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Management and Conservation

Invasive Bullfrog Larvae Lack Developmental Plasticity to Changing Hydroperiod

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ABSTRACT Determining the mechanisms responsible for the success of invasive species is critical for developing effective management strategies. Artificially draining managed wetlands to maintain natural ephemeral conditions is a common practice in the Pacific Northwest and is assumed to kill invasive American bullfrog (*Lithobates catesbeianus*) larvae, which typically overwinter in permanent wetlands before metamorphosis. Bullfrogs in the Willamette Valley, Oregon, however, have invaded ephemeral wetland sites with confirmed metamorphosis within 4 months after hatching at 1 site. We hypothesized that plasticity in growth and development rates in response to hydroperiod facilitated bullfrog invasion in Oregon. We tested this hypothesis by quantifying larval bullfrog development and growth in response to 3 hydroperiod conditions in a mesocosm setting. We tested clutches collected from both ephemeral ($n = 3$) and permanent ($n = 3$) wetlands. We found no differences in development or growth due to hydroperiod treatments (body length, $P = 0.48$; mass, $P = 0.27$), but we found differences in growth among clutches ($P \leq 0.001$). These differences likely represent natural variation in growth rates because clutches collected from the same wetland type did not respond with similar growth and geographic barriers between collection sites did not account for the differences. These results indicate a lack of plasticity to hydroperiod and suggest that artificial hydroperiod manipulation in the Pacific Northwest will not induce rapid metamorphosis by invasive bullfrog larvae, although some genotypes may be capable of rapid growth and metamorphosis. © 2013 The Wildlife Society.

KEY WORDS amphibian larvae, bullfrog, development, growth, hydroperiod, invasive species, management, Pacific Northwest, plasticity.

Effective control of biological invaders requires understanding the mechanisms that lead to successful establishment (Schlaepfer et al. 2005). Numerous hypotheses attempt to explain why some species successfully invade particular systems and others do not, yet relatively few studies have conducted manipulative experiments to test these potential mechanisms of invasion success (notable exceptions include Petren et al. 1993, Kupferberg 1997, Holway 1999, Kiesecker et al. 2001, Rehage and Sih 2004). Rapid adaptation to local conditions or phenotypic plasticity to a range of environmental conditions may explain successful invasion across a large geographic region or environmental gradient (Sexton et al. 2002). Adapted versus plastic trait response must be considered when developing management strategies for invasive species because the nature of the response could influence the speed and extent of range expansion and novel habitat use, and the efficacy of a particular management strategy.

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The American bullfrog (*Lithobates catesbeianus*) is among the world's worst invaders based on its negative impacts to native amphibians and reptiles and its wide distribution (Lowe et al. 2000). These impacts include competition (Kiesecker and Blaustein 1997, Kupferberg 1997, Lawler et al. 1999), predation (Kats and Ferrer 2003), habitat displacement (Pearl et al. 2004), disease transmission (Daszak et al. 2004, Garner et al. 2006), and possibly breeding interference (D'Amore et al. 2009, Pearl et al. 2005*b*). These effects, however, have often been difficult to separate from correlated factors such as the presence of invasive fish (Hayes and Jennings 1986) and habitat change (Adams 1999). In their native range, bullfrogs breed during the summer months and larvae typically overwinter before metamorphosing, which requires permanent hydroperiods (Bury and Whelan 1984). Invasive bullfrogs in the northwestern United States are thus expected to breed in permanent hydroperiod habitat (Nussbaum et al. 1983). Based on this assumption, land managers often artificially drain wetlands at the end of the summer to promote natural ephemeral hydroperiod conditions and native vegetation and to kill bullfrog larvae (M. T. Cook, Oregon State University, personal observation). Recent observations indicate that bullfrogs are successfully reproducing in ephemeral habitats

in the southern Willamette Valley of Oregon. Bullfrog egg masses and larvae have been observed in multiple ephemeral wetlands, and larvae at 1 site successfully reached metamorphosis in less than 4 months after hatching (S. S. Heppell, Oregon State University, unpublished data; Cook 2011). If sites dry before bullfrog larvae can complete metamorphosis, those sites can become population sinks. If bullfrogs can plastically increase development in response to drying, however, artificial draining requires careful evaluation (Adams and Pearl 2007). If the artificial draining parameters (e.g., timing and speed) fall within the bullfrog's ability to respond plastically, this management practice could inadvertently benefit the invasive bullfrog.

These recent observations of invasive bullfrogs breeding in ephemeral habitats are concerning because this does not typically occur in their native range (Bury and Whelan 1984, Gahl et al. 2009). Experimental evidence from the native range, however, demonstrates plasticity in larval bullfrog development rates in response to hydroperiod and larval density. Overwintered (i.e., second-year) bullfrog larvae accelerated development and reduced time to metamorphosis in response to drying conditions (Boone et al. 2004) and bullfrog larvae from Ohio metamorphosed in a single season across a range of densities (Provenzano and Boone 2009). Developmental plasticity in response to hydroperiod is well documented for many amphibians that breed in ephemeral wetlands and risk desiccation before metamorphosis (e.g., Newman 1989, Denver et al. 1998, Laurila and Kujasalo 1999). Natural variation in larval development could have facilitated bullfrogs in successfully invading these novel environments in the western United States, although data on single season metamorphosis of invasive bullfrogs is largely anecdotal (Cohen and Howard 1958, Flores-Nava and Vera-Muñoz 1999).

Bullfrog invasion into ephemeral habitats could have significant conservation and management repercussions, such as increased bullfrog occupancy across the landscape and removal of habitat refuges for native amphibians that breed in ephemeral habitat. Bullfrogs can lay egg masses with up to 40,000 eggs, which is 20 times larger than the largest native clutch size in the Pacific Northwest (northern red-legged frog [*Rana aurora*], Jones et al. 2005). This extreme difference in reproductive output would likely exacerbate any negative interactions, including competition and predation impacts. In addition, artificially draining wetlands could accelerate development rates of bullfrog larvae, unintentionally selecting for a rapidly metamorphosing population of bullfrogs (Adams and Pearl 2007).

Exploring plasticity in larval bullfrog development rate will provide insight into the mechanisms behind invasive bullfrog population persistence and spread in the northwestern United States and help determine whether new habitats are at risk of bullfrog invasion. This, in turn, will help identify potential management actions and their expected effectiveness, such as artificial hydroperiod manipulation (i.e., draining). We hypothesized that invasive bullfrog larvae in the Willamette Valley, Oregon, would plastically respond to hydroperiod by altering development and growth rates. To test this, we quantified larval bullfrog development and growth in response to 3 hydroperiod conditions in a mesocosm setting. Secondarily, we compared responses between bullfrog larvae collected from ephemeral and permanent wetlands. By testing 1 clutch per site, we were able to determine genetic variation in development and growth between clutches and relate variation to source wetland type.

STUDY AREA

We collected bullfrog egg masses in 2010 from 6 sites in the Willamette Valley in western Oregon—3 permanent wetland sites and 3 ephemeral wetland sites. Collection sites were within a 35-km radius from Corvallis, Oregon (city coordinates 44.5708, −123.2760) and were a combination of federal refuge land (Finley and Ankeny National Wildlife Refuges), mitigation wetlands (Evergreen and One Horse Slough Mitigation Banks), and private land. We determined wetland hydroperiod classifications through personal communication with land managers and subsequently ground-truthing the classifications. For our study, we defined ephemeral wetlands as those that had a history of annual drying and were confirmed to completely dry in the summer or fall of 2009 (M. T. Cook, personal observation), corresponding with the typical drying times for amphibian-inhabited ephemeral wetlands in the Willamette Valley. Weather conditions during this time were typical for the region (NOAA National Climatic Data Center 2009). We selected our study sites based on a preliminary survey of many sites in 2009 where we documented dates of bullfrog egg deposition. We sampled a subset of those sites in 2010 that had active bullfrog breeding by early May.

METHODS

Field Collection and Animal Care

We collected 1 bullfrog egg mass (hereafter clutch) from each of 3 ephemeral wetlands (E1, E2, and E3) and 3 permanent wetlands (P1, P2, and P3), for a total of 6 clutches, from 24 June to 11 July 2010 (Table 1, Oregon Department of Fish and Wildlife Scientific Taking Permit # 099-10 and U.S. Fish and Wildlife Service Special Use Permit # 13590-10-04). We selected multiple sites for each habitat type to minimize the chance that more than 1 clutch was fertilized by the same male. We transported clutches to Oregon State University, Corvallis, Oregon (Animal Care and Use Protocol # 3915) and held them in 30-L high-density polyethylene plastic tubs filled with filtered tap water that had been treated with NovAqua Plus® and Amquel Plus® (Kordon, LLC, Hayward, CA). All tubs were located in an environmental chamber with constant temperature and photoperiod (18° C, 16L:8D) under full-spectrum lighting (Philips Natural Sunshine fluorescent bulbs, Philips, Andover, MA). After larvae hatched and absorbed yolk sacs, we fed them a 3:1 ratio of ground spirulina algae and brine shrimp flakes ad libitum (Brine Shrimp Direct, Ogden, UT). We performed water changes every 3–4 days, each time rotating tubs to different locations in the

Table 1. Bullfrog clutch collection sites and size estimates of larvae at the beginning of a mesocosm experiment that tested the effects of clutch and hydroperiod on larval bullfrog growth and development in the Willamette Valley, Oregon, USA, 2010. We collected clutches from ephemeral (E) and permanent (P) wetlands. Coordinates refer to clutch collection locations. Hatching date refers to when all larvae in the clutch completed hatching. Initial clutch body length and mass were based on 10 larvae per clutch measured immediately prior to the experiment.

					Body length (mm)		Mass (mg)	
Clutch	Latitude	Longitude	Collection date	Hatching date	$\bar{x}$	SE	$\bar{x}$	SE
E1	44.3282	−123.3497	24 Jun	02 Jul	4.2	0.4	15.7	4.6
E2	44.5071	−123.3394	25 Jun	02 Jul	4.8	0.5	23.6	8.2
E3	44.4278	−123.3098	11 Jul	17 Jul	3.2	0.2	7.8	0.7
P1	44.4097	−123.3355	24 Jun	02 Jul	4.5	0.3	16.8	2.9
P2	44.7760	−123.0862	25 Jun	02 Jul	4.2	0.3	17.3	3.4
P3	44.5627	−122.8710	29 Jun	08 Jul	3.9	0.2	13.5	3.2

environmental chamber to minimize the effects of differential heat distribution.

We held hatched larvae for 25–43 days in the lab at high densities (approximately 10–40 larvae per liter) before placing them in experimental mesocosms. We controlled for larval density as individuals grew larger by separating them into additional tubs. Variation in lab rearing time across all clutches was due to differences in breeding times at the 6 sites, with some clutches collected up to 17 days apart (Table 1). We reared all clutches until Gosner (1960) stage 25 in the lab to control for hatchling mortality (which we expected based on preliminary work in 2009) and initial development stage in the experiment. We estimated initial mean body size for each clutch by measuring 10 haphazardly chosen larvae per clutch; we did not use these larvae in the experiment. Clutch estimates of body length ranged from 3.2 mm to 4.8 mm, whereas wet mass ranged from 7.8 mg to 23.6 mg (Table 1). These size differences generally corresponded to larval age.

Experimental Design

We employed a 6 × 3 fully factorial mesocosm design with 6 bullfrog clutches and 3 hydroperiod treatments (slow-draining, fast-draining, and permanent). This was a reciprocal transplant experimental design, which is a type of common garden experiment. We exposed individuals from multiple populations to 3 hydroperiod treatments in an effort to identify potential genetic differences in developmental and growth rates. We tested genetic differences by keeping clutches separate, and secondarily tested source wetland type by using clutches from both permanent and ephemeral habitats. The slow-draining hydroperiod treatment mimicked a naturally drying wetland, whereas the fast-draining hydroperiod treatment mimicked a managed wetland in which water level decreases rapidly over a short period because of the removal of drainage barricades. We replicated each clutch and hydroperiod treatment combination 3 times and randomly assigned clutch-hydroperiod combinations to mesocosms (54 mesocosms total).

The experiment took place outdoors at the Willamette Research Station in Corvallis, Oregon (site coordinates 44.5361, −123.2486). Mesocosms were 120-L white high-density polyethylene tubs arranged in a 6 × 9 grid. We filled each mesocosm with well water and added 15 g of oak leaves (*Quercus kelloggii*), 2.5 g of ground alfalfa pellets, and 1.2 L of pond water to establish algal and macroinvertebrate communities. Mesocosms sat undisturbed for 12 days to allow algal growth and were covered with 3-mm mesh fiberglass frames to deter potential predators and colonists. We added temperature loggers in waterproof casings (ibutton model DS1922L®, accuracy ± 0.5° C; Maxim Integrated Products, San Jose, CA) to 2 randomly chosen mesocosms within each treatment combination; we logged water temperature every 60 minutes for the duration of the experiment. Each mesocosm had a half-inch diameter external standpipe to control water levels; lengths cut from the top of the standpipe allowed water to drain until the mesocosm water level matched the height of the standpipe. Water levels for all mesocosms initially began at 30.5 cm, which we maintained throughout the permanent hydroperiod treatment. Water level in the slow-draining hydroperiod treatment decreased by 1.5 cm every 3 days, whereas water level in the fast-draining hydroperiod treatment remained at 30.5 cm for 33 days and then decreased an average of 6.0 cm every 6 days.

We added larvae to mesocosms on 11 August 2010 (experimental day 0) at about 35 days old and Gosner stage 25; the experiment ended 6–7 October 2010 (experimental days 56–57). Each mesocosm contained 20 larvae from its assigned clutch. During the experiment, we sub-sampled 5 larvae from each mesocosm every week to measure development and growth over time. We averaged responses within mesocosms and measured Gosner developmental stage, body length (mm), and wet mass (g). We staged larvae with a dissecting scope, measured body length and wet mass, and then returned larvae to their mesocosm. At the end of the experiment, we measured all larvae and recorded the proportion of individuals that survived. We humanely euthanized surviving larvae in a solution of tricaine methanesulfonate (MS-222, 3 g/L water).

Comparison to Field Sites

To compare mesocosm results with field conditions, we surveyed each clutch collection site on 13–14 September 2010 (experimental days 33 and 34). Ephemeral sites E1 and E2 were completely dry on the sampling dates and we did not catch or observe any bullfrog larvae at site E3, despite extensive dipnetting (>100 sweeps). We recorded surface water temperature for E3, P1, and P2 at time of sampling. We collected bullfrog larvae using dipnet sweeps while

wading throughout the sites, completing at least 25 dipnet sweeps or until we caught at least 30 individuals per site. We humanely euthanized larvae with MS-222 and preserved them in 95% ethanol. Staging and body length measurements took place at a later date (we did not measure mass because of water loss from preservation). We differentiated second-year larvae from first-year larvae based on size, coloration, and developmental stage and excluded second-year larvae from comparisons with mesocosm larvae.

Statistical Analysis

To account for the variation in initial size of each clutch, we analyzed growth, defined as the increase in body length and mass during the experiment. We subtracted the initial mean value for each clutch from the corresponding mesocosms' final mean body length and mass measurements. We used residual plots and normal *Q–Q* plots to examine data for normality and homogenous variance. We angularly transformed proportion survived to normalize and homogenize variance; no other variables required transformation. We used analysis of variance (ANOVA) tests with clutch and hydroperiod treatment as fixed effects to determine their effects on proportion survived and growth in body length and mass. The ANOVA used the overall growth of the larvae (not the weekly sub-samples). We also used ANOVA to compare mean body length and Gosner stage among permanent field sites. For pairwise comparisons, we used Tukey's Honestly Significant Differences (HSD). We performed all analyses using the R statistical program (R Development Core Team 2010).

RESULTS

Mean daily mesocosm temperatures were similar among the 3 hydroperiod treatments (slow-draining: 19.5 ± 2.7° C, fast-draining: 19.8 ± 2.6° C, permanent: 20.0 ± 2.5° C; means ± SD). As water levels in the drained treatments decreased toward the end of the experiment, however, the drained hydroperiod treatments reached greater maximum temperatures and lower minimum temperatures than the permanent hydroperiod treatment (Fig. 1).

We found no interaction between hydroperiod treatment and clutch for any of the analyses (Table 2). Larvae exhibited no biologically meaningful differences in development due to hydroperiod treatment or clutch (Table 3), progressing from Gosner stage 25 (when independent feeding begins) at the beginning of the experiment up to Gosner stage 26.4 ± 0.3 (mean ± 2 SE; the beginning of hind limb bud development). We found no differences in body length and mass growth due to hydroperiod treatment (Fig. 2) but we found significant differences in growth as a function of clutch (Fig. 3). Clutches E2, P1, and P2 exhibited approximately twice as much growth as clutches E1 and P3, whereas clutch E3 overlapped both groups (from pairwise comparisons with Tukey HSD; Fig. 3). The pairwise groupings demonstrate that these clutch differences did not correspond to differences in source wetland type (Fig. 3). In addition, the differences in growth among clutches were consistent throughout the weekly sub-sampling (Fig. 4). We found no differences in

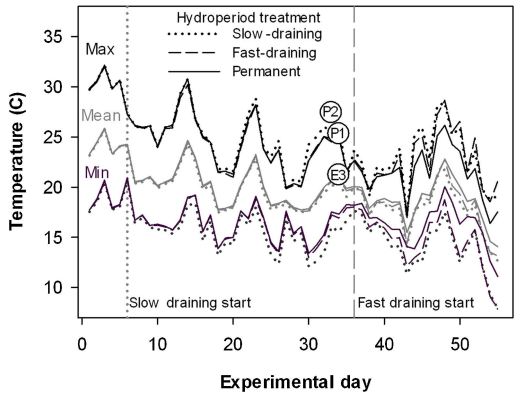

Figure 1. Mesocosm temperatures (° C) per hydroperiod treatment throughout our mesocosm experiment that tested the effects of clutch and hydroperiod on larval bullfrog growth and development in the Willamette Valley, Oregon, USA, 2010. Temperatures are averaged hourly readings from 0000 to 2400 hours every day from loggers in two-thirds of the mesocosms. Temperature profiles are similar between hydroperiod treatments for most of the experiment. Towards the end of the experiment, the drained treatments experienced higher maximum and lower minimum temperatures than the permanent hydroperiod treatment. A sub-sample of temperatures from field sites E3, P1, and P2 on experimental Days 33 and 34 suggests that mesocosm temperatures were comparable to field conditions.

proportion survived due to hydroperiod treatment or clutch (Table 2). Mean survivorship was 0.75 ± 0.06 (grand mean ± 2 SE).

Water temperatures taken at sites P1 and P2 were higher than the maximum mesocosm temperatures for all treatments on the corresponding dates (Fig. 1). We found little variation in mean Gosner stage for first-year bullfrog larvae among the permanent sites (26.1 ± 0.7 at site P1, $n = 27$; 27.3 ± 1.6 at site P2, $n = 47$; and 27.4 ± 1.6 at site P3, $n = 73$; means ± SD). These measurements were similar to the sites' respective clutches in the mesocosm experiment and were not meaningfully different from the stages measured in the hydroperiod treatments (Table 3). Larval size differed, however, with greater mean body length at site P3 (23.0 ± 4.4 mm) than at sites P1 and P2 (18.7 ± 3.3 mm and 19.4 ± 3.6 mm, respectively; means ± SD; ANOVA, $F_{2,145} = 17.03$, $P \leq 0.001$).

DISCUSSION

Our prediction of phenotypic plasticity in response to hydroperiod treatment was not supported. Instead, we found differences in larval bullfrog growth as a function of clutch, which suggests a genetically based response. These were not unique clutch-specific levels of growth; rather, clutch growth formed a gradient (pairwise comparisons separated the 6 clutches into 3 groupings, Fig. 3). This gradient in growth did not correspond to the source wetland types; clutches collected from both permanent and ephemeral wetlands were included at both ends of the gradient. We did not find geographical patterns between the 6 field sites, such as distance or barriers, that corresponded to the gradient. This suggests a genetic basis to growth rate, regardless of environmental conditions.

Table 2. Analysis of variance results for a mesocosm experiment that tested the effects of clutch and hydroperiod on larval bullfrog growth and development in the Willamette Valley, Oregon, USA, 2010. We found no differences in growth due to hydroperiod treatment for body length or mass, but we found differences in growth among clutches. We found no differences in survivorship to the end of the experiment. SS represents sum of squares and MS represents mean square.

Variable	Source	df	SS	MS	*F*	*P*-value
Body length growth (mm)	Clutch	5	257.72	51.55	11.36	≤0.001
	Hydroperiod treatment	2	6.86	3.43	0.76	0.477
	Clutch × hydroperiod	10	48.44	4.84	1.07	0.411
	Residuals	36	163.34	4.54		
Mass growth (g)	Clutch	5	2.25	0.45	9.86	≤0.001
	Hydroperiod treatment	2	0.12	0.06	1.35	0.272
	Clutch × hydroperiod	10	0.45	0.04	0.98	0.478
	Residuals	36	1.64	0.05		
Proportion survived (arcsine square root transformed)	Clutch	5	0.30	0.06	0.75	0.591
	Hydroperiod treatment	2	0.21	0.10	1.31	0.283
	Clutch × hydroperiod	10	0.48	0.05	0.61	0.798
	Residuals	36	2.86	0.08		

Numerous models of larval amphibian growth propose that greater growth rates confer higher fitness in nearly any environmental condition; thus, growth rate can relate to competitive ability (Wilbur and Collins 1973, Smith-Gill and Berven 1979, Travis et al. 1987). The Wilber–Collins model predicts that a minimum size threshold must be reached before metamorphosis can occur. In an ephemeral hydroperiod, individuals with a greater growth rate will reach the size threshold faster and be able to undergo metamorphosis sooner, which will help avoid habitat drying-induced mortality. In a permanent hydroperiod, greater growth rates would allow individuals to maximize growth before metamorphosis, potentially increasing size at metamorphosis and reducing age at first reproduction, which both influence lifetime fitness (Berven and Gill 1983, Smith 1987, Semlitsch et al. 1988, Chelgren et al. 2006). The clutch differences we observed could indicate that the clutches with greater growth would be more competitive and more likely to survive in a range of hydroperiod conditions. Werner (1986) expanded on the growth-only models, however, and proposed that the optimum size at metamorphosis will change depending on the ratio of size-specific mortality and growth between the aquatic and terrestrial habitats. Under this model, metamorphosing at a smaller size is not necessarily disadvantageous if the ratio of mortality to growth is greater in the aquatic habitat than the terrestrial habitat. In an ephemeral hydroperiod, smaller size would need to correspond with faster development, which we did not observe.

Table 3. Mean Gosner stage by clutch and hydroperiod treatment at the end of a mesocosm experiment that tested the effects of clutch and hydroperiod on larval bullfrog growth and development in the Willamette Valley, Oregon, USA, 2010. We collected clutches from ephemeral (E) and permanent (P) wetlands. The number of mesocosms is indicated by *n*. We found no biologically meaningful differences in Gosner stage among clutches or hydroperiod treatments.

		Gosner stage	
	n	$\bar{x}$	2 SE
Clutch			
E1	9	25.7	0.3
E2	9	26.1	0.1
E3	9	25.9	0.2
P1	9	26.2	0.2
P2	9	26.4	0.3
P3	9	25.6	0.3
Hydroperiod treatment			
Slow-draining	18	26.0	0.2
Fast-draining	18	26.0	0.3
Permanent	18	26.0	0.2

Balancing trade-offs between drying-induced mortality and size at metamorphosis can maintain genetic variation in growth rate (Alford 1999). This variation in larval bullfrog growth could result in local variation in bullfrog invasion risk and bullfrog success in different hydroperiod conditions. Our experimental design prevents us from determining whether clutch effects are due to additive genetic variation, nonadditive genetic variation, or maternal effects that could be due to nongenetic factors (Harris 1999). In amphibians, maternal effects largely manifest via offspring egg size. Egg size can influence larval growth and development, with larger eggs producing larvae that grow larger and develop faster (Loman 2002). In some instances, these effects may depend on environmental conditions, with initial larval size influencing growth and development more in lower quality (e.g., drying) environments than in higher quality environments (Parichy and Kaplan 1992), although we did not observe this in our study. The source origin of introduced bullfrog populations could also contribute to genetic differences. Recent genetic work found that bullfrogs in the Willamette Valley are likely attributed to more than 1 introduction from the Mississippi River basin and the Great Lakes region, though further differentiation was not possible (Funk et al. 2011). Regardless of the underlying mechanism, however, the implications of clutch differences in growth on larval survival and adult fitness remain.

Ephemeral wetland habitats are vital for native amphibian reproduction. Even if bullfrog larvae cannot metamorphose in a single season, their presence in ephemeral habitats still negatively affects native amphibians through competition for food and refuge. Understanding which habitats are at greater risk of bullfrog invasion can help target management and conservation efforts for native amphibians. Although native amphibians initiate seasonal breeding before bullfrogs, native

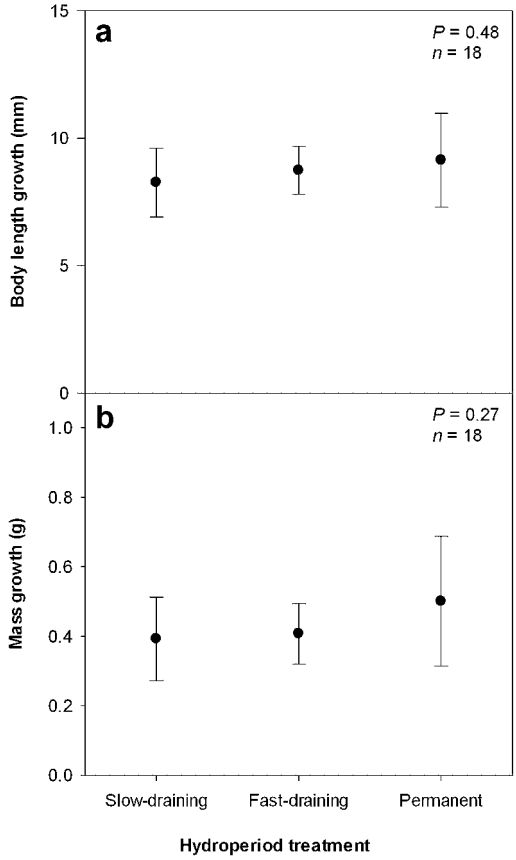

Figure 2. Growth in body length and mass (means ± 95% individual CI) per hydroperiod treatment during a mesocosm experiment that tested the effects of clutch and hydroperiod on larval bullfrog growth and development in the Willamette Valley, Oregon, USA, 2010. We found no differences in body length growth (a) or mass growth (b) due to hydroperiod treatment.

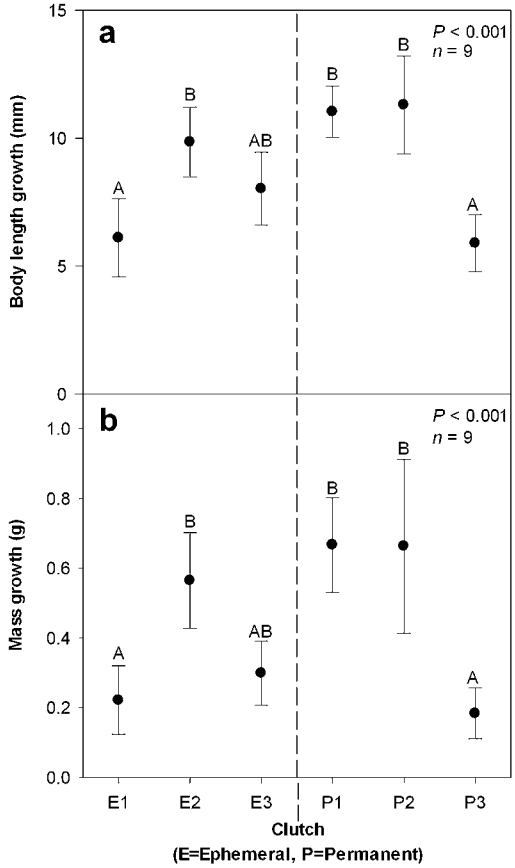

Figure 3. Growth in body length and mass (means ± 95% individual CI) per clutch during a mesocosm experiment that tested the effects of clutch and hydroperiod on larval bullfrog growth and development in the Willamette Valley, Oregon, USA, 2010. We found significant growth differences in body length (a) and mass (b) among clutches that did not correspond to source wetland type. The dashed line separates the clutches collected from ephemeral wetlands and clutches collected from permanent wetlands. A and B labels represent results from pairwise comparisons made with Tukey's Honestly Significant Differences; clutches denoted with the same letter are not statistically different.

amphibians and bullfrogs can overlap in wetlands during the summer months. Managed draining tends to occur in late summer, after the native amphibians have completed metamorphosis but before bullfrog larvae have fully developed.

We chose an outdoor mesocosm design for its strengths in hybridizing field and laboratory conditions. Our experimental mesocosms were exposed to natural outdoor conditions (e.g., weather, temperature) and hydroperiod and density were carefully manipulated, which allowed for causal inferences and identification of interactions between factors (Rowe and Dunson 1994). Further, we compared larval development during our 2-month mesocosm experiment to development observed in our permanent wetland field sites (Table 3) to validate that our mesocosm conditions roughly approximated conditions in our field sites over the same period.

Although 3–4 months is the minimum length of time in which bullfrog larvae have been documented undergoing metamorphosis (Bury and Whelan 1984, Provenzano and Boone 2009; S. S. Heppell, unpublished data), we predicted larvae would develop faster than the 1–3 Gosner stages we measured. A potential explanation could be that the draining cue was not delivered early enough in development. Multiple studies, however, have shown that development rate is changeable until late in the larval period (e.g., Newman 1994, Tejedo and Reques 1994, Audo et al. 1995, Beck 1997). Additionally, many factors are confounded with natural pond draining and our experimental setup may not have adequately reproduced the appropriate indirect cue (Denver et al. 1998). Our draining treatments increased larval density and decreased swim time to the top of the water, but did not increase concentration of compounds in the water (as would occur with natural evaporation) and food was unlikely to be limiting in the drained treatments. Most importantly, the temperature of the drained treatments may not have realistically imitated temperature profiles of naturally or artificially drying sites. Average temperature as well as diurnal maximum and minimum temperatures are closely tied to growth and development for larval amphibians (Ultsch et al. 1999) and may be the key proximate cause of increased growth and development in response to hydroperiod. Temperatures were nearly identical among all hydroperiod treatments for the majority of the experiment with greater fluctuations in the drained treatments in the last third of the experimental

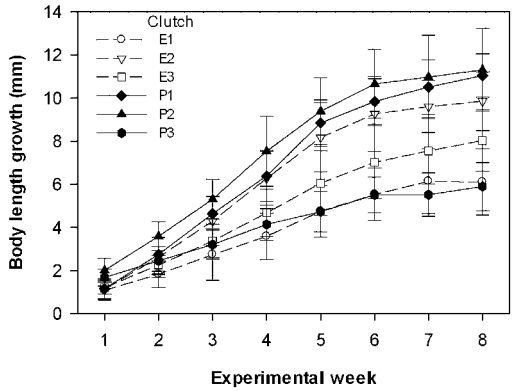

Figure 4. Growth in body length over time for each clutch in a mesocosm experiment that tested the effects of clutch and hydroperiod on larval bullfrog growth and development in the Willamette Valley, Oregon, USA, 2010. Each point represents a clutch's weekly mean body length (±95% CI), averaged across all mesocosms ($n = 9$). Weeks 1–7 were based on a sub-sample of 5 larvae per mesocosm, whereas week 8 was based on measurements of all surviving larvae. Clutches with the greatest growth at the end of the experiment exhibited greater growth throughout the experimental timeline. Growth differences did not correspond to source wetland type (ephemeral [E] or permanent [P]).

timeline (Fig. 1). The sub-sample of temperatures from field collection sites (1 ephemeral and 2 permanent sites) suggest mesocosm temperatures were similar to field conditions on those sampling dates, but we lack complete temperature data for the field sites.

We found significant differences in growth among bullfrog clutches and an unexpected lack of plasticity in response to hydroperiod. This suggests that the bullfrog's widespread invasion success has not been facilitated by plasticity to changing hydroperiods. Pre-existing genetic variation in life history traits such as growth rate along with multiple introduction events and tolerance of disturbed habitats could be sufficient to explain the bullfrog's ability to spread throughout the western United States. Our results indicate that invasive bullfrogs in the Pacific Northwest are unlikely to complete metamorphosis in ephemeral or drained wetlands, suggesting these sites could act as population sinks.

MANAGEMENT IMPLICATIONS

The lack of plasticity in response to hydroperiod in this study suggests that artificially draining wetlands to simulate ephemeral hydroperiods would not induce rapid metamorphosis in invasive bullfrogs. We do not propose, however, that bullfrog eradication should be the primary motivation for draining wetlands because high recolonization rates from nearby populations result in a low chance of successfully eradicating bullfrogs. Previous bullfrog eradication and control efforts have focused on pond draining and culling adults, but the bullfrog's high fecundity and dispersal and recolonizing abilities have made these efforts largely ineffective (Adams and Pearl 2007). Govindarajulu et al. (2005) suggested that culling newly emerged metamorphs would be optimal for reducing population growth rate in an invasive bullfrog population in Canada. Other research suggests that maintaining complex emergent vegetation and riparian habitat would promote coexistence between invasive bullfrogs and native amphibians (Schlaepfer et al. 2005, Adams and Pearl 2007, Adams et al. 2011) and that managing non-native fish species might have greater benefits for native amphibians than managing bullfrogs (Hayes and Jennings 1986, Adams et al. 2003, Pearl et al. 2005*a*). If pond draining is pursued as part of a management scheme beyond bullfrog eradication, our results suggest these efforts would not inadvertently benefit invasive bullfrogs. Other reasons for draining include reducing invasive vegetation, removing non-native fish, and restoring historical hydroperiods in ephemeral wetlands. We recommend monitoring larval growth and development during draining to confirm it does not accelerate bullfrog development in field conditions, particularly in regions outside the Willamette Valley, Oregon.

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