

Interpopulation Variability in Evasive Behavior in the Oregon Spotted Frog (*Rana pretiosa*)

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ABSTRACT.—Few data exist that quantify evasive behavior in postmetamorphic anurans. On the basis of our casual observations that Oregon Spotted Frogs (*Rana pretiosa*) from one of two different populations appeared to exhibit a more pronounced evasive response, we used a pseudopredator stimulus to quantify the evasive response of juveniles from the two populations. We drew test animals from a pool of animals for each population that were captive-reared under identical conditions. Using latency to initial response, we compared the distribution of response times between the two populations at two sequential intervals over the rearing period. In both experiments, the Conboy Lake population had shorter latency-to-response times than the Black River population. However, we also found that latency-to-response times were shorter during the second test interval than during the first test interval for each population. The basis of population differences in response times may reside in differences in the predator set influencing each population, contaminants differentially influencing the Black River population in a negative manner, or some combination of both. Explanation for the faster latency-to-response times in the second experiment is unclear since both maturation and the length of the captive rearing interval may contribute to the effect, but they are confounded in this study.

Antipredator responses represent behaviors basic to species survival. Moreover, understanding intraspecific variation in antipredator behavior is basic to elucidating the functional significance of alternative behavioral patterns in specific ecological contexts (Dowdey and Brodie, 1989; Ingle and Hoff, 1990; Brodie et al., 1991; Gomes et al., 2002). Our casual observations that juveniles of the aquatic ranid frog, *Rana pretiosa*, from one of two populations seemed to have a more rapid evasive behavior than individuals from the second population led us to a systematic evaluation of evasive behavior in this species. These observations were made during captive rearing of frogs for a pilot translocation program on Joint Base Lewis McChord (JBLM), Pierce County, Washington for this federal candidate (USFWS, 2010) and Washington State endangered species (McAllister and Leonard, 1997). Our primary interest was to evaluate the hypothesis that captive-reared frogs from the Conboy population exhibited faster escape responses (reduced latency to respond) than those similarly reared from the Black River population. However, this exploratory investigation was also intended to: 1) move toward developing a system that could be used ultimately to evaluate whether captive rearing, because it is a predator-free environment, might dilute antipredator responses; and 2) determine whether frogs from one source population are more likely to exhibit behaviors that improve survival than those from other populations and as a result, potentially be more successful in re-establishing a population.

MATERIALS AND METHODS

Study Animals.—In 2009 and 2010, we drew study animals from pools of juvenile Oregon Spotted Frogs that were obtained from each of two populations (Conboy Lake National Wildlife Refuge [Klickitat County] and the upper Black River [Thurston County], Washington). These animals were being reared at the Oregon Zoo for translocation to JBLM. Frogs were collected as eggs (ca. 400/yr) from their respective source populations in March of each year; reared in plastic containers (34.3 cm × 20.3 cm × 12.7 cm) to an approximately 2-cm total larval length on a diet of kale, romaine lettuce, and *Spirulina*; transferred to

Rubbermaid® plastic cattle tanks (1,136 L) for rearing to metamorphosis; and fed calcium and vitamin-dusted crickets during rearing as postmetamorphic juveniles. Frogs from each population were reared at the same densities in each year, but the sizes of the reared populations from which frogs were drawn in 2009 were less than one-third the size of those in 2010 ($n = 75$).

Behavioral Experiments.—We conducted all experiments using a ball-drop apparatus (Fig. 1) in a climate-controlled room at the Oregon Zoo where the temperature was maintained at 24.0 to 26.6°C. The presentation stage of this apparatus consisted of a white plastic container 61 cm long × 46 cm wide × 31 cm high filled with water to a depth of about 10 cm. Water was aged, had a pH of 6.8 to 7.1, and a temperature of 21.1 to 23.3°C. Aging water involved treating a separate 1,136-L tank with AmQuel® (Kordon LLC, 2242 Davis Court, Hayward, California 94545), a dechlorinating and denitrifying agent, at the appropriate dosage (13.2 ml of AmQuel per 100 L of water) and letting it stand for no less than 22 h before use. We used a water temperature range mimicking active-season daytime conditions for *R. pretiosa* because suboptimal temperatures have the potential to impair both physiological performance (Pough et al., 1992; Brodie and Russell, 1999) and influence antipredator response (Gomes et al., 2002). Water was deep enough to allow frogs to float with their front limbs off the bottom, but shallow enough that their hind limbs could touch bottom, which limited unnecessary energy expenditure (Pough et al., 1992). The pseudopredator stimulus in this apparatus was an orange-colored 10.2-cm-diameter plastic ball tethered to a rope strung through pulleys attached to the ceiling above the presentation stage to permit an investigator to move the ball from a concealed location; an opaque visual barrier about 85 cm high surrounding the stage concealed investigators. The ball could be released from about 60 cm over the water surface and a knot in the tether made the released ball stop ~1.3 cm above the water. We mounted a Canon® Vixia HF 200 video camera at an angle over the tank to enable the recording of test trials with a broad overhead view and produce an archive for subsequent scoring with a 30-frames/sec (0.03 sec) resolution. We selected frogs randomly from each population for each experiment.

We conducted an initial test of this apparatus on 14 juveniles selected from each of the aforementioned populations in August 2009 and measured latency to initial response. We elected to measure latency to initial response as we anticipated that this

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FIG. 1. Apparatus used in experiments in 2009–2010.

was the measure most likely to correspond to our casual observations of responsiveness. This test revealed that frogs from Conboy Lake had faster average latency-to-initial-response times ($x = 0.50$ sec, $SD = 0.11$ sec, range: 0.33–0.70 sec) than those from Black River ($x = 0.70$ sec, $SD = 0.35$ sec, range: 0.43–1.80 sec; t -test: $t = 1.970$, $P = 0.0303$). We used the same apparatus in 2010 with further refinements to the test protocol. First, similar to Pearl et al. (2004), we normalized metabolic output by withholding food from frogs in the selection pool for 12–18 h. Second, after selecting frogs, but before trials, we housed frogs for at least 20 min in closed but ventilated 0.95-L opaque plastic containers that contained water about 1 cm deep to minimize pretrial disturbance. Third, selection of the first frog in an experiment (e.g., which source population the frog was from) was determined by coin flip. We alternated source population for all subsequent frogs in an experiment. Last, after placing a frog in the apparatus, we began the trial (dropping the ball) only after a frog became stationary with its eyes positioned above water to ensure that the pseudopredator stimulus would immediately be perceived (body position was not controlled for in the initial 2009 experiment). After placement in the apparatus, we left a frog undisturbed for 60 sec, after which it was monitored through peepholes in the barrier to determine whether it had achieved the desired position.

We conducted two sequential experiments in 2010, one on 3 August, when frogs ranged from 2 to 6 g in mass, and one on 4 September, when frogs ranged from 8 to 15 g in mass. For each experiment frogs were selected arbitrarily from the rearing tanks; some frogs used in the second experiment may also have been used in the first. As frogs were not marked this was unavoidable.

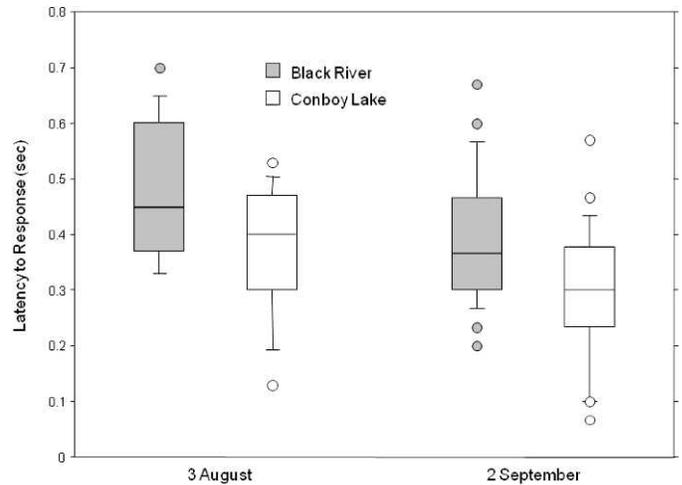


FIG. 2. Latency-to-response results (in seconds) from early (3 August) and late (2 September) experiments in 2010. Boxes encompass the median, and are bounded by the 25th (lower) and 75th (upper) percentiles; whiskers denote the 10th (lower) and 90th (upper) percentiles, and dots are data points falling below the 10th or above the 90th percentiles. See Table 1 for the sample sizes in each experimental population.

In the second experiment, we obtained individual body size (snout-to-vent length [SVL]), shank (fibulotibia), and mass measurements; in the first experiment, we had only an estimate of the range of body sizes and masses in each population sample.

Data Handling and Analyses.—We used Ulead Video© software (Ulead Systems North America, 970 West 190th Street, Suite 480, Torrance, California 90501) to review the video archive. We measured latency to response by counting video frames to determine the amount of time between the initial movement of the dropping ball and the initial frog evasive action. We defined initial evasive action as the first leg movement a frog made after the ball was dropped. Though the distribution of latency-to-response times was skewed in some cases (skewness values were $\leq |0.72|$), we considered these departures from normality within the range for which t -tests are robust (Zar, 1999). Because our preliminary experiment suggested that Conboy Lake frogs would have shorter latency-to-response times than Black River frogs, the 2010 tests examined the one-tailed null hypothesis that latency-to-response time for Conboy Lake frogs would be greater than or equal to that for Black River frogs. We also used one-tailed tests to examine the differences implied by the mean latency-to-response times within each population between our early and late experiments in 2010. As noted above, early vs. late experiments in 2010 were also constrained by differences in body size as a consequence of growth of the juvenile cohort, so we also examined whether a relationship existed between body size parameters (SVL and mass) and latency-to-response time using Pearson correlations. Last, our serial trial sequence resulted in frogs used later in an experiment being held for progressively longer periods of time in the opaque containers, so we examined whether a relationship existed between the sequence of when a frog was tested and the latency-to-response time using a simple linear regression.

RESULTS

Notwithstanding our more refined experimental approach in 2010, we obtained the same results in both 2010 experiments (Fig. 2) as in our preliminary experiment in 2009. In all cases,

TABLE 1. Variation in latency-to-response time (seconds) to a pseudopredator stimulus for juvenile Oregon Spotted Frogs (*Rana pretiosa*) in two experiments conducted in 2010. The reduced sample for the Black River population in the 3 August experiment resulted from four nonrespondent juveniles that were removed from calculation of the summary statistics. All tests are one-tailed (see text for details).

Experiment	Population	Latency-to-response time					Contrasts			
		<i>n</i>	<i>x</i>	SD	Minimum	Maximum	Intraexperiment		Intrapopulation	
							<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
3 August	Black River	10	0.48	0.13	0.33	0.70			1.98	0.028
	Conboy Lake	14	0.37	0.12	0.13	0.53	2.21	0.019	1.75	0.044
2 September	Black River	25	0.39	0.12	0.20	0.67				
	Conboy Lake	25	0.29	0.12	0.07	0.57	2.74	0.004		

Conboy Lake frogs had significantly shorter latency-to-response times than Black River frogs (Table 1), and the mean difference between populations in both 2010 experiments was about 0.1 sec. Four juveniles failed to respond to the pseudopredator stimulus in the early experiment; all four were from the Black River population. We had no practical way to include these animals when calculating the difference in latency-to-response time in the early experiment (Table 1). In contrast, all 25 frogs from each population responded in the late experiment.

The 2010 experiments also revealed significantly shorter latency-to-response times in the later experiment for both populations (Fig. 2, Table 1). This difference was similar in magnitude to the difference between populations. In the 2 September experiment, we recorded no significant differences among the three measures of body size between the Conboy Lake and Black River frogs (SVL: $t = 1.78, P = 0.082$; mass: $t = 0.40, P = 0.695$; shank length: $t = 0.40, P = 0.695$) and found no significant correlations between any of the three measures of body size and latency-to-response times ($r = |0.290|, P \geq 0.161$).

Regression of latency-to-response times on the sequence in which frogs were tested revealed no significant relationship for any population sample in the 2010 experiments (Table 2).

DISCUSSION

The consistency of our results gives us confidence that our hypothesis is supported, that juvenile *R. pretiosa* from the Conboy Lake population are on average faster than those from the Black River population. Rearing conditions between the two populations were identical, so we expect that the basis of the difference lies in some environmental difference to which the two populations are exposed in their natal habitat. One possible basis for the differences is in the predator set to which each population is exposed. Though details of the full predator set for both populations are not known, the Conboy Lake population is the only *R. pretiosa* population known that has been able to coexist with American Bullfrogs (*Lithobates catesbeianus*), and has

done so for over 60 yr (M. Hayes, unpubl. data). However, it needs to be acknowledged that the dropping-ball stimulus exhibits little resemblance to *L. catesbeianus* or other frogs that might approach floating aquatic prey (Werner et al., 1995; Pearl and Hayes, 2002; Wu et al., 2005), and is closer to what one might anticipate from an approaching avian predator (Martin and Lopez, 1990; Poulin et al., 2001). Alternatively or in addition, the slower latency-to-response behavior in the Black River population could reflect some impairment in antipredator response that reflects local conditions. Contaminants are known to impair antipredator responses, and impairment can sometimes be manifest as either the level or speed of response (Bridges, 1999; Punzo, 2005; Boone et al., 2005; Relyea and Edwards, 2010). That four Black River juveniles in the earlier 2010 experiment, when the juveniles were smaller, did not respond was a concern to us precisely because of such a potential scenario; the Black River population occurs in a more urbanized location where the likelihood of contamination is increased (Sprague and Nowell, 2008; Wittmer et al., 2010). Clearly, investigation of the environmental conditions influencing these two populations is a prerequisite to understanding the difference in response behavior.

The reduced latency to response that we observed in the second vs. the first experiment in both populations also has three potentially confounded explanations. A more rapid response linked to ontogeny may be the simplest. Documentation of a more rapid response time with growth and maturation is well known (Choi and Park, 1996; Gomes et al., 2002; Martin et al., 2005). Though the lack of relationship between metrics of body size and latency-to-response time might seem to contradict this possibility, the size range of frogs in the experiment was small and perhaps not large enough to show the correlation we might expect with an ontogenetic change in response. However, it is also possible that husbandry activities such as cleaning and feeding made the frogs progressively warier. Last, a more rapid response could also reflect a second exposure to the stimulus. Though we believe the latter two explanations less likely,

TABLE 2. Relationship between the sequence in which Oregon Spotted Frogs (*Rana pretiosa*) were tested and latency in response time for all population samples used in 2010 experiments.

Experiment	Population	Category	df	Mean square	<i>F</i>	<i>P</i>	Adjusted r^2
3 August	Black River	Regression	1	0.019	1.23	0.300	0.082
		Residual	8	0.015			
	Conboy Lake	Regression	1	0.002	0.13	0.729	0.010
		Residual	12	0.016			
2 September	Black River	Regression	1	0.027	1.94	0.177	0.038
		Residual	23	0.014			
	Conboy Lake	Regression	1	0.002	0.11	0.746	0.005
		Residual	23	0.015			

disentangling these explanations is of paramount importance to the captive-rearing effort for frogs such as these, which are targeted for translocation and intended to establish a new population. If husbandry is shown to effect predator escape behavior in captivity it may be possible to manipulate husbandry protocols to increase postrelease survival.

We envision rich opportunities in the study of postmetamorphic frog responses to predators or stimuli mimicking predators, a depauperate research area. Our finding of significant variability in such a simple behavioral parameter as latency to response in the first two *R. pretiosa* populations we examined leads us to believe that this general model has promise for evaluating antipredator responses. Notably, we see it as valuable in distinguishing among rearing regimes where frogs are drawn from the same populations. It also has promise for comparing wild and captive-reared frogs for dilution (or augmentation) in antipredator response. The dropping-ball pseudopredator stimulus is useful to evaluate rearing regimes or captive vs. wild frog responses. However, an evaluation of how the pseudopredator stimulus compares with the stimulus from actual predators from different *R. pretiosa* populations is a critical next step.

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