Individual Behavioral Variability Across Time and Contexts in *Dendrobates tinctorius* Poison Frogs

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Abstract

Consistent individual differences in behavior (aka, “animal personality”) have consequences for individual fitness, adaptive trajectories, and species’ persistence. Such differences have been documented across a wide range of animals, though amphibians are generally underrepresented in this research area. The aim of our study was to examine consistent individual differences in poison frogs (*Dendrobates tinctorius*). We evaluated repeatability in behaviors including activity, exploration, and boldness to assess consistency of behaviors across different temporal, experimental, and environmental contexts. We found repeatability in activity and exploration across time and contexts. In contrast, we observed context-specific behavior for our metrics of boldness, with consistent individual differences only for some measures. Further, while activity and exploration displayed consistent correlations across contexts, relationships between activity and boldness were context dependent. Our findings document the presence of consistent individual differences in behavior in poison frogs, challenging historic assumptions about the simplicity of amphibian behavior. Nonetheless, our approach testing the same individuals across multiple time points and assays also reveals context-dependent differences, highlighting the complex relationship between consistent individual differences and context-specific responses in animal behavior.

Statement of Significance

The concept of animal personality centers on the existence of consistent individual differences in behavior. However, behavioral responses can also depend on the context, and consistent individual differences in one context do not guarantee consistent differences in another. To address this question, we assessed activity, exploration, and boldness in captive-bred poison frogs (*Dendrobates tinctorius*) across time and environmental contexts. Our comprehensive approach revealed consistent individual differences in some behaviors and context-specificity in

others. While activity and exploration were generally repeatable and correlated with one another, boldness was not. Especially in view of the emphasis on measures of boldness in the animal personality literature, our findings emphasize the importance of reiterative and holistic approaches in the study of animal behavior.

Keywords: behavioral syndromes, animal personality, repeatability, individual variation, Dendrobatid

53 **Introduction**

54 There is growing evidence that consistent individual differences in behavior exist across
55 taxa, and that these differences can have important consequences for individual fitness,
56 adaptive trajectories, and species' persistence (Bell et al. 2009). For example, bold and
57 aggressive individuals may be superior at territory defense but inferior at parental care
58 (Duckworth 2006). Such trade-offs can have consequences for individual fitness, leading to the
59 establishment of alternative behavioral strategies within populations and have been argued to
60 constrain adaptive trajectories (Smith and Blumstein 2008; Dochtermann and Dingemanse
61 2013). Moreover, certain personality types may fare better in the face of increasing human-
62 induced global change, for example if they are better able to adapt to increasingly urban
63 habitats (Pamela et al. 2015; Murgui and Hedblom 2017) or more likely to invade novel
64 environments (Brodin et al. 2013; Carvalho et al. 2013). Increased recognition of the complex
65 consequences of consistent individual behavioral differences have led to an increase in the
66 study of animal personalities across taxa. Animal personality is increasingly invoked to explain
67 behavioral differences in natural and captive populations, and to speculate on how these
68 differences may influence survival and adaptation. However, many studies do so without
69 confirming consistent individual differences across multiple contexts and over time. Assuming –
70 rather than testing – that individual behavioral variation is stable across contexts and time can
71 introduce bias, leading to adaptive inferences based on isolated incidents or context-specific
72 behavior (Bell et al. 2009).

73 Animal personality is broadly defined as consistent individual differences in behavior
74 (Réale et al. 2010; Carere and Maestripieri 2013). These differences are typically characterized
75 by quantifying behavioral repeatability, defined as the proportion of variance explained by
76 within-individual variation compared to between-individual variation (Bell et al. 2009; Bergmüller
77 2010; Garamszegi and Herczeg 2012). Animal personality encompasses an array of behavioral
78 traits, often with a particular focus on boldness:shyness, exploration:avoidance, and activity

(Réale et al. 2007; Garamszegi and Herczeg 2012; Michelangeli et al. 2019). Further, these traits can become "packaged together" into behavioral syndromes (Sih et al. 2004). For instance, studies spanning diverse taxa such as common voles (*Microtus arvali*) (Herde and Eccard 2013), convict cichlids (*Amatitlania nigrofasciata*) (Mazué et al. 2015), sea turtles (*Chelonia mydas)* (Kudo et al. 2021), and great tits (*Parus major*) (Cole and Quinn 2011) document positive correlations between boldness, activity, and exploration.

While the presence of animal personalities and behavioral syndromes have been documented in animals from primates to beetles, there is bias in taxonomic representation. While there is extensive research in non-human animals including birds (e.g., Réale et al. 2007; Carere and Locurto 2011) and fish (e.g., Bell 2005; Smith and Blumstein 2010; Mazué et al. 2015), amphibians have received comparatively less attention (but see Peignier et al. 2022; Sonnleitner et al. 2022). These biases arise in part from historical assumptions about the (behavioral) complexity of different animals. Amphibians were long viewed as behaviorally simple and stereotyped (Stamps 2007; Sih and Del Giudice 2012), yet evidence is accumulating that these creatures make complex, context dependent behavioral decisions (Kelleher et al. 2018). Studying individual behavioral variation in amphibians is critical for advancing our understanding of how these animals navigate risks and opportunities, such as the trade-off between mating advertisement boldness and predator avoidance (Stamps 2007; Sih and Del Giudice 2012). Importantly, this knowledge has broader implications for understanding adaptive strategies, ecological interactions, and conservation needs of amphibians, which is of particular importance given the alarming global decline of amphibian populations (Kelleher et al. 2018).

Among anurans (frogs and toads), poison frogs (family Dendrobatidae) offer compelling study subjects for exploring individual variation and correlations in behavior. Poison frogs are known for their bright coloration, toxicity, and interesting behaviors. These species' vivid hues are an honest signal of their toxicity, and both traits have coevolved with bold behavior and a conspicuous, diurnal lifestyle as compared to most anurans (Summers 2003; Maan and

105 Cummings 2012). Poison frogs exhibit a rich array of complex behaviors, encompassing anti-
106 predator defenses (Breed 2008), aggressive territoriality (Crothers and Cummings 2015),
107 parental care (Roland and O'Connell 2015; Fischer et al. 2019), spatial learning and navigation
108 (Liu et al. 2016; Pašukonis et al. 2022), and behavioral plasticity (Ringler et al. 2017; Peignier et
109 al. 2023). Taken together, these characteristics have garnered poison frogs increasing attention
110 in the realm of animal personality and behavioral syndromes. Existing studies in poison frogs
111 have examined consistent individual differences in activity (Sonnleitner et al. 2022), antipredator
112 behavior (Peignier et al. 2023), and aggression (Chaloupka et al. 2022). Despite these initial
113 insights, there remains a substantial knowledge gap regarding the extent of consistent individual
114 differences across time and context in poison frogs specifically, and amphibians more generally.
115 We used the Dyeing Poison Frog, *Dendrobates tinctorius*, to investigate personality and
116 behavioral syndromes across contexts and timeframes. We repeatedly assessed activity,
117 exploration, and boldness – in the same and in different assays – to test whether commonly
118 used personality metrics are robust to differences in assay design and timing (Fig. 1). Following
119 the methodologies of Kelleher et al. (2018), our experiments included an open field test, a threat
120 stimulus test, a novel environment test, and an activity in a familiar vs novel environment test
121 (Fig. 1). All experiments were built upon the open field test, a widely utilized method for
122 assessing activity levels, exploratory behavior, and boldness across taxa (Bell et al. 2009;
123 Carere and Locurto 2011).
124 In Experiment 1, we conducted an open field test in an artificial environment to establish
125 baseline activity, exploration, boldness, and the relationships among them (Fig. 1). This test
126 reflects the standard, basic design used for open field tests. In Experiment 2, we transitioned to
127 an open field test in a naturalistic environment, additionally assessing behavior before, during,
128 and after exposure to a threat stimulus (Fig. 1). In Experiment 3, we maintained the naturalistic
129 environment, but extended the duration of each trial, and directly compared behavior in a
130 familiar, naturalistic versus a novel, artificial environment (Fig. 1). Importantly, individuals were

assayed multiple times in each experiment (i.e., experienced multiple 'rounds' of each 'trial type' - terminology we use throughout the manuscript). This design allowed us to assess repeatability of commonly used behavioral metrics within and across contexts, and in response to multiple stimuli.

We predicted consistent individual differences in activity, exploration, and boldness across multiple rounds and the three distinct experiments. Further, we anticipated a behavioral syndrome, in which frogs with higher activity levels would also display increased boldness and exploration. Finally, we expected frogs to perceive the threat stimulus and the novel environment as stressful and exhibit context-dependent behavioral responses. Our overarching goal was to provide a comprehensive, nuanced understanding of how consistent individual differences manifest across diverse time and context, and to reflect on the implications of these patterns for extrapolation to ecological and evolutionary outcomes.

Methods

Animals

D. tinctorius were housed in our animal facility at the University of Illinois Urbana-Champaign. Terraria contained soil, moss, leaf litter, live plants, coconut shelters, and a small pond. For housing and all experiments, we maintained terraria and test arenas at 21°–23° C and 80%–90% humidity on a 12L:12D light cycle. We fed frogs *Drosophila hydei* or *Drosophila melanogaster* dusted with vitamins three times weekly. All animal care and experimental procedures were approved by the University of Illinois Animal Care and Use Committee (IACUC protocol #20147).

All behavioral experiments were conducted between March and April 2022. We used a total of 48 captive bred frogs (N= 20 adult males, N= 12 adult females, and N= 17 juveniles of unknown sex). All frogs were assayed in Experiments 1 and 2. In Experiment 3, we used a

157 subset of 19 frogs (N= 6 adult males, N= 8 adult females, and N= 5 juveniles; see additional
158 details below). For Experiments 1 and 2, we ran frogs sequentially in groups of five per week,
159 excluding the last group which had only four frogs. We followed the same protocol in a second
160 round of trials with the same groups of frogs. In essence, each individual underwent two
161 identical rounds, with a 5-week interval in between. For Experiment 3, we ran frogs in groups of
162 9 per week through a series of sequential trials that lasted a combined 25 hours (details below).
163 These trials were repeated three times with three weeks between each round. Although frogs
164 varied in age, all individuals were sexually inexperienced. Previous studies have demonstrated
165 that fed or digesting amphibians exhibit decreased levels of activity compared to fasted
166 individuals (Andrade et al. 2005). To ensure that the frogs were not actively digesting during the
167 experiments, we fasted frogs for 24 hours before behavioral trials.
168
169 *Experiment 1: Artificial open field test*
170 In Experiment 1, we investigated exploration, activity, and boldness in an open field test
171 in a novel, artificial environment (Fig. 1). The open field arena was a circular enclosure with a
172 diameter of 108 mm. To maintain humidity and moisture, we lined the arena with white bench
173 paper soaked in reversed-osmosis (RO) water. For each observation, we placed a single frog at
174 the center of the arena as a standardized starting point and recorded behavior for 15 minutes
175 using an overhead camera (DVC, HDV-604S). Individuals were immediately returned to their
176 home terraria following trials. We quantified exploration as the proportion of areas a frog visited,
177 activity as the number of times a frog crossed the line between areas, and boldness as time
178 spent in the center of area (Fig. 2) (additional details below).
179
180 *Experiment 2: Naturalistic open field and threat stimulus test*
181 In Experiment 2, we quantified activity and exploration in a naturalistic open field. The
182 behavioral arena was a 305 x 205 x 203 mm plexiglass tank that mimicked the frogs' home

183 terraria, including soil substrate, moss, leaves, and a live plant positioned in one corner. The
184 arena was completely covered from the outside with white contact paper, excluding a 240 x
185 169.5 mm exposed area opposite the plant for the presentation of the threat stimulus. As in
186 Experiment 1, we placed a single frog at the center of the arena at the beginning of each
187 observation and used an overhead camera to record behavior. We conducted three trials to
188 investigate behavior (1) pre-stimulus open field test (15 minutes after introduction to arena), (2)
189 during threat stimulus test (3 minutes of looming stimulus), and (3) post-stimulus open field test
190 (for 15 minutes after the stimulus).

191 We assessed activity and exploration using the same methods as in Experiment 1 (Fig.
192 2). Additionally, we measured boldness by observing the frogs' responses to a looming stimulus
193 (Fig. 2). Previous studies using leopard frogs (*Lithobates pipiens*) have shown reliable
194 avoidance behavior in response to looming objects (Ingle 1990; Waldeck and Gruberg 1995).
195 To present the looming stimulus, we used an iPad (Apple, 6th generation). The iPad was
196 covered in white tape, except for the display screen, and placed in the exposed area of the
197 arena (as shown Fig. 1). The looming stimulus was an expanding black circle projected
198 repeatedly onto the iPad screen for a duration of three minutes. To prevent habituation, random
199 pauses ranging from 1 to 30 seconds were incorporated into the stimulus presentation. During
200 the pre- and post- exposure trials, the iPad displayed a white screen to match the surrounding
201 environment. During the looming stimulus, we recorded activity and exploration, as well as
202 boldness based on whether the frogs approached or displayed avoidance behavior in response
203 to the stimulus.

204

205 *Experiment 3: Naturalistic open field and novel environment test*

206 In Experiment 3, we increased the duration of each trial to capture more prolonged
207 patterns in the frogs' behavior. To conduct these longer trials concurrently with Experiments 1
208 and 2 while also maintaining our repeated measures design, we selected a subset of individuals

209 (N= 19) from the previous experiments. The behavioral arena was constructed using the same
210 materials as Experiment 2. However, the dimensions of the arena were twice as long (610 x 305
211 x 203 mm) and the arena split into two halves. One side of the arena mimicked the frogs' home
212 terraria as in Experiment 2. The other side mimicked the novel, artificial environment of
213 Experiment 1, with only white, moistened paper flooring. The two sides of the arena were
214 separated by a white cardboard barrier, which could be removed without disturbing the frog or
215 the soil structure of the natural chamber.

216 We conducted three trials to investigate the frogs' activity and boldness: (1) baseline
217 activity in the novel, naturalistic environment (the first 90 minutes after introduction to the area),
218 (2) activity in after familiarization to the naturalistic environment (60 minutes after a 24 hour
219 acclimation period in the arena), and (3) exposure to a novel, artificial environment (60 minutes
220 after the removal of the barrier between the familiar and novel environment) (Fig. 1). Trials were
221 conducted sequentially, with each frog remaining in the arena for 25 hours while the trials
222 progressed. We conducted two subsequent rounds of trials for each individual, with three weeks
223 between each round. Due to the extended duration of each trial, we focused our analyses on
224 activity and boldness. We quantified activity using the same methods as in Experiment 1 and 2,
225 number of line crosses. To assess boldness, we measured the frogs' latency to cross into the
226 novel environment when the barrier was lifted (Fig. 2).

227

228 *Data analysis*

229 We describe general methods here with modifications for each experiment below. We
230 coded videos using Behavioral Observation Research Interactive Software (BORIS) ver. 8.13
231 (Friard and Gamba 2016). Experiment type was not blinded because assay identity was obvious
232 from videos, but all analyses were blind to individual identity. Behavioral data was analyzed with
233 R v.4.2.4 (R Core Team, 2022) in Rstudio v.4.2.2. (RStudio Team 2021). For all response
234 variables that were normally distributed, we used the raw data for analysis. Response variables

235 that did not meet expectations of normality were transformed as described below. For all
236 analyses, outcomes were considered significant at $p <= 0.05$.
237 We used the lmer function in the lmerTest package (Kuznetsova et al. 2017) for all linear
238 models, and always included frog ID as a random effect and round (i.e., repeated
239 measurements in the same assay) as a fixed effect. Additional fixed effects varied based on
240 experiment (see below). We tested for the effects of sex on behavior and found no significant
241 results. Therefore, and because not all individuals could be sexed at the time of the experiment,
242 we did not include sex as a factor in the analyses described below. We ran Type III ANOVAs on
243 linear models to generate test statistics and p-values for main effects and interactions.
244 Subsequently, we used the emmeans package (Lenth 2021) to run pairwise post-hoc
245 comparisons where appropriate.
246 We performed repeatability analyses using the rptR package (Stoffel et al. 2017), with
247 95% confidence intervals (CI) based on 1000 bootstrapping runs. Frog ID was used as a
248 random effect for repeatability (R) among individuals. We used Pearson correlation for normally
249 distributed data and Kendall correlation for non-normally distributed data to assess relationships
250 between behaviors. The correlation coefficients were interpreted using these criteria: ≈0.1 a
251 small effect, ≈0.3 a moderate effect, and>0.5 a strong effect (Garamszegi et al. 2013).
252
253 *Analysis Experiment 1: Artificial open field test*
254 We coded videos in BORIS using a transparent overlay that divided the behavioral arena
255 into 40 areas: 24 on the ground and 16 on the wall. From videos, we quantified exploration as
256 the proportion of areas a frog visited and activity as the number of times a frog crossed a line
257 between areas. Movement between areas was recorded when the majority of the frogs' body
258 entered into a new area. We measured boldness as time spent in the center four areas of the
259 arena (Fig. 2). We log transformed the activity and boldness data to improve normality. We ran
260 linear models for each behavior (activity, exploration, boldness) as described above, including

261 round as a fixed effect and frog ID as a random effect. We performed repeatability analysis with
262 Gaussian distributions for exploration and Poisson distributions for activity and boldness (Stoffel
263 et al. 2017). Data from 11 individuals was lost due to equipment failure, leaving a final sample
264 size of N=38 individuals. Analyses and results are based on these samples.
265
266 *Analysis Experiment 2: Naturalistic open field threat stimulus test*
267 We coded videos in BORIS using a transparent overlay that divided the behavioral arena
268 into 32 areas: 16 on the ground and 16 on the wall. We quantified and analyzed activity and
269 exploration as in Experiment 1, with the addition of a fixed effect of trial (pre-stimulus, during
270 stimulus, and post-stimulus) to test for pre- and post- stimulus behavior differences. To compare
271 trials of different duration, we divided each behavior by the total trial time.
272 For response to stimulus, which we used as a measure of boldness, we divided the
273 arena into three sections: (1) at stimulus (the two wall areas overlaying the iPad, the two floor
274 areas directly in front the iPad, and one wall area immediately to the left of the iPad), (2) near
275 stimulus (the two wall areas and the four floor areas surrounding the “at stimulus” area), and (3)
276 away from stimulus (the remaining areas) (Fig. 2). We noted the frogs' position before and after
277 stimulus presentation. We used a Chi-Squared test in the Tidyverse package (Wickham et al.
278 2019) for pairwise comparisons of responses (moved away, moved toward, or froze) (R Core
279 Team 2022). We fit rptR models with Gaussian distributions to test for repeatability of each
280 behavior across rounds and across trials (Stoffel et al. 2017).
281
282 *Analysis Experiment 3: Naturalistic open field novel environment test*
283 We coded videos in BORIS using a transparent overlay that divided the behavioral arena
284 into 96 areas: 16 areas on both the natural and novel arena floor, and 16 areas on the walls of
285 each chamber. We recorded activity as the number of times a frog crossed the line between
286 areas as in Experiments 1 and 2. After the divider was removed, we recorded the time it took for

287 a frog to move into the novel area as latency to explore, which we interpreted as a measure of
288 boldness (Fig. 2). We recorded activity on both sides of the chamber after the barrier was
289 removed. We ran linear models for activity and latency to explore, including round (i.e., repeated
290 measurements in the assay), trial (baseline, after acclimation, in a novel environment), and trial:
291 round interaction as fixed effects, and frog ID as a random effect. As in Experiment 1, behaviors
292 were divided by trial duration when analyzing trial effects.

293 To determine whether activity predicted an individual's latency to cross into a new area,
294 we filtered the data to only include observations taken during the novel environment test. We
295 then fit a similar linear mixed effect model to predict latency to enter the novel area with fixed
296 effects of activity, round, and their interaction, and frog ID as a random effect. We fit rptR
297 models with Gaussian distributions to test for repeatability of each behavior across rounds and
298 across trials (Stoffel et al. 2017).

299

300 *Cross-experiment analysis of activity and exploration*

301 In addition to behavioral analyses within experiments, we were interested in the behavior
302 of individuals across experiments. To compare exploration between Experiments 1 and 2, which
303 had different arena sizes, we calculated the proportion of areas explored. We did not quantify
304 exploration in Experiment 3, and it was therefore excluded from this analysis (Fig. 2). We
305 compared the ratio of areas explored in Experiment 1 to both the pre-stimulus and post-stimulus
306 exploration in Experiment 2 using linear mixed models, with exploration rate and experiment as
307 fixed effects (Bates et al. 2015). We tested repeatability using a Gaussian distribution (Stoffel et
308 al. 2017).

309 In all experiments, we measured activity by counting the number of line crosses (Fig. 2).
310 To compare across experiments of different duration, we divided the number of lines crossed by
311 the total trial time to calculate a line crossing rate. We compared the subset of individuals (n=
312 19) in Experiment 3 with the same individuals from Experiments 1 and 2. Baseline activity

measurements were similar across all experiments, taken prior to either the presentation of a stressor (looming stimulus test) or an environmental stressor (novel environment test) (Fig. 1). We also compared the post-stimulus activity in Experiment 2 to the activity observed during the novel environment test in Experiment 3, reasoning that these trials were similar in representing responses following an environmental change. We analyzed behavioral differences using activity rate and experiment as fixed effects and frog ID as a random effect (Bates et al. 2015). We calculated post hoc comparisons and repeatability as before.

Results

Experiment 1: Artificial open field test

Frogs displayed repeatability in exploration (R= 0.278, p= 0.029) (Fig. 3a) and activity (R= 0.279, p= 0.032) (Fig. 3b) and across both rounds separated by five weeks. However, time in the center of the arena, which we interpret as a measure of boldness, was not repeatable (R= 0.044, p= 0.395). No significant effect of round was observed for activity ($F_{1,37}$= 3.390, p= 0.074) or exploration ($F_{1,37}$= 1.392, p= 0.245, or boldness ($F_{1,74}$= 0.628, p= 0.431), suggesting that frogs' behavior did not change in response to previous experience in the arena. Activity and exploration exhibited a strong positive correlation (corr= 0.767, p< 0.001). Boldness was not correlated with either activity (corr= -0.150, p= 0.196) or exploration (corr= -0.119, p= 0.307) (Table 1).

Experiment 2: Naturalistic open field and threat stimulus test

Activity and exploration were repeatable across pre- and post- stimulus trials in both round 1 (activity: R= 0.593, p< 0.001; exploration: R= 0.421, p< 0.001) and round 2 (activity: R= 0.529, p< 0.001; exploration: R= 0.449, p< 0.001). We observed the same trend across rounds within trial (activity: R= 0.123, p= 0.006; exploration: R= 0.177, p< 0.001), aligning with the

results from Experiment 1 (Table 1). In contrast, during the looming stimulus presentation, there was no repeatability of activity (R= 0.201, p= 0.069) or exploration (R= 0.175, p= 0.145) across rounds.

We found an effect of trial on exploration ($F_{2,230}$= 112.143, p< 0.001) (Fig. 4a) and activity ($F_{1,234}$= 170.128, p< 0.001) (Fig. 4b), with frogs decreasing behaviors during the stimulus and then increasing post-stimulus (Fig. 4). We observed no significant round effect for activity ($F_{1,232}$= 0.570, p= 0.451) or exploration ($F_{1,234}$= 0.292, p= 0.590), nor an interaction between round and trial ($F_{2,228}$= 0.506, p= 0.604; $F_{2,228}$= 0.216, p= 0.801), indicating that frogs behaved consistently across multiple experiences in the arena. Activity and exploration were highly correlated across all trials (pre-stimulus, during stimulus, and post-stimulus) (corr= 0.7–0.8, p< 0.001).

We evaluated boldness by quantifying frogs' responses to the looming stimulus. Frogs' responses to the looming stimulus were not repeatable across rounds (R= 0, p= 1). We note this is likely because overall variation was low, as the majority of frogs demonstrated a freezing response during stimulus presentation rather than moving away ($\chi^2_{1,96}$= 19.755, p< 0.001) or towards ($\chi^2_{1,96}$= 41.796, p< 0.001) the stimulus. No significant effect of round was observed for boldness ($\chi^2_{2,96}$= 22.429, p= 0.401).

We found significant correlations between boldness (response to stimulus) and frogs' activity (tau= 0.155, p= 0.002) and exploration (tau= 0.155, p= 0.002) during the pre- and post-stimulus trials. These trends suggest that frogs displaying higher levels of activity and exploration prior to and after the stimulus were also more inclined to approach the stimulus during the threat stimulus trial. These correlations between boldness, activity, and exploration contrast with the results obtained in Experiment 1 (Table 1).

Experiment 3: Naturalistic open field and novel environment test

Frogs were repeatable for activity, both across trials (R= 0.321, p<0.011) and across

365 rounds (R= 0.651, p= 0.001). We found that frogs moved more with repeated experience in the
366 arena ($F_{2,143}$= 5.971, p= 0.003), with Round 1 having significantly less movement than Round 2
367 (t_{36}=-3.423, p=0.004) and Round 3 (t_{36}= -3.784, p= 0.002), but no significant difference between
368 Round 2 and 3 (t_{36}= -0.360 p=0.931) (Fig. 5a). Similarly, we found that frogs took less time to
369 cross the boundary into the novel area as rounds progressed ($F_{2,36}$= 13.100, p< 0.001), with
370 frogs taking longer to cross in Round 1 compared to Round 2 (t_{36}= 2.837, p= 0.019) and Round
371 3 (t_{36}= 5.108, p< 0.001), and was approaching significance between Round 2 and 3 (t_{36}= 2.270,
372 p= 0.073). We found significant repeatability for latency to explore across rounds (R= 0.388, p=
373 0.003) suggesting all frogs responded to repeated experience similarly.
374 We found a significant negative correlation between activity and boldness (*e.g.,* latency
375 to cross into novel environment; corr= -0.665, p< 0.001). Additionally, we found that boldness
376 was influenced by both activity ($F_{1,38}$= 28.795, p<0.001) and round ($F_{2,38}$= 6.546, p= 0.004),
377 indicating that more active frogs moved into the novel area more quickly, and all frogs moved
378 into the novel area more quickly with repeated experience.
379
380 *Cross-experiment analysis of activity and exploration*
381 Frogs exhibited consistent individual differences in exploration (R= 0.171, p= 0.005)
382 across Experiments 1 and 2. For activity, baseline activity was repeatable between the
383 naturalistic environments of Experiment 2 and Experiment 3 (R= 0.207, p< 0.001), and between
384 the artificial environment of Experiment 1 and the naturalistic environment of Experiment 3 (R=
385 0.171, p= 0.041). Activity was not repeatable between the artificial environment of Experiment 1
386 and the naturalistic environment of Experiment 2 (R= 0.029, p= 0.401). Activity was repeatable
387 across stressor trials (Fig. 1) (i.e., threat stimulus in Experiment 2 and novel environment in
388 Experiment 3) (R= 0.243, p= 0.003) (Fig. 5b).
389
390

Discussion

We investigated animal personality and behavioral syndromes in captive-bred poison frogs (*Dendrobates tinctorius*) by characterizing behavioral differences across multiple environmental contexts and timepoints. We conducted three experiments to assess activity, exploration, and boldness in the same individuals across varying conditions. We found repeatability in activity and exploration across time and contexts. In contrast, we observed context-specific behavior for our metrics of boldness, with consistent individual differences only for some measures of boldness. The stability of individual differences in behavior can carry profound implications for how species adapt to shifting environmental conditions (Wong and Candolin 2015; Merrick and Koprowski 2017), thereby shaping the evolutionary trajectory and ecological interactions of the species (Shaw 2020; Stiegler et al. 2022). Despite their declining populations, amphibians remain relatively understudied in this research area. The results of our work help to fill this gap, address broader issues in the field of “animal personality”, and set the stage for future work in *D. tinctorius*.

Our study revealed widespread repeatability for activity and exploration, evident across time, trials, and experimental contexts. These findings suggest that measures of activity and exploration are indeed reliable, and measurements in one assay or at one timepoint are likely to predict an individual’s behavior in another context or at another time. We also recovered the commonly described activity:exploration behavioral syndrome (Réale et al., 2007). While we expected a positive relationship between activity and exploration (i.e., exploration is impossible without movement), we note that there is room for variability in this relationship. For instance, frogs might have displayed a lot of movement but only within a restricted area, leading to heightened activity but limited exploration. The strong, significant relationships we recover (correlations > 0.76), suggest that these behaviors are indeed tightly linked and can therefore predict one another as well as some other behaviors (e.g., latency to explore a novel area, see below) under certain circumstances.

The above outcomes align with prior research on animal personality across taxa (Réale et al. 2007; Kelleher et al. 2018), and complement a recent investigation on another Dendrobatid, *Allobates femoralis*, which demonstrated the efficacy of both artificial and naturalistic setups in capturing consistent individual differences in behavior (Bégué et al. 2023). However, a study on *D. tinctorius* froglets by Sonnleitner et al. (2022) yielded somewhat different findings: the authors found significant repeatability at the family level but no significant repeatability at the individual level. These results suggests that genetic factors influence behavior and this effect outweighs individual differences when related individuals are reared under similar conditions. We were unable to control for relatedness in our study, but assume genetic differences explain at least some of the behavioral variation we document. A non-mutually exclusive explanation is that individuals in the study of Sonnleitner et al. (2022) were much younger than those used here, and individual differences in behavior may become more pronounced with age and experience (Bell et al. 2009). In any case, while consistent individual differences are being increasingly documented across diverse taxa, contrasting results even in the same species underscore the importance of considering various contexts and timepoints when extrapolating from single studies to evolutionary and ecological outcomes.

While we observed a high degree of repeatability in activity and exploration across time and contexts, the behaviors we considered as measures of boldness demonstrated contextual dependence. Our first measure was the commonly used metric of time spent in the center of a novel arena (Carter et al. 2012; Yuen et al. 2017; Baker et al. 2018; Rudolfová et al. 2022). We did not observe consistent individual differences in this measure, nor was it correlated with other behaviors. As our next measure of boldness, we assessed response to a threat stimulus in a familiar environment, aligning with prior studies (Ingle 1990; Waldeck and Gruberg 1995; Carter et al. 2012; Yuen et al. 2017). Once again, we did not find repeatability for boldness, although we did observe a correlation between this measure of boldness, activity, and exploration. In our final approach, we measured boldness as the latency to enter a novel environment, a less

443 common yet established method for evaluating boldness (White et al. 2013; Magnhagen et al.
444 2014; Theódórsson and Ólafsdóttir 2022). With this approach, we observed both repeatability
445 and a correlation between boldness and activity. Taken together, our results suggest that
446 commonly used boldness methodologies may not measure the same personality trait.

447 Despite the popularity of measuring boldness in animal personality studies, our findings
448 align with those of others who have explicitly compared outcomes across different metrics
449 categorized as 'boldness' and found conflicting results. For example, our approach and
450 outcomes are similar to that of Yuen et al. (2017) in their study on African striped mice
451 (*Rhabdomys pumilio*). The authors investigated whether distinct experimental methods were
452 indeed measuring boldness. Similar to our approach, they employed both artificial and natural
453 environments, and conducted two boldness tests, an open field, and a startle test. Yuen et al.
454 (2017) highlighted the distinction between these methods. The startle test evaluated risk-taking
455 behavior after threat exposure, potentially measuring anxiety rather than boldness, while the
456 open field test did not incorporate a preceding threat and proved to be a more precise method
457 for gauging boldness in their species. Carter et al. (2012) found related results when examining
458 chacma baboons (*Papio ursinus*), concluding that threatening situations may measure anxiety,
459 not boldness. Moreover, the use of artificial open field tests, particularly in evaluation of time
460 spent in the center, may not consistently capture boldness. Indeed, a recent study on Zebrafish
461 (*Danio rerio*) suggests that thigmotaxis may not effectively measure boldness or anxiety-like
462 behavior in this species (Rajput et al. 2022).

463 Translating these observations to our own results, our open field test focused on
464 evaluating time in center, the threat stimulus test observed behavior in the presence of a threat,
465 whereas the novel environment test examined behavior in a new and unfamiliar environment.
466 While we initially considered all tests as measures of boldness, our data suggest that the
467 specific nature of the challenges presented in each experiment and the environmental context
468 led to varying types and intensities of behavioral responses, and in turn distinct patterns of

repeatability and behavioral correlations.

Our findings also confirm the importance of considering the environment when measuring behavioral correlations: we observed correlations between boldness, activity, and exploration (i.e., behavioral syndromes) in naturalistic environments (Experiments 2 and 3) but not in artificial settings (Experiment 1) (Table 1). Alongside those of others, our observations highlight that the environment in which individuals are assessed can exert a profound influence on their behavior (e.g., Niemelä and Dingemanse 2014; Bégué et al. 2023) and the necessity of evaluating individual variation across diverse contexts (Bell et al. 2009).

Conclusions

Our findings demonstrate consistent individual differences in behavior across time and context in *D. tinctorius*, while also highlighting the subtle complexities of studying animal personality. While we found strong evidence for individual consistency in activity and exploration, our measures of boldness were variable and context dependent. Our results demonstrate the value of assessing multiple behavioral traits and the relationships among them to build a comprehensive view of behavioral repeatability and variability within and across species (Sih et al. 2004; Bell et al. 2009; Sih and Del Giudice 2012). Broadly, our results add to the growing literature emphasizing the importance of evaluating context-dependent patterns of individual behavioral variation, even for assay commonly considered as measuring the same trait (Bell et al. 2009; Carter et al. 2012, Yuen et al. 2017). More specifically, our findings contribute to the growing recognition of behavioral complexity in anuran amphibians, with implications for amphibian research, including their role as ecological indicators amid habitat degradation and climate change (Grant et al. 2019). Our findings here pave the way for future research on the impact of consistent individual differences on reproductive success, survival, and community dynamics.

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Code availability

All raw data and analysis code will be available on Dryad upon publication.

Author Contributions

KMS, FOH, HPA, and EKF conceived of and planned the experiments. KMS, FOH, and HPA carried out the experiments. KMS, FOH, and EKF contributed to data analysis and interpretation of the results. KMS wrote the manuscript with assistance from all authors.

Conflict of Interest

None.

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Tables

Table 1. Overview of statistical results for behavioral repeatability, correlations, and linear models. Exploration was not quantified in Experiment 3 and therefore no results are presented. Significance interpreted as: R= 0.2-0.4 moderate repeatability, corr≥0.5 strong correlation, p< 0.05 significant. Pearson correlation (corr) used for normal data, Kendall Rank correlation (tau) used for non-parametric data

Repeatability			
Behavior	**Experiment 1**	**Experiment 2**	**Experiment 3**
Activity	**R= 0.279, p= 0.032**	**R= 0.123, p= 0.006**	**R= 0.321, p= 0.011**
Exploration	**R= 0.278, p= 0.029**	**R= 0.177, p< 0.001**	NA
Boldness	R= 0.044, p= 0.395	R= 0, p= 1	**R= 0.388, p= 0.003**
Behavioral Syndromes			
Syndrome	**Experiment 1**	**Experiment 2**	**Experiment 3**
Activity:Exploration	**corr= 0.767, p< 0.001**	**corr= 0.911, p< 0.001**	NA
Activity:Boldness	corr= -0.150, p= 0.196	**tau= 0.155, p= 0.002**	**corr= -0.665, p< 0.001**
Exploration:Boldness	corr= -0.119, p= 0.307	**tau= 0.155, p= 0.002**	NA
ANOVAs			
Activity	**Experiment 1**	**Experiment 2**	**Experiment 3**
Trial	NA	**$F_{1,234}$= 170.128, p< 0.001**	**$F_{2,143}$= 6.312, p= 0.002**
Round	$F_{1,37}$= 3.390, p= 0.074	$F_{1,232}$= 0.570, p= 0.451	**$F_{2,143}$=5.971, p= 0.003**
Trial:Round	NA	$F_{2,228}$= 0.216, p= 0.801	$F_{4,,143}$ = 1.356, p= 0.252
Exploration	**Experiment 1**	**Experiment 2**	**Experiment 3**
Trial	NA	**$F_{2,230}$= 112.143, p< 0.001**	NA
Round	$F_{1,37}$= 1.392, p= 0.245	$F_{1,234}$= 0.292, p= 0.590	NA
Trial:Round	NA	$F_{2,228}$= 0.506, p= 0.604	NA
Boldness	**Experiment 1**	**Experiment 2**	**Experiment 3**
Round	$F_{1,74}$= 0.628, p= 0.431	$\chi^2_{2,96}$= 22.429, p= 0.401	**$F_{2,36}$ = 13.100, p< 0.001**

Figures

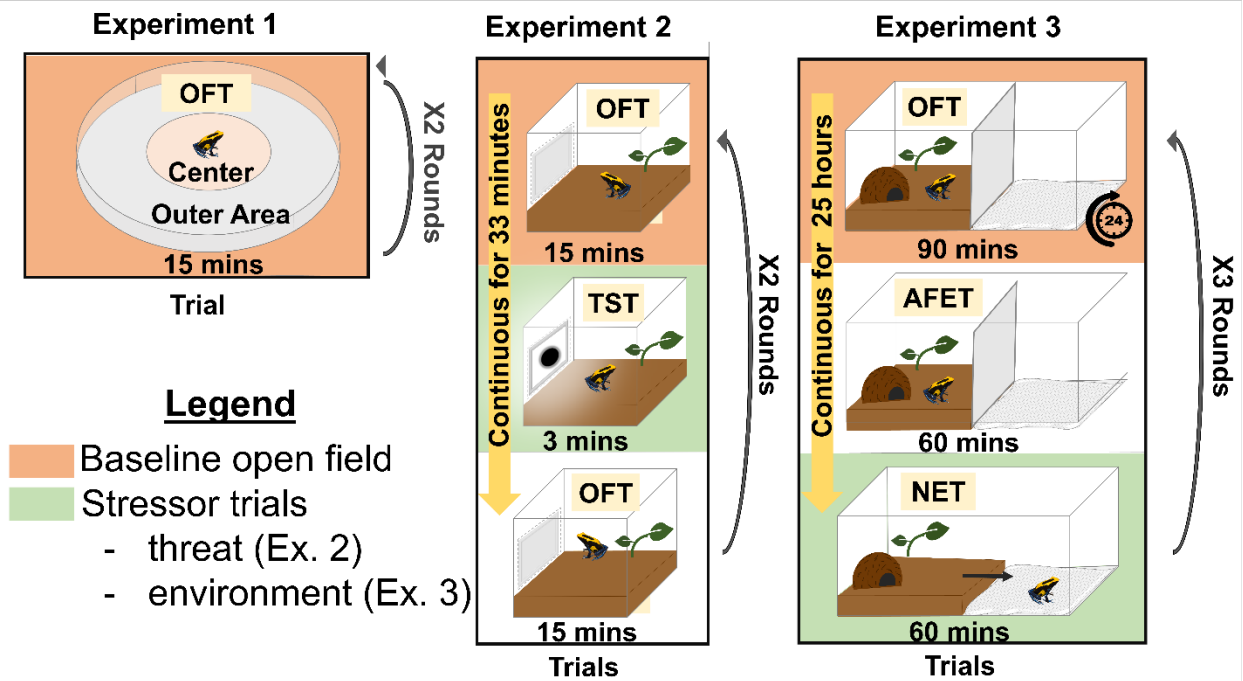

Figure 1. Overview of experimental design. All experiments were built on the framework of an open field test and expanded to explore differences in time and context. For each experiment, trial type (i.e., different phases within each experiment), trial duration, and number of rounds (i.e., repeated experiences in the same assay) are shown. OFT = open field experiment, TST = threat stimulus test, AFET = activity in a familiar environment test, NET = novel environment test. Colors represent corresponding trial types that were compared across experiments (See "Cross Analysis" Methods)

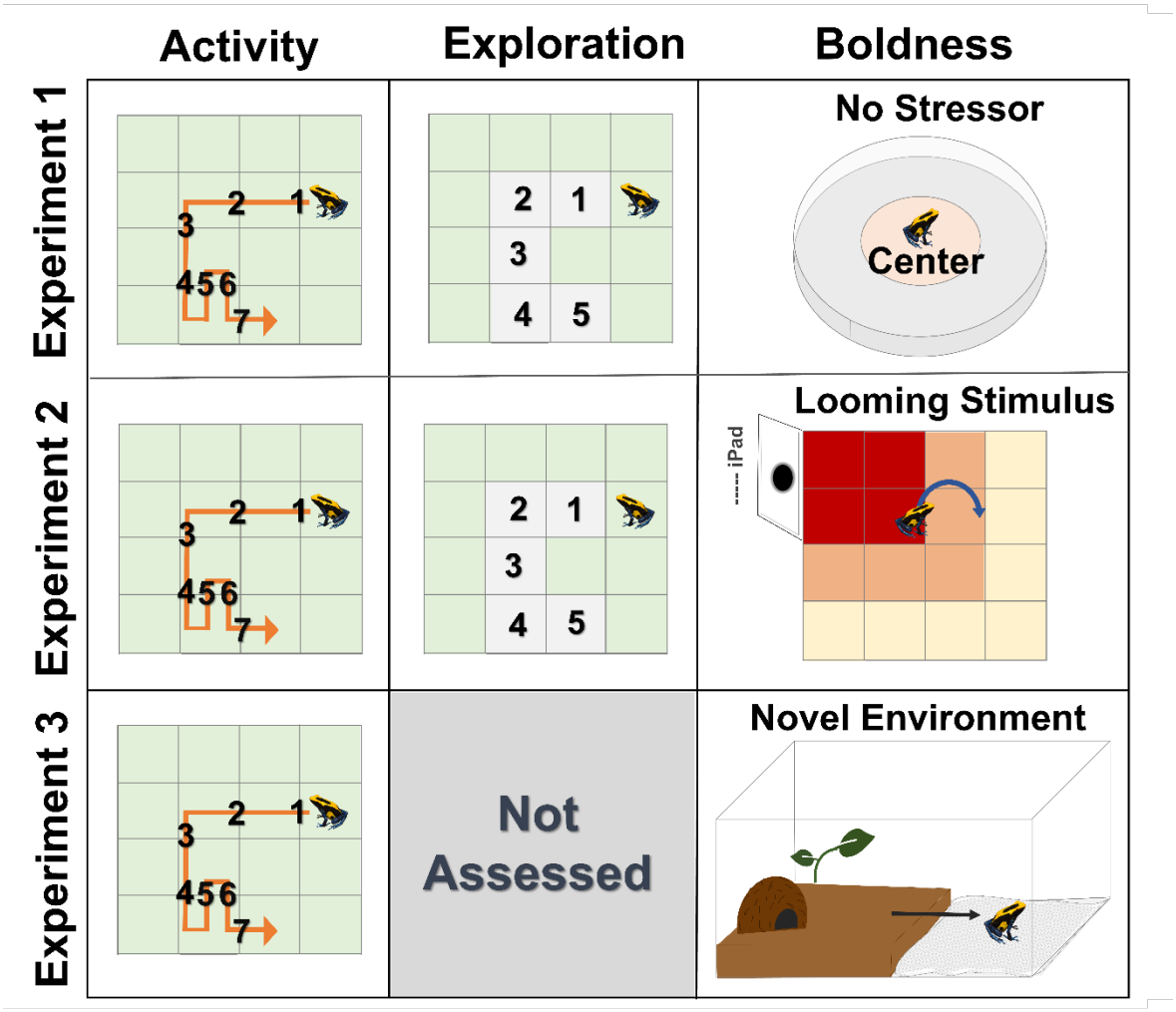

Figure 2. Overview of analysis methods. For all experiments, we quantified activity as the number of lines crossed from one area into the next and exploration as the total number of areas visited (not assessed in Experiment 3). Note that crossing back and forth from one area to another increases our measure of activity but not exploration. We quantified boldness as time in center (Experiment 1), response to a looming stimulus (Experiment 2), and latency to cross into a novel environment (Experiment 3)

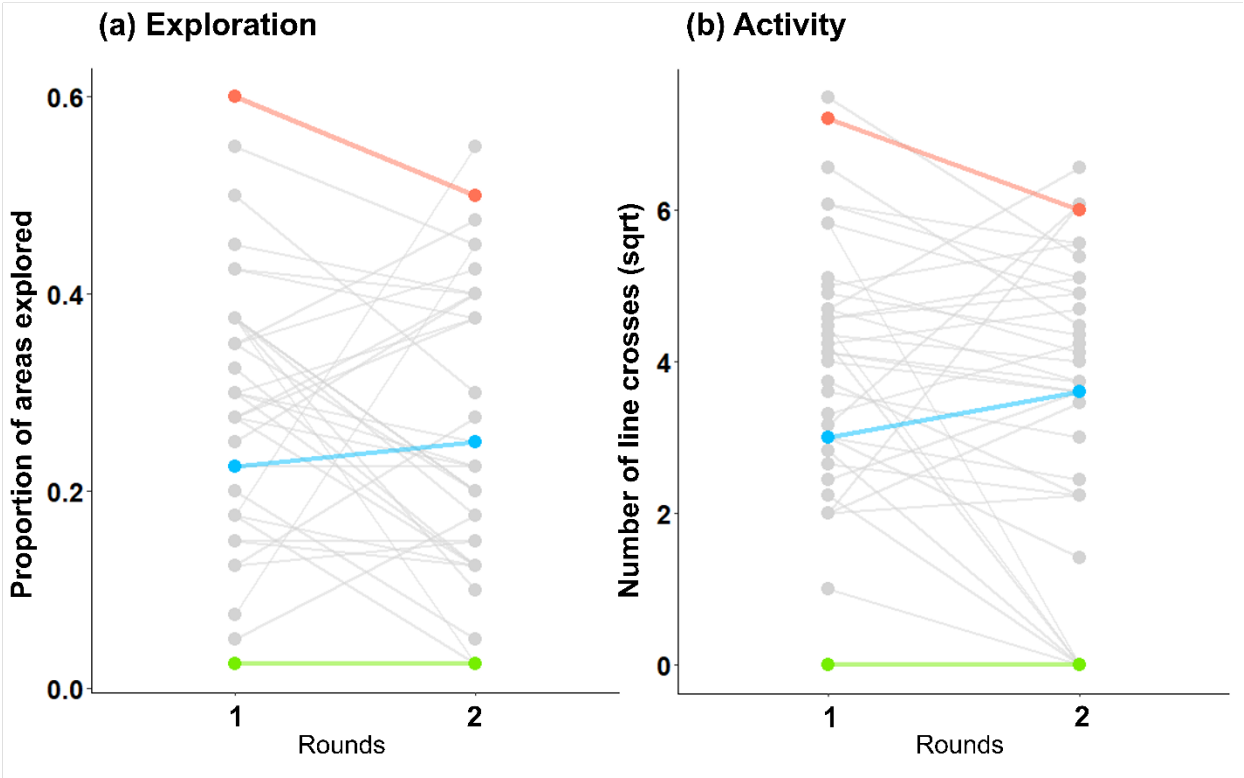

Figure 3. Behavior in an artificial environment open field test (Experiment 1). Frogs exhibited repeatability in (a) exploration (R= 0.278, p= 0.029) and (b) activity (R= 0.279, p= 0.032) across two rounds separated by five weeks. Three frogs are highlighted to showcase consistent individual differences in and the relationship between exploration and activity

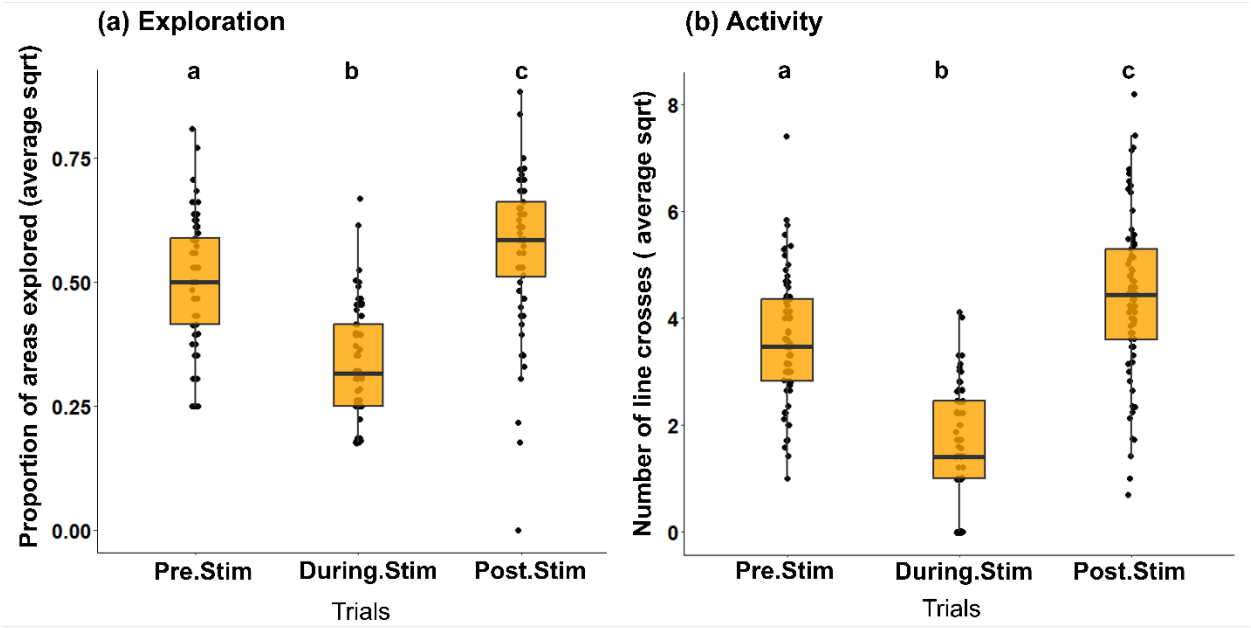

Figure 4. Behavior in a naturalistic open field before, during, and after a threat stimulus (Experiment 2). Exploration (a) and activity (b) decrease during stimulus presentation compared to pre-stimulus and post-stimulus ($F_{2,230}$= 112.143, p< 0.001; $F_{1,234}$= 170.128, p< 0.001), and these patterns were repeatable across the two rounds of the experiment. Data are collapsed across rounds, as no significant effect of round or a trial by round interaction were observed for exploration or activity

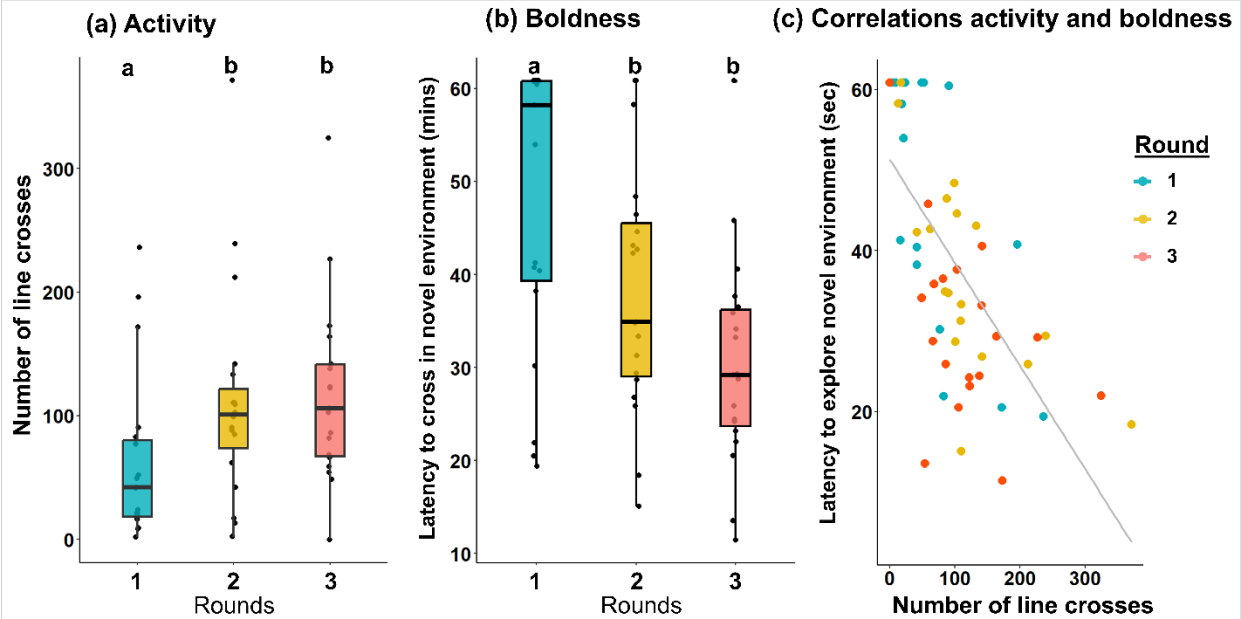

Figure 5. Behavior in a familiar, naturalistic environment versus a novel environment (Experiment 3). (a) Activity increased with repeated experience in the assay (i.e., across rounds; ($F_{2,143}$= 5.971, p= 0.003). (b) All frogs explored the novel area more quickly in subsequent rounds ($F_{2,36}$ = 13.100, p< 0.001), and (c) more active frogs explored the novel area sooner (corr = -0.655, p< 0.001). Letters in (a) and (b) indicate significant pair-wise differences between rounds