Restoring Genetic Diversity of Endangered Mountain Yellow-legged Frogs in Extirpated Watersheds

Final Project Performance Report
Cooperative Agreement P18AC01415

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Background

This study was originally developed by Drs. Roland Knapp and Erica Bree Rosenblum in 2018 and funded by the National Park Service (Cooperative Agreement P18AC01415; UCSB: $71,602, UCB: $25,000). The objective of the study was to reestablish *Rana sierrae* at several sites in Yosemite and Kings Canyon National Parks from which they were extirpated, and do so using multiple donor populations instead of the usual single donor population. The use of multiple donor populations was motivated by the fact that in some situations the available donor populations were relatively small and individually could support the removal of only a few frogs. One alternative to using a single donor population was to collect small numbers of frogs from multiple populations. Such a multi-donor reintroduction had never been conducted with mountain yellow-legged frogs (*Rana muscosa*, *R. sierrae*); in addition, it provided an opportunity to use genomic methods to (1) compare the contribution of each donor population to post-reintroduction recruitment into the adult population, and (2) describe selection in the recruited cohorts resulting from the mixing of genomes from the donor populations.

Study design

The original study design was to translocate adult *R. sierrae* into two lakes in Yosemite and two lakes in Kings Canyon (Figure 1). Based on the availability of suitable donor populations, frogs for the Yosemite and Kings Canyon translocations would be collected from two and three donor populations, respectively. We planned to assess survival of translocated adults, reproduction, and recruitment of new adults (i.e., progeny of translocated individuals) using visual encounter surveys (VES) and capture-mark-recapture (CMR) surveys. To provide samples for genomic analyses, toe tips would be collected from the translocated individuals and from subadults and adults produced at the recipient sites. Unfortunately, in 2018 when this study got underway, the number of adults in the donor populations was smaller than expected and precluded any collection of adults. Therefore, we collected tadpoles from the donor populations which were then raised to adulthood at the San Francisco and Oakland Zoos before being reintroduced into the recipient sites. This use of reintroductions instead of translocations delayed the availability of subadults and adults at the recipient sites for tissue sampling by several years.

The donor populations were all characterized by high prevalence and moderate median load of *Batrachochytrium dendrobatidis* (Bd), as is typical for enzootic *R. sierrae* populations (Knapp et al. 2011). All contained *R. sierrae* populations that were in various stages of recovering from presumed or known Bd-caused declines that occurred in recent decades. In Yosemite (Figure 2), the recipient sites are two lakes in a basin in the Merced River watershed, but bordering the Tuolumne River watershed. *R. sierrae* populations were present in this basin historically (until the early 2000s) and their extirpation is assumed to be the result of Bd outbreaks. When the current study was initiated, no extant *R. sierrae* populations remained in the basin or in the immediate vicinity. The two donor populations are in two lakes in a basin located in the Merced River headwaters (site id = 70284, 70567; separated by only 100 m) and a pond in the Tuolumne River headwaters (70397). We collected *R. sierrae* tadpoles from the donor populations in 2018 and 2019. Tadpoles were raised to adulthood in the zoos and reintroduced to the two recipient lakes (70470, 70481) in 2019, 2020, and 2021 (Table 1; Table 2). For details on the genetic structure of *R. sierrae* populations in Yosemite, see Poorten et al. (2017).

In Kings Canyon (Figure 3), the recipient sites are two lakes in a basin located in the Middle Fork Kings River watershed (10223, 10225). *R. sierrae* populations were extirpated from these sites in the mid-2000s following a well-documented Bd epizootic (Vredenburg et al. 2010). The three donor populations are located in two lakes in the Middle Fork Kings River watershed (10206, 10593) and one lake in the South Fork San Joaquin River watershed (10055). The 10206 donor population is located in the same basin as the recipient sites and also experienced the Bd-caused epizootic described above. However, unlike most populations in the basin, the 10206 population was not extirpated by the epizootic. To date, this population has persisted for more than 15 years post-epizootic, but has not yet shown much evidence of recovery (i.e, few adults present). We collected *R. sierrae* tadpoles from the donor populations in 2018 and 2019. These animals were raised to adulthood in the zoos and reintroduced to the recipient sites in 2020 and 2021 (Table 1; Table 2). Genetic structure of *R. sierrae* populations in Kings Canyon is described in Rothstein et al. (2020).

Following the reintroductions, we assessed the *R. sierrae* populations at all four recipient sites one or more times per summer using CMR surveys and VES. The VES counts provide information on the relative abundance of tadpoles and subadults, and the frog capture histories produced by CMR surveys allow estimation of adult survival, population size, and recruitment of new adults (i.e., progeny of reintroduced frogs). In addition to conducting CMR and VES, we collected skin swabs from every adult frog recaptured during CMR. Skin swabs were used for the standardized Bd diagnostic assay, and placed in the Smith/Knapp lab swab archive. When we encountered subadult *R. sierrae* in the four recipient sites, we collected toe-tips for future evaluation of contributions of different source populations.

Results

Following frog reintroduction into the study lakes (see Table 2 for release years), introduced adults were observed in all CMR surveys conducted through 2023 (Figure 4 A). Tadpoles were observed during VES conducted during the 2-4 year post-reintroduction period (Figure 4 B), and the first subadults were observed during VES conducted in 2022 (Figure 4 C). Notably, the number of all *R. sierrae* life stages counted in the two Yosemite sites is considerably lower than the number counted in the two Kings Canyon sites, likely due to differences in detectability and/or survival. In the Kings Canyon sites, a declining trend in captures of reintroduced adults is conspicuous; this pattern parallels trends observed in many post-reintroduction cohorts, as reintroduced individuals experience mortality (due to senescence, predation, or disease). Significant recruitment has not occurred by 2023; one recruit (a young frog exceeding >40 mm snout-vent-length) was PIT tagged at 70470, and no recruits have observed/tagged in the other release sites.

An interesting result is that at 10223 and 10225, three very large frogs were captured and PIT tagged in 2020 and 2021 (snout-vent-lengths 60-76 mm). Although we cannot rule out that these frogs lost their PIT tags post-reintroduction, it is our suspicion that these frogs may have survived the initial Bd outbreaks and were living in these lakes undetected until they were joined by the reintroduced frogs. This phenomenon has occurred at other reintroduction or translocation sites.

To provide a preliminary assessment of the survival of frogs from each of the donor populations, for the total number of frogs released into the study lakes and subsequently recaptured during CMR surveys, we compared the proportion of frogs that originated in each of the donor sites. Although the proportion released versus captured was generally similar between the donor sites (Figure 5), there were some small but notable differences. In 10223 and 10225, frogs from 10055 and 10206 were underrepresented in captures compared to releases (e.g. at 10225, just two frogs from 10055 have been recaptured). In contrast, frogs from 10593 were overrepresented in captures. This suggests that survival of frogs from 10593 was higher than that of frogs from 10055 and 10206. In 70470, frogs from 70567 appeared to have higher survival than frogs from 70397, but in 70481 frog survival from both donor populations was similar. Across all sites, the pattern seems stable over time, with no detectable change between 2022 and 2023.

Tissue samples (toe-tips) from the first cohort of subadults were collected from three of the four recipient sites in 2022 (number of samples: 10223 = 20; 10225 = 19; 70481 = 16) and 2023 (10223 = 20; 10225 = 4; 70481 = 4). To date, subadults have not been captured or sampled at 70470. Samples from newly-recruited adults will be collected from populations in all four recipient sites whenever new recruits are captured during CMR surveys. In addition, we also have tissue samples (buccal or toe-tip) from four of the five donor populations, and toe-tips from the one unsampled source population (70284 / 70567) will be collected in 2024.

Bd loads.

In reintroduced frogs captured in 2019-2021 at sites 10223, 10225, 70470, and 70481, Bd loads display a trend that we have also observed following other frog reintroductions: median loads were low following reintroduction, and increased dramatically in the year following reintroduction. Those elevated loads are similar to loads seen on wild frogs at Bd persistent sites. Between the recipient sites, there are no substantive differences between overall median Bd loads; within each site, the median Bd loads do not differ with respect to source sites. Bd loads for 2022-2023 were not available as of writing. This result is reassuringly unremarkable.

Additional Sites.

In 2020, a new reintroduction effort at two additional sites used a similar multi-donor strategy. These sites are located in the South Fork San Joaquin River watershed (Kings Canyon: 10109, 10114, Figure 3). *R. sierrae* tadpoles were collected in 2020 from two donor populations (10037, 10055) located in the South Fork San Joaquin watershed, and raised to adulthood in the zoo. Adults were reintroduced into the two recipient sites in 2021, 2022, and 2023; an additional cohort from 10055 will be reintroduced in 2024. Detectability of frogs during CMR is low in these large, very rocky lakes, and overall recapture rates remain very low. As in sites 10223, and 10225, the proportion of frogs from each source site differs in our CMR captures. Although we have captured just 7 and 13 reintroduced frogs at 10109 and 10114 respectively, the proportion of recaptured frogs from 10055 is lower than their proportion upon release - none have been recaptured, to date (Figure 5). Nonetheless, a hopeful sign for success of these reintroductions is that second year tadpoles were observed in 10109 in 2023 (outside of VES, and therefore not reflected in the tadpole counts in (Figure 4 C),

Summary and Next Steps

In summary, we released zoo-reared *Rana sierrae,* collected from seven Bd persistent populations (source lakes), into six recently frogless lakes (release, recipient, or reintroduction lakes; two in Yosemite National Park, four in Kings Canyon National Park). These reintroductions are unique relative to previous and other ongoing actions, because we simultaneously released frogs from two or three sources - versus a single source - into each release lake. Cohorts were released between 2019 and 2022. These multidonor reintroduction actions appear to be on track to reestablish *Rana sierrae* populations in most if not all of the reintroduction sites. Reproduction has occurred at all six sites, and metamorphosis has occurred at all four where sufficient time has elapsed. Per the original conservation goals of the project, this work demonstrates that the use of multiple source (donor) populations can employ multiple small cohorts to establish populations.

In addition to potentially reestablishing six populations, this project addresses several research questions: 1) Are there genetic, fitness, and evolutionary benefits or pitfalls from allowing frogs from different donor populations to reproduce, 2) Do frogs from one donor population vary in survival or disease dynamics across sites that vary in habitat characteristics, 3) Do frogs from different donor populations vary in survival or disease dynamics when placed in a common, Bd-occupied habitat?

The first question will take time and additional funds to resolve, relying heavily on molecular biology and bioinformatics. The latter two questions can be addressed with already collected habitat, population and Bd data, pending future statistical analysis. Frogs from each source population were released in multiple lakes simultaneously (e.g. in the case of 10055, frogs were released into four sites). Variation of habitat characteristics (e.g. depth, elevation) in these several sites may further refine our understanding of the relative effects that source population, habitat characteristics, and year have on frog population reestablishment (Knapp et al. 2011, Joseph and Knapp 2018, Wilber et al. 2022). With regard to the third question, preliminary evaluation of frog recaptures suggests that survival was not equal for frogs from each source population, within each release lake. These open questions and recent results motivate further work and underscore the need for research-driven frog recovery actions.

Future work should focus on several areas: 1) evaluating the outcome of ongoing recoveries such as those described here, 2) identifying additional potential donor sites, 3) discovering how much frog fitness and Bd tolerance/resistance varies in previously used and potential donor sites, and how these frog characteristics relate to successful population reestablishment, 4) continuing frog reintroductions from single and multiple donor sites.

In future projects, our research group will continue to visit these six reintroduction sites and continue CMR surveys. Analysis of the success of these population reestablishments and the underlying mechanisms may rely on a decade or more of capture-mark-recapture surveys, visual encounter surveys, disease diagnostics, and statistical analysis. Secondly, we will continue to identify additional frog (or, tadpole) donor sites that are characterized by high adult and tadpole abundance, long-term trends of moderate Bd loads in adult frogs, and represent a broad range of the *Rana sierrae* landscape genetic structure (Byrne et al. 2023), based on past and future visual encounter survey and Bd load data. Third, we will continue to collect genomic samples from metamorphs or new recruits at lakes 10223, 10225, 70470, and 70481. Note that future analysis of genomic samples will rely on additional funding for the molecular biology work and intensive computer analysis required to answer questions of genetic introgression and donor population fitness. That said, in 2024, we will begin detailed statistical analysis of capture-mark-recapture data for the release sites described here, assessing the contributions of frog source population, Bd load, site characteristics, and annual variation in snowpack on frog survival and recruitment. When genomic and fitness data become available, we will incorporate these into our analyses and future recommendations.

Acknowledgements

This work was performed under the following permits: Yosemite National Park: Study#: YOSE-00666 and most recent Permit#: YOSE-2022-SCI-0075, and Study#: YOSE-00953 and most recent Permit#: YOSE-2023-SCI-0034. Sequoia-Kings Canyon National Park: Study#: SEKI-00501 and most recent Permit#: SEKI-2023-SCI-0017. US Fish and Wildlife Service section 10(a)(1)(A) permit TE-40090B-1. UCSB IACUC protocols #478.3 and #478.4. We are also grateful for the support of the University of California Sierra Nevada Aquatic Research Laboratory.

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Tables

Table 1: Total number of zoo-reared adult *R. sierrae* released into each of the four recipient sites. Note that collect_siteid = 70567 includes frogs collected from the adjacent 70284. The missing information for 10223 will be added when it is obtained from the responsible zoo.

jurisdiction	site_id	collect_siteid	release_total
NA	10109	10037	30
NA	10109	10055	131
NA	10114	10037	27
NA	10114	10055	126
kings_canyon	10223	10055	41
kings_canyon	10223	10206	57
kings_canyon	10223	10593	61
kings_canyon	10223	NA	20
kings_canyon	10225	10055	33
kings_canyon	10225	10206	59
kings_canyon	10225	10593	55
yosemite	70470	70397	33
yosemite	70470	70567	75
yosemite	70481	70397	46
yosemite	70481	70567	80

Table 2: Description of collection and release locations for all cohorts of zoo-reared *R. sierrae* reintroduced into each of the four recipient sites. Note that collect_siteid = 70284 and 70567 are immediately adjacent and constitute a single donor population. The missing information for 10223 will be added when it is obtained from the responsible zoo.

site_id	collect_siteid	collect_year	zoo	release_year	n_release
10109	10037	2020	oaz	2021	11
10109	10037	2020	sfz	2021	16
10109	10037	2020	sfz	2022	3
10109	10055	2021	sfz	2022	73
10109	10055	2022	sfz	2023	58
10114	10037	2020	oaz	2021	10
10114	10037	2020	sfz	2021	17
10114	10055	2021	sfz	2022	68
10114	10055	2022	sfz	2023	58
10223	10055	2019	oaz	2020	11
10223	10055	2019	sfz	2020	22
10223	10055	2019	oaz	2021	8
10223	10206	2019	oaz	2020	15
10223	10206	2019	sfz	2020	42
10223	10593	2018	sfz	2020	6
10223	10593	2019	sfz	2020	36
10223	10593	2019	oaz	2020	15
10223	10593	2019	oaz	2021	4
10223	NA	NA	sfz	2021	20
10225	10055	2019	oaz	2020	11
10225	10055	2019	sfz	2020	22
10225	10206	2019	sfz	2020	43
10225	10206	2019	oaz	2020	16
10225	10593	2019	sfz	2020	35
10225	10593	2018	sfz	2020	6
10225	10593	2019	oaz	2020	14
70470	70284	2019	sfz	2020	30
70470	70397	2019	sfz	2020	26
70470	70397	2018	sfz	2020	7
70470	70567	2018	sfz	2019	45
70481	70284	2019	sfz	2020	28
70481	70284	2019	sfz	2021	8
70481	70397	2019	sfz	2020	27
70481	70397	2018	sfz	2020	6
70481	70397	2019	sfz	2021	13

Table 2: Description of collection and release locations for all cohorts of zoo-reared *R. sierrae* reintroduced into each of the four recipient sites. Note that collect_siteid = 70284 and 70567 are immediately adjacent and constitute a single donor population. The missing information for 10223 will be added when it is obtained from the responsible zoo.

site_id	collect_siteid	collect_year	zoo	release_year	n_release
70481	70567	2018	sfz	2019	44

Figures

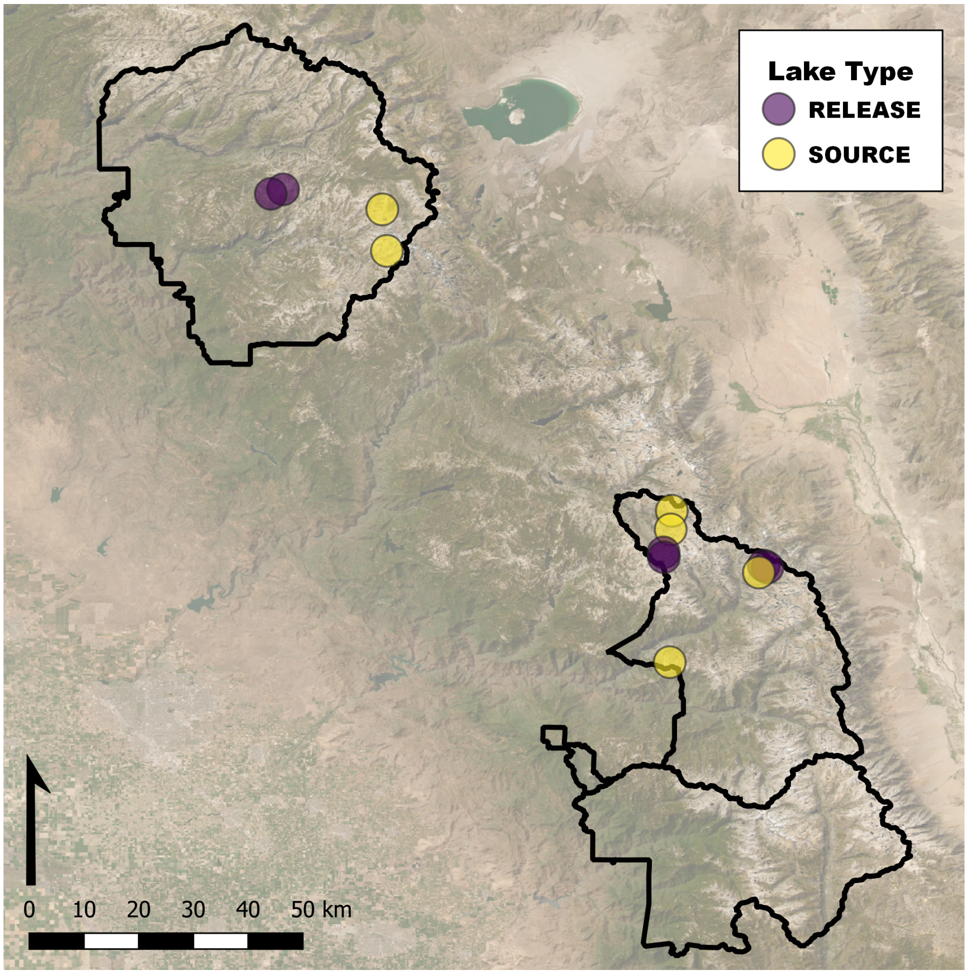

Figure 1: Overview map of project sites across Yosemite National Park and Sequoia and Kings Canyon National Park.

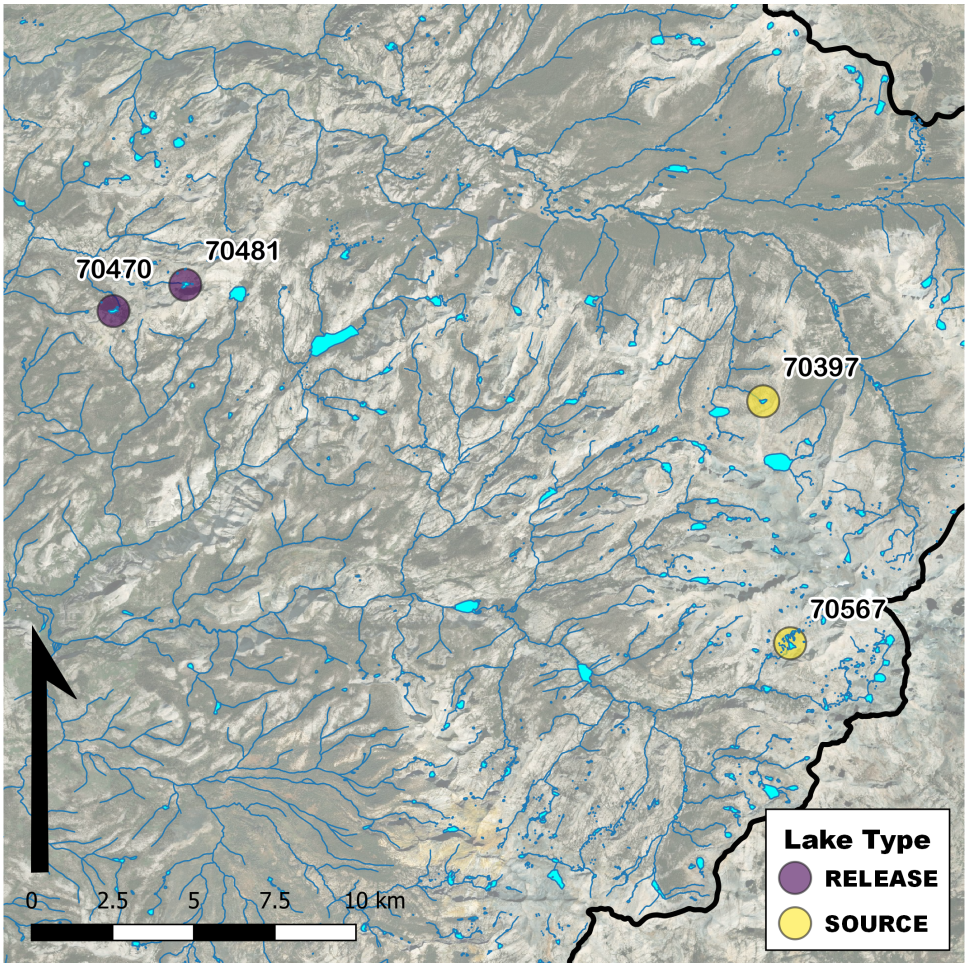

Figure 2: Map of source and release sites in Yosemite National Park.

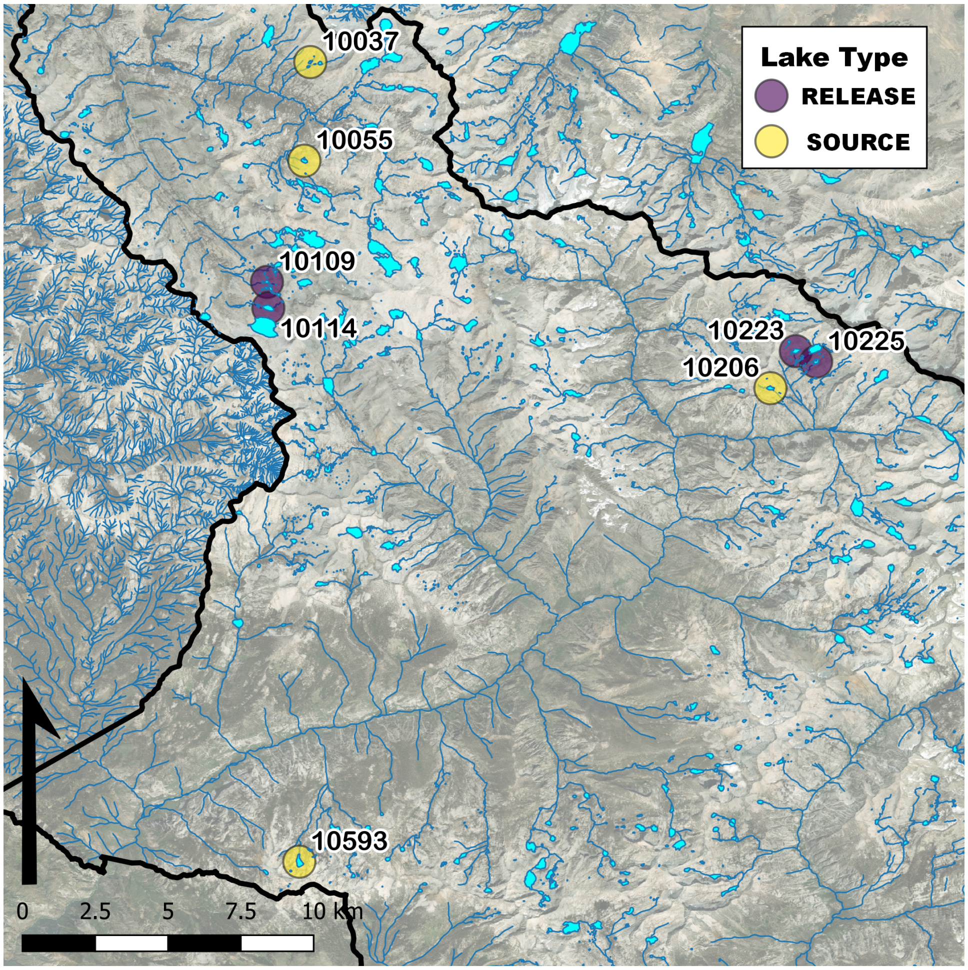

Figure 3: Map of source and release sites in Kings Canyon National Park.

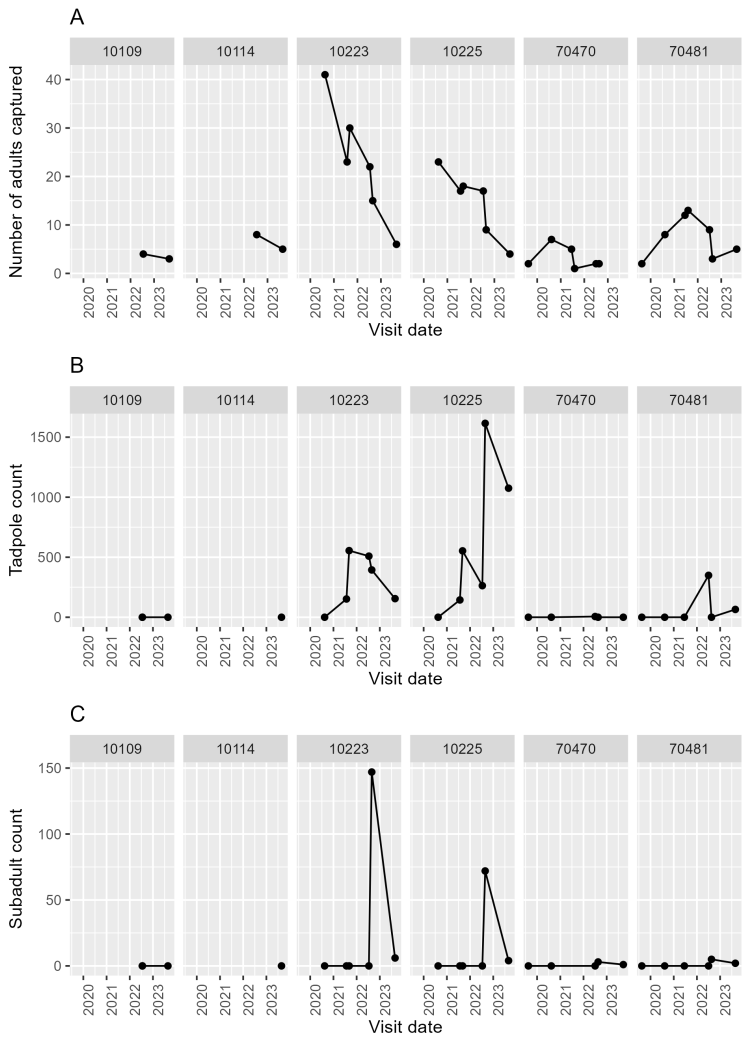

Figure 4: For the reintroduced frog populations, (A) number of adults captured during CMR surveys, (B) number of tadpoles counted during VES, and (C) number of subadults counted during VES.

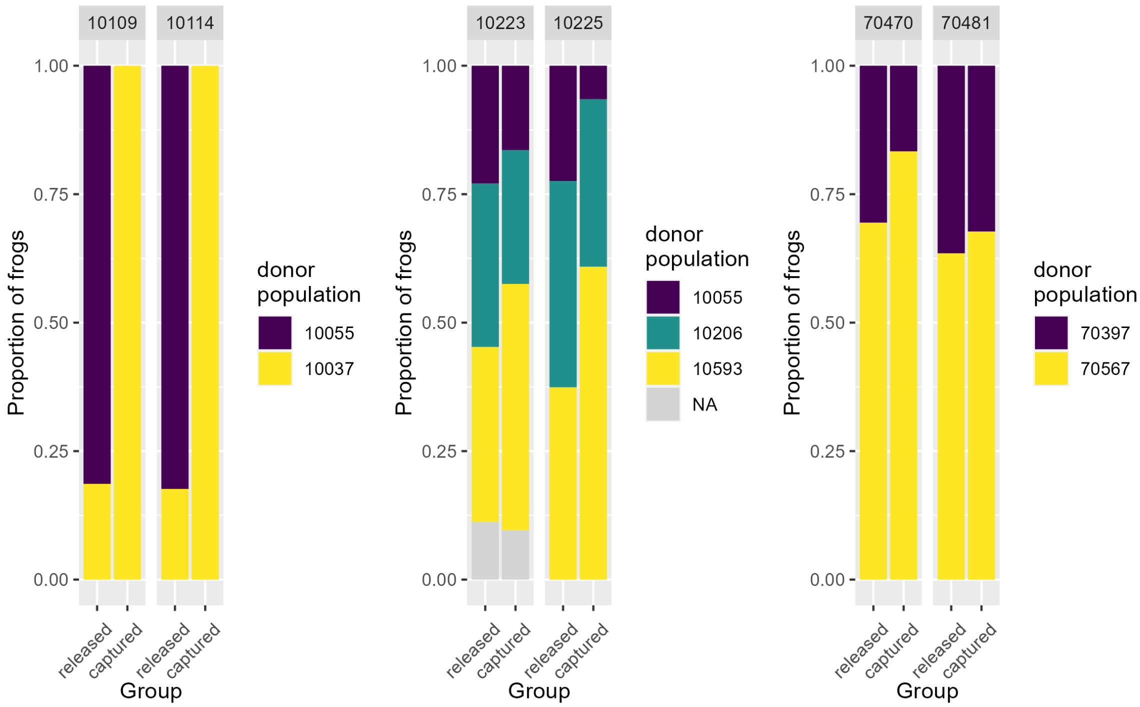

Figure 5: For frogs reintroduced into each of the study lakes, the proportion from each of the donor populations that were reintroduced ("released") versus captured during subsequent CMR surveys. Captures are pooled across all years (2019-2023).