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ORIGINAL PAPER

Effective removal of the American bullfrog (*Lithobates catesbeianus*) on a landscape level: long term monitoring and removal efforts in Yosemite Valley, Yosemite National Park

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Abstract Invasive alien species are a major threat to freshwater ecosystems, and American bullfrogs are among the world's 100 most prominent aquatic invasive species causing negative direct and indirect effect on native aquatic fauna worldwide. Bullfrogs were intentionally introduced into Yosemite Valley, Yosemite National Park in the 1950s where they became well established in the subsequent years. Starting in 2005, the National Park Service (NPS) began bullfrog removal, targeting various life stages using hand, net, and spear techniques. Starting in 2015, the NPS conducted environmental DNA (eDNA) surveys and deployed audio recordings devices to ensure adequate detection of bullfrogs. During the first year of cencerted effort in the Valley in 2005, the NPS removed 86% of all recorded bullfrog. The subsequent decade was spent searching for individuals with lower return on effort. In 2012, the NPS removed the last observed signs of bullfrog breeding, and the last observed bullfrog in 2019. Following removal of the breeding bullfrog population, the NPS began restoration projects for species of special concern. The NPS introduced the federally threatened California red-legged frogs (*Rana draytonii*) into Yosemite Valley beginning in 2016. This is the first published successful eradication of bullfrogs on a landscape level. National Parks and Monuments often provide refuges for imperiled wildlife and should be managed to remove invasive species. Our work highlights effective bullfrog removal is obtainable and can lead to local recovery of endangered species.

Keywords Alien invasive species · Aquatic restoration · California red-legged frog · Conservation · Environmental DNA · eDNA · Wildlife management

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Introduction

Freshwater aquatic ecosystems are among the most imperiled ecological communities worldwide (Dudgeon et al. 2006). Invasive alien species are a major threat to freshwater ecosystems, and American bullfrogs (*Lithobates* (*Rana*) *catesbeianus*) are among the

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world's 100 most prominent aquatic invasive species (Lowe et al. 2000; Rahel et al. 2008; Bucciarelli et al. 2014). Bullfrogs, native only to eastern North America, occupy over 40 countries across 4 continents and Oceania (Adams and Pearl 2007). Human land-use changes, such as modifying aquatic habitats to support perennial water, helped facilitate the spread of invasive bullfrog populations (Ficetola et al. 2010; Fuller et al. 2011). Bullfrogs are incredibly fecund; females can lay multiple clutches of > 20,000 eggs per season (Emlen 1977; Bury and Whelan 1985). As a result, bullfrogs become quickly established in new areas (Luja and Rodríguez-Estrella 2010; Orchard 2011). Bullfrog invasion has been linked to negative direct and indirect impacts on native species, including special status species, as well as lowering overall diversity and species richness in native amphibian communities through competition, predation and/or spreading parasites or diseases in areas around the globe (Moyle 1973; Kupferberg 1997; Adams and Pearl 2007; Bai et al. 2010; Schloegel et al. 2010; Da Silva et al. 2011; Li et al. 2011).

Bullfrogs were likely intentionally introduced into Yosemite National Park's valley in the 1950s. The first recorded observation of bullfrogs in Yosemite Valley was in 1955 at the Ahwahnee Hotel's reflection pond (Cunningham 1960). By the 1990s, bullfrog populations were well established throughout Yosemite Valley (Drost and Fellers 1996). Although Yosemite National Park is protected public land, it has experienced extirpations or declines of California red-legged frogs (*R. draytoni*), Foothill yellow-legged frogs (*R. boylii*), Western toads (*Anaxyrus* (*Bufo*) *boreas*), Sierra newts (*Taricha sierra*), and Western pond turtles (*Actinemys* (*Emys*) *marmorata*) that are likely linked to the presence of invasive bullfrogs (Drost and Fellers 1996; Yosemite National Park unpublished data). In order to restore aquatic ecosystems, Yosemite National Park took management actions to remove all bullfrogs from Yosemite Valley.

Methods

Study area

Yosemite Valley (the Valley) is a glacially-carved valley on the western slope of the Sierra Nevada within Yosemite National Park (Fig. 1). The Valley elevation ranges from 900 to 1280 m, spans across ~ 1500 ha, and contains the Merced Wild and Scenic River. The north and south sides of the Valley are bounded by steep granitic cliffs and the east and west sides of the valley are bounded by steep waterfalls or cascades. Yosemite Valley is a particularly good place for bullfrog eradication as bullfrogs do not inhabit the high elevation (> 6000 ft.) aquatic tributaries that feed into the Merced River in the Valley. As a result, the only possible way for the non-assisted colonization of bullfrogs to the Valley would be from downstream source populations. The dramatic geological features of the Merced River canyon downstream from the Valley likely impede any natural migration of bullfrogs from neighboring populations.

The National Park Service (NPS) collected data on bullfrog removal efforts sporadically. The history of bullfrog removal in the Valley is a patchwork of raw data as well as anecdotal accounts (history of eradication effort in the Valley is summarized in Table 1). Bullfrog removal started opportunistically in the mid-1990s by a single NPS employee who sporadically removed bullfrogs at breeding locations for less than a month a year until 2004. Bullfrog efforts began in earnest in the Valley in 2005 when the NPS hired 2 full time restoration technicians specifically for bullfrog removal. From 2005 to 2015, a 1 or 2-person crew worked 1–4 mo./year surveying and removing bullfrogs throughout the Valley. During this time, the NPS collected data on the number of bullfrogs removed and did not record negative bullfrog sightings, the sex of bullfrogs removed, survey covariates, or the presence or densities of native anurans. After 2015, the NPS continued surveying for bullfrogs using traditional visual surveys as well as environmental DNA (eDNA) techniques. All bullfrog surveys and removal efforts occurred during the onset of breeding (mid-May) until the end of the summer (late August).

Removal techniques

To identify breeding bullfrog populations, the NPS conducted visual surveys for egg masses and larvae (tadpoles) around all available breeding habitat: slack water, ponds, or stagnant streams (Fig. 1). Bullfrog egg masses, which are formed as large gelatinous mats, are unlike native anuran egg masses of other species in Yosemite Valley, and therefore distinguishing species was possible via visual detection. Crews

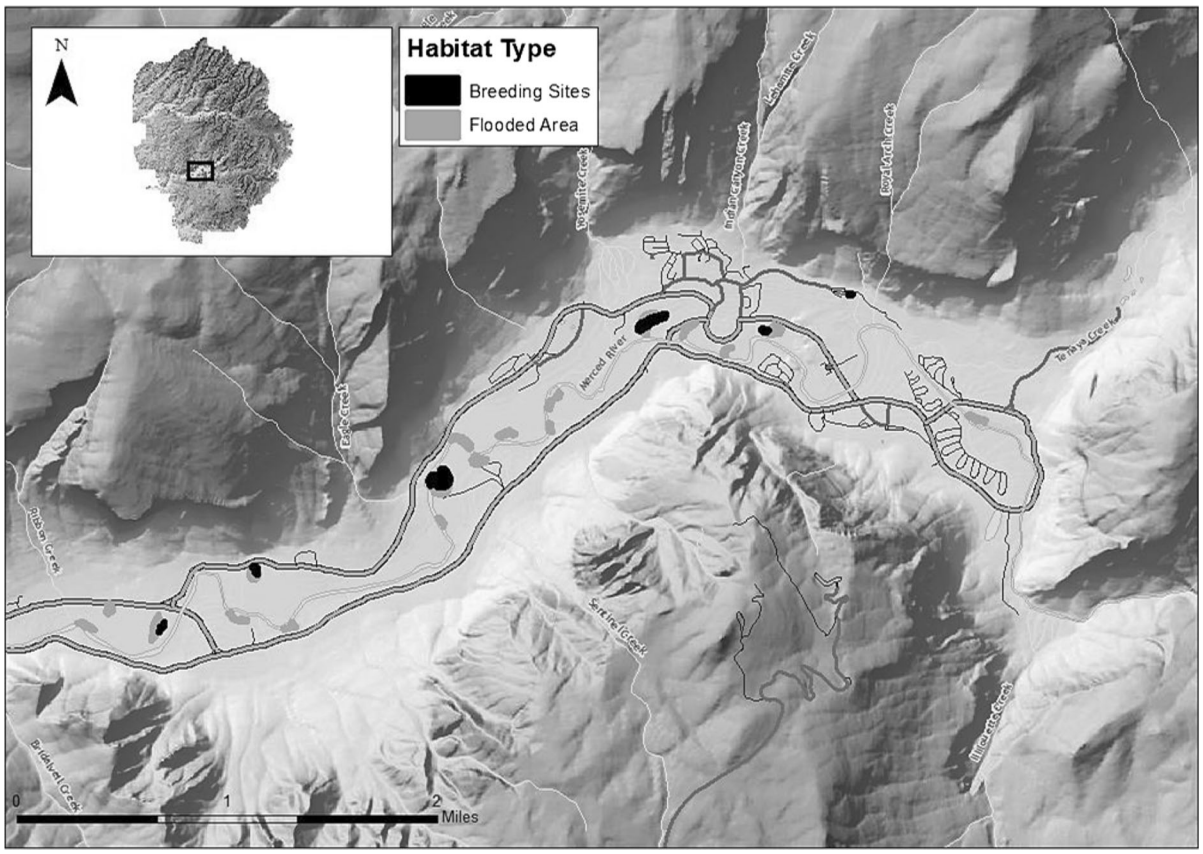

Fig. 1 Map of Yosemite Valley in Yosemite National Park, located in central California. The black breeding sites are areas where American bullfrog larvae and/or egg masses were observed. The grey flooded areas are potential bullfrog habitat. Figure created in ArcMap

conducted breeding surveys in late spring and early summer (mid May to early June) during daylight hours (0900–1600 h; see Table 1 for yearly survey effort). During surveys, crews walked slowly through the water bodies removing any egg masses and larvae observed using dip nets, paint strainers, or 5-gallon buckets. Occasionally, crews would use a backpack electrofisher (Smith-Root, Vancouver, WA) or seine nets to remove larvae.

The NPS surveyed for and removed adult and sub-adult bullfrogs during night visual surveys from the onset of bullfrog breeding (May/June) until the end of summer (August/September; see Table 1 for yearly survey effort). Crews used 200+ lumen flashlights to locate and stupefy bullfrogs via eyeshine. Crews captured bullfrogs using a variety of methods: hand grabbing, spearing (trident pole spears outfitted with rubber sling), dip netting, seine netting, or shooting with pellet rifles. On occasion, crews caught frogs using hook and line methods. Crews also attempted to trap individuals using funnel traps with "Alive Lure™", a mechanical lure that looks and vibrates like an insect placed inside the traps as an attractant. (The funnel trap method was not successful in the Valley as no frogs were captured.)

The Ahwahnee Hotel reflection pond was an artificial concrete structure that sustained a breeding population of bullfrogs. Along with eradication activities stated above, in spring 2006, NPS drained the pond to remove all life stages of bullfrogs. This is the only water body that could be drained in the Valley. All other water bodies were naturally occurring.

We humanely euthanized all bullfrogs depending on life stage and capture method. Crews placed all collected egg masses on shorelines to dry. For adults and sub-adults captured via pole spear, crews would immediately euthanize the frog using skull blunt force trauma and pithing (Underwood et al. 2013). Crews

Table 1 Survey and removal efforts of the American bullfrog (BF) in Yosemite Valley (the Valley) from 1990 to 2018

Year	Description of staff[a]	Months/year[b]	Description of activities[c]
Mid 1990s–2004	1 part time employee and occasionally volunteers	< 1 month	Crews worked irregularly targeting and removing eggs and larvae at BF breeding sites (Fig. 1), and opportunistically removed adults
2005–2006	2 full time employees	~ 4 month	Crews worked from onset of breeding season to the end of summer. Crews targeted all life stages with particular attention to egg masse, sub-adult, and adult BF. Crews removed the majority of BF during this time
2007–2011	1 full time employee and 1 volunteer	< 1 month	Crews surveyed and removed all life stages of BF for less than 4 weeks per year
2012–2013	2 full time employees	~ 4 months	Crews worked from onset of breeding season through the summer. Crews spent most of their time looking for BF (without many encounters), including conducting transects for BF across the Valley
2014	2 part time employees	~ 4 month	Crews worked from onset of breeding season through the summer. Crews spent most of their time surveying for BF (without many encounters). Surveys included conducting transects through the Valley
2015–2016	1 or 2 part time employees	< 1 month	Crews surveyed for bullfrogs at known bullfrog breeding location (Fig. 1) and collected eDNA samples for ~ 2–4 weeks each year
2017	–	–	No surveys or eDNA collected
2018	4–8 part time employees	< 1 month	Crews surveyed for bullfrogs at known bullfrog breeding location (Fig. 1) and collected eDNA samples

[a]Number of individuals assigned to removal efforts and their work schedule (full time or part time)

[b]Number of months surveying and removing BFs in the Valley

[c]The area and life stages of focus during that time period

euthanized all other adult and sub-adult bullfrogs using a buffered solution of MS 2-22 at a concentration of 2–3 g/l solution at pH 7.0–7.5 and/or skull blunt force and pithing protocol.

Environmental DNA surveys and long term monitoring

In 2015, Yosemite NPS began surveying for bullfrogs using aquatic eDNA sampling, an alternative survey technique sensitive to species at low densities. Species detection using eDNA methods is accomplished by collection and identification of trace DNA particles originating from shed skin cells, feces, etc., that are extracted from water samples (Taberlet et al. 2012). Environmental DNA methods have been used to detect aquatic amphibian species at low densities (Rees et al. 2014) and have been found to be more effective at detecting bullfrogs than traditional survey methods (Dejean et al. 2012; Goldberg et al. 2018).

For eDNA surveys, crews collected filtered water samples from suitable bullfrog habitats where breeding or presence was previously known (Fig. 1). The amount of water filtered as well as samples collected varied over the years as the NPS refined eDNA collection techniques. In 2015 and 2016, we collected a 50 ml samples every 40 m around the perimeter or length of each site as this sampling strategy was proven effective for trout species (Kamoroff and Goldberg 2018; see also Dunker et al. 2016; Table 2). In 2018, we collected 250–500 ml water samples every 40 m around the perimeter or length of each site to attempt to collected 1–2 l sample from each site (a standard eDNA sampling amount; Rees et al. 2014). However filter clogging and time limitation limited volume collected at some sites (Table 2). To detect any contamination from field equipment, we collected a 250–500 ml field blank using distilled or deionized water per site or per sampling day. We filtered all water samples using a 0.45 µm cellulose nitrate filter membrane with a 47 mm diameter filter funnel

Table 2 Metadata for bullfrog environmental DNA (eDNA) sampling and visual survey sites in Yosemite Valley beginning in 2015

Site type[a]	Site name	Survey year	Total amount filtered (ml)	# eDNA samples	LICA DNA detected[b]	Total min. surveyed[c]	LICA observed
Breeding	Awahnee pond	2015	500	2	Yes	8	No
Breeding	Camp 6	2015	200	3	Yes	338	No
Breeding	Cathedral east	2015	200	2	No	814	Yes
Breeding	Cooks meadow	2015	–	–	–	56	No
Breeding	El Cap. meadow	2015	–	–	–	28	No
Breeding	V6	2015	> 300	7	Yes	98	No
Flooded	Waski	2015	–	–	–	14	No
Breeding	Awahnee pond	2016	750	3	No	–	–
Breeding	Camp 6	2016	1250	5	No	–	–
Breeding	Cathedral east	2016	> 500	6	No	106	No
Breeding	Cooks meadow	2016	750	3	No	–	–
Breeding	El cap. meadow	2016	NA	3	No	–	–
Other	Merced river	2016	750	3	No	–	–
Flooded	Mirror lake	2016	> 750	5	Yes	–	–
Breeding	Sentinel bridge	2016	1000	3	No	–	–
Breeding	V6	2016	1025	5	Yes	242	No
Flooded	Waski	2016	–	–	–	165	No
Flooded	Yellow pine	2017	–	–	–	165	No
Breeding	Awahnee pond	2018	1200	4	–	66	No
Flooded	Backpacker's CG	2018	–	–	–	604	No
Breeding	Camp 6	2018	650	4	No	192	No
Breeding	Cathedral east	2018	1170	4	Yes	20	No
Breeding	Cooks meadow	2018	–	–	–	574	No
Flooded	Mirror lake	2018	1200	4	No	3303	No
Breeding	Sentinel bridge	2018	–	–	–	50	No
Breeding	V6	2018	550	4	No	–	–
Flooded	Yellow pine	2018	420	4	No	212	No

[a] Breeding sites are areas where NPS observed bullfrogs breeding at some point during removal efforts, and flooded areas are suitable habitat for bullfrogs where NPS never observed breeding

[b] A "Yes" denotes the detection of bullfrog DNA in at least 1 of the samples collected

[c] Total min. surveyed is the combined survey duration of all observers minus any breaks taken

(Thermo Fisher Scientific). We used the same collection, filtration, storage, and DNA extraction method described in Kamoroff and Goldberg (2018). To determine if bullfrog DNA was present in the samples collected, we analyzed the extracted DNA from the filtered water samples in triplicate using a quantitative polymerase chain reaction (qPCR) and a previously published American bullfrog qPCR assay (Strickler et al. 2015). We included an exogenous internal positive control to ensure no PCR inhibition had occurred (IPC; Applied Biosystems) and that DNA would indeed amplify if it were present in a sample. We ran inhibited samples though OneStep^{tm} PCR Inhibitor Removal spin columns (Zymo Research). If inhibition was still present, we diluted the samples 1:10 and re-analyzed. We created and analyzed negative extraction and qPCR controls with every batch and plate.

We considered the species to have been detected in a sample if all 3 qPCR reaction replicates tested positive. If 1 or 2 of the technical replicates tested positive, we reanalyzed the samples in triplicate. We confirmed the presence of DNA if any reanalyzed replicates tested positive during the 2nd round. We stored samples at 4 °C between qPCR runs to minimize DNA degradation caused by heat or multiple freeze–thaw events. We considered a technical replicate to be positive if an exponential increase occurred at any point of the 50 cycles (as described by Goldberg et al. 2013, see also Ellison et al. 2006).

Additional survey methods included the installation of song meters at known bullfrog sites in the Valley. From 2016 to 2018 we deployed 1 song meter at 1–2 locations where we had previously detected bullfrogs via eDNA surveys. The NPS set the song meters in early spring (April–June) during bullfrog breeding and retrieved them late summer (August–September).

The focus of the removal efforts was to directly manage for non-native species. The project was not set up as a research experiment, rather effort and time was spent when resources were available. Over the past $\sim$ 15 years, the project has been managed by different personnel. All project managers had the same goal for removal/eradication of non-native bullfrogs, however methods, efforts, and protocols fluctuated. As a result, we did not conduct visual surveys or eDNA surveys in a systematic approach that would allow for the determination of detection probability of either method.

Results

We found bullfrog breeding at 6 locations, 5 natural waterbodies and 1 manmade pond, the Ahwahnee Hotel (Fig. 1). Additionally, we found > 15 flooded areas within Yosemite Valley that we characterized as potential bullfrog habitat (Fig. 1). From 2005 to 2018, we concentrated surveys and removal efforts at those breeding and flooded areas. Available data of individuals removed are likely low estimates (i.e. not all bullfrogs removed were recorded; Fig. 2).

Of the recorded 8126 individuals removed (44 egg masses, 7462 larvae, 67 sub-adults, and 553 adults), the NPS removed 86% in the first year of concerted effort (2005). When specified, the majority of the bullfrogs removed were from breeding locations (98% of specified locations; Fig. 2). The subsequent decade was primarily spent searching for individuals with lower return on effort (Fig. 2). We removed the last observed bullfrog egg mass in 2012 and last observed larvae in 2013; since then, the NPS has not seen signs of bullfrog breeding. We captured two adult bullfrogs in 2014 and another two adult bullfrogs in 2015. In 2016, Valley residents (non NPS staff) reported observing two adult bullfrogs in the Merced River (non-breeding location). We were unable to capture the two frogs reported by residents in 2016, and we did not detected any other bullfrogs at the Merced River site or any other site in the Valley during visual surveys or on song meters from 2015 to 2018. In 2019, we located and captured one male bullfrog that was heard calling by an off duty NPS ranger.

We collected eDNA samples from 4 bullfrog breeding sites in 2015, 11 breeding sites and slack water areas in 2016, and 6 breeding and slack water areas in 2018. We conducted visual surveys at 7, 3, 1, and 8 breeding and slack water area in 2015, 2016, 2017, and 2018 respectively. We focused survey efforts on bullfrog "hotspots" or, later in the project, where we previously detected bullfrog DNA. We detected bullfrog DNA at 3 of 4 sites in 2015, 2 of 10 sites in 2016, and 1 of 6 sites in 2018 (Table 2). We did not observe bullfrogs in any areas where we detected bullfrog DNA, but DNA detects were in areas of previously known bullfrog breeding or occupied sites. We did not detect bullfrog DNA at 1 site where we observed bullfrogs in 2015 (Table 2). We collected eDNA 1 month prior to the bullfrog observations at the site. All eDNA negative control samples,

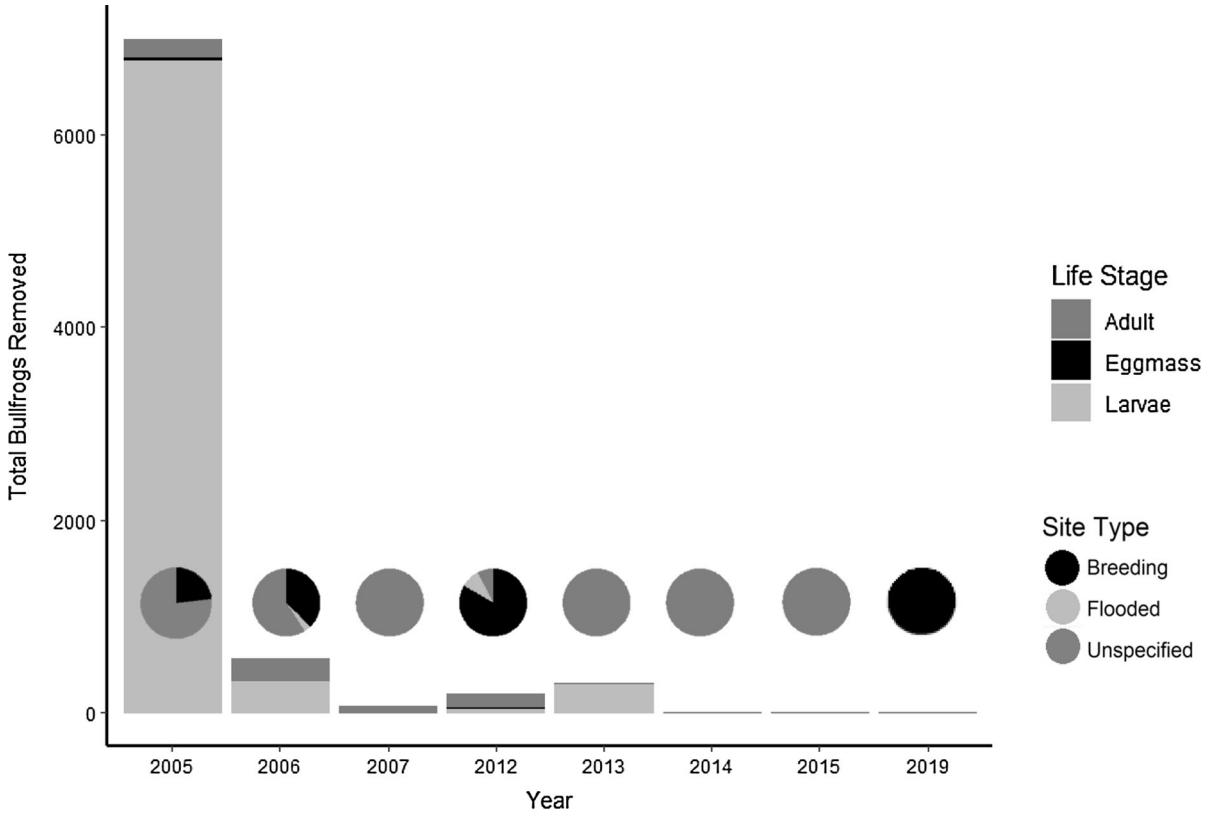

Fig. 2 Bar graph depicts the number American bullfrogs removed in Yosemite Valley from 2005 to 2019 (available numbers of individuals removed are likely low estimates due to limited data collected and recorded throughout the legacy of the bullfrog removal effort). We included all animals removed between 2008 and 2012 in the 2012 column as number of individuals removed was not differentiated per year at this time. Bullfrogs removed are split up by life stage: adult, larvae (characterized by having a tail), and egg mass. Corresponding pie charts are the percentage of animals removed from each site type from 2005 to 2019. Site types include: breeding (areas where egg masses or larvae were observed), flooded areas (potential bullfrog habitat), and unspecified (unknown or other locations). Figure created in R version 3.3.0 (R Core Team 2016)

extraction negatives, and qPCR negatives tested negative. We treated 4 samples for inhibition.

Discussion

This is the first peer reviewed journal documenting successful eradication of bullfrogs on a landscape level. Previous studies have been successful in removing bullfrogs from isolated ponds (Orchard 2011), minimizing population size (Ficetola et al. 2007; Orchard 2011), or removing bullfrogs in areas with few individuals (D'Amore et al. 2009). We effectively removed over 8000 individual bullfrogs from 6 breeding sites and > 15 flooded areas throughout Yosemite Valley, an area of ~ 1500 ha. In a review of bullfrog management efforts, Adams and Pearl (2007) explained that there are few practical control methods for bullfrogs. Our work highlights that the removal of bullfrogs in a confined area is possible by targeting breeding populations, using a variety of mechanical removal methods, and monitoring via traditional (visual surveys and audio recording devices) and eDNA survey techniques. Additional factors such as limited sites (n = 6) that supported bullfrog breeding and limited time when temperatures and conditions are conducive for breeding due to the Valley's elevational gradient (900–1280 m; Sepulveda and Layhee 2015), contributed to the successful removal of bullfrogs in Yosemite National Park's Valley.

Our eDNA surveys were sensitive to low densities of individuals, a critical component to invasive species management. From 2015 to 2018, we detected bullfrogs using eDNA methods but did not detect bullfrogs using traditional visual surveys. We even detected bullfrog DNA at Mirror Lake in 2016, a site where we have never observed bullfrogs during visual surveys nor have we observed any evidence of bullfrog breeding. While it is possible that this detection was from exogenous DNA deposited by a predator (e.g., Merkes et al. 2014), the consistent detection indicates bullfrogs have not been eradicated from the area or bullfrogs could be migrating upstream from downstream sources during low-water years. In 2016, Valley residents observed two adult bullfrogs that were never caught, and in 2019, after 4 years of non-detects via visual surveys, NPS found and removed one male bullfrog. The eDNA surveys consistently detected bullfrogs in the valley while it took 4 years of survey effort to find an individual bullfrog via visual surveys. In 2015, we observed 2 bullfrogs in an area we collected eDNA samples 1 month prior. We did not detect bullfrog DNA in the samples indicating that the bullfrogs were either not present at the time of sampling or that our sampling methods failed to detect bullfrog occupancy. At the site, we filtered 200 ml of water (Table 2). Increasing eDNA sample volume, number of samples collected, as well as frequency of sample collection would increase detection and confidence in negative sample results. Using similar methods, Goldberg et al. (2018) found an average per-sample detection rate of 0.75. We are unsure the detection probability for bullfrog eDNA in this study as we did not conduct systematic visual surveys and eDNA surveys. However, the consistent detection of bullfrog eDNA and the absence of observations of bullfrog post-2015 suggest that eDNA methods are capable of detecting bullfrogs at very low densities, and eDNA methods are more sensitive to traditional visual surveys.

Our results are consistent with previous findings that eDNA survey methods surpass traditional bullfrog surveys in terms of sensitively (Dejean et al. 2012; Goldberg et al. 2018). Sensitive survey techniques are essential to prevent re-colonization of alien invasive species and particularly bullfrogs, as they are incredibly fecund and can become quickly (re-)established in novel habitats. The use of eDNA to detect invasive species at low density is a promising tool for land managers (Darling and Mahon 2011; Kamoroff and Goldberg 2018). The consistent detection of bullfrog eDNA indicates that low densities of bullfrogs are still be present in the Valley or that bullfrogs may migrate upstream from downstream locations during low water years (Sepulveda and Layhee 2015), therefore, the NPS will continue monitoring the Valley using visual surveys and eDNA techniques in order to catch any new or remaining individuals and prevent re-establishment. However, we have not observed bullfrog breeding since 2012 suggesting that there are not enough individuals to support a population in Yosemite Valley.

After bullfrog removal, the NPS began restoration projects to support California species of special concern. Beginning in 2016, the NPS took action to introduce California red-legged frogs (*Rana draytonii;* hence red-legged frog) into the Valley. Invasive bullfrogs are one of the main threats to red-legged frogs (Lawler et al. 1999; D'Amore et al. 2009), a federally threatened species endemic to California and in decline across most of its range (U.S. Fish and Wildlife Service 2002). The park captively reared red-legged frog egg masses at the San Francisco Zoo, a program funded by the Yosemite Conservancy. The captive rearing program was extremely successful, and the NPS released approximately 400 adult red-legged frogs into the Valley from 2016 to 2018. During the 2018 fall surveys, the park staff observed the first gravid red-legged frogs, and spring 2019, we observed red-legged frog egg masses at multiple locations. The observation of multiple red-legged frog egg clutches is a promising sign for a successful introduction program.

National Parks and Monuments often provide refuges for imperiled wildlife and should be managed to remove invasive species. The presence of nonnative species, like bullfrogs, can inhibit or completely suppress native herpetofuana and other wildlife. Furthermore, climate change is likely to result in conditions that will favor increased spread of bullfrog invasion (Loyola et al. 2012), underscoring the importance of eradicating bullfrogs in a timely fashion. Our work highlights that effective bullfrog removal is obtainable in contained circumstances and can lead to local recovery of endangered species.

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Authors' contribution CK led the writing of manuscript, collected and analyzed eDNA aquatic samples, and contributed to field work. ND, RG, TE, and RR contributed to writing of the manuscript, compiling of data, and overseeing field work as well as obtaining funding and resources for project. CG oversaw the processing and analysis of all eDNA samples and contributed to manuscript writing. All authors contributed critically to the drafts and gave final approval for publication.

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