

EVOLUTIONARY EXPERIENCE WITH THE INVASIVE *LITHOBATES CATESBEIANUS* PREDICTS LOWER SURVIVAL OF LARVAL *RANA DRAYTONII*

RACHEL B. ANDERSON^{1,3}, JONATHAN P. ROSE², AND SHARON P. LAWLER¹

¹Department of Entomology and Nematology, University of California at Davis, One Shields Avenue, Davis, California 95616, USA

²Department of Wildlife, Fish & Conservation Biology, University of California at Davis, One Shields Avenue, Davis, California 95616, USA

³Corresponding author; e-mail: rachel.betts.anderson@gmail.com

Abstract.—Invasive intraguild predators can have particularly strong negative effects on native species due to the combined pressures of predation and competition. American Bullfrogs (*Lithobates catesbeianus*) can consume and compete with native anurans, including the California Red-legged Frog (*Rana draytonii*). *Rana draytonii* co-occur with *L. catesbeianus* in some areas but were likely extirpated by this intraguild predator in others. Previous work has shown that larvae of *R. draytonii* from populations that had coexisted with invasive *L. catesbeianus* responded behaviorally to their chemical cues whereas evolutionarily naïve individuals did not. To determine whether co-occurrence also affected larval body metrics and survival, we raised larval *R. draytonii* from populations evolutionarily experienced with *L. catesbeianus* (syntopic) and from naïve populations (allotopic) in replicated mesocosms with or without overwintered larval *L. catesbeianus*. We also varied habitat heterogeneity to test whether responses were habitat dependent. Surprisingly, individuals from evolutionarily experienced populations had lower survival across all treatments, indicating that responses to *L. catesbeianus* may have substantial costs to populations of *R. draytonii*. We also found that allotopic *R. draytonii* experienced reduced mass in the presence of overwintered *L. catesbeianus*, whereas syntopic individuals did not. Future research is needed to determine whether these costs are mitigated in natural habitats that are larger and more complex than the mesocosms, or if costs of responses to *L. catesbeianus* in the larval stage are balanced by benefits later in the life history.

Key Words.—American Bullfrog; California Red-legged Frog; conservation; intraguild predation; invasive species; mesocosms; survival

INTRODUCTION

Intraguild predators both consume and compete with their prey, and this combined pressure can drive strong selection (Polis et al. 1989; Anderson et al. 2013). Invasive intraguild generalist predators may spread quickly and have severe impacts on native prey (Polis et al. 1989; Polis and Holt 1992; Crowder and Snyder 2010; Hall 2011). Intraguild predation (IGP) is common among larval amphibians, where asynchronous breeding leads to size differences (Sours and Petranka 2007). Many larval anurans consume smaller individuals of other species (Ehrlich 1979; Petranka and Kennedy 1999; Schiesari et al. 2009) and compete within life stages for food (Petranka and Thomas 1995; Burley et al. 2006). Few studies have tested the extent to which natural selection might modulate the effects of IGP in these systems (Ingram et al. 2012).

Invasive American Bullfrogs (*Lithobates catesbeianus*) are large bodied in aquatic and terrestrial stages and can cause population declines of native anurans through IGP in both stages (Ehrlich 1979; Bury and Whelan 1984; Schwalbe and Rosen 1986; Pearl et al. 2004). The largest native ranid in California is the

California Red-legged Frog (*Rana draytonii*), which shares similarities with *L. catesbeianus*, including a generalist diet and highly aquatic lifestyle (Hayes and Tennant 1985). Populations of this federally listed threatened species decline in areas invaded by *L. catesbeianus* (Moyle 1973; Hayes and Jennings 1986; Fisher and Shaffer 1996; Davidson et al. 2001), and these declines could in part be due to larval interactions. *Lithobates catesbeianus* typically breeds in summer and the larvae overwinter, whereas *R. draytonii* breeds in late winter, such that its larvae are subject to IGP from older and larger larval *L. catesbeianus*. In a prior experiment, overwintered larval *L. catesbeianus* reduced survival of larval *R. draytonii* from 34% to < 5% and delayed their metamorphosis (Lawler et al. 1999).

Rapid evolution in response to species introduction is known for multiple vertebrate taxa and could mitigate the negative impacts of larval *L. catesbeianus* (Reznick and Endler 1982; Thompson 1998; Stockwell et al. 2003; Carroll et al. 2007; Stuart et al. 2014). Selection from *L. catesbeianus* can produce trait changes via contemporary evolution in native intraguild prey (Kiesecker and Blaustein 1997; Chivers et al. 2001; Anderson and Lawler 2016). A study of Northern Red-

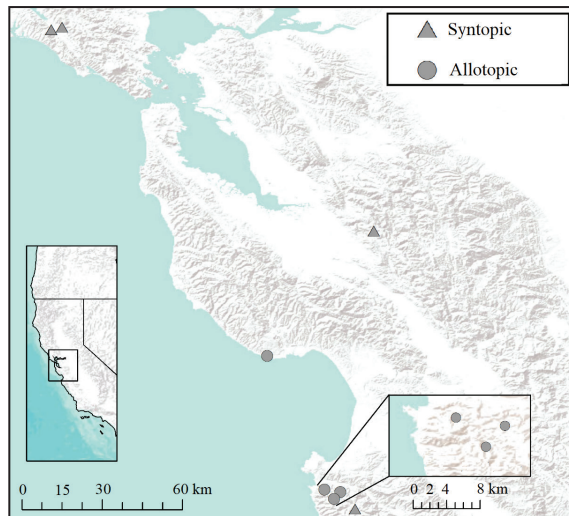


Figure 1. Collection sites for populations of California Red-legged Frog (*Rana draytonii*) in central California, USA. Triangles indicate populations syntopic with invasive American Bullfrog (*Lithobates catesbeianus*) and circles indicate allotopic populations. (Image source: ArcGIS, Esri, Redlands, California, USA).

legged Frogs (*Rana aurora*) found that populations syntopic to invasive *L. catesbeianus* experienced decreased predation from both larval and adult forms, compared to allotopic populations, likely due to changes in activity (Kiesecker and Blaustein 1997). Selection from competition and predation can cause opposing pressures on activity and morphology (Relyea 2004); however, recent evidence indicates that selection for ontogenetic shifts in behavior (e.g., reducing foraging activity when smaller body size increases risk of predation) might ameliorate both effects, as suggested by Werner and Anholt (1993). We found that larval *R. draytonii* from ponds that had been invaded by *L. catesbeianus* responded to cues from *L. catesbeianus* with reduced activity early in development, which could reduce predation, whereas larvae from uninvaded ponds did not respond (Anderson and Lawler 2016). Larvae from invaded ponds also became more active later in development. These larger larvae may have been less vulnerable to predation, and activity is associated with competitive ability in anuran larvae (Lawler 1989; Laurila 2000).

Rapidly evolved traits relevant to IGP could also include induced morphological changes, which are known to occur in larval amphibians in response to the presence of certain predators or competitors. Responses to predators can include shorter bodies, smaller trunks, and deeper tail fins, which may allow larvae to elude and/or better survive attacks (Lima and Dill 1990; Álvarez and Nicieza 2006; Nunes et al. 2014). Responses to competitors include shorter tails and longer bodies, which can facilitate foraging and rapid

growth (Smith and Van Buskirk 1995; Relyea 2000). Plasticity in morphology or behavior can also modify responses to predators (Van Buskirk 2002; Capellán and Nicieza 2007). When they occur, measurement of these plastic traits can give an indication of the strength of competitive or predatory interactions in a habitat (Relyea 2004). Selection by predators can cause evolution in trait plasticity (Nunes et al. 2014; Lewis et al. 2018), although plasticity can be both adaptive and non-adaptive (Ghalambor et al. 2007).

In addition to trait evolution, habitat complexity might mediate co-existence of *L. catesbeianus* and *R. draytonii*. Larvae of *R. draytonii* prefer areas with floating and emergent vegetation (Reis 1999) and increased plant cover is generally associated with amphibian diversity and decreased probability of extinction (Adams et al. 2011; Holzer 2014; Rowe and Garcia 2014). Increasing habitat complexity can minimize impacts of both predators and competitors (Devore and Maerz 2014). Complexity can reduce predation by providing more prey refuges or reducing encounter rates (Nelson 1979; Pope and Hannelly 2013; Hartman et al. 2014; Grutters et al. 2015) and may allow for amphibian populations to persist in the face of invasive predators (Hartel et al. 2007; Hartman et al. 2014). Complex habitats can reduce competition via similar mechanisms (MacArthur and Levins 1964). Conversely, anthropogenic simplification of the environment can facilitate competitive displacement (Petren and Case 1998).

We examined whether evolutionary experience and habitat complexity could mitigate the impacts of larval *L. catesbeianus* on larval *R. draytonii* by conducting a mesocosm experiment with *R. draytonii* that were either syntopic or allotopic with *L. catesbeianus*, varying presence of *L. catesbeianus* and habitat complexity. Our working hypotheses were that syntopic larval *R. draytonii* would have higher survival and body mass than allotopic larvae in the presence of overwintered larval *L. catesbeianus*, and complex habitat structure would allow for greater growth and survival of larval *R. draytonii* subject to IGP. We also examined the morphology of larval *R. draytonii* nearing metamorphosis to determine if morphological differences were associated with presence of the intraguild predator, evolutionary experience, or with habitat complexity.

MATERIALS AND METHODS

We collected eggs of *R. draytonii* in February–March 2014 from eight permanent ponds along the central coast of California (Fig. 1), taking one partial egg mass/population to minimize disturbance and limit collection to $\leq 5\%$ of reproductive output per pond, according to our permits. We collected partial egg

masses of four populations each from allotopic and syntopic locations, choosing sites that were ≥ 2 km apart to promote independence of populations. These were the same collections that we used for larval behavioral assays in a previous study (Anderson and Lawler 2016). We considered sites allotopic if *R. draytonii* was recorded at the locality and they were > 10 km from known occurrences of *L. catesbeianus*, or syntopic if *L. catesbeianus* had been recorded for ≥ 40 y based on a combination of sources including historical data, existing survey data, and interviews with land managers. However, we did not perform systematic annual surveys to confirm presence/absence throughout this entire period and thus our study represents the best possible estimate of their evolutionary history, given realistic logistical constraints. Surveys by RBA confirmed presence/absence of species during the previous 2 y. The above requirements limited the number of accessible allotopic sites such that full interspersions of allotopic and syntopic sites was not possible.

Egg masses were at Gosner (1960) Stage 15 or less when collected. We put egg masses in pond water and transported them to the lab in a food-grade plastic container on ice. We transferred egg masses into 2 L of R/O Right® (Kent Marine, Franklin, Wisconsin, USA) conditioned deionized water in the lab (2.5 ml/L), and kept them in a growth chamber until hatching, at 12° C (day; 14 h) and 9° C (night; 10 h). Upon hatching, we moved larvae to the Center for Aquatic Biology and Aquaculture (CABA) of the University of California at Davis, USA. We split individuals from each egg mass into two groups of 35 individuals and housed them in 30-L round flow-through tanks, fed by well water at 18–20° C. Larvae had *ad libitum* access to rabbit chow (Kaytee Products, Chilton, Wisconsin, USA), algae wafers (Hikari, Kyorin Food Inc. Ltd., Tokyo, Japan), and frozen spinach; we removed uneaten food and waste daily. We moved the larvae to these holding tanks on 28 March 2014, 4 d before the beginning of the experiment, when they weighed 0.11–0.35 g and were at Gosner stage 25.

All mesocosms consisted of 1,136-L round plastic cattle watering tanks (AquaBundance, Longmont, Colorado, USA). We arranged 32 tanks in four blocks of eight in a grassy field with scattered oaks outside the CABA facility. We filled each tank with well water, 22.68 kg of clean sand, 1 kg of oak leaf litter, and 50 mL of mixed zooplankton, including *Daphnia* and Ostracoda, from plankton net tows taken from wetlands in Davis, California, USA, on 8 March 2014. We added 100 g of rabbit chow to each mesocosm at the start of the experiment and again every three weeks until the experiment concluded to simulate resource inputs from land adjacent to ponds. To prevent colonization by other frogs, we coated the outside lip of each tank with

Vaseline® (Unilever, London, UK), and kept nearby grass trimmed. We left mesocosms uncovered to allow natural colonization by aquatic insects.

We used a $2 \times 2 \times 2$ factorial experimental design: simple/complex habitat, presence/absence of larval *L. catesbeianus*, and syntopic/allotopic larval *R. draytonii*, with the four populations of *R. draytonii* per experience category serving as replicates (32 mesocosm tanks in total). We added 35 larval *R. draytonii* per mesocosm. For the treatments with *L. catesbeianus* present, we added five overwintered, similarly sized larvae to each tank. Average size of overwintered larval *L. catesbeianus* was 7.54 g and Gosner stage 30–35. We collected larval *L. catesbeianus* by dip netting and seining Putah Creek in Davis, which is outside the current range of *R. draytonii*. Use of a single source population of predators is an established method (e.g., Kiesecker and Blaustein 1997; Lawler et al. 1999; Smith and Smith 2015) and serves as a common garden condition of predator exposure for populations of *R. draytonii*. Use of a variety of populations of *L. catesbeianus* would have required a large experimental design, and collection of many more threatened *R. draytonii*. We added larvae of both species on 2 April 2014; by this time, initial algal and bacterial blooms in the mesocosms had subsided.

To generate the habitat complexity treatment, we added vegetation to each tank on 15 March 2014. We used 7.5-L black plastic pots filled with clean sand for all live plants. The simple habitat treatment (approximately 1% cover) consisted of one potted Hardstem Bulrush plant (also known as Tule, *Scheuchzeria palustris*) harvested from Davis wetlands. The complex treatment (approximately 30% cover) consisted of four potted bulrushes as well as one potted Cattail (*Typha latifolia*) from a Davis wetland. We also added two submerged artificial plastic pine trees, 91 cm height and 35.5 cm diameter at base, to further increase habitat complexity while minimizing nutrient addition or competition between algae and macrophytes (Fig. 2). These habitat treatments are within the range of habitat complexities we observed in the field, in which some ponds almost entirely lacked vegetation whereas others had $> 90\%$ cover of submersed and emergent plants.

We monitored mesocosms daily to assess the general appearance and health of surviving larvae, and took water quality measurements at least weekly (temperature, dissolved oxygen, salt, pH, ammonia, nitrite, and nitrate) using a YSI Model 85 Handheld meter (YSI Incorporated, Yellow Springs, Ohio, USA) and Aquarium Pharmaceuticals Freshwater Master Test Kit (Mars Fishcare North America, Chalfont, Pennsylvania, USA). We added well water to mesocosms weekly, or as needed to offset losses from evaporation. We ended the experiment on 24 May 2014, when we first observed larval *R. draytonii* at Gosner stage 40 (forelimbs about



Figure 2. Habitat treatments of mesocosms for a study on survival and growth of larval California Red-legged Frogs (*Rana draytonii*): simple habitat (left) and complex habitat (right). Artificial plastic trees in the complex treatment are submerged and not visible. (Photographed by Matthew Savoca).

to emerge). We captured and counted all individuals in each tank over 3 d via hand net, checking all tanks daily until tanks were empty. We euthanized larvae in a buffered solution of tricaine mesylate (MS-222; Syndel, Ferndale, Washington, USA). We measured the snout-vent length (SVL) of larvae, weighed them to obtain wet mass, laid them flat, and photographed them laterally with a ruler for scale, ensuring the camera was at the same angle for all photos. We then preserved larvae in 90% alcohol. We used ImageJ (Rasband 2018) to measure photographs of larvae, including the linear variables of body height (ventral to dorsal) and SVL, total length, tail muscle height, and tail length as described in Relyea (2002). From these we calculated ratios of body height to total length, tail muscle height to body height, and tail length to total length. We used these proportions to ensure that morphological variables were independent of mass and overall size of individuals.

We collected samples of insect predators and competitors in mesocosms on 24 May 2014. We used a 10.6-cm aquarium net with 16-mm mesh and took three L-shaped sweeps from the center of the mesocosm tank along the substrate and up the side. We preserved all insects in 70% alcohol and identified them to family, using the dichotomous key in Merritt et al. (2008). Additionally, we collected any odonate exuvia present in or on each tank on that date.

We measured survival as the proportion of individuals of *R. draytonii* remaining at the end of the experiment. We created a Generalized Linear Mixed-effects Model with binomial distribution (binomial GLMM) that included the three interactive predictive factors of habitat complexity, evolutionary experience (allotopic/syntopic), and presence/absence of *L. catesbeianus*, a fixed effect of insect predator count, as well as random effects of tank (to account for non-independence of individuals within the same tank) and source population. We also tested for differences between treatments in individual body mass and morphological measurements of surviving larvae, using a Linear Mixed-effects Model

(LMM) with three interactive predictive factors of habitat complexity, evolutionary experience (allotopic/syntopic), and presence/absence of *L. catesbeianus*, as well as random effects of tank and population. We also included a fixed effect of predator count in the body mass and morphological models and included body mass as a covariate in the morphological models to account for possible changes in allometric relationships with body size.

To further explore the complex relationship between presence of *L. catesbeianus*, evolutionary experience of *R. draytonii*, and larval body mass, we calculated contrasts for the four *L. catesbeianus* presence \times evolutionary experience treatment groups. We performed post-hoc pairwise comparisons of the estimated means for these four treatments (averaging over the two habitat treatments) using the emmeans R package (Lenth 2019), while accounting for multiple comparisons using the Tukey method. We tested for differences in insect predator count among treatments to assess whether this uncontrolled variable may have influenced our results. We fit a GLMM with a Poisson response and interactive predictive factors of habitat complexity, evolutionary experience, and presence/absence of *L. catesbeianus* on insect predator counts, with random effects of tank and population. We used R v. 3.5.1 (R Core Team 2018) for all analyses and, specifically, the lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2017) packages to implement and evaluate the mixed effects models.

RESULTS

Evolutionary experience best explained survival of *R. draytonii*, with syntopic cohorts having the lowest survival under all treatments (Table 1; Fig. 3). The survival model estimated 9.7% survival for syntopic and 55.3% survival for allotopic larval *R. draytonii* ($Z = -2.447$, $df = 1,21$, $P = 0.027$). There were no detectable effects of other factors on survival, including presence of *L. catesbeianus*, habitat type, predator count, or

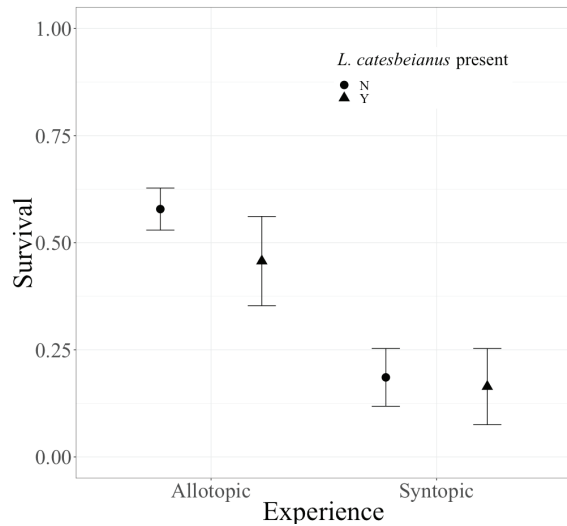


Figure 3. Mean survival of larval California Red-legged Frogs (*Rana draytonii*) from populations syntopic and allotopic to American Bullfrogs (*Lithobates catesbeianus*) in mesocosms with and without overwintered larval *L. catesbeianus*. Error bars represent \pm one standard error.

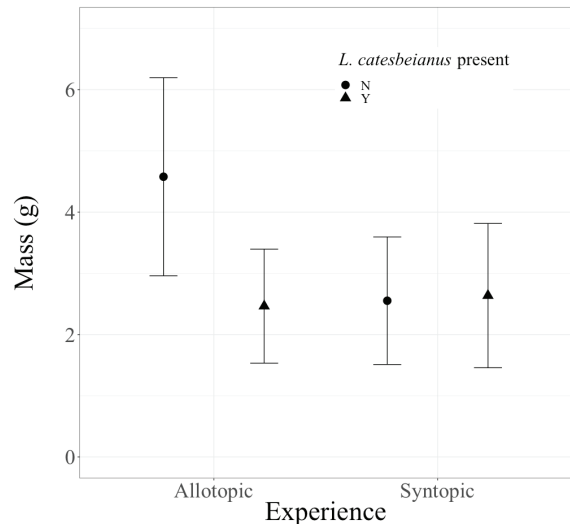


Figure 4. Mean mass of larval California Red-legged Frogs (*Rana draytonii*) from populations syntopic and allotopic to American Bullfrogs (*Lithobates catesbeianus*) in mesocosms with and without overwintered larval *L. catesbeianus*. Mass was averaged within individual mesocosms. Error bars represent \pm one standard error.

interactive effects. Survival of *L. catesbeianus* was uniformly high and did not vary across treatments, with all five individuals surviving in most tanks. Two tanks experienced mortality of larval *L. catesbeianus* (one and two larvae, respectively).

Larval *R. draytonii* had lower body mass in the presence of *L. catesbeianus* ($t = -2.146$, $df = 17$, $P = 0.023$; Table 2; Fig. 4). No other main effects or interaction terms were significant, indicating that presence of *L. catesbeianus* alone affected body mass of larval *R. draytonii*; however, although body mass was much lower for allotopic larval *R. draytonii* when *L. catesbeianus* were present, body mass appeared similar for syntopic larval *R. draytonii* with *L. catesbeianus* present or absent (Fig. 4). To elucidate this variation, we conducted *post hoc* pairwise comparisons among the four *L. catesbeianus* presence \times evolutionary experience groups. The only significant difference in body mass

was between allotopic larval *R. draytonii* with and without *L. catesbeianus* (difference in mean body mass = 2.23 g, $t = 3.79$, $df = 10.2$, $P = 0.015$). There were no detectable differences in mass between syntopic groups raised with or without *L. catesbeianus* (difference in mean body mass = 1.10 g, $t = 0.92$, $df = 14.4$, $P = 0.796$), nor between either syntopic group and either allotopic group.

The effect of habitat complexity on morphology of larval *R. draytonii* depended on evolutionary experience with *L. catesbeianus*. The ratio of body height to total length of allotopic larvae increased in simple habitat but decreased for syntopic larvae in simple habitat (Table 3). The ratio of maximum tail muscle height relative to body height significantly increased in syntopic larvae grown in simple habitat (Table 4). There were no detectable differences in tail length relative to total length across treatments (Table 5). There were no

Table 1. Estimates of influence of evolutionary experience (syntopy or allotopy), presence of overwintered larval American Bullfrogs (*Lithobates catesbeianus*), habitat type, and interactions on survival of larval California Red-legged Frogs (*Rana draytonii*) in a mesocosm study. Model residual degrees of freedom = 21. Bold type indicates significance ($P \leq 0.05$).

Fixed Effects (survival):	Estimate	Standard Error	Z value	$P (> Z)$
(Intercept)	0.215	0.746	0.288	0.774
Simple Habitat	0.218	0.728	0.299	0.765
Syntopy	-2.447	1.110	-2.206	0.027
<i>L. catesbeianus</i> Present	-0.812	0.732	-1.110	0.267
Predator Count	-0.089	0.240	-0.370	0.711
Simple Habitat \times Syntopy	-0.200	1.124	-0.178	0.859
Simple Habitat \times <i>L. catesbeianus</i> Present	0.285	1.064	0.268	0.789
Syntopy \times <i>L. catesbeianus</i> Present	0.879	1.115	0.788	0.431
Simple Habitat \times Syntopy \times <i>L. catesbeianus</i> Present	-1.276	1.631	-0.782	0.434

Table 2. Estimates of influence of evolutionary experience (syntopy or allotopy), presence of overwintered larval American Bullfrogs (*Lithobates catesbeianus*), habitat type, and interactions on body mass of larval California Red-legged Frogs (*Rana draytonii*) in a mesocosm study. Model residual degrees of freedom = 378. Bold type indicates significance ($P \leq 0.05$).

Fixed effects (mass)	Estimate	Standard Error	<i>t</i> value	<i>P</i> (> <i>t</i>)
(Intercept)	4.748	0.705	6.737	< 0.001
Simple Habitat	-0.242	0.799	-0.303	0.768
Syntopy	-1.351	1.157	-1.168	0.260
<i>L. catesbeianus</i> Present	-2.146	0.814	-2.636	0.023
Predator Count	0.137	0.438	0.312	0.759
Simple Habitat × Syntopy	-0.712	1.319	-0.540	0.599
Simple Habitat × <i>L. catesbeianus</i> Present	-0.162	1.191	-0.136	0.894
Syntopy × <i>L. catesbeianus</i> Present	1.750	1.349	1.297	0.215
Simple Habitat × Syntopy × <i>L. catesbeianus</i> Present	-1.244	2.350	-0.530	0.605

detectable differences in insect predator abundances across treatments (Habitat complexity, $Z = -0.404$, $df = 1,22$, $P = 0.600$; Evolutionary experience, $Z = -0.551$, $df = 1,22$, $P = 0.479$; presence of *L. catesbeianus*, $Z = -0.429$, $df = 1,22$, $P = 0.580$).

DISCUSSION

Surprisingly, syntopic larval *R. draytonii* had lower survival even when raised without *L. catesbeianus*, whereas the negative effect of the presence of *L. catesbeianus* on mass of *R. draytonii* was restricted to allotopic groups. The low survival of larval *R. draytonii* from populations syntopic with *L. catesbeianus* indicates that coexistence with this invasive intraguild predator may come at a high cost. Populations that coexist with *L. catesbeianus* may be more susceptible to other stressors. Indeed, field surveys indicate that the presence of *L. catesbeianus* predicts the absence of *R. draytonii* at breeding ponds (Anderson 2019).

Lithobates catesbeianus are strong interspecific competitors so their effect on mass of larval *R. draytonii* is unsurprising, though the mechanism by which they affected only allotopic groups is unknown. Anderson and Lawler (2016) showed that allotopic larvae did not respond to odor cues from *L. catesbeianus*, but larval *R. draytonii* may have modified their behavior

in mesocosms with *L. catesbeianus* where direct interactions were likely, reducing foraging in the presence of overwintered *L. catesbeianus*. Also, the capacity for growth may have been higher in the absence of *L. catesbeianus* due to less exploitative competition. In contrast, syntopic *R. draytonii* in lab trials were less active in early stages, so even when raised in the absence of *L. catesbeianus*, they may have been unable to capitalize on available resources.

The lower survival of larval *R. draytonii* from populations syntopic with *L. catesbeianus* could be explained by several factors including greater vulnerability to other predators, egg-borne infections, or maternal effects. In contemporaneous lab trials, we observed that syntopic larval *R. draytonii* suppressed their activity in response to cues from *L. catesbeianus* early in development, whereas allotopic larvae were less active and did not respond to cues (Anderson and Lawler 2016). Later in development, syntopic larvae were generally more active than allotopic larvae whether cues were present or not (Anderson and Lawler 2016). Decreased foraging in early development stages is associated with slow growth, which could lengthen time to reach a size refuge from predation (McCollum and Leimberger 1997, and references therein). Increased activity in later stages could increase predator encounters (Skelly 1994), thereby decreasing survival. Our insect

Table 3. Estimates of influence of evolutionary experience (syntopy or allotopy), presence of overwintered larval American Bullfrogs (*Lithobates catesbeianus*), habitat type, and interactions on body height relative to total length for larval California Red-legged Frogs (*Rana draytonii*) in a mesocosm study. Bold type indicates significance ($P \leq 0.05$).

Fixed effects (body height: total length)	Estimate	Standard Error	<i>t</i> value	<i>P</i> (> <i>t</i>)
(Intercept)	0.177	0.008	22.366	< 0.001
Simple Habitat	0.024	0.009	2.588	0.024
Syntopy	0.021	0.011	1.815	0.086
<i>L. catesbeianus</i> Present	0.003	0.010	0.271	0.790
Body Mass	0.001	0.001	1.247	0.213
Predator Count	-0.007	0.005	-1.381	0.185
Simple Habitat × Syntopy	-0.044	0.016	-2.785	0.014
Simple Habitat × <i>L. catesbeianus</i> Present	-0.021	0.014	-1.520	0.153
Syntopy × <i>L. catesbeianus</i> Present	-0.023	0.016	-1.470	0.160
Simple Habitat × Syntopy × <i>L. catesbeianus</i> Present	0.022	0.024	0.909	0.378

Table 4. Estimates of influence of evolutionary experience (syntopy or allotopy), presence of overwintered larval American Bullfrogs (*Lithobates catesbeianus*), habitat type, and interactions on maximum tail muscle height relative to body height for larval California Red-legged Frogs (*Rana draytonii*) in a mesocosm study. Bold type indicates significance ($P \leq 0.05$).

Fixed effects (tail length: total length)	Estimate	Standard Error	<i>t</i> value	<i>P</i> (> <i>t</i>)
(Intercept)	0.598	0.031	19.237	< 0.001
Simple Habitat	-0.054	0.040	-1.349	0.199
Syntopy	-0.074	0.046	-1.593	0.131
<i>L. catesbeianus</i> Present	-0.009	0.041	-0.218	0.830
Body Mass	0.002	0.003	0.765	0.445
Predator Count	0.007	0.021	0.352	0.730
Simple Habitat × Syntopy	0.154	0.066	2.346	0.032
Simple Habitat × <i>L. catesbeianus</i> Present	0.033	0.060	0.552	0.589
Syntopy × <i>L. catesbeianus</i> Present	0.074	0.064	1.141	0.270
Simple Habitat × Syntopy × <i>L. catesbeianus</i> Present	-0.041	0.102	-0.397	0.697

samples showed that predatory beetles and odonates readily colonized the mesocosms, but abundances did not differ among treatments.

Lithobates catesbeianus are known to carry pathogens, including *Batrachochytrium dendrobatidis* and *Ranavirus*, and their presence at breeding ponds could have contaminated egg masses (Schloegel et al. 2009; Martel et al. 2013). Syntopic groups, however, performed poorly whether or not they were exposed to *L. catesbeianus*. No disease symptoms were evident in individuals from the same egg masses in our laboratory study, where survival was uniformly high (60–70%; Anderson and Lawler 2016).

Maternal effects could influence survival and mass by influencing the resources allotted to each egg or the initial larval morphology (Relyea 2002). In a prior laboratory study of the same populations raised under ideal conditions, body condition and survival were similar between syntopic and allotopic individuals, suggesting that maternal effects or a genetic bottleneck are unlikely (Anderson and Lawler 2016). Such effects, however, might be exaggerated under more natural conditions in which animals must forage for food, survive natural enemies, and cope with a wider range of competitors and environmental conditions. It would require several years to raise the multiple generations of *R. draytonii* needed to eliminate maternal effects.

Habitat complexity alone had no detectable effect on mass or survival of larval *R. draytonii*, but habitat complexity interacted with evolutionary experience to affect morphology. In simple habitats, allotopic larvae showed increased body height relative to total length. A larger and taller body may be indicative of competitive pressure, which drives the formation of increased gut mass, allowing for increased foraging (Nunes et al. 2014). Simple habitats may have had lower productive surface area for periphyton growth, and thus increased competition for algal resources. In contrast, syntopic individuals in simple habitat showed a reduction in body height relative to total length, and deeper tail fin and tail muscle, both typically indicative of predatory pressure (Relyea and Werner 2000; Relyea 2004; Nunes et al. 2014). Habitat complexity may decrease the effectiveness of predators and allow small prey to more easily reach a size refuge from predation (Babbitt and Tanner 1998).

Because we only measured morphology at the conclusion of the experiment, and phenotypic responses can be plastic in amphibian larvae, differences among treatments may reflect conditions faced by later-stage larvae (Relyea 2003). Previous work has shown that when amphibian larvae reach later stages, they rely less on behavioral defenses (e.g., reduced foraging), and they may show more morphological responses to predators

Table 5. Estimates of influence of evolutionary experience (syntopy or allotopy), presence of overwintered larval American Bullfrogs (*Lithobates catesbeianus*), habitat type, and interactions on tail length relative to total length for larval California Red-legged Frogs (*Rana draytonii*) in a mesocosm study. Bold type indicates significance ($P < 0.05$).

Fixed effects (tail length: total length)	Estimate	Standard Error	<i>t</i> value	<i>P</i> (> <i>t</i>)
(Intercept)	0.640	0.014	46.676	< 0.001
Simple Habitat	-0.008	0.017	-0.490	0.638
Syntopy	-0.011	0.020	-0.531	0.604
<i>L. catesbeianus</i> Present	-0.009	0.017	-0.496	0.632
Body Mass	0.003	0.002	1.650	0.100
Predator Count	0.015	0.008	1.814	0.095
Simple Habitat × Syntopy	0.038	0.028	1.347	0.208
Simple Habitat × <i>L. catesbeianus</i> Present	0.011	0.025	0.437	0.673
Syntopy × <i>L. catesbeianus</i> Present	0.017	0.028	0.626	0.544
Simple Habitat × Syntopy × <i>L. catesbeianus</i> Present	-0.014	0.043	-0.331	0.748

(Relyea 2003). Additionally, morphological responses may have been more pronounced as a result of the experimental venue: phenotypic response to predation risk has been shown to be strongest in the mesocosm, where cues do not dissipate (Winkler and Van Buskirk 2012). Finally, it is possible that these differences in morphology are due to natural selection rather than plasticity: the differences observed could be in part a fixed response to the presence of *L. catesbeianus*, and/or only individuals with a particular morphology may have survived. Both plasticity and natural selection can act simultaneously to produce differences in morphology, and the relative contribution of each is currently unknown. Further work is needed to determine if these changes in morphology occur in pond-dwelling populations and how morphology may change during development when *R. draytonii* are exposed to *L. catesbeianus*.

Reduced survival of larval *R. draytonii* syntopic with *L. catesbeianus* was consistent across populations included in this study. We collected some egg masses from spatially clustered populations, with two syntopic populations only 2 km from each other in the north, and four populations (three allotopic, one syntopic) clustered in the southernmost portion of the study area, such that we cannot entirely rule out unknown, potentially confounding geographical effects. Additionally, use of a limited number of partial egg masses of *R. draytonii* due to conservation concerns may have reduced generalizability of the results due to low associated genetic variation. We note, however, that response variables were similar between our most distant pairs of syntopic and allotopic populations of *R. draytonii*. Further field study will show whether the presence of *L. catesbeianus* is associated with changes in other metrics, such as body size of terrestrial life stages, or fecundity of adult females, and the relative role that geography or human activity may have (e.g., Anderson 2019).

Acknowledgments.—We thank undergraduate interns Thomas Christoffel, Katie Clarkson, Isabella Fenstermaker, Gabriella Garcia, Dylan Hickey, and Andrea Montalvo for assisting with data collection, and Dr. Matthew Savoca and the staff of the Center for Aquatic Biology and Aquaculture for assisting with larval husbandry. We also thank Drs. Evan Eskew, Peter Moyle, and Brian Todd for improving the manuscript. A University of California Davis Jastro Research Award (2013–2014) funded this project. Specimens were collected under U.S. Fish and Wildlife Service permit TE63330A-2, California Department of Fish and Wildlife permit SC-11950, and University of California, Davis IACUC Protocol 17892.

LITERATURE CITED

- Adams, M.J., C.A. Pearl, S. Galvan, and B. McCreary. 2011. Non-native species impacts on pond occupancy by an anuran. *Journal of Wildlife Management* 75:30–35.
- Álvarez, D., and A.G. Nicieza. 2006. Factors determining tadpole vulnerability to predators: Can prior experience compensate for a suboptimal shape? *Evolutionary Ecology* 20:523–534.
- Anderson, R.B. 2019. Human traffic and habitat complexity are strong predictors for the distribution of a declining amphibian. *PLoS One* 14:e0213426. <https://doi.org/10.1371/journal.pone.0213426>.
- Anderson, R.B., and S.P. Lawler. 2016. Behavioral changes in tadpoles after multigenerational exposure to an invasive intraguild predator. *Behavioral Ecology* 27:1790–1796.
- Anderson, T.L., C.L. Mott, T.D. Levin, and H.H. Whiteman. 2013. Life cycle complexity influences intraguild predation and cannibalism in pond communities. *Copeia* 2013:284–291.
- Babbitt, K.J., and G.W. Tanner. 1998. Effects of cover and predator size on survival and development of *Rana utricularia* tadpoles. *Oecologia* 114:258–262.
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1–48.
- Burley, L.A., A.T. Moyer, and J.W. Petranka. 2006. Density of an intraguild predator mediates feeding group size, intraguild egg predation, and intra- and interspecific competition. *Oecologia* 148:641–649.
- Bury, R.B., and J.A. Whelan. 1984. Ecology and management of the Bullfrog. U.S. Fish and Wildlife Service Resource Publication 155:1–24.
- Capellán, E., and A.G. Nicieza. 2007. Trade-offs across life stages: Does predator-induced hatching plasticity reduce anuran post-metamorphic performance? *Evolutionary Ecology* 21:445–458.
- Carroll, S.P., A.P. Hendry, D.N. Reznick, and C.W. Fox. 2007. Evolution on ecological time-scales. *Functional Ecology* 21:387–393.
- Chivers, D.P., E.L. Wildy, J.M. Kiesecker, and A.R. Blaustein. 2001. Avoidance response of juvenile Pacific Treefrogs to chemical cues of introduced predatory Bullfrogs. *Journal of Chemical Ecology* 27:1667–1676.
- Crowder, D.W., and W.E. Snyder. 2010. Eating their way to the top? Mechanisms underlying the success of invasive insect generalist predators. *Biological Invasions* 12:2857–2876.
- Davidson, C., H.B. Shaffer, and M.R. Jennings. 2001. Declines of the California Red-legged Frog: climate, UV-B, habitat, and pesticides hypotheses. *Ecological Applications* 11:464–479.

- Devore, J.L., and J.C. Maerz. 2014. Grass invasion increases top-down pressure on an amphibian via structurally mediated effects on an intraguild predator. *Ecology* 95:1724–1730.
- Ehrlich, D. 1979. Predation by Bullfrog tadpoles (*Rana catesbeiana*) on eggs and newly hatched larvae of the Plains Leopard Frog (*Rana blairi*). *Bulletin of the Maryland Herpetological Society* 15:25–26.
- Fisher, R.N., and H.B. Shaffer. 1996. The decline of amphibians in California's Great Central Valley. *Conservation Biology* 10:1387–1397.
- Ghalambor, C., J. McKay, S. Carroll, and D. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* 21:394–407.
- Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190.
- Grutters, B.M.C., B.J.A. Pollux, W.C.E.P. Verberk, and E.S. Bakker. 2015. Native and non-native plants provide similar refuge to invertebrate prey, but less than artificial plants. *PLoS One* 10:e0124455. <https://doi.org/10.1371/journal.pone.0124455>.
- Hall, R.J. 2011. Eating the competition speeds up invasions. *Biology Letters* 7:307–311.
- Hartel, T., S. Nemes, D. Cogălniceanu, K. Öllerer, O. Schweiger, C.I. Moga, and L. Demeter. 2007. The effect of fish and aquatic habitat complexity on amphibians. *Hydrobiologia* 583:173–182.
- Hartman, R., K. Pope, and S. Lawler. 2014. Factors mediating co-occurrence of an economically valuable introduced fish and its native frog prey. *Conservation Biology* 28:763–772.
- Hayes, M.P., and M.R. Jennings. 1986. Decline of ranid frog species in western North America: Are Bullfrogs (*Rana catesbeiana*) responsible? *Journal of Herpetology* 20:490–509.
- Hayes, M.P., and M.R. Tennant. 1985. Diet and feeding behavior of the California Red-legged Frog, *Rana aurora draytonii* (Ranidae). *Southwestern Naturalist* 30:601–605.
- Holzer, K.A. 2014. Amphibian use of constructed and remnant wetlands in an urban landscape. *Urban Ecosystems* 17:955–968.
- Ingram, T., R. Svanback, N.J.B. Kraft, P. Kratina, L. Southcott, and D. Schluter. 2012. Intraguild predation drives evolutionary niche shift in threespine stickleback. *Evolution* 66:1819–1832.
- Kiesecker, J.M., and A.R. Blaustein. 1997. Population differences in responses of Red-legged Frogs (*Rana aurora*) to introduced Bullfrogs. *Ecology* 78:1752–1760.
- Kuznetsova, A., P.B. Brockhoff, and R.H.B. Christensen. 2017. lmerTest Package: tests in linear mixed effects models. *Journal of Statistical Software* 82(3) doi.org/10.18637/jss.v082.i13.
- Laurila, A. 2000. Competitive ability and the coexistence of anuran larvae in freshwater rock-pools. *Freshwater Biology* 43:161–174.
- Lawler, S.P. 1989. Behavioural responses to predators and predation risk in four species of larval anurans. *Animal Behaviour* 38:1039–1047.
- Lawler, S.P., D. Dritz, T. Strange, and M. Holyoak. 1999. Effects of introduced Mosquitofish and Bullfrogs on the threatened California Red-legged Frog. *Conservation Biology* 13:613–622.
- Lenth, R. 2019. emmeans: estimated marginal means, aka least-squares means. R package version 1.3.3. <https://cran.r-project.org/package=emmeans>.
- Levis, N.A., A.J. Isdaner, and D.W. Pfennig. 2018. Morphological novelty emerges from pre-existing phenotypic plasticity. *Nature Ecology & Evolution* 2:1289–1297.
- Lima, S.L., and L.M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68:619–640.
- MacArthur, R., and R. Levins. 1964. Competition, habitat selection, and character displacement in a patchy environment. *Proceedings of the National Academy of Sciences of the United States of America* 51:1207–1210.
- Martel, A., C. Adriaensen, M. Sharifian-Fard, M. Vandewoestyne, D. Deforce, H. Favoreel, K. Bergen, A. Spitzen-van der Sluijs, S. Devisscher, T. Adriaens, et al. 2013. The novel “*Candidatus Amphibiichlamydia ranarum*” is highly prevalent in invasive exotic Bullfrogs (*Lithobates catesbeianus*). *Environmental Microbiology Reports* 5:105–108.
- McCollum, S.A., and J.D. Leimberger. 1997. Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. *Oecologia* 109:615–621.
- Merritt, R.W., K.W. Cummins, and M.B. Berg. 2008. An Introduction to the Aquatic Insects of North America. 4th Edition. Kendall/Hunt Publishing Group, Dubuque, Iowa, USA.
- Moyle, P.B. 1973. Effects of introduced Bullfrogs, *Rana catesbeiana*, on the native frogs of the San Joaquin Valley, California. *Copeia* 1973:18–22.
- Nelson, W.G. 1979. Experimental studies of selective predation on amphipods - consequences for amphipod distribution and abundance. *Journal of Experimental Marine Biology and Ecology* 38:225–245.
- Nunes, A.L., G. Orizaola, A. Laurila, and R. Rebelo. 2014. Rapid evolution of constitutive and inducible defenses against an invasive predator. *Ecology* 95:1520–1530.
- Pearl, C.A., M.J. Adams, R.B. Bury, and B. McCreary.

2004. Asymmetrical effects of introduced Bullfrogs (*Rana catesbeiana*) on native ranid frogs in Oregon. *Copeia* 2004:11–20.
- Petranka, J.W., and C.A. Kennedy. 1999. Pond tadpoles with generalized morphology: Is it time to reconsider their functional roles in aquatic communities? *Oecologia* 120:621–631.
- Petranka, J.W., and D.A.G. Thomas. 1995. Explosive breeding reduces egg and tadpole cannibalism in the Wood Frog, *Rana sylvatica*. *Animal Behaviour* 50:731–739.
- Petren, K., and T.J. Case. 1998. Habitat structure determines competition intensity and invasion success in gecko lizards. *Proceedings of the National Academy of Sciences of the United States of America* 95:11739–11744.
- Polis, G.A., and R.D. Holt. 1992. Intraguild predation: the dynamics of complex trophic interactions. *Trends in Ecology & Evolution* 7:151–154.
- Polis, G.A., C.A. Myers, and R.D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annual Review of Ecology and Systematics* 20:297–330.
- Pope, K.L., and E.C. Hannelly. 2013. Response of benthic macroinvertebrates to whole-lake, non-native fish treatments in mid-elevation lakes of the Trinity Alps, California. *Hydrobiologia* 714:201–215.
- R Core Team. 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>.
- Rasband, W.S. 2018. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA. <https://imagej.nih.gov/ij/>.
- Reis, D.K. 1999. Habitat characteristics of California Red-legged Frogs (*Rana aurora draytonii*): ecological differences between eggs, tadpoles, and adults in a coastal brackish and freshwater system. M.Sc. Thesis, San Jose State University, San Jose, California, USA. 70 p.
- Relyea, R.A. 2000. Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. *Ecology* 81:2278–2289.
- Relyea, R.A. 2002. Local population differences in phenotypic plasticity: predator-induced changes in Wood Frog tadpoles. *Ecological Monographs* 72:77–93.
- Relyea, R.A. 2003. Predators come and predators go: the reversibility of predator-induced traits. *Ecology* 84:1840–1848.
- Relyea, R.A. 2004. Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology* 85:172–179.
- Relyea, R.A., and E.E. Werner. 2000. Morphological plasticity in four larval anurans distributed along an environmental gradient. *Copeia* 2000:178–190.
- Reznick, D., and J.A. Endler. 1982. The impact of predation on life history evolution in Trinidadian Guppies (*Poecilia reticulata*). *Evolution* 36:160–177.
- Rowe, J.C., and T.S. Garcia. 2014. Impacts of wetland restoration efforts on an amphibian assemblage in a multi-invader community. *Wetlands* 34:141–153.
- Schiesari, L., E.E. Werner, and G.W. Kling. 2009. Carnivory and resource-based niche differentiation in anuran larvae: implications for food web and experimental ecology. *Freshwater Biology* 54:572–586.
- Schloegel, L.M., A.M. Picco, A.M. Kilpatrick, A.J. Davies, A.D. Hyatt, and P. Daszak. 2009. Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American Bullfrogs (*Rana catesbeiana*). *Biological Conservation* 142:1420–1426.
- Schwalbe, C.R., and P.C. Rosen. 1986. Preliminary report on effect of Bullfrogs on wetland herpetofaunas in southeastern Arizona. Pp. 166–173 *In* R. C. Szaro, K.E. Severson, and D.R. Patton (Eds.). *Management of Amphibians, Reptiles, and Small Mammals in North America*. U.S. Forest Service, Fort Collins, Colorado, USA.
- Skelly, D.K. 1994. Activity level and the susceptibility of anuran larvae to predation. *Animal Behaviour* 47:465–468.
- Smith, G.R., and L.E. Smith. 2015. Effects of Western Mosquitofish (*Gambusia affinis*) on tadpole production of Gray Treefrogs (*Hyla versicolor*). *Herpetological Conservation and Biology* 10:723–727.
- Smith, D.C., and J. Van Buskirk. 1995. Phenotypic design, plasticity, and ecological performance in two tadpole species. *American Naturalist* 145:211–233.
- Sours, G.N., and J.W. Petranka. 2007. Intraguild predation and competition mediate stage-structured interactions between Wood Frog (*Rana sylvatica*) and Upland Chorus Frog (*Pseudacris feriarum*) larvae. *Copeia* 2007:131–139.
- Stockwell, C.A., A.P. Hendry, and M.T. Kinnison. 2003. Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution* 18:94–101.
- Stuart, Y.E., T.S. Campbell, P.A. Hohenlohe, R.G. Reynolds, L.J. Revell, and J.B. Losos. 2014. Rapid evolution of a native species following invasion by a congener. *Science* 346:463–466.
- Thompson, J.N. 1998. Rapid evolution as an ecological process. *Trends in Ecology & Evolution* 13:329–332.
- Van Buskirk, J. 2002. A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. *American Naturalist* 160:87–102.

Herpetological Conservation and Biology

Werner, E., and B. Anholt. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *American Naturalist* 142:242–272.

Winkler, J.D., and J. Van Buskirk. 2012. Influence of experimental venue on phenotype: multiple traits reveal multiple answers. *Functional Ecology* 26:513–521.



RACHEL B. ANDERSON received her Ph.D. in the Ecology Graduate Group at the University of California, Davis, USA, and her B.S. in Biology and Environmental Science from the College of William & Mary in Williamsburg, Virginia, USA. Her dissertation research focused on the effects of introduced American Bullfrogs on the native California Red-legged Frogs in Central California, USA. Her research interests include freshwater ecology, invasion biology, and amphibian conservation. (Photographed by Matthew Savoca).



JONATHAN P. ROSE is a Population Ecologist who studies invasive, threatened, and endangered amphibians and reptiles. Jonathan received his Ph.D. from the University of California, Davis, USA, in 2016. His dissertation research focused on the distribution, population dynamics, and eradication of non-native watersnakes in the genus *Nerodia* introduced to California, USA. Jonathan earned an M.A. in Geography and a B.S. in Biology from the University of Iowa, Iowa City, USA. (Photographed by Emily A. Miller).



SHARON P. LAWLER is a Professor of Entomology and Nematology at the University of California, Davis, USA. She is on the Editorial Board of *Ecology and Ecological Monographs* and is a contributing member of Faculty of 1000. Professor Lawler earned a M.S. in Ecology and a Ph.D. in Ecology and Evolution at Rutgers University, Piscataway, New Jersey, USA. Her thesis research demonstrated that innate activity level in tadpoles is an important predictor of how well different anurans withstand predation. For her dissertation research, she used microcosm as a model system to test various areas of food web theory. She extended this work as a Postdoctoral Researcher at the Natural Environmental Research Council Centre for Population Biology, Imperial College, London, UK, and at the University of Kentucky, Lexington, USA. Professor Lawler currently has research programs on the ecological and evolutionary effects of invasive species on herpetofauna, on freshwater ecotoxicology, and on how aquatic community structure affects mosquito populations. (Photographed by Scott Carson).