Invasive species shifts ontogenetic resource partitioning and microhabitat use of a threatened native amphibian

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ABSTRACT

1. Invasive species may have direct effects on native species, such as predation or competition, as well as indirect effects such as altering habitat or the behaviour of a native species. Indirect effects can be difficult to detect, but whole-system removal of invasive species presents an excellent opportunity for revealing these more subtle effects.

2. American bullfrogs were introduced to California in the late 1800s and have been implicated in the declines of native ranid species. Invasive American bullfrogs were removed from a series of 12 ponds to test for changes in microhabitat use of federally threatened California red-legged frogs, using a neighbouring field site with six ponds as a control.

3. There was a marked increase in the total number of adult California red-legged frogs seen in all of the ponds after the first year of bullfrog removal, suggesting that these adults were in the ponds, but hiding when invaders were present. Ontogenetic partitioning of habitat in this species was documented, as well as a shift in that partitioning and increased hiding behaviour with adult bullfrog presence. California red-legged frogs used willows significantly more as cover, and were found on bare shores half as often when adult bullfrogs were present.

4. These results support recent suggestions that behavioural responses of prey to predators can play a substantial role in total predator effects and suggest that careful management of invasive species may be necessary to accurately evaluate population sizes of this threatened amphibian species. Copyright © 2009 John Wiley & Sons, Ltd.

INTRODUCTION

Ecologists have long examined the strong role that predators may play in controlling the numbers of their prey species (Sih *et al.*, 1985). More recently, attention has turned to the indirect effects that predators may have on prey species (Sih *et al.*, 1985; Lima and Dill, 1990; Lima, 1998; Brown *et al.*, 1999) by shifting prey behaviour, changing prey habitat use (Creel *et al.*, 2005), altering abundance of a competing species, and changing timing of life-history events. Examples of these effects include: frog-eating bats altering mating call complexity in Tungara frogs (Ryan *et al.*, 1982), wolf presence shifting grazing patterns in Yellowstone elk populations (Laundre *et al.*, 2001; Creel *et al.*,

2005; Fortin *et al.*, 2005) and predator presence driving life history evolution in guppies (Reznik *et al.*, 1990).

As with native predators, the total impact of invasive predators is almost certain to involve both direct and indirect effects on native species (White *et al.*, 2006). Direct effects of invasive species on populations of native species have been well characterized, including the extinction of numerous avian species on Guam by the invasive brown tree snake (Savidge, 1987; Fritts and Rodda, 1998) and endangerment of flightless birds in New Zealand by introduced mammals (Clout and Craig, 1995). Indirect effects have been shown more rarely, and have the potential to outweigh direct predation in terms of population-level impacts. Some examples include: increasing

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shell thickness of native snails in response to invasive green crab predation (Vermeij, 1996); altering foraging strategies of mayflies in response to introduced trout (McIntosh and Townsend, 1994) and reduced activity levels and increased refuge-seeking behaviour by native frog larvae in the presence of an invasive predator (Kiesecker and Blaustein, 1997). As these indirect effects can either partially mitigate or amplify predator impacts on population viability, they are of clear concern. This is especially true in instances where rare species are declining and additional negative impacts, such as reduced growth rates or declines in reproductive success, could lead to extinction.

Given this concern, the means by which introduced predators affect the behaviour of native species has been recognized as a key area for research (Holway and Suarez, 1999; Blumstein and Fernandez-Juricic, 2004). The least ambiguous way to understand these responses is in combination with whole-system invasive species removal, which can be a highly effective conservation tool and method for large-scale experimentation (Donlan et al., 2002). A removal experiment of this nature is valuable for the development of scientific understanding of potentially cryptic behavioural effects and how these subtle effects combine with direct effects to generate population-wide impacts on native prey (Zavaleta et al., 2001).

The objective of this study was to determine whether a non-native predator, the American bullfrog, Rana catesbeiana, indirectly affects habitat use by a native, threatened species, the California red-legged frog, Rana draytonii. Bullfrogs, introduced from the eastern USA in the 1800s, are known predators of California red-legged frogs, as well as dominant competitors (Lawler et al., 1999). They have been introduced globally and are listed on the IUCN's List of 100 Worst Invasive Species (Lowe et al., 2000). This eradication work was a large-scale experiment built around conservation action, as California red-legged frog have declined from over 70% of their former range in California and were listed under the Endangered Species Act in 1996 (US Fish and Wildlife Service, 1996). Their decline has been attributed to the effects of habitat destruction and fragmentation (Fisher and Shaffer, 1996), introduced predators, such as bullfrogs, and potentially wind-born agrochemicals (Davidson et al., 2000; Davidson, 2004). Controlling invasive bullfrogs and understanding their impacts are actions called for in the California red-legged frog recovery plan (US Fish and Wildlife Service, 2002) and are a high priority for conservation of this species.

Working in sites with and without this invader present gave preliminary support for the idea that California red-legged frogs are more difficult to locate in the presence of bullfrogs. The difficulty in locating California red-legged frogs in sites where bullfrogs were present led to the hypothesis that these native frogs had changed their habitat use or were hiding and therefore harder to detect when bullfrogs were present. The goal was to remove invasive bullfrogs and concurrently compare habitat use by California red-legged frogs in the same ponds as bullfrog presence decreased.

MATERIALS AND METHODS

Study system

The study site encompassed 1,000 acres (400 ha) of private land in the western half of the Elkhorn Slough catchment in northern Monterey County, California, USA. The land is surrounded on two sides by salt marsh and by a major highway and agricultural fields on the other sides, leaving the ponds relatively isolated from secondary invasion by bullfrogs. All of the ponds within the site are man-made, but have been present for at least 30 years. Bullfrogs were introduced to this site shortly after the ponds were created, approximately 25 years ago, from a population in a neighbouring catchment. The ponds are primarily permanent water bodies, making them suitable for reproduction of both California red-legged frogs and bullfrogs.

Site visits began in 2003 to establish which amphibian species inhabited each pond and found California red-legged frogs, Pacific chorus frogs (Pseudacris regilla) and invasive American bullfrogs. The site initially had a bullfrog population that was estimated in the thousands, with an average of six large, adult bullfrogs in each pond and many juvenile bullfrogs. There are 20 ponds on the property, only the 12 ponds in which both California red-legged frog populations and bullfrog adults were observed were included in the analyses.

Research time-line

Bullfrog removal began in June 2004 and bullfrogs were removed until October 2007. All data on microhabitat use were collected from June 2004 until the end of September, 2005 when California red-legged frogs began to be PIT-tagged for mark-recapture. At that point the collection of microhabitat use data was stopped because capture by researchers was likely to influence California red-legged frog behaviour as well. Bullfrog eradication and the counts of both species seen within each pond were continued until October 2007.

Bullfrog removal experiment

While an experimental design involving control ponds where bullfrog populations were left intact would have been desirable from a statistical hypothesis-testing perspective, this approach was not considered ethical given the low population numbers of California red-legged frogs in this region, the well documented direct effects of bullfrogs on their populations, and the potential for movement by bullfrogs between ponds. The design, therefore, involved two complementary components. First, California red-legged frog behaviour within ponds was compared, using times when adult bullfrogs were seen and when they were not as treatments. Second, annual census data on California red-legged frog abundances at the experimental site were compared with data from a neighbouring field site without bullfrogs, the Elkhorn Slough National Estuarine Research Reserve (ESNERR). The second approach was used to determine whether temporal population trends existed among years at non-manipulated sites that resembled trends observed at removal sites. These comparisons allowed for understanding if trends at the experimental site were potentially attributable to weather or other factors apart from bullfrog removal.

To evaluate changes in the dynamics of habitat use in response to bullfrog numbers, microhabitat use of the California red-legged frog was evaluated night-by-night, establishing with each survey the number of bullfrog adults and juveniles seen. Microhabitat refers to the different types of

vegetation and near-shore habitat used by individual frogs. This approach allowed evaluation of both the effects of multiyear decline in bullfrogs (did the number of California red-legged frogs seen in a pond change from year to year of the eradication?) and also of immediate bullfrog presence (does the microhabitat use of California red-legged frogs change between visits when adult bullfrogs were seen or not?).

Bullfrogs were removed concurrently with California red-legged frog censuses, with bullfrogs generally captured by hand and then humanely killed. Hawaiian slings (gigs) were also employed to capture and remove this species. In addition, male bullfrog breeding calls were used to identify male location throughout the breeding season (May–July) and increase the rate of adult capture. Ponds were seined in June of 2005 and 2006 to remove bullfrog tadpoles when encountered.

Assessment of microhabitat use

Regular site visits began in June 2004, with approximately three night-time visits per week. The technique was to locate frogs using high-powered lights to spot their eyeshine, and then carefully approach them for identification. The inner perimeter of each pond was surveyed using chest-waders. When the water was too deep to wade, a small inflatable boat or float tubes were used to conduct surveys. The total number of frogs of both species and all age classes was tallied at the end of each site visit. California red-legged frogs were not captured or handled during the period of assessment of microhabitat, as regular capture by researchers could also alter their behaviour and habitat use. Owing to this lack of contact, it was assumed that researcher surveys did not affect California red-legged frog behaviour.

When a California red-legged frog was observed during the surveys, it was placed into one of several categories using visual estimation of its size. Individuals were categorized as: large adult (>8 cm snout-vent); small adult (7–8 cm snout-vent); or juvenile (<7 cm snout-vent). For a total of 214 individuals, the distance the frog was from shore, the depth of the water the frog was occupying, what percentage of the frog was visible from overhead and what kind of vegetation it was utilizing, if any, was estimated. Frog microhabitat use was assessed as the circumference of the pond surveyed. The starting point of each survey was noted to avoid assessing the same individual's habitat use several times in a night. Finally, the number of adult and juvenile bullfrogs was counted during the survey and whether or not bullfrogs were removed during the same site visit was noted. The microhabitat use of individuals in the pond was then compared with microhabitat use during later visits when no adult bullfrogs were encountered. Sites were scored as having adult bullfrogs 'absent' versus 'present' based on whether adults were detected during that night of the survey, visually or by call. The words 'present' and 'absent' are used as shorthand for the rest of the paper, even though it is possible that visits scored as 'absent' had some cryptic individuals present. The number of adult, not juvenile, bullfrogs was used as the factor determining California red-legged frog behaviour, because there was repeated evidence of adult bullfrogs consuming all three age classes of California red-legged frog but no evidence of such predation by juvenile bullfrogs (D'Amore, unpublished data).

California red-legged frog males take two years to reach maturity and females take three years. Owing to this time-lag,

any increase in adult California red-legged frog numbers seen during the first two years of this work are probably due to behavioural changes induced by bullfrog removal, rather than an increase in recruitment. California red-legged frogs move between these ponds at a rate of about 17% per year (D'Amore, unpublished data), so this change may also reflect some increased dispersal to sites with lower bullfrog densities. As all of the ponds had invasive bullfrogs at the start of the eradication, it seems unlikely that any one pond represented better habitat.

Statistical analyses

Two-way ANOVA was used to test for effects of adult bullfrog presence and of individual size class on three continuous measures of microhabitat use of California red-legged frog. These habitat use measures were distance from shore, depth of water utilized, and how much of the frog was visible from overhead. The vegetation the frog was occupying, if any, was also noted. A general linear model was used to test whether there was a significant effect of 'year', 'pond' or 'year $\times$ pond' on the average number of California red-legged frogs seen in each year in each pond. As there were not sufficient degrees of freedom to run this as one larger model, an interaction of 'year $\times$ pond' and 'year' effects were tested. If the 'year $\times$ pond' interaction was not significant, it was dropped and then a general linear model testing for separate effects of 'year' and 'pond' was run. This series of tests was repeated with bullfrog abundance and with the California red-legged frog abundance estimates for the ESNERR ponds, which lack bullfrogs (Table 2).

Linear regression was used to test for correlation between the number of adult bullfrogs in a pond and the total number of California red-legged frogs seen in the pond during a given survey night. A separate test was then used to examine the effect of bullfrogs on different California red-legged frog life stages: adults, small adults and juveniles. This was in case bullfrogs predominately affected one life stage. A chi-squared test was used to look for a difference in California red-legged frog presence/absence in sites with and without bullfrogs, allowing for determination of whether bullfrog presence was correlated with California red-legged frog absence from a site, as well as a change in abundance. A chi-squared test was used to test for differences in use rates by California red-legged frogs of the six main vegetation types (willow, tule, pennywort, duckweed, other and none) when adult bullfrogs were present and when they were absent. 'Other' was a category including a mix of low frequency aquatic and terrestrial vegetation.

RESULTS

No significant relationship between the number of bullfrogs and California red-legged frogs seen in a pond was found ($R^2 = 0.005$, $P = 0.356$), with each pond-night acting as a replicate. No significant interaction between California red-legged frog and bullfrog presence/absence (chi-squared $= 0.281$, df $= 1$, $P = 0.596$) was found either. However, very strong effects of bullfrog removal on the annual counts of California red-legged frog numbers were seen between 2004 and 2005/2006 at the removal site (Figure 1). The general linear model found no significant 'year $\times$ pond' interaction for California red-legged frog abundance at either
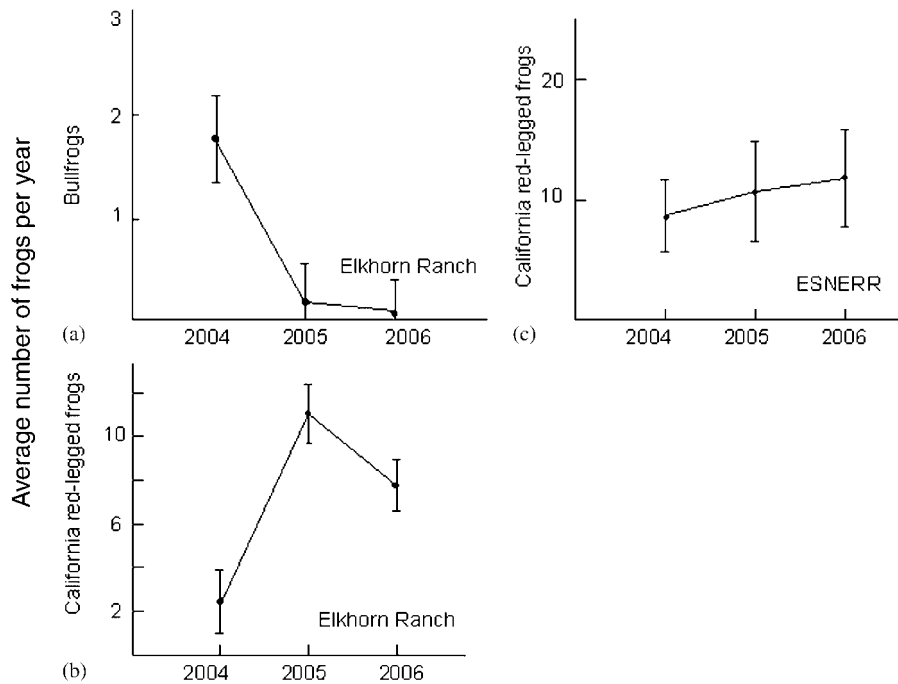
Figure 1. (a) Change in the average number of adult American bullfrogs during the bullfrog eradication in all Elkhorn Ranch ponds. (b) Change in the average number of adult California red-legged frogs during the bullfrog eradication in all Elkhorn Ranch ponds. (c) Change in the average number of adult California red-legged frogs in the Elkhorn Slough National Estuarine Research Reserve (ESNERR) control ponds.

Table 1. Output from general linear models testing for a year, pond by year and pond effect California red-legged frog abundance (CRLF) and bullfrog abundance on Elkhorn Ranch. Tests repeated for Elkhorn Slough National Estuarine Research Reserve (ESNERR) ponds, CRLF only

Model # (description)	DF	F-statistic	P-value
1. CRLF abundance (Elkhorn R.)			
Year	2	2.043	0.180
Year X pond	22	0.252	0.997
2. CRLF abundance (Elkhorn R.)			
Year	2	6.264	0.007
Pond	11	4.083	0.003
3. Bullforg abundance (Elkhorn R.)			
Year	2	1.354	0.430
Year X pond	22	0.908	0.597
4. Bullforg abundance (Elkhorn R.)			
Year	2	3.073	0.067
Pond	11	3.159	0.011
5. CRLF abundance (ESNERR)			
Year	2	0.083	0.922
Year X pond	5	0.063	1.00
6. CRLF abundance (ESNERR)			
Year	2	1.313	0.312
Pond	5	15.838	0.000

site, or for bullfrog abundance (Table 1). Subsequent models with pond and year as separate effects found significant effects of both pond and year for California red-legged frogs at the study site and significant pond effects and marginal year effects for bullfrog abundance. There was no significant effect of year on California red-legged frog abundance in the control ponds on the ESNERR property, though there was a significant effect of the pond (Table 1). Generally, there were significantly

fewer adult bullfrogs after the initial year of removal (Figure 1(a), (c)), and there were significantly more adult California red-legged frogs seen in the years following the bullfrog removal than in the previous year (Figure 1(b), (c)).

A shift in microhabitat use of California red-legged frogs was found when in the presence of adult bullfrogs, but this effect was strongly mediated by an interaction with age class. The two-way ANOVA was used to examine the effects of adult bullfrog presence within the pond, as well as the effect of age class of California red-legged frog observed with respect to distance from shore, depth of water, and percentage of the frog that was visible (Table 2). When bullfrogs were present, the California red-legged frog adults moved closer to shore (Figure 2(a), marginal significance), sharing habitat with the small adult and juvenile frogs. There was no significant effect of bullfrogs on the water depth used, although age classes used different depths on average (Figure 2(b)). Adult California red-legged frogs were less visible when bullfrogs were present (Figure 2(c)), although the other age classes did not show a significant shift in percentage of the frog that was visible. Overall, significant habitat partitioning between age classes for every index of microhabitat use was found, with adults generally occupying deeper water, sitting further from shore and being more visible than the other size classes.

Significant differences were found between use of the six main vegetation types (willow, tule, pennywort, duckweed, other and no vegetation) when adult bullfrogs were present or absent (χ^2, (DF = 5) = 37.672, $P < 0.001$) (Figure 3). California red-legged frogs were primarily found using bare shore and tule (*Scirpus californicus*) when there were no adult bullfrogs seen in the pond and found primarily using willows *(Salix* spp.) and tule when bullfrogs were present. They inhabited bare shore much less frequently when adult bullfrogs were seen concurrently in the pond.

DISCUSSION

Ontogenetic habitat-partitioning in California red-legged frogs

Body size influences energetic demands and resource use, as well as susceptibility of an individual to predation (Werner and Gilliam, 1984). In organisms such as amphibians, which increase in size with age, impacts on individuals of certain size classes necessarily mean impacts on individuals at different ontogenetic stages. Clear evidence of ontogenetic habitat-partitioning was found for all metrics of microhabitat

Table 2. Results of two-way ANOVA showing effect of adult bullfrog (ABF) presence and California red-legged frog size class on use of water depth, distance from shore and percentage of the frog that was visible

Variable	Factor	DF	F-stastistic	*P*-value
Water depth	Size class	2	18.055	0.000
Water depth	ABF presence	1	0.173	0.678
Water depth	ABF presence X size class	2	0.169	0.845
Distance from shore	Size class	2	6.523	0.002
Distance from shore	ABF presence	1	2.340	0.193
Distance from shore	ABF presence X size class	2	2.751	0.066
% visible	Size class	2	3.798	0.024
% visible	ABF presence	1	0.090	0.764
% visible	ABF presence X size	2	3.183	0.043

use examined, specifically the depth of water the frog used, the distance from shore the frog occupied and how visible the frog was. Resource partitioning by amphibians of different size and age classes has been shown to evolve in response to the varying selective pressures of predation, competition, and physiological constraints (Toft, 1985). Larval size class affects the microhabitat use of larval ranids (Alford and Crump, 1982), but this sort of ontogenetic habitat partitioning has not been demonstrated in post-metamorphic individuals. Post-metamorphic frogs of different ontogenetic stages may specialize on different prey species, which suggests either that there may be resource partitioning in these species, or simply a relationship between gape size and prey size (Christian, 1982; Hirai, 2002). These results clearly demonstrate habitat partitioning by post-metamorphic individuals according to their ontogenetic class.

Different size classes are likely to respond to predator presence in different ways, and this ontogenetic pattern has been demonstrated with larval anurans in other studies. In a study of larval American bullfrogs and green frogs, *Rana clamitans*, larger larval size classes were positively affected by predator presence (through reduced competition with smaller classes), while smaller size classes were negatively affected (Werner and Anholt, 1996). In this case, it may be that when predators are not present, adults utilize habitat away from shore and small adults and juveniles avoid them, reducing opportunistic cannibalism by adult frogs (Polis and Myers, 1985). California newt populations demonstrate a similar ontogenetic change in refuge-seeking behaviour, driven by avoidance of cannibalistic adults (Kats *et al.*, 1994). Some reptile (Keren-Rotem *et al.*, 2006) and amphibian species
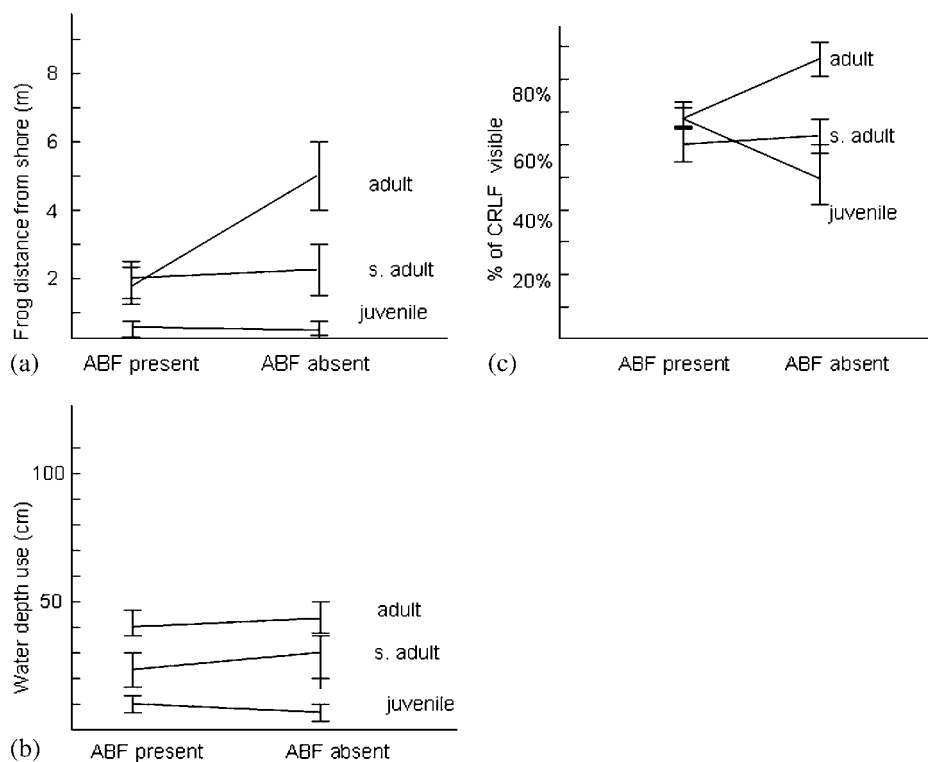

Figure 2. Interaction between adult bullfrog (ABF) presence and (a) distance from shore used by age class of California red-legged frog (CRLF), (b) depth of water used by CRLF age class, and (c) the percentage of the frog that was visible by CRLF age class.
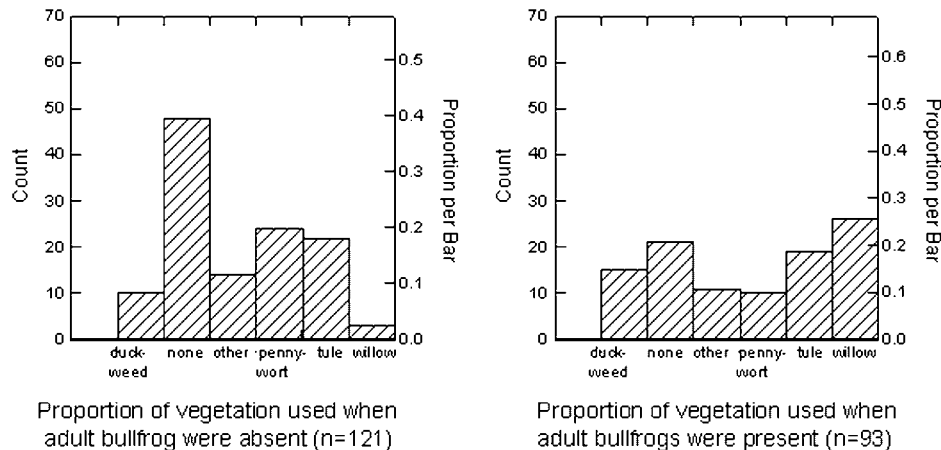
Figure 3. Differential use of vegetation by California red-legged frog depending on adult bullfrog presence or absence. The same ponds were searched before and after bullfrog removal.

(Polis and Myers, 1985) exhibit complete habitat partitioning between adults and juveniles.

Indirect effects of bullfrog presence on California red-legged frogs

This work suggests that bullfrogs influence the behaviour of native amphibians, in addition to the negative direct effects that have previously been documented with this and other ranids (Moyle, 1973; Hammerson, 1982; Hecnar and M'Closkey, 1997; Kupferberg, 1997; Schwalbe and Rosen, 1999; Doubledee et al., 2003). As described above, California red-legged frogs altered microhabitat use in response to bullfrog presence and this effect was generally strongest for the largest California red-legged frog individuals. The striking in-pond shifts are probably only a small part of the total habitat shift. We suspect that some portion of the population may leave the ponds altogether or become entirely cryptic when adult bullfrogs are there. The conservation implications of this are discussed in detail below.

The finding of California red-legged frogs shifting nearer to shore is consistent with laboratory work that has shown bullfrogs to be highly aquatic (Stewart and Sandison, 1972) and to have greater effects on native species with more aquatic habitat use (Pearl et al., 2004). By changing their habitat use, the California red-legged frogs may be partly mitigating the impact of bullfrog presence. Although there was no significant effect of bullfrog presence on the water depth preferred by the California red-legged frogs, this may be due to the non-uniform shoreline topography at the study sites. To our knowledge, this is the first example of indirect effects of predator presence on post-metamorphic anuran habitat use.

California red-legged frogs were most frequently found on bare shore or in tule when adult bullfrogs were absent and they used primarily willow for cover when adult bullfrogs were present. There are two possible interpretations of this pattern: (1) the available vegetation changed significantly across the period of bullfrog removal; or (2) the California red-legged frogs shifted habitat use in response to bullfrog removal. As work has been continuing in these ponds for a number of years and there have been no changes in vegetation type, we strongly support the second interpretation. Bare shore was used 38% of

the time when bullfrogs were absent and 16% of the time in the presence of bullfrogs, suggesting that some sort of cover may be needed for protection from bullfrog predation and that appropriate management of bullfrog-invaded habitat should take this into consideration.

The effect of bullfrog presence on the percentage of the frog that was visible arises from changes in the behaviour of the different size classes. The California red-legged frog adults were less visible when adult bullfrogs were present, while the other size classes showed no significant reduction in visibility in response to this invasive predator. It may be that California red-legged frog adults have learned to become less conspicuous when in the presence of bullfrogs, recognizing them as a threat, while smaller size classes have not developed such behaviour. Alternatively, smaller size classes may simply always hide more, while adults generally have significant benefits (through increased foraging success or breeding opportunities) when in more open habitat.

It is commonly assumed that species use their habitat in an optimal manner (Mittelbach, 1981; Werner et al., 1983). A shift in habitat use in response to a predator may increase survivorship, but affect other activities detrimentally, such as acquiring resources for growth or procuring mates (Schoener, 1974; Losos et al., 2004). A frog shifting habitat use in response to predator presence may result in trait-mediated indirect interactions (TMII) through feeding less efficiently (Relyea and Werner, 1999; Peacor and Werner, 2000) or changing competitive interactions. In this case, changing ontogenetic habitat partitioning with bullfrog presence may increase the number of conspecifics a California red-legged frog consumes (Foster et al., 1988), keep it from occupying key territories during the breeding season or make it more susceptible to predation from terrestrial predators. These behavioural changes, in combination with direct predation from bullfrogs, may substantially increase the negative impact of these invaders.

The differing impact of adult bullfrogs across California red-legged frog size classes highlights a need to evaluate the impacts of invasive species with specific attention to life history stages. These findings have parallels to studies that found grazing mammals altering their habitat selection to increase protective cover upon reintroduction of wolves to Yellowstone

National Park (Creel *et al.*, 2005). Species, sex and reproductive status all influenced how strongly wolves affected habitat selection of the grazers and these indirect effects may be more costly to prey populations than the direct predation events (Laundre *et al.*, 2001).

Conservation implications of bullfrog effects on threatened amphibians

This study is the first demonstration of indirect effects of bullfrogs on post-metamorphic individuals, but previous research has found indirect effects on the larval stages of native ranids. Native foothill yellow-legged frog (*Rana boylii*) larvae in California are not only much less abundant in the presence of bullfrog larvae, they also have reduced survivorship and reduced size at metamorphosis (Kupferberg, 1997). Northern red-legged frog (*Rana aurora*) larvae from sites with adult or larval bullfrog presence will reduce activity levels and increase refuge-seeking behaviour when the invader is present, thereby reducing bullfrog predation levels. Naïve larvae, however, showed no such change in behaviour (Kiesecker and Blaustein, 1997). The behavioural effects of predators on larval ranids translate to reduced growth rates in the field and the magnitude of this effect varies with predator type (Relyea and Werner, 1999).

Bullfrogs are likely to pose a real threat to native frog populations if they are affecting larvae and adults in both a direct and indirect manner. These indirect effects such as shifting ontogenetic habitat partitioning and changing the cover used may then alter food web dynamics or growth rates through trait-mediated indirect pathways (Relyea and Werner, 1999; Peacor and Werner, 2000, 2001). Indirect effects can easily be mistaken for direct effects and the two may be difficult to distinguish without careful surveys and the collection of appropriate behavioural data. For example, it may be assumed that a decline in California red-legged frog abundance in ponds with bullfrog is entirely due to increased predation and a direct reduction in numbers, while it may be due partly to indirect effects.

While introduced bullfrogs clearly have a broad range of detrimental effects, the behavioural shifts found in this study also mean that simple census data may overestimate their direct effects on native frog populations. This reliance on census data without properly accounting for cryptic individuals may also help to explain the mixed messages in the literature about the impact of bullfrog populations (Hayes and Jennings, 1986; Adams, 1999). These data provide an excellent case study of this dilemma. There was a significant increase in the total number of California red-legged frogs seen in the field site as the number of adult American bullfrogs decreased. The control sites showed no difference in California red-legged frog abundance between years. As it was primarily adult individuals that accounted for the increase in number at the experimental site, these individuals were necessarily in the pond network or immediate vicinity already. California red-legged frogs take two years as males and three years as females to reach adult status (USFWS, 2002) and these increased adult counts could not result from increased reproduction or survival.

This trend indicates that avoidance of sites with bullfrogs, more reliance on terrestrial habitat, or other changes in behaviour made California red-legged frogs appear more abundant when bullfrogs were removed. Standard, time-intensive protocols were used to survey frogs, but the results suggest that the numbers seen are nonetheless not a constant fraction of the population size depending on predator presence. In other words, simple count data with and without adult bullfrogs can give a misleading estimate of effects. Low numbers of California red-legged frogs spending more time out of the pond or obscured in the vegetation may not be detected and therefore absence data for this species in sites with bullfrog presence may be suspect. This has significant consequences for how habitat assessments and mitigation measures are determined and may mean that an 'absent' finding for California red-legged frogs at a site with bullfrogs should only be credited once bullfrog control measures have been implemented.

Lastly, this work suggests that the presence of vegetation like willows and other shoreline vegetation may help California red-legged frogs to hide more effectively from their predators, invasive or otherwise. While there is often dispute about the amount of vegetation that is ideal in a given piece of freshwater habitat, it seems clear that having at least some patches of dense vegetation in invaded sites will be important. Although the full measure of the population-level impact that American bullfrogs have on California red-legged frogs is yet to be described, this research indicates that such a description will necessitate inclusion of both direct and indirect effects.

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