

Short pond hydroperiod decreases fitness of nonnative hybrid salamanders in California

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Abstract

Human-facilitated introductions of nonnative taxa have changed species' geographic ranges and increased the prevalence of secondary contact and the potential for hybridization, with a host of effects on ecological systems. Land managers increasingly face these complex situations, tasked with developing strategies to preserve biodiversity in the face of such changes. We conducted a management-relevant experiment to inform the development of strategies for management of wild populations. We examined the fitness consequences of genetic admixture in a hybrid swarm between native California tiger salamanders and nonnative barred tiger salamanders. Variation in hydroperiod within the hybrid zone has previously been observed to affect landscape patterns of genetic introgression, with modified permanent ponds harboring salamanders with a greater proportion of nonnative genes. Our study experimentally examines the relationship between hydroperiod and fitness of three classes of salamanders: native, nonnative and hybrid. Using experimental pond mesocosms, we implemented three pond drying regimes and recorded survival and secondary determinants of salamander fitness. Our results indicate native-genotype advantages in rapidly drying mesocosms relative to other genetic classes. Furthermore, our results indicate that management of aquatic habitat to minimize the success of hybrid individuals may be a viable strategy to reduce the spread of nonnative genotypes. Overall, our approach demonstrates how controlled experiments can be designed to provide meaningful information for the development of real-world conservation strategies.

Introduction

The ease with which humans move wild organisms across our planet has increased the likelihood that formerly isolated species may suddenly occur in sympatry. The ecological risks to native species associated with human-mediated introductions include predation by and competition with nonnative species (Simberloff & Stiling, 1996). These risks have complex conservation implications including the disruption of native food webs and physical modification of habitats (Vitousek *et al.*, 1997). A less frequently recognized threat is hybridization (Rhymer & Simberloff, 1996; Allendorf *et al.*, 2001; Mooney & Cleland, 2001). Hybridization can result in a wide range of evolutionary outcomes including reinforcement and character displacement, formation of one or more hybrid taxa, and local genetic extinctions within the zone of contact, as well as a host of immediate ecological effects (Arnold, 1992; Dowling &

Secor, 1997; Barton, 2001). These scenarios present complex challenges to land managers tasked with preserving biodiversity, often amidst a dearth of quantitative data to guide the development of on-the-ground conservation strategies.

The effects of hybridization on biological communities depend upon the degree and form of phenotypic differences between hybrids and native species. Likewise, the effectiveness of conservation strategies depends in part on how different genetic classes respond to habitat modification and disturbance. Hybridization following secondary contact is a frequent event in nature and need not necessarily be viewed in a positive or negative light. However, human-mediated hybridization involving introduced species often leads to loss of genetic and species diversity (Ellstrand & Schierenbeck, 2000). Ultimately, to manage hybridizing populations for the prevention of biodiversity loss, we need to understand fitness differences between native and hybrid phenotypes along habitat gradients that occur in nature.

Furthermore, in cases when hybridization is caused by human activities and has demonstrable negative effects on native species, then a clear goal for conservation and management is to quantify the nature of those effects and identify strategies that may reverse or mitigate them. The development of these strategies often falters in the transition between basic research and implementation, so here we present the results of an experiment designed to help bridge the gap between the two.

There are many studies of anthropogenic vertebrate hybrid zones in which native species are adversely affected by introduced ones (e.g. Barilani *et al.*, 2007; Gunnell *et al.*, 2008; McDonald *et al.*, 2008; Muhlfeld *et al.*, 2009; Chazara *et al.*, 2010; Senn *et al.*, 2010). Often researchers suggest identification of hybrid populations and subsequent protection of native populations (e.g. Barilani *et al.*, 2007) or eradication of introgressed populations (e.g. Muhlfeld *et al.*, 2009) as a strategy to slow the spread of introgression. However, these strategies are often not feasible in natural landscapes and sometimes lead to adverse outcomes. Some studies offer management suggestions such as reducing anthropogenic impacts or reversions to natural habitat characteristics (e.g. Gunnell *et al.*, 2008; Muhlfeld *et al.*, 2009) as mechanisms to reduce the extent of introgression in populations that are already admixed. These recommendations are derived largely from natural history observations, but are rarely experimentally tested to quantitatively explore their potential to alter the fitness of native and hybrid individuals. This highlights a more general problem that much research has 'little direct bearing on real-world conservation outcomes' (Laurance *et al.*, 2012).

Here we exploit a well-characterized salamander system to evaluate the effect(s) of habitat manipulations on the survival and growth of native salamanders relative to nonnative-introduced salamanders and their hybrids, with the goal of evaluating hydroperiod manipulation as a tool for conservation management. Hybridization between California tiger salamander (CTS) *Ambystoma californiense* and barred tiger salamander (BTS) *Ambystoma tigrinum marmoratum* began 6 decades ago when bait dealers transferred thousands of BTS larvae from Texas and New Mexico into ponds within the Salinas Valley, California (Riley *et al.*, 2003; Johnson *et al.*, 2011). Although the introductions have ceased, hybridization within the Salinas Valley has been extensive and hybrid populations occupy at least 20% of the entire range of CTS. Most hybrid salamanders are phenotypically distinct (e.g. larger body size) and ecologically distinct (e.g. capacity for facultative paedomorphosis) from native CTS (Ryan, Johnson & Fitzpatrick, 2009; Johnson, Fitzpatrick & Shaffer, 2010a).

Previous work has demonstrated that the degree to which introgression has proceeded across the hybrid swarm is correlated with attributes of aquatic breeding sites. Individuals from more natural breeding sites that dry annually tend to have genomes with a higher proportion of native CTS alleles than those from more modified perennial breeding sites (Fitzpatrick & Shaffer, 2004, 2007a). It is important to note that large permanent ponds do not occur naturally in the

range of CTS; rather, they are recent anthropogenic constructs usually created to provide water for livestock and agriculture. These perennial ponds are more similar to the breeding habitats utilized by BTS in their natural range than to the shallow vernal pools that CTS have evolved to exploit during their 5+ million years in isolation (Fisher & Shaffer, 1996; Shaffer & McKnight, 1996). Furthermore, hybrid tiger salamanders generally experience higher fitness than native individuals both in the laboratory (Johnson *et al.*, 2010a) and in the field (Ryan *et al.*, 2013; Fitzpatrick & Shaffer, 2007b), and appear to have the capacity for more extensive terrestrial movements (Johnson, Johnson & Shaffer, 2010b). The cumulative evidence paints a bleak picture for halting the advance of admixed CTS populations, at least within the modified landscapes that favor hybrids.

This system provides a case study for examining links between important environmental variables and fitness in hybrid and native genotypes that could inform the development of conservation strategies to prevent or slow the rate of hybrid spread. We designed our experiment to test whether the manipulation of hydroperiod in aquatic breeding sites could provide a mechanism by which CTS could be selectively favored in nature. The primary objectives of our experiment were to identify phenotypic and fitness differences among CTS, BTS and wild-caught hybrid genetic classes under three pond mesocosm hydroperiod regimes – nondrying (i.e. perennial), slow drying, and fast drying – to determine which conditions might favor CTS. The CTS hybrid system presents an excellent opportunity to experimentally evaluate the fitness consequences of potential management actions on genotypic categories observed in the wild.

Materials and methods

Experimental animals

We included four genotypic classes: (1) CTS collected as eggs from wild populations; (2) BTS that were captive bred from individuals derived from the original introduction stock (Johnson *et al.*, 2011); (3 and 4) hybrid salamanders (*A. californiense* × *tigrinum*) collected as eggs from six locations in the Salinas Valley (Supporting Information Table S1) during February 2008. No 100% native CTS have been recorded from these sites since the discovery of the hybrid swarm (see Riley *et al.*, 2003; Fitzpatrick & Shaffer, 2007a) so we presumed that each individual was a hybrid of unknown genomic admixture; subsequent analyses confirmed this assumption (Supporting Information Table S1). Hybrid salamanders were further divided into those collected from perennial and seasonal ponds (Supporting Information Table S1). Previous field genotyping suggested that on average individuals collected from seasonal ponds have a greater proportion of native-ancestry genes than those collected from perennial ponds (Fitzpatrick & Shaffer, 2004). However, genotyping of the specific ponds used in this study revealed similar high levels

of nonnative ancestry (Supporting Information Table S1). Nevertheless, the pond type category provides information regarding the natural variation in hydroperiod typically encountered by individuals from that pond type. See supporting materials for more information.

Experimental design

We performed a randomized complete block experiment in which we examined the response of salamander genotypic class to variation in hydroperiod. We added 8 salamander larvae and 50 Pacific chorus frog *Pseudacris regilla* tadpoles to each mesocosm as prey (see supporting material). Salamander hatchlings were reared in the laboratory following the protocol of Johnson *et al.* (2010a), and larvae were transferred to mesocosms on 30 April 2008, about 63 days post hatching. Mesocosms were arranged into 5 blocks containing 1 replicate of each treatment combination (genotype by hydroperiod, $n = 12$) for a total of 60 mesocosms. Genetic classes ($n = 4$) consisted of natives (100% CTS), nonnatives (100% BTS), hybrids from perennial ponds (Hyb_P), and hybrids from seasonal ponds (Hyb_S). Pond drying regimes ($n = 3$) consisted of a 90-day 'fast' (~1.3-cm decrease in depth/day) treatment, a 120-day 'slow' (~0.8 cm/day) treatment, and a >150-day 'none' treatment. Published reports of CTS larval period indicate a range of 90–180 days with an average of 120–150 days (Petranka, 1998) in natural vernal pools that reliably dry early each summer. Our intention was to test the leading edge of the larval period for CTS with the fast drying treatment (90 days), and mimic an 'average' hydroperiod with the slow drying treatment (120 days). At least 90% of California's vernal pool habitat has been destroyed (Holland, 1998), and human-modified ponds within the hybrid swarm have hydroperiods that span this range. Water was periodically added in small amounts to mesocosms in the 'none' drying category to counteract evaporation. Drying down of ponds began after larvae were introduced into the mesocosms, but day 1 of the hydroperiod was calculated as the date of hatching in the laboratory.

We checked for metamorphosing salamanders daily and conducted counts of larvae in each mesocosm weekly to estimate mortality that was not attributable to pond drying. When the mesocosm water depth was effectively zero (i.e. a few centimeters, owing to the position of the drain), any remaining larvae were transferred to the laboratory and allowed 48 h to metamorphose. Larvae that had not metamorphosed were euthanized and considered victims of 'drying-related' mortality. The nondrying treatment was terminated at day 150 from hatching and all the remaining larvae were transferred to the laboratory. Some larvae metamorphosed as a result, and those that did not were categorized as paedomorphs. The experiment ended when the final larva metamorphosed in the laboratory on 4 August 2008.

Data analysis

Each individual was weighed and measured for snout-vent length (SVL) as they completed metamorphosis or, in the

case of paedomorphic individuals, upon termination of the experiment. We calculated time to metamorphosis (T_{met}) for each metamorphosed salamander as the number of days that had elapsed between hatching and metamorphosis. Mass and T_{met} were log transformed. For each treatment, we calculated salamander survival, the mean mass of surviving salamanders, and the mean time to metamorphosis (T_{met}). Differences between means were investigated using pairwise t -tests and evaluated at $\alpha = 0.05$ following a sequential Bonferroni correction. We also estimated effect sizes for comparisons using Cohen's d (Cohen, 1988), and recorded the number of tadpoles surviving in each mesocosm (see supporting material).

To identify the relationship among hydroperiod, genetic class and salamander fitness, we evaluated linear mixed-effects models fitted with restricted maximum likelihood in the lme4 package of R (Bates & Maechler, 2009). 'Genotype' and 'hydroperiod' were fixed categorical effects and block was a random effect. Blocks were arbitrary and we assumed no interaction between treatments and blocks. Results are presented as likelihood ratio tests of (1) each single-factor model versus the model including only the random block effect (i.e. the null model), and (2) each higher order model compared with the best-fitting reduced model. To test the effect of hydroperiod on survival, we modeled individual survival as a categorical variable (e.g. alive or dead) using a logit link and binomial error term. This tested the association between the survival of salamanders and the hydroperiod and the interaction of genetic class and hydroperiod. Mass and T_{met} were used to test the effect of pond drying regime on life history patterns using a Gaussian error term.

Results

Hydroperiod treatments generated large variation in survival, size and larval duration (Table 1). All tiger salamander genetic classes showed within-class differences in response to drying regime (Figs 1 and 2), and comparisons among genotype categories showed that the pattern and magnitude of CTS responses across treatments differed strongly from those of all nonnative types (Figs 3 and 4).

Salamander survival

Survival of CTS versus nonnative salamanders was roughly inverted in response to drying. CTS survival was high in both the fast and slow drying treatments (Fig. 1a) but was significantly lower in the nondrying treatment (Supporting Information Table S2). CTS in our experiment experienced 16-fold and 20-fold decreases in survival in the nondrying treatment relative to the fast and slow drying treatments, respectively (Supporting Information Table S3). In contrast, survival of BTS and Hyb_S was significantly lower (Supporting Information Table S2) in the fast drying treatments than in the slow or nondrying treatments (Fig. 1a). Survival of Hyb_P did not differ significantly among drying

Table 1 Mean (\bar{x}), standard deviation (s) and sample size (n) for each hydroperiod treatment and genotype combination. Time to metamorphosis is presented here as days from hatching to metamorphosis or termination of the experiment in the case of paedomorphic individuals

Trt	Cross type	Salamander survival (%)		Drying mortality (%)		Paedomorph (%)		Mass (g)		Condition (g mm ⁻¹)		T _{met} (d)		n
		\bar{x}	s	\bar{x}	s	\bar{x}	s	\bar{x}	s	\bar{x}	s	\bar{x}	s	
Fast	CTS	90.0	10.46	5.0	11.18	–	–	6.51	1.42	0.11	0.02	105.77	7.74	35
Fast	BTS	75.0	15.31	22.5	16.30	–	–	8.93	0.99	0.13	0.01	111.80	3.39	30
Fast	Hyb_P	67.5	14.25	25.0	12.50	–	–	10.52	1.99	0.15	0.02	117.07	4.21	27
Fast	Hyb_S	50.0	19.76	40.0	13.69	–	–	10.07	2.50	0.14	0.03	105.56	3.35	18
Slow	CTS	95.0	6.85	0.0	0.00	–	–	7.88	1.84	0.12	0.02	116.42	7.75	36
Slow	BTS	100.0	0.00	0.0	0.00	–	–	10.14	1.65	0.14	0.02	126.65	5.73	40
Slow	Hyb_P	87.5	17.68	2.5	5.59	–	–	11.80	3.49	0.16	0.03	132.77	10.28	35
Slow	Hyb_S	77.5	16.30	7.5	6.85	–	–	12.27	3.74	0.17	0.04	120.34	8.91	32
None	CTS	30.0	18.96	–	–	0.0	0.00	8.69	1.96	0.13	0.02	114.00	6.73	12
None	BTS	92.5	6.85	–	–	30.0	22.71	24.31	8.83	0.27	0.08	161.05	13.58	37
None	Hyb_P	80.0	22.71	–	–	25.0	17.68	22.50	18.13	0.25	0.15	145.44	24.46	32
None	Hyb_S	72.5	16.30	–	–	22.5	5.59	29.37	16.40	0.31	0.14	142.93	19.52	29

CTS, California tiger salamander; BTS, barred tiger salamander; Hyb_P, hybrids from perennial ponds; Hyb_S, hybrids from seasonal ponds.

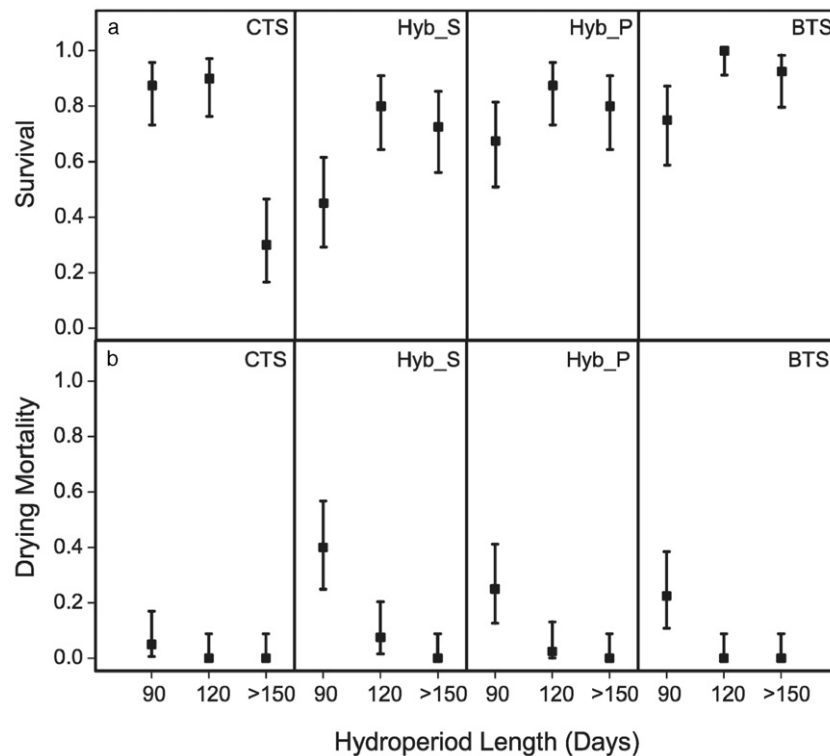


Figure 1 Salamander survival and mortality grouped by genetic class for comparison among hydroperiod treatments. Hyb_P and Hyb_S represent the hybrid salamanders originating from perennial and seasonal ponds, respectively. Error bars represent binomial 95% confidence intervals. (a) Survival of salamander larvae to metamorphosis. These data include paedomorphic individuals from the nondrying treatment. (b) Mortality of salamander larvae. We included only mortality due to pond drying in these data. See Table S2 for pairwise comparisons of mean survival and drying mortality values, and Supporting Information Table S3 for odds ratios and confidence intervals of comparisons.

treatments (Supporting Information Table S2) but showed a trend matching that of BTS and Hyb_S (Fig. 1a).

Considering only the direct effect of pond drying on survival (i.e. drying-related mortality, Fig. 1b), CTS showed no significant differences among treatments (Supporting Information Table S2). In contrast, BTS and both hybrid types showed significant increases (Supporting Information Table S2) in drying-related mortality in the fast drying treatment. Hyb_S and Hyb_P salamanders experienced an 8-fold and a 13-fold increase, respectively, in mortality due to drying in the fast drying versus the slow drying treatment (Supporting

Information Table S3). Significant drying-related mortality was limited to the fast drying treatment (Supporting Information Table S2). Very low rates of drying-related mortality occurred in the slow drying treatment (Fig. 1b). Potential sources of mortality other than pond drying include intrinsic developmental mortality, cannibalism [not observed in CTS but a likely occurrence within nonnative categories (Ryan *et al.*, 2009), and unknown environmental sources of mortality as occurred in the nondrying treatment for CTS (see supporting material).

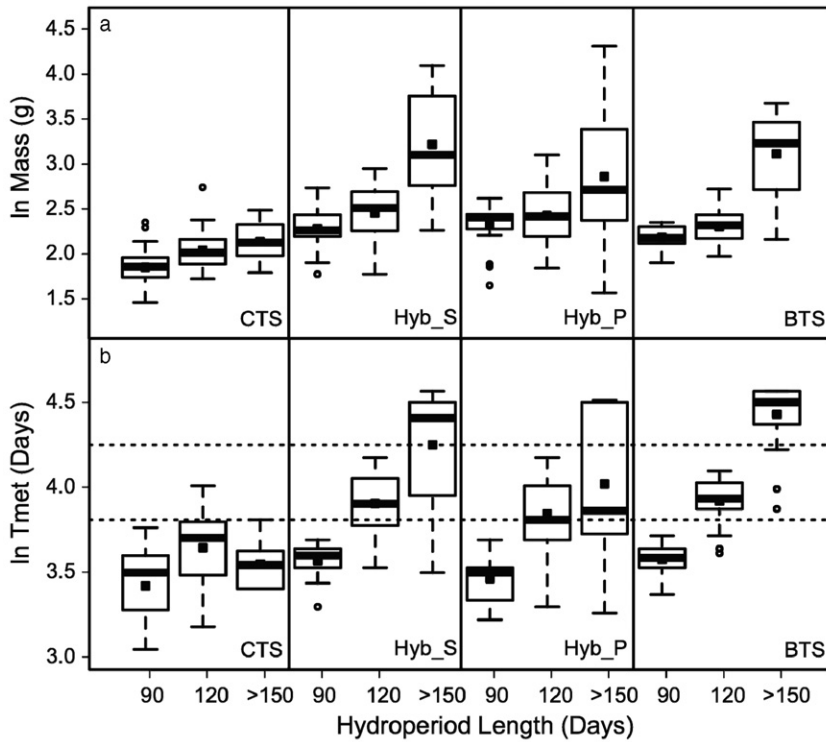


Figure 2 Mass at, and time to metamorphosis, grouped by genotype category for comparisons among hydroperiod treatments. Hyb_P and Hyb_S represent the hybrid salamanders originating from perennial and seasonal ponds, respectively. Mean values are denoted by black squares, median values by bold lines, and outliers by open circles. Outliers are data points falling outside of 1.5 times the interquartile range, which is represented by the boxplot whiskers. (a) Average mass of surviving salamanders. These data include both metamorphosed and paedomorphic individuals. (b) Average time to metamorphosis (T_{met}) measured as the number of days elapsed between the start of the experiment and completion of metamorphosis. These data do not include paedomorphic individuals. See Table S4 for pairwise comparisons of mean mass and T_{met} values, and Supporting Information Table S5 for the effect sizes of comparisons.

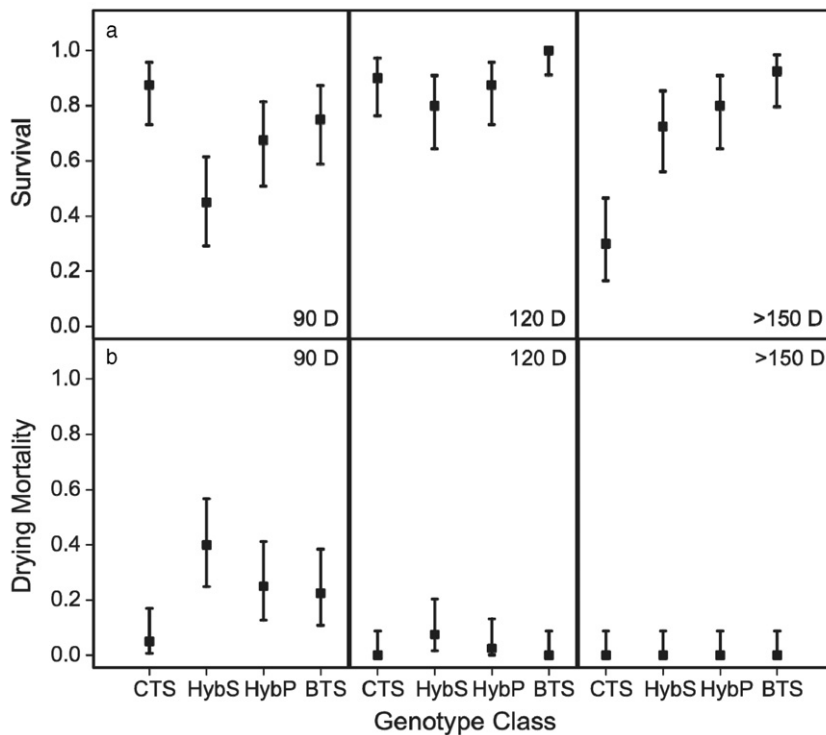


Figure 3 Salamander survival and mortality grouped by hydroperiod treatment for comparison among genetic classes. Hyb_P and Hyb_S represent the hybrid salamanders originating from perennial and seasonal ponds, respectively. Error bars represent binomial 95% confidence intervals. (a). Survival of salamander larvae to metamorphosis. These data include paedomorphic individuals from the nondrying treatment. (b) Mortality of salamander larvae. We included only mortality due to pond drying in these data. See Table S2 for pairwise comparisons of mean survival and drying mortality values, and Supporting Information Table S3 for odds ratios and confidence intervals of comparisons.

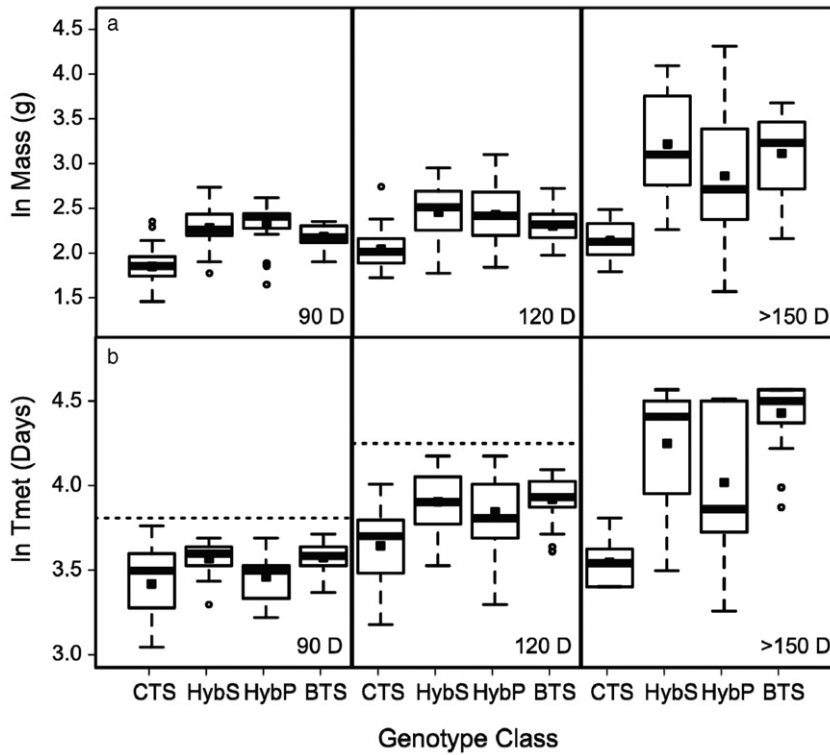


Figure 4 Mass at, and time to metamorphosis, grouped by hydroperiod treatment for comparisons among genotype categories. Hyb_P and Hyb_S represent the hybrid salamanders originating from perennial and seasonal ponds, respectively. Mean values are denoted by black squares, median values by bold lines, and outliers by open circles. Outliers are data points falling outside of 1.5 times the interquartile range, which is represented by the boxplot whiskers. (a) Average mass of surviving salamanders. These data include both metamorphosed and pedomorphic individuals. (b) Average time to metamorphosis (T_{met}) measured as the number of days elapsed between the start of the experiment and completion of metamorphosis. These data do not include pedomorphic individuals. See Table S4 for pairwise comparisons of mean mass and T_{met} values, and Supporting Information Table S5 for the effect sizes of comparisons.

Relative differences in survival of CTS versus other genotypic classes were greatest in the fast drying treatment (Fig. 3a), and CTS experienced significantly less mortality related to drying overall (Fig. 3b). Interestingly, Hyb_S performed poorly compared with those from perennial ponds in the fast drying treatment (see supporting material).

Salamander size and time to metamorphosis

The mass of surviving animals increased with the length of hydroperiod for all genetic classes (Fig. 2a). CTS showed a significant trend toward larger mass with longer hydroperiod (Supporting Information Table S4); however, the effect sizes of these comparisons were relatively small (Supporting Information Table S5). All nonnative genetic classes also showed significant increases in mass (Supporting Information Table S4) in the nondrying hydroperiod treatment (Fig. 2a), with much larger effect sizes than those seen in CTS (Supporting Information Table S5). It is important to note that the data from nondrying treatments include pedomorphic individuals (see Supporting Information Table S6) that were weighed at the termination of the experiment and tend to be large relative to metamorphosed animals. We included these animals because they exist in wild populations and illustrate the dramatic phenotypic difference between CTS and hybrids. When we evaluated the differences in mass of metamorphosed animals only, our results were qualitatively identical (compare Supporting Information Figure S1 with Figs 2a and 4a), but the mag-

nitudes of the effects were reduced (compare Supporting Information Table S7 with Supporting Information Tables S4 and S5).

All three nonnative genotypic classes demonstrated highly plastic responses to hydroperiod (Fig. 2b), significantly extending the aquatic stage (and continuing to grow) as hydroperiod was prolonged (Supporting Information Table S4). In contrast, while CTS showed some plasticity in metamorphic timing based on hydroperiod (Fig. 2b), the significant difference between the fast and slow drying regimes (Supporting Information Table S4) was associated with a very small effect size (Supporting Information Table S5).

Differences between CTS and all nonnative classes (hybrids and BTS) in size and T_{met} were significant (Supporting Information Table S4): hybrids and BTS emerged significantly larger than CTS in all treatments (Fig. 4a). The average size at metamorphosis for CTS [46–114 mm (Petranka, 1998)] is naturally smaller than estimates for BTS [80–150 mm (Petranka, 1998)]. However, we feel that these comparisons are meaningful because there is a general relationship between increased fitness and reproductive capacity and increased size (e.g. Semlitsch, Scott & Pechmann, 1988) that should have direct effects (numerical output and competition and predation) on the smaller CTS given their forced association with the introduced BTS and subsequent hybrids. A more conservative metric might be salamander condition (mass/total length), and we provide those results (which are nearly identical to our mass data) in the supporting material (Supporting Information Table S8; Supporting Information Figure S2). All nonnative genetic

classes emerged significantly later (Supporting Information Table S5) than CTS in all but the fast drying treatment (Fig. 4b). This difference corresponds with an elevated drying-related mortality for hybrids and BTS in the fast drying treatment (Fig. 3b).

Hydroperiod \times genotype interactions

Linear mixed-effect regression model comparisons indicate that both hydroperiod and genotype influence survival and secondary fitness estimates of tiger salamanders (Table 2). Comparisons of single-factor models containing either hydroperiod or genotype demonstrate that each performs better than the null model. Furthermore, the effect of hydroperiod alone provides a better fit to our data than genotype alone for survival and secondary measurements of fitness. Comparisons of two-factor additive or interaction models indicate a significant interaction between hydroperiod and genotype in determining the survival of larval salamanders, but drying-related mortality alone does not show an interactive effect. Model comparisons also suggest that mass [and condition (Supporting Information Table S9)] and T_{met} are affected by an interaction between salamander genotype and hydroperiod treatment (Table 2). Likelihood ratio tests reveal that these interactions are all significant when compared with the null model containing only random effects (Table 2).

Discussion

The calls for management-relevant research (Laurance *et al.*, 2012) and the extensive literature on adaptive management highlight the importance of targeted experimentation in the development of successful conservation strategies. However, managers tasked with preserving biodiversity are often faced with a dearth of relevant quantita-

tive data to inform the development of specific strategies. The need for management-relevant data is particularly great in the case of complex scenarios involving hybridization, which involves rapid overlapping ecological and evolutionary dynamics. Our results illustrate how targeted controlled experiments can be valuable in testing mechanisms associated with potential conservation strategies.

Our experiment demonstrates that reductions in pond hydroperiod enhance the survival and fitness of native CTS relative to hybrids. Therefore, the creation or restoration of short-hydroperiod habitat is a promising option for maintaining native populations and allelic diversity at the edge of the hybrid zone where CTS and hybrids directly interface. Among our hydroperiod treatments, we found extensive differences in salamander fitness components based on genetic class. Most importantly, the survival of native CTS was significantly greater than that of hybrids and nonnative BTS when hydroperiod was shortest, with nonnative classes experiencing the highest rates of mortality due to drying in the shortest hydroperiod treatment. In contrast, the significant interaction between salamander genotype and hydroperiod treatment, and the poor performance of CTS in the nondrying treatment, shows that longer hydroperiods favor hybrids. Active management of short pond hydroperiods should therefore increase selection for native genotypes and reduce the fitness advantages of hybrid and nonnative salamanders entering CTS populations peripheral to the hybrid zone. This strategy would also eliminate the capacity for hybrid populations to harbor paedomorphs, which likely act as a reservoir for nonnative genes and are likely to have stronger negative effects on native communities (Ryan *et al.*, 2009). When we remove paedomorphic individuals from our analyses of the size differences between CTS and nonnative salamanders, the relative fitness (at least based on size) is less biased against CTS.

Table 2 Linear mixed-effect regression model comparison for (A) salamander survival and (B) salamander mass and time to metamorphosis

A. Model	d.f.	Survival				Drying mortality			
		AIC	Δ AIC	X^2	P	AIC	Δ AIC	X^2	P
1. Null	2	533.95	79.16			284.13	79.71		
2. Hydroperiod	4	510.23	55.44	27.72	9.57E-07	218.46	14.04	69.67	7.43E-16
3. Genotype	5	516.58	61.79	23.37	3.38E-05	272.66	68.24	17.47	5.66E-04
4. Hydroperiod + genotype	7	491.63	36.84	24.61	1.87E-05	204.42*		20.04	1.67E-04
5. Hydroperiod \times genotype	13	454.79*		48.84	8.04E-09	214.03	9.61	2.40	0.88
B. Model	d.f.	Mass				T_{met}			
		AIC	Δ AIC	X^2	P	AIC	Δ AIC	X^2	P
1. Null	3	593.87	293.83			-404.94	347.49		
2. Hydroperiod	5	411.49	111.45	186.38	<2.20E-16	-571.28	181.15	170.34	<2.20E-16
3. Genotype	6	501.79	201.75	98.08	<2.20E-16	-475.13	277.30	76.19	<2.20E-16
4. Hydroperiod + genotype	8	324.21	24.17	93.28	<2.20E-16	-664.13	88.30	98.85	<2.20E-16
5. Hydroperiod \times genotype	14	300.04*		36.17	2.56E-06	-752.43*		100.30	<2.20E-16

Asterisks denote the models with the lowest AIC score. P -values indicate significance of likelihood ratio tests for each single-factor model (models 2 and 3) versus the null (model 1), the additive model (model 4) versus the single-factor model with the lowest AIC score, and the model containing an interaction term (model 5) versus the additive model. AIC, Akaike Information Criterion.

A common challenge in developing conservation strategies that promote native species over introduced hybrids is the transgressive nature of many hybrid phenotypes that confers a competitive advantage (Rieseberg, Archer & Wayne, 1999; Ellstrand & Schierenbeck, 2000). This is true in our case, as we consistently observe a deficit in CTS mass relative to the nonnative and hybrid genetic classes (see also Ryan *et al.*, 2009; Johnson *et al.*, 2010a), particularly in the long-hydroperiod treatment. These discrepancies in size result in part from intrinsic size differences between CTS and BTS, hybrid transgression, and also an apparent lack of plasticity in time to metamorphosis in CTS, which prevents them from exploiting the full larval growth period afforded by extended pond hydroperiod. In this experiment, size differences between CTS and hybrids were most exaggerated in the long-hydroperiod treatment. In contrast, the condition ($g\ cm^{-1}$) of metamorphosing hybrids was significantly reduced under shorter drying regimes. Therefore, we expect that reduced hydroperiod will limit post-metamorphic hybrid advantages as well. While direct competition with hybrids can reduce CTS advantages in drying habitats, reductions in hybrid condition in fast-drying ponds will help preserve CTS advantage (Ryan *et al.*, 2009). Preliminary results from field experiments including fast-drying ponds suggest that CTS survival advantage in these habitats remains even if the size of individuals is reduced (Ryan *et al.*, 2013).

Cumulatively, these results suggest that reduction in hydroperiod may be a viable conservation strategy, which is likely to be most effective at the edge of the hybrid zone. Pond drying, or any period during which the aquatic environment deteriorates beyond the physiological limitations of gilled salamanders (e.g. high temperature, low dissolved O_2), should eliminate paedomorphic adults for that season. Annual cycling through such conditions should render paedomorphosis an unviable life history strategy, although individuals carrying paedomorphosis alleles would still exist in the terrestrial adult population. Alternatively, an early drying regime ~90 days subsequent to the first detection of hatchling larvae in the pond could serve to select against hybrids or at least eliminate hybrid advantage with respect to size at metamorphosis. If such a strategy was implemented along the periphery of the hybrid swarm, the potential for spread of alleles associated with hybrid advantage could be minimized.

Second, if resources were available, hydroperiod manipulation strategies could also be implemented deeper within the hybrid zone to test their ability to promote the maintenance of native allelic diversity within hybrid populations. Existing landscape genetic patterns within the hybrid zone suggest that shorter hydroperiod habitats favor individuals with lower frequencies of nonnative BTS alleles (Fitzpatrick & Shaffer, 2007a). Native individuals are unlikely to enter populations at the core of the hybrid zone, nor to compete well with large numbers of hybrids if they did (Ryan *et al.*, 2009; Johnson *et al.*, 2010a), but periodic drying of habitats may promote the maintenance of native alleles associated with metamorphic timing that remain in

hybrid populations. It is also possible that pond drying may select for hybrid phenotypes that more closely resemble native CTS and play more similar ecological roles.

The most effective conservation approaches integrate knowledge from primary research with experimentation on the ground, while working with the ecological and evolutionary dynamics of the system itself to promote genetic and species diversity (Holling, 1978; Black, Groombridge & Jones, 2011). We can always do more research; for example, in our system additional experiments examining the quantitative relationship among hydroperiod, selection, and genetic composition for specific loci including those involved with generating the paedomorphic phenotype would be beneficial. However, we must also find the balance between controlled experimentation and action on the ground. Focused management-informative experiments can help in this transition by testing hypotheses relevant to potential conservation actions. In our system, the currently available evidence from our experiment and prior work suggests that shortening hydroperiods to natural levels is likely to promote relatively more native genotypes in nature. Land use needs such as cattle watering will certainly maintain hybrid-favoring ponds in the landscape, but modifications of pond hydroperiod to shorter duration, combined with the creation of new shallow sites, are a promising direction in which to focus management and conservation efforts, particularly at the edges of the hybrid zone where large native CTS populations still thrive. More broadly, we hope that our approach illustrates a way of bridging the gap between management and primary research to facilitate the development of informed conservation strategies.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Salamander mass excluding paedomorphic individuals. Mean values are denoted by black squares, median values by bold lines, and outliers by open circles. Boxplot whiskers denote the maximum and minimum quartiles. (a) Data grouped by hydroperiod treatment for comparison among genotypes. (b) Data grouped by genetic class for comparison among treatments. See Supporting Information Table S7 for significance tests of pairwise comparisons of mean values.

Figure S2 Body condition of surviving salamanders calculated as the final mass divided by SVL. Mean values are denoted by black squares, median values by bold lines, and outliers by open circles. Boxplot whiskers denote the maximum and minimum quartiles. (a) Means arranged by hydroperiod treatment for comparison among genotype categories. These data include both metamorphosed and paedomorphic individuals. (b) Means arranged by genotype category for comparison among hydroperiod treatments. See Supporting Information Table S8 for significance tests of pairwise comparisons of mean values.

Table S1 Hybrid salamander collection site information. Pond-level Hybrid Index Score (HIS) is calculated as the proportion of non-indigenous alleles present in the population.

Table S2 *P*-values for Fisher's exact tests for survival (below diagonal) and drying-related mortality (above diagonal). Asterisks denote significance at $\alpha = 0.05$ after sequential Bonferroni correction. See Supporting Information Table S3 for odds ratios and confidence intervals.

Table S3 Odds ratios and 95% confidence intervals (in parentheses) for Fisher's exact tests for salamander survival (below diagonal) and drying-related mortality (above diagonal). See Supporting Information Table S2 for *P*-values.

Table S4 *P*-values for pairwise *t*-tests for mass (above diagonal) and time to metamorphosis (below diagonal). Asterisks denote significance at $\alpha = 0.05$ after sequential Bonferroni correction. See Supporting Information Table S5 for effect sizes of comparisons.

Table S5 Effect sizes (Cohen's *d*) for pairwise *t*-tests for salamander mass (below diagonal) and time to metamorphosis (above the diagonal). Daggers indicate effect sizes exceeding 0.3. See Supporting Information Table S4 for *P*-values.

Table S6 Summary data for paedomorphic individuals.

Table S7 *P*-values (above diagonal) and effect sizes (below diagonal) of pairwise *t*-tests for salamander mass excluding paedomorphs. Only comparisons involving the 'none' (non-drying) treatment are shown. Asterisks denote significance at $\alpha = 0.05$ after sequential Bonferroni correction; daggers denote effect sizes greater than 0.3.

Table S8 *P*-values (above diagonal) and effect sizes (below diagonal) of pairwise *t*-tests for salamander condition at metamorphosis excluding paedomorphs. Asterisks denote significance at $\alpha = 0.05$ after sequential Bonferroni correction; daggers indicate effect sizes greater than 0.3.

Table S9 Linear mixed-effect regression model comparison for salamander condition. Asterisks denote the models with the lowest AIC score.