were transferred to their respective holding containers (see holding protocol above) and allowed to acclimate for 24 h. After this initial acclimation period, each individual was observed 3 times (10:00, 13:00, and 16:00) daily for 3 days. During each observation session, we recorded whether the focal individual was active or sedentary and if the individual was in the open or near/under refuge. These parameters were meant to ascertain individual differences in risk-taking (i.e. active in the open vs. sedentary in refuge).

Data analyses

First, comparisons of behavioral traits within each individual's life-history period (larvae and frog) were made using the nonparametric Spearman rank correlation test. These correlations were made to assess individual consistency in measures of activity, exploration, and risk-taking in both larval tadpoles and juvenile frogs prior to any comparisons of individual behavior across metamorphosis. To avoid an inflated chance of type 1 error, we adjusted our alpha level to be more

Table 1

PCA loadings of within-context behavioral variables used to generate first principal component scores (PC1) to assess across-context correlations in activity/exploration, refuge use, and antipredator behavior in larval and juvenile frogs (*Rana ridibunda*)

Behavioral context	Behaviors within each context	Loadings for PC1	Percentage of variation explained
Activity/exploration	Larvae: latency to first movement (T1)	0.5123	75.7
	Larvae: latency to first movement (T2)	0.4973	
	Larvae: total activity (T1)	−0.4948	
	Larvae: total activity (T2)	−0.4954	
	Frogs: latency to first movement (T1)	0.5107	74.6
	Frogs: latency to first movement (T2)	0.5148	
	Frogs: total activity (T1)	−0.4784	
	Frogs: total activity (T2)	−0.4953	
Refuge use	Proportion active (D1)	−0.0912	32.19
	Proportion active (D2)	−0.1406	
	Proportion active (D3)	−0.1859	
	Proportion in open (D1)	0.6110	
	Proportion in open (D2)	0.5120	
	Proportion in open (D3)	0.5496	
Antipredator behavior	Larvae: latency to resume activity (T1)	−0.4256	59.0
	Larvae: latency to resume activity (T2)	−0.4714	
	Larvae: flight initiation distance (T1)	0.5515	
	Larvae: flight initiation distance (T2)	0.5409	
	Frogs: latency to resume activity (T1)	−0.0402	42.2
	Frogs: latency to resume activity (T2)	−0.1209	
	Frogs: flight initiation distance (T1)	0.7051	
	Frogs: flight initiation distance (T2)	0.6976	

T, test; D, day.

conservative ($P < 0.0018$, both larvae and frogs independently) for these analyses using the Bonferroni correction.

Second, we took the mean value of repeated measures of behavior for both larvae and frogs to allow comparison to morphological (total length/snout-vent length and wet weight) and ontogenetic characters (larval stage of development during testing). To compare refuge use and activity during holding to ontogenetic and morphological traits, we used the relative proportion of overall activity across all 3 days of observation. Our level of significance for these tests was also adjusted using the Bonferroni correction ($P < 0.0016$, larvae; $P < 0.0033$ frogs).

Lastly, to test for consistency of behavioral traits across metamorphosis we collapsed individual traits best representing general activity/exploration, refuge use, and antipredator contexts into first principal component scores for each axis using principal components analysis (PCA) (Table 1). The PC1 scores for general activity/exploration and refuge use were characterized separately for 3 reasons. First, although both measures have an estimate of activity level, the activity/exploration axis represents behavior in a novel environment and the refuge axis represents behavior in a familiar environment in conjunction with foraging and refuge use (risk-taking behavior). Secondly, the behavioral measures making up the activity/exploration axis were performed identically for both larvae and frogs and therefore allowed direct comparison. Thirdly, the refuge use axis was only performed in the larval stages, although across multiple stages of development. As such we represented activity/exploration and refuge use as separate traits. Correlations in behavior across metamorphosis were then calculated using Spearman rank correlation tests as above. Similarly, our alpha level for these analyses was adjusted to be more conservative using the Bonferroni correction ($P < 0.005$).

RESULTS

All correlations that were significant, or suggested strong trends ($P < 0.05$), are shown in Tables 2–5 as appropriate.

Only those correlations that were significant at the appropriate adjusted alpha levels (see MATERIALS AND METHODS) or are otherwise important for subsequent analyses are discussed in further detail below.

Behavioral correlations within life-history stage (larvae and frogs)

Larvae: behavioral correlations
Individuals that were more exploratory (had shorter latencies to move in a novel experimental arena) were also more active overall. These behavioral measures were all highly intercorrelated and consistent across observations. These more active, exploratory individuals were also bolder in terms of spending more time in the open away from refuge than less exploratory individuals when observed in a familiar environment. Similarly, individuals were also highly consistent in both their FID and their latency to resume activity after a threat stimulus. Although these 2 measures were not significantly correlated with each other or with measures of activity, strong trends suggest a potential relationship in some instances (Table 2).

Larvae: behavioral correlations with morphological or ontogenetic traits
Larvae that were larger (in terms of total length but not weight) for their given life-history stage had longer latencies to initial movement and were less active on average than smaller tadpoles (Table 3). However, individuals in later stages of development (Gosner 1960) tended to have shorter latencies to initial movement, shorter FIDs, and were on average more active in a novel environment than individuals in early stages of development (Table 3).

Frogs: behavioral correlations
Individuals that were more exploratory in terms of having shorter latencies to first movement were also more active overall. General activity, latency to first movement, and

Table 2

Spearman (r_s) correlations in activity, exploration, risk-taking, and antipredator behavioral contexts in larval lake frogs

Behavioral trait 1	Behavioral trait 2	r_s	P value
Larvae: total activity (T1)	Larvae: total activity (T2)	0.8041	<0.0001*
Larvae: latency to first movement (T1)	Larvae: latency to first movement (T2)	0.8528	<0.0001*
Larvae: total activity (T1)	Larvae: latency to first movement (T1)	−0.8531	<0.0001*
Larvae: total activity (T1)	Larvae: latency to first movement (T2)	−0.7803	<0.0001*
Larvae: total activity (T2)	Larvae: latency to first movement (T1)	−0.7314	<0.0001*
Larvae: total activity (T2)	Larvae: latency to first movement (T2)	−0.8583	<0.0001*
Larvae: flight initiation distance (T1)	Larvae: flight initiation distance (T2)	0.5641	0.0003*
Larvae: latency to resume activity (T1)	Larvae: latency to resume activity (T2)	0.6466	<0.0001*
Larvae: total activity (T2)	Larvae: latency to resume activity (T1)	−0.4572	0.0085
Larvae: total activity (T2)	Larvae: latency to resume activity (T2)	−0.3594	0.0369

All Spearman correlations at $P < 0.05$ are shown; correlations that are significant at Bonferroni-adjusted alpha = 0.0018 are marked with an asterisk.

latency to resume activity after a potential threat stimulus were also all highly independently correlated across observations. Time spent active in a novel experimental arena was also strongly correlated with latency to resume activity (risk-taking behavior) after a simulated predation event. FID, however, was not significantly correlated across observations due to the adjusted level of significance, although a trend in the data suggests a positive relationship across observations (Table 4).

Frogs: behavioral correlations with morphological traits
Frogs that were larger (in terms of snout to vent length) were also heavier, more active and had shorter latencies to first movement on average than smaller frogs. That said larger frogs on average also had longer latencies to resume activity after a threat stimulus than smaller frogs (Table 5). Body size was not correlated with FID in juvenile frogs.

Principal components analysis (across metamorphosis consistency in behavior)

Spearman rank correlation tests across the first PCA scores for each axis of behavior suggests strong consistency in aspects of anuran behavior across metamorphosis. Larval refuge use was highly negatively correlated with both larval ($r_s = -0.5953$, $P = 0.0001$; Figure 1) and frog ($r_s = -0.4982$, $P = 0.0032$; Figure 1) activity/exploration suggesting that more risky behaviors in novel environments are also related to individual willingness to

spend time in the open in a familiar environment. Individual differences in exploration/activity were also strongly positively correlated between larval and juvenile frog life-history stages ($r_s = 0.5586$, $P = 0.0007$; Figure 1). Antipredator behavior was not significantly correlated with any other behavioral axis when considered under the appropriate Bonferroni-adjusted alpha level of significance ($P < 0.005$).

DISCUSSION

Our study addresses several important areas of current research interest in behavioral ecology: personality in wild caught animals, consistency of behavior across ontogenetic niche shifts and metamorphosis. In this study, we sought to ascertain whether individual-level differences in personality (activity, exploration, and boldness) were consistent within a given life-history stage and across metamorphosis in the common lake frog. Most importantly, we found that most behaviors of interest were highly consistent within a given life-history stage (i.e. larva and frog ecomorphs) and at least some traits were consistent across metamorphosis (e.g. activity and exploration). Generally, more active, exploratory individuals in novel experimental arenas were also bolder and more likely to spend time in more risky open areas of a familiar environment. To our knowledge, our study is the first to both characterize personality traits across anuran development and provide evidence of consistency of those traits across metamorphosis in any vertebrate species.

Table 3

Spearman (r_s) correlations in comparing mean measures of personality (activity, exploration, risk-taking, and antipredator behavior) with morphological and ontogenic characters in larval lake frogs

Trait 1	Trait 2	r_s	P value
Larval: total body length	Larval: wet weight	0.3513	0.0356
Larval: total body length	Stage of development	−0.4636	0.0044
Larval: total body length	Proportion in open area of familiar refuge	−0.6984	<0.0001*
Stage of development	Proportion in open area of familiar refuge	0.5360	0.0008*
Stage of development	Proportion active in familiar refuge	−0.4682	0.0040*
Larval: total body length	Larval: mean latency to first movement	0.5528	0.0005*
Stage of development	Larval: Mean latency to first movement	−0.5501	0.0005*
Larval: total body length	Larval: mean total activity	−0.6385	<0.0001*
Stage of development	Larval: mean total activity	0.4261	0.0096
Larval: mean latency to first movement	Larval: mean total activity	−0.8730	<0.0001*
Stage of development	Larval: mean flight initiation distance	−0.5107	0.0015*
Larval: total body length	Larval: mean latency to resume activity	0.3585	0.0477

All Spearman correlations at $P < 0.05$ are shown; correlations that are significant at Bonferroni-adjusted alpha = 0.0016 are marked with an asterisk.

Table 4

Spearman (r_s) correlations in activity, exploration, risk-taking, and antipredator behavioral contexts in juvenile lake frogs

Behavioral trait 1	Behavioral trait 2	r_s	P value
Frogs: total activity (T1)	Frogs: total activity (T2)	0.9143	<0.0001*
Frogs: latency to first movement (T1)	Frogs: latency to first movement (T2)	0.9567	<0.0001*
Frogs: flight initiation distance (T1)	Frogs: flight initiation distance (T2)	0.4241	0.0437
Frogs: latency to resume activity (T1)	Frogs: latency to resume activity (T2)	0.6084	0.0003*
Frogs: total activity (T1)	Frogs: latency to first movement (T2)	−0.5183	0.0017*
Frogs: total activity (T2)	Frogs: latency to first movement (T1)	−0.4293	0.0127
Frogs: total activity (T2)	Frogs: latency to first movement (T2)	−0.4852	0.0042
Frogs: total activity (T2)	Frogs: flight initiation distance (T1)	0.4939	0.0103
Frogs: total activity (T1)	Frogs: latency to resume activity (T1)	0.3948	0.0279
Frogs: total activity (T2)	Frogs: latency to resume activity (T1)	0.4758	0.0068
Frogs: latency to resume activity (T2)	Frogs: latency to first movement (T1)	−0.4371	0.0110
Frogs: latency to resume activity (T2)	Frogs: latency to first movement (T2)	−0.4113	0.0174
Frogs: latency to resume activity (T2)	Frogs: total activity (T1)	0.5494	0.0009*
Frogs: latency to resume activity (T2)	Frogs: total activity (T2)	0.6115	0.0002*

All Spearman correlations at $P < 0.05$ are shown; correlations that are significant at Bonferroni-adjusted alpha = 0.0018 are marked with an asterisk.

As predicted, individual larvae and frogs were highly consistent in their behavior when measured multiples times within a given life-history stage. This is an important result for several reasons. First, observations conducted within life-history periods may offer insight into the mechanistic bases of behavioral consistency and further, may reflect adaptive temporal variation in trait combinations that are influenced by individual experience or ecological circumstances over time (Bell and Stamps 2004). Second, because many studies of personality typically focus solely on a single life-history stage (i.e. juvenile or adult) or are conducted over short time periods (Stamps and Groothuis 2010b); our novel findings with regard to anuran behavior in both larval tadpole and frog stages of development are suitable for comparison to an abundant existing body of literature on other taxa. Lastly, finding consistency in behavioral traits within each life-history stage was critical to our experimental design. If larval behavior was not repeatable, any comparison with post-metamorphosis behavior would be erroneous.

Our initial prediction that behavioral consistency should be disrupted across metamorphosis was based on the notion that selection should uncouple behavioral correlations (or syndromes) across ontogeny when environmental conditions experienced as larvae vary greatly from those experienced as adults (Bell and Stamps 2004; Sih et al. 2004). Larval tadpoles and frogs differ significantly in most aspects of their ecology (Wilbur 1980; Rowe and Ludwig 1991). Tadpoles in our study system are herbivorous, completely aquatic, live in high densities and are subject to limited predation from aquatic insects in their earliest stages of development. Frogs on the other hand, are geared primarily for dispersal, are semi-terrestrial and carnivorous. Frogs are also subject to comparatively higher levels of predation by fish, birds (i.e. herons), snakes, and mammalian predators (i.e. foxes). Many amphibians are also able to adaptively modify their developmental rate in response to changes in ambient environmental conditions. For example, frogs have been known to metamorphose faster (although at a smaller size) if food is scarce, competition is high, or environmental conditions (i.e. pool drying out) are unfavorable (Nicieza 2000). Thus, it is reasonable to predict that behavior, as with morphological characters would be highly plastic and thus adaptive in terms of trait development. Certain behavioral types and processes are also thought to be influenced by state-dependent factors (i.e. hunger level) and asset protection principles that would suggest that individuals should modify their behavior based on current status and future needs (Wolf et al. 2007). However, contrary to our initial predictions, general activity and exploration tendency were consistent across metamorphosis and related to refuge use over development, in spite of environmental differences between habitats.

Although this result may be surprising from an adaptive standpoint, it is not altogether unexpected. Sih et al. (2003)

Table 5

Spearman (r_s) correlations in comparing mean measures of personality (activity, exploration, risk-taking, and antipredator behavior) with morphological characters in juvenile lake frogs

Trait 1	Trait 2	r_s	P value
Frog: S–V body length	Frog: wet weight	0.9423	<0.0001*
Frog: S–V body length	Frog: mean latency to first movement	−0.4519	0.0083
Frog: S–V body length	Frog: mean latency to resume activity	0.4845	0.0057
Frog: S–V body length	Frog: mean total activity	0.5137	0.0022*
Frog: wet weight	Frog: mean latency to first movement	−0.4008	0.0208
Frog: wet weight	Frog: mean total activity	0.4043	0.0196
Frog: mean total activity	Frog: mean latency to first movement	−0.4871	0.0040
Frog: mean total activity	Frog: mean latency to resume activity	0.4724	0.0073

All Spearman correlations at $P < 0.05$ are shown; correlations that are significant at Bonferroni-adjusted alpha = 0.0033 are marked with an asterisk. Developmental stage was not used for comparison in juvenile frogs as all individuals who had completed metamorphosis and were, therefore, in the same stage of development.
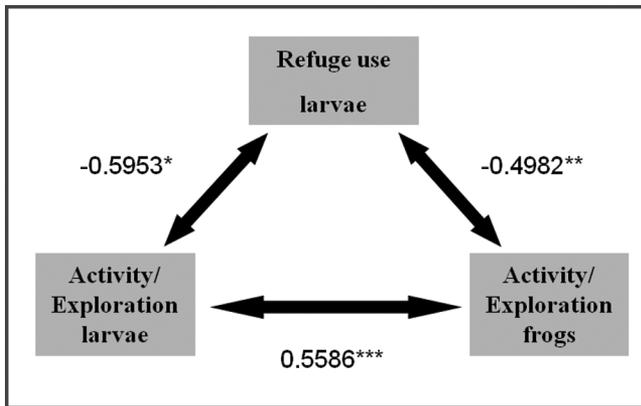
Figure 1
Behavioral syndrome showing relationships between principal component scores for larval refuge use and activity/exploration in both larval and juvenile *Rana ridibunda*. The correlation between activity/exploration in both larvae and frogs represents an across metamorphosis relationship. Numbers shown indicate results from Spearman rank correlation tests. Levels of significance are indicated by (*) for each pair of traits (*P = 0.0001, **P = 0.0032, ***P = 0.0007). Antipredator behavior was not significantly correlated with any other behavioral axis when considered under the appropriate Bonferroni-adjusted alpha level of significance (P < 0.005).

found that salamander larvae (*Ambystoma barbouri*) maintained similar levels of activity both in the presence and absence of predators, even when seemingly maladaptive. Further, what little information there is available about consistency in personality traits across metamorphosis comes from research on insects and seems to support our findings. For example, Brodin (2009) investigated the relationship between different predator treatments (familiar vs. unfamiliar predator cues) and physiological states (food deprived vs. satiated) on personality in the damselfly (*Lestes congener*) and found that individual differences in activity were consistent across metamorphosis. A recent study on field crickets (*Gryllus integer*) also found that females that were tested for their latency to emerge from refuge (boldness) once as juveniles and once as adults were consistent across metamorphosis; although this was not the case for males (Hedrick and Kortet 2012). The measures used in these previous studies, as with our tests, all incorporate some measure of activity in conjunction with exploration and/or risk-taking (boldness) behavior. One potential explanation for the observed consistency in behavioral traits across metamorphosis (even if seemingly maladaptive) is that some traits may have a genetic (i.e. pleiotropic) or mechanistic (i.e. hormonal and metabolic) basis that is difficult to uncouple over ontogeny (Sih et al. 2004). Individual differences in activity in particular may be indicative of a variety of different mechanistic underpinnings that may remain robust and carryover across metamorphosis. For example, there may be hormonal or metabolic factors governing activity through individual differences in growth rate and thus foraging needs. These factors may independently be responsible for such consistency or be correlated in some way with other fitness-related traits (i.e. behavioral syndromes) that persist through metamorphosis, in spite of the dramatic morphological and physiological changes occurring.

Although this study is important for providing the first vertebrate example of consistency in personality across metamorphosis, it is important to note some limitations of the study and identify areas of future research. Our use of individuals that were from multiple pools and that had already begun

exogenous feeding introduces several strengths but also some weaknesses. Our experimental design presumably allowed for greater genetic diversity and heterogeneity in individuals sampled. Similarly, by using individuals that had already begun exogenous feeding we had a much higher probability of having individuals acclimate successfully to laboratory conditions as they were being fed with natural food with which they were already familiar. Our design also allowed for individual differences in experience to arise during development, which has been proposed to be an important component of studies interested changes in behavior over ontogeny (Stamps and Groothuis 2010a).

A downside to our approach and to the collection of test subjects haphazardly in general is that we cannot know the genetic composition of individuals sampled (i.e. all individuals from 1 egg clutch) or know the exact age of individuals tested. Because amphibians can modulate the timing of metamorphosis based on ambient conditions (i.e. metamorphose early if resources are limited; Nicieza 2000), estimates of age and growth rate are difficult to ascertain. This information would have been valuable in terms of estimating underlying causal mechanisms of consistency in behavior across metamorphosis in anurans. Nonetheless, this factor is mitigated by being able to accurately stage individuals based on stereotyped changes in morphological characters (which follow set stages of development; Gosner 1960) and by the fact that few studies of wild animals know the genetic makeup and exact ages of test animals in general.

To our knowledge, our study documents consistency in personality across metamorphosis in a vertebrate species for the first time. In general, very few studies have documented the relationship between personality and the complex developmental processes that occur during metamorphosis or ontogenetic niche shifts. This is true in spite of the near taxonomic ubiquity of such phenomena across taxa (Werner 1988) and the rare and valuable opportunity they represent. Animals that undergo metamorphosis represent a type of in situ experimental opportunity by which to study the mechanistic underpinnings of behavioral traits and the processes that shape them. It is possible to study suites of behavioral traits in animals adapted to 1 particular ecological niche and then track and characterize these traits in the same animals as they undergo dramatic hormonal, physiological, and morphological changes as they adapt to an entirely new set of environmental conditions. Understanding how and why behaviors change and perhaps more importantly, why some do not change, represents both a considerable research challenge and a unique opportunity for future study.

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