BUILD IT AND THEY WILL COME: RESPONSE OF THE CALIFORNIA RED-LEGGED FROG (*RANA DRAYTONII*) TO HABITAT RESTORATION IN BAJA CALIFORNIA

by

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DEDICATION

This paper is dedicated to my wife, Caitlin, and my son, Lukas. Thank you, Caitlin, for being the light that guides me forward, the support I need, and the inspiration that pushes me to do my best.

ACKNOWLEDGEMENTS

I would like to acknowledge the hard work, dedication, and steadfast commitment to this project and the flora and fauna of northern Baja California by those I was able to work with on this paper. I had the good fortune to be able to analyze this data set and join a round of field surveys, but all the hard work on this project was done by others. Dr. Anny Peralta-García, Jorge H. Valdez Villavicencio, and their team at Fauna del Noroeste have led this conservation work for years, and they also directed the strategy and on-the-ground work for this project. Anny graciously worked closely with me on all the data analysis and background information, and was a critical resource for this work, and I am greatly appreciative. Jeff Alvarez of the Wildlife Project, Dr. Brad Hollingsworth and the team at the San Diego Natural History Museum, the United States Geological Survey, the United States Fish and Wildlife Service, and The Nature Conservancy have all been integral partners in this work, and again, I thank them for allowing me to conduct this research.

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HERITAGE

I am proud to be a member of the Citizen Potawatomi Nation and a descendant of the LaFromboise family. The *Bodewadmi* (Potawatomi) known as the Keepers of the Fire, originally inhabited the coast of Lake Michigan and surrounding regions, until they were forcibly relocated via the Indian Removal Act of 1830 to present-day central Kansas where they have built a thriving community.

TABLE OF CONTENTS

INTRODUCTION 1

Species Information 2
Conservation Status 6
Study Areas 8
La Grulla 10
El Potrero 11
Rancho Meling 12

METHODS 14

Field Surveys 14
Field Restoration 15
La Grulla 16
El Potrero 16
Rancho Meling 16
Data Analysis 18

RESULTS 21

Population - Abundance 21
Population – Percentage Change 23
Population – Age Classes 24
Population - Egg Masses 26

DISCUSSION 27

Population Abundance and Habitat Restoration 27
Age Class 30
California Red-Legged Frog Translocation in the United States and Baja California 31
Future Restoration Actions and Recommendations 33
Study Limitations 35
Summary and Conclusions 36

REFERENCES CITED 38

LIST OF TABLES

Table Page

Table 1. Annual estimated population abundance (number of frogs observed) at survey sites in Baja California, 2013-2023. 21

Table 2. ANOVA table showing model results for the relationship between frog abundance and year at the three survey sites in Baja California, 2013-2023 23

Table 3. Average percent of annual frog populations for each age class at survey sites in Baja California, 2013-2017 and 2019-2023. 25

LIST OF FIGURES

Figure Page

Figure 1. California red-legged frog approximate range map 2

Figure 2. California red-legged frog juvenile. Rancho Meling, Baja California. (Photo by Author) 3

Figure 3. California red-legged frog adult. Rancho Meling, Baja California. 4

Figure 4. California red-legged egg mass. Rancho Meling, Baja California. 5

Figure 5. California red-legged genetic lineages showing a separation around the Los Angeles Basin. 7

Figure 6. Survey locations for California red-legged frog in Baja California. 10

Figure 7. La Grulla Meadow, Baja California (Fauna Del Noroeste 2021). 11

Figure 8. El Potrero, Baja California (Photo by Author) 12

Figure 9. Rancho Meling, Baja California (Fauna Del Noroeste 2021). 13

Figure 10. California red-legged frog field processing and PIT-tagging. Rancho Meling, Baja California (Photo by author). 15

Figure 11. Aerial image of newly created ponds in 2018. Rancho Meling, Baja California (Peralta-Garcia 2023) 17

Figure 12. Pond restoration - before. Rancho Meling, Baja California (Fauna Del Noroeste 2021). 18

Figure 13. Pond restoration - after. Rancho Meling, Baja California (Fauna Del Noroeste 2021). 18

Figure 14. Annual estimated population abundance (number of frogs observed) at survey sites in Baja California, 2013-2023. 22

Figure 15. Scatterplot showing frog abundance at three survey locations in Baja California, 2013-2023. 23

Figure 16. Annual population estimated year-over-year percentage change at survey sites in Baja California, 2013-2023. 24

Figure 17. Annual count, egg masses observed at survey sites in Baja California, 2020-2023. 26

ABSTRACT

The California red-legged frog (*Rana draytonii*) is a large frog native to California, USA and northern Baja California, Mexico that predominantly inhabits slower-moving permanent water sources such as streams, lakes, marshes, ponds, and ephemeral drainages in valley bottoms and foothills, as well as adjacent upland habitats. However, range-wide population decline of the species has been substantial, and today it is estimated that the species occupies less than 30% of its historical range. In Mexico, California red-legged frog populations are declining dramatically, primarily from anthropogenic stresses, including ground-water mining, habitat conversion, and spread of exotic species. To improve habitat conditions for the species in the Sierra San Pedro Martir mountain range, a local non-profit conservation organization, along with international partners, created new pond and wetland habitat, and enhanced habitat along an existing stream with California red-legged frogs in 2018, and annual wetland management has occurred since then. Annual field surveys for California red-legged frogs and their egg masses were conducted from 2013 to 2023 at multiple locations. The annual population surveys suggest California red-legged frog populations have been increasing at the restoration site since 2018. From these data, I found that habitat restoration and maintenance may effectively increase local populations of California red-legged frog in Baja California. These observations suggest that funding from international partners to support conservation efforts like this one may be an effective means of positively impacting threatened species over a relatively short period of time.

CHAPTER ONE

INTRODUCTION

Amphibian populations are globally in decline, with negative impacts coming from a variety of inputs, including loss and fragmentation of habitat, disease, invasive species, urbanization, and many other factors (Tatarian 2008; North et al. 2022). In northern Baja California, Mexico, along the foothills of the Peninsular Ranges, one of the region's most threatened frog species, the California red-legged frog (*Rana draytonii*), maintains its existence, albeit under many of the same stressors that globally threaten amphibian populations. Due to declining local populations, a conservation effort was started in 20103 by a variety of organizations, including non-profits, research institutions, and governmental organizations, to increase California red-legged frog populations in the region. As part of this conservation effort, the research team, including Conservación de Fauna del Noroeste A.C. (Fauna del Noroeste), the San Diego Natural History Museum, the Wildlife Project, the United States Geological Survey, the United States Fish and Wildlife Service, and The Nature Conservancy, have undertaken extensive efforts to protect the species. The conservation efforts include threat management, genetics research, and habitat restoration and maintenance to increase available habitat for the species (North et al. 2022). Annual surveys for California red-legged frog have been performed intermittently in the region since 2013 at three locations. These three locations have received differing amounts of restoration effort. At Rancho Meling, successful habitat restoration and management activities have been performed from 2018 through 2023. Habitat restoration was attempted at El Potrero but was unsuccessful, and no restoration efforts were performed at La

Grulla. My study here seeks to help understand how restoration efforts may be influencing the population dynamics of California reg-legged frog at these three study areas. My efforts focus on estimated frog abundance as the primary data source, and available data on life stage (age class) distributions and egg mass production to further assess potential influences on frog abundance.

Species Information

The California red-legged frog is a federally endangered frog species in the United States that was formerly present in western North America from northwestern Baja California, Mexico north to Mendocino County and the Redding area in Shasta County, California (Jennings and Hayes 1984; USFWS 2002; Shaffer et al. 2004; Schulz 2022; Figure 1).

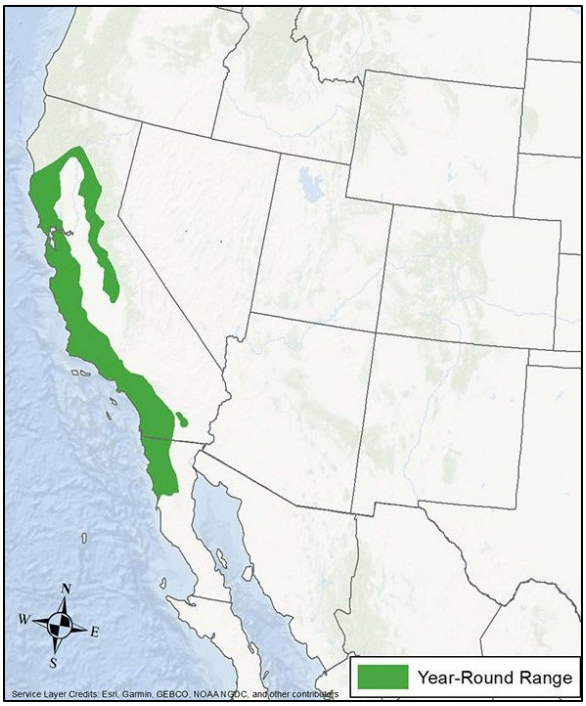

Figure 1. California red-legged frog approximate range map (adapted from Schulz 2022).

California red-legged frogs are 1.5 to 5 inches in length as adults with prominent dorsolateral folds, reddish hind legs, and darkly colored dorsal spotting (Figures 2-3; USFWS 2002). They predominantly inhabit slower-moving permanent water sources such as streams, lakes, marshes, natural and man-made ponds, and ephemeral drainages in valley bottoms and foothills up to 2,065 m in elevation (Jennings and Hayes 1994; Bulger et al. 2003; Peralta-Garcia et al. 2022).

Figure 2. California red-legged frog juvenile. Rancho Meling, Baja California. (Photo by Author)

Adults breed in a variety of aquatic habitats, while larvae and metamorphs use streams, deep pools, backwaters of streams, ponds, marshes, sag ponds, dune ponds, stock ponds, lagoons and other lentic habitats. Permanent ponds with sufficient depth (at least 0.6 m in depth) that contain emergent and shoreline vegetation for both breeding and cover from predators are particularly important to California red-legged frog (Tatarian 2008; Jennings and Hayes 1994).

Figure 3. California red-legged frog adult. Rancho Meling, Baja California.

Breeding occurs between November and April within still or slow-moving water, with light to dense riparian or emergent vegetation, such as cattails (*Typha* spp.), tules (*Scirpus* spp.) or overhanging tree branches (Jennings and Hayes 1984; USFWS 2002). Egg masses are attached to vegetation below the surface and hatch after 6 to 14 days (Storer 1925; Jennings and Hayes 1994; Figure 4). Larvae undergo metamorphosis 3.5 to 7 months following hatching and reach sexual maturity at two to three years of age (Jennings and Hayes 1984; Jennings and Hayes 1994).

Figure 4. California red-legged egg mass. Rancho Meling, Baja California.

California red-legged frogs also use non-breeding aquatic habitats, such as freshwater ponds or streams that may not hold water long enough for the frog to complete its breeding life cycle. These non-breeding uses can include shelter, foraging, predator avoidance, and aquatic dispersal over the landscape. During the dry season, California red-legged frogs may use refugia in upland habitat, such as small mammal burrows or adjacent moist vegetation, particularly during nocturnal movement (USFWS 2002; Alvarez & Wilcox 2021).

California red-legged frog predators include a variety of mammalian species, avian species, larger fish species, snakes, and other amphibian species, particularly the American bullfrog (*Lithobates catesbeianus;* USFWS 2002; North et al. 2022; Doubledee et al. 2003). In Baja California, two predators known to have a significant negative impact on California red-

legged frog populations are the San Pedro Martir rainbow trout (*Oncorhynchus mykiss nelsoni*) and the American bullfrog.

Conservation Status

The California red-legged frog is California's largest native frog, and prior to population declines, the species was prolific throughout its range (Backlin et al. 2018; USFWS 2002). Population decline of the species was rapid following Euromerican colonization of the west coast. The cause of decline range-wide includes habitat conversion and fragmentation, modified hydrology, pollution, mining, poorly managed livestock grazing, overharvesting for food in the late 19th and early 20th centuries, proliferation of non-native predators across taxa (in particular, American bullfrog and larger fish species), prolonged drought, and possibly the amphibian fungal disease chytridiomycosis (North et al. 2022; USFWS 2002; Peralta-Garcia et al. 2016; Adams et al. 2017). Range-wide population decline of the species has been significant, and current estimates suggest that the species occupies less than 30% of its historical range (North et al. 2022; Thomson et al. 2016).

California red-legged frog was considered extirpated in the United States from areas south of Los Angeles County to the United States-Mexico border, with the last extant population from this area occurring on the Santa Rosa Plateau in western Riverside County, where it was extirpated in the early 2000s (Backlin et al. 2018). However, approximately 10 localities with California red-legged frog remain in Baja California's Sierra de San Pedro Martir mountain range, leaving a 418 km gap in the southern part of the species range (North et al. 2022; Peralta-Garcia et al. 2016).

The population genetics and ancestry of California red-legged frog in northern Baja California and southern California has been examined using mitochondrial, microsatellite, and genomic data in a number of recent studies (Shaffer et al. 2004; North et al. 2022; Backlin et al. 2018). These studies confirm that all populations in Baja California, Riverside County, California, and San Diego County, California form a single, connected lineage within the species (known as the Mexican Lineage or Baja California Lineage). This lineage is considered distinctive from other lineages north of the Los Angeles Basin in California (known as the United States Lineage, Figure 5). The genetic analyses also confirmed that the Baja populations of California red-legged frog are the most genetically similar to the recently extirpated Riverside County and San Diego County populations (North et al. 2022; Backlin et al. 2018).

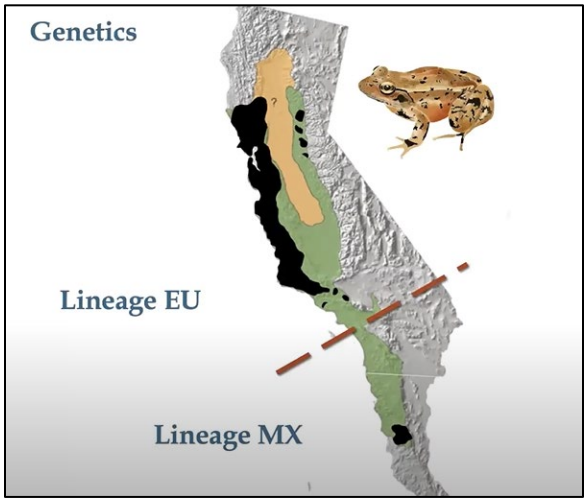

Figure 5. California red-legged genetic lineages showing a separation around the Los Angeles Basin. Extant populations of the species are in black, the former range is green, and unknown populations are in orange (Adapted from Shaffer et al. 2004).

The genetic lineages for the species have been used by the project partners (Fauna del Noroeste, United States Geological Survey, United States Fish and Wildlife Service, San Diego

Natural History Museum, and The Nature Conservancy) to inform California red-legged frog reintroduction efforts in the field. When United States-based conservation organizations along with federal and states agencies determined in 2019 that reintroduction of California Red-legged frog would take place in Riverside and San Diego County, they determined the frogs should come from Baja California due to the direct genetic link to the former populations in Riverside and San Diego County and the extant populations in Baja California (North et al. 2022).

Study Areas

This study was conducted in the Sierra San Pedro Martir mountain range, approximately 250 km south of the United States' border in the vicinity of San Quintín, from the Pacific Ocean coastline to 80-100 km east into higher elevations of the mountain range (North et al. 2022). The Sierra San Pedro Martir and Peninsular Ranges of the Baja California Peninsula create a unique ecosystem in the region, characterized by a temperate to cold climate, surrounded by desert and coastal ecosystems (Peralta-Garcia 2016). The Sierra San Pedro Martir mountain range ecosystem is isolated on the landscape by the surrounding xeric habitat at lower elevations. Such "sky island" habitats are disconnected from other similar habitats and represent important biodiversity hot spots (Davis et al. 2015; Wiens et al. 2019). Due to their isolation, these habitats and the species they host are at particularly high risk from the negative effects of climate change. The threats include overall higher winter temperatures, increased fire risk, and increased intense precipitation events resulting in flooding (Davis et al. 2015; Wiens et al. 2019).

The Sierra San Pedro Martir is a batholithic formation (a large mass of intrusive igneous rock, also called plutonic rock) extending from southern California to the southern peninsula of

Baja California, Mexico (Ruiz-Campos et al. 2022). The headwaters of the Sierra San Pedro Martir are characterized by a series of meadows, springs, and perennial streams that drain into the Pacific Ocean and are intermittent in their middle and lower sections during the dry seasons (Ruiz-Campos et al. 2022). High elevation sites in the Sierra San Pedro Martir are characterized by coniferous forest consisting of Jeffrey pine (*Pinus jeffreyi*), white fir (*Abies concolor*), and quaking aspen (*Populus tremuloides*), as well as meadows containing shrubs, forbs, and grasses (Welsh 1988). Mid-elevation sites suitable for California red-legged frog are found in riparian areas with moist soil, perennial and ephemeral streams, and vegetation consisting of willows (*Salix* spp.), oaks (*Quercus* spp.), California coffeeberry (*Frangula californica*), western cottonwood (*Populus fremontii*), and California sycamore (*Platanus racemosa*). The surrounding drier habitat is largely characterized as chaparral and coastal sage scrub communities (Welsh 1988). Lower-elevation sites suitable for California red-legged frog are similar to mid-elevation sites, but are more commonly impacted by urban development, agriculture, grazing, and groundwater extraction, such that many potentially suitable streams remain dry except for short periods of time during above-average rainy years (Peralta-Garcia et al. 2016).

Here, I analyzed California red-legged frog abundance and assessed life stage distributions and reproductive success at three sites that have received different degrees of conservation activity. California red-legged frog population surveys have been underway at all three locations since 2013 (Figure 6; Fauna del Noroeste 2021; North et al. 2022).

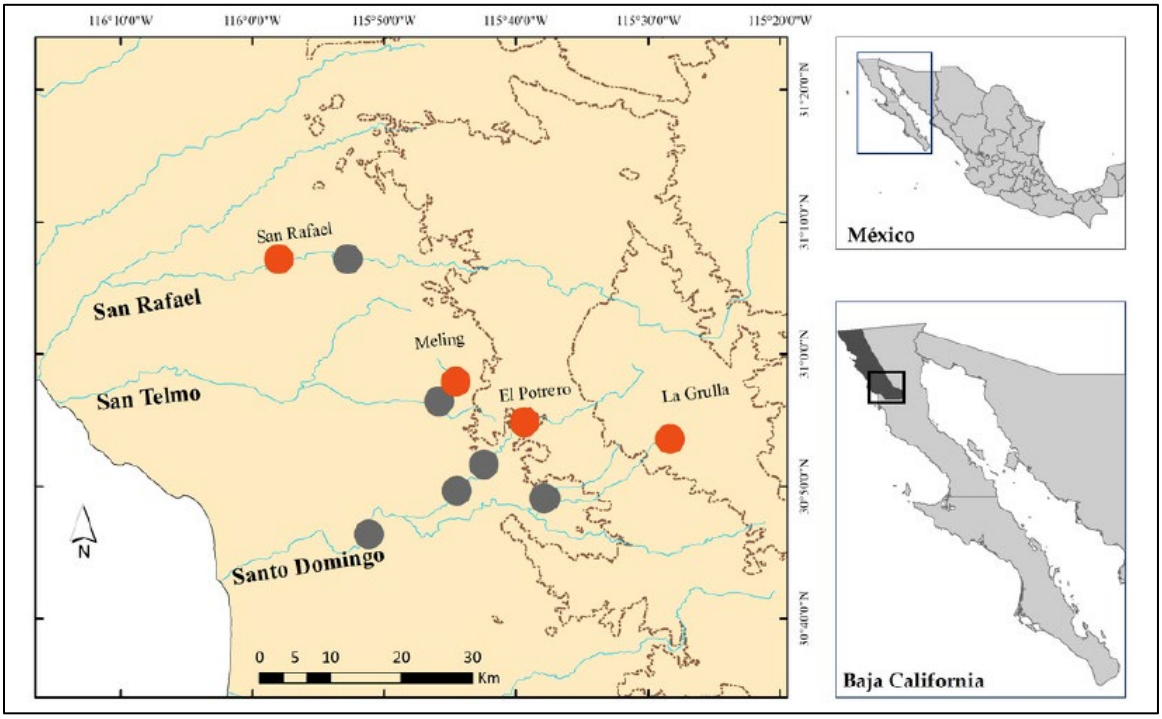

Figure 6. Survey locations for California red-legged frog in Baja California. Grey points represent sites with the presence of the species; orange points indicate the survey sites with recent survey efforts taking place. San Rafael is not part of this study (Adapted from Fauna del Noroeste 2021).

La Grulla

La Grulla Meadow is in Sierra San Pedro Martir National Park in the Santo Domingo watershed and is a large mountain meadow surrounded by coniferous forest at an elevation of 2,060 m (30.89374° N, -115.47016° W, Figure 7). The meadow contains a complex of ponds and sinkholes along its western edge that eventually flow into a channel downstream where the water descends through multiple rocky pools before becoming a stream flowing through the pine forest (Peralta-Garcia et al. 2016). The meadow ponds contain emergent vegetation and surface vegetation such as floating-leaf pondweed (*Potamogeton natans*). This is one of the highest elevation populations of California red-legged frog known to exist (Peralta-Garcia et al. 2022). Genetic diversity in this population is low and San Pedro Martir rainbow trout are abundant

approximately 50 m downstream from the edge of the meadow (Peralta-Garcia et al. 2016; Fauna del Noroeste 2021). No restoration has taken place at this location (see Chapter Two, Field Restoration).

Figure 7. La Grulla Meadow, Baja California (Fauna Del Noroeste 2021).

El Potrero

El Potrero[1] is on a privately owned ranch and is an upper tributary within the Santo Domingo watershed that exists in a mid-elevation portion (885 meters) of the Sierra de San Pedro Martir (30.917995° N, -115.649861° W, Figure 8). Upland locations are primarily chapparal and coastal sage scrub, with some oak woodlands and grasslands. Riparian areas consist of open washes and thick canopies of willow, with interspersed cottonwood (*Populus* spp.) and California sycamore trees along with other riparian vegetation. The stream is a perennial waterway with wide shallow areas, as well as narrow deeper runs and pools with some off-channel ponding. The most suitable habitat for California red-legged frog is on-channel habitat (Peralta-Garcia et al. 2016; Fauna del Noroeste 2021). In 2019, restoration efforts were

[1] El Potrero is known by one other place name from literature in the region, Santo Domingo. See Peralta-Garcia et al. 2016, Peralta-Garcia et al. 2022, and North et al. 2022.

undertaken to build two ponds, but these were filled back in with silt during winter high-flow rainstorms. San Pedro Martir rainbow trout are abundant in the stream ecosystem at this location (Peralta-Garcia et al. 2016; Fauna del Noroeste 2021; Ruiz-Campos et al. 2022). No successful restoration has taken place at this location, which is further described below (see Chapter Two, Field Restoration).

Figure 8. El Potrero, Baja California (Photo by Author)

Rancho Meling

Rancho Meling[2] is on a privately owned ecotourism ranch in the San Jose watershed and exists in a mid-elevation portion (625 m) of the Sierra de San Pedro Martir (30.972077° N, -115.742778°W, Figure 9). Similar to El Potrero, upland locations at Rancho Meling are chapparal and coastal sage scrub with some oak woodlands and grasslands. Riparian areas consist of open washes, sandy substrate, and thick canopies of willow, with interspersed

[2] Rancho Meling is known by two other place names from literature in the region, San Telmo and San Jose. See Peralta-Garcia et al. 2016, Peralta-Garcia et al. 2022, and North et al. 2022.

cottonwood and sycamore trees. Off-channel habitat is more prevalent at this site, in part due to ponds built by the ranch management, and from restoration activities (see Chapter Two, Field Restoration).

Figure 9. Rancho Meling, Baja California (Fauna Del Noroeste 2021).

CHAPTER TWO

METHODS

Field Surveys

To estimate the populations at each site, night visual encounter surveys were carried out from 2013 to 2023 by biologists from Fauno del Noroeste in Baja California and their partners. Survey teams consisted of a minimum of two biologists, and the surveyors waded or walked the shoreline and shallow-water habitats where possible, scanning ahead approximately 10 m at a time, searching for all lifestages (eggs, larvae, juveniles, adults) of California red-legged frog. The number of surveyors varied from two to more than twenty, depending on availability of surveyors. The searches were performed along streams and ponds at each of the sites, with surveyors searching carefully between the aquatic vegetation and the shores of the water bodies, following the guidelines of Heyer et al. (1994). All microhabitats that could be used by amphibians were searched (water surface, between vegetation, along the stream, ponds, and adjacent uplands).

When adult and juvenile frogs were detected, individuals were captured by hand or with dip nets. For each individual captured, data on age, sex, weight, length, and GPS location were recorded. Captured adult and juvenile individuals with greater than 60 mm of snout-vent length were marked with an individually numbered passive integrated transponder tag (PIT-tag). The tags were placed under the skin of each frog using a sterile needle to insert the tag through a "V"-shaped notch made at the level of the sacral hump with a pair of sterile sharp scissors (Fauna del Noroeste 2021). All frogs were released at their place of capture. All frogs captured during each

survey from approximately 2017 onwards were scanned for a PIT-tag, and if present, their tag number was recorded along with relevant data described above. If a PIT-tag was absent and the frogs were of suitable size, a PIT-tag was inserted as described above (Figure 10). The use of PIT-tags and subsequent scanning over the two-night survey period allows the scientist to track individual frogs over time, as well as estimate population abundance using a Mark-Release-Recapture method. For this study, Mark-Release-Recapture data analysis was not conducted due to a lack of consistent data across years at each study site, but the researchers have used these data with other studies.

Figure 10. California red-legged frog field processing and PIT-tagging. Rancho Meling, Baja California (Photo by author).

Field Restoration

Recognizing the threats to California red-legged frog in the region, Fauna del Noroeste and their partners began to develop a habitat restoration strategy to increase abundance of the

species across its extant locations. This multi-pronged approach includes invasive species control, habitat restoration and maintenance, translocation of the species to locations in the United States, and future translocation of the species to different locations within Baja California, Mexico (North et al. 2022). As part of the habitat restoration and management approach, the project partners began their efforts with pond restoration (increasing pond depth), creation of new pond features, and habitat maintenance at certain study locations, as described below.

La Grulla

Due to its remote location in Sierra de San Pedro Martir National Park and regulations imposed by the Mexican Federal Government on natural protected areas, no restoration activity has been conducted at La Grulla. This site is not currently being considered for habitat restoration or maintenance and for this analysis, this site is considered unrestored.

El Potrero

At El Potrero, restoration efforts included the attempted construction of two 50-m^2 ponds built in off-channel locations in 2019. However, these ponds partially filled in with sediment during high stream flow events and did not create breeding habitat for aquatic species. Pond design is being reconsidered at this location and future habitat restoration is likely at El Potrero. For this analysis, this site is considered unrestored.

Rancho Meling

At Rancho Meling, two ponds measuring approximately 200 m^2 and 300 m^2 were built in 2018 under the guidance of Jeff Alvarez at the Wildlife Project and others (Figure 11).

Additionally, one pond (250 m^2) was restored by increasing pond depth and removing overgrown pond vegetation in 2018. At Rancho Meling, ponds can become dominated by cattail reeds, which can remove standing water from the pond and render the ponds unsuitable for California red-legged frog reproduction. To manage these ponds and maintain their potential use by California red-legged frogs and other aquatic species, Fauna del Noroeste and their volunteer partners perform annual maintenance visits where 70-80% of the aquatic vegetation is removed (Fauna del Noroeste 2021). To complete this work, the team uses hand tools and removes the vegetation each year from September to November, once tadpoles have become metamorphs and habitat disturbance does not affect them (Figures 12, 13). Pond maintenance is a critical part of the restoration and management program to maintain the ponds at Rancho Meling in suitable condition for California red-legged frog reproduction.

Figure 11. Aerial image of newly created ponds in 2018. Rancho Meling, Baja California (Peralta-Garcia 2023).

Figure 12. Pond restoration - before. Rancho Meling, Baja California (Fauna Del Noroeste 2021).

Figure 13. Pond restoration - after. Rancho Meling, Baja California (Fauna Del Noroeste 2021).

Data Analysis

Survey data were recorded in the field using a combination of physical data sheet completion and transcription to Microsoft Excel spreadsheets, or data were entered into ArcGIS

Survey123 and exported to Excel. Field surveys at various locations were not conducted in some years due to lack of access and/or surveyor availability. No surveys were conducted in 2018.

Annual population abundance was estimated using the highest number of frogs observed in a single night in each year. At some locations, multiple nights of surveys were conducted in a single year, and for those locations we have selected the single night with the highest number of frogs observed. I assessed the estimated annual population percent change year-over-year for all three sites, each year comparing the annual population to the prior year. I assessed age class distributions for each year's population by determining the percentage of adults, subadults, and juveniles per site, per year. I then averaged these same percentages at each site across two time periods, 2013-2017 (pre-restoration at Rancho Meling) and 2019-2023 (post-restoration at Rancho Meling). Age class data were unavailable at select times and locations, including from La Grulla in 2013, Rancho Meling in 2020, and La Grulla in 2021.

For statistical analysis the program R, version 4.3.1, was utilized (R Core Team 2023). I conducted a multiple linear regression analysis to test for differences in the relationship between frog abundance and year at each of the three study sites. We included a quadratic term for the explanatory variable "year" after visually assessing frog abundance data across years, and performed an Analysis of Variance (ANOVA) to test for the significance of a non-linear relationship between frog abundance and year. Model assumptions of normality and equal variance were assessed by plotting the fitted values against the residuals and using normal QQ plots.

My statistical analysis focused on estimated frog abundance, as this data set was the most complete across years and study sites. Life stage data and egg mass data were not available

across enough years and sites to allow a formal analysis, and thus life stage and egg mass data was not used for statistical analysis. Instead, we used the available date on life stage distributions and egg mass production to supplement our statistical results, and to support our conclusions and recommendations regarding the influence of restoration on frog populations at the focal study sites. In doing so, we comment on general trends in population stage structure and reproductive success among sites.

CHAPTER THREE

RESULTS

Population - Abundance

Annual estimated frog abundance varied across all sites, from a low of four frogs to a high of 178 frogs (Table 1, Figure 14). The frog population at La Grulla decreased overall during the study period, reaching a high estimate of 52 frogs in 2013, and ranged from an estimated 22 to 37 from 2014 to 2023. At El Potrero, estimated population abundances were low overall, but saw a slight increase during the study period, ranging from 7 to 15 frogs from 2013 to 2015 and then 9 to 20 frogs from 2019 to 2023. Rancho Meling saw a marked increase in estimated frog population abundance, ranging from 9 to 34 frogs in 2013 to 2016, and increasing to 58 in 2021, 82 in 2022, and 178 frogs in 2023. Due to the disconnected nature of habitat availability in the Sierra San Padro Martir and the lack of observed movement of PIT-tagged frogs, the research team's current understanding is that each site contains one population of frogs that is disconnected from other populations in the Sierra San Pedro Martir (Peralta-Garcia pers. comm.).

Table 1. Annual estimated population abundance (number of frogs observed) at survey sites in Baja California, 2013-2023.

Year	La Grulla- NR	El Potrero- NR	Rancho Meling- R
2013	52	7	9
2014	27	4	34
2015	27	15	6
2016	22	-	30
2017	37	-	-
2018	-	-	-

2019	-	9	18
2020	-	10	24
2021	25	16	58
2022	27	20	82
2023	-	13	178

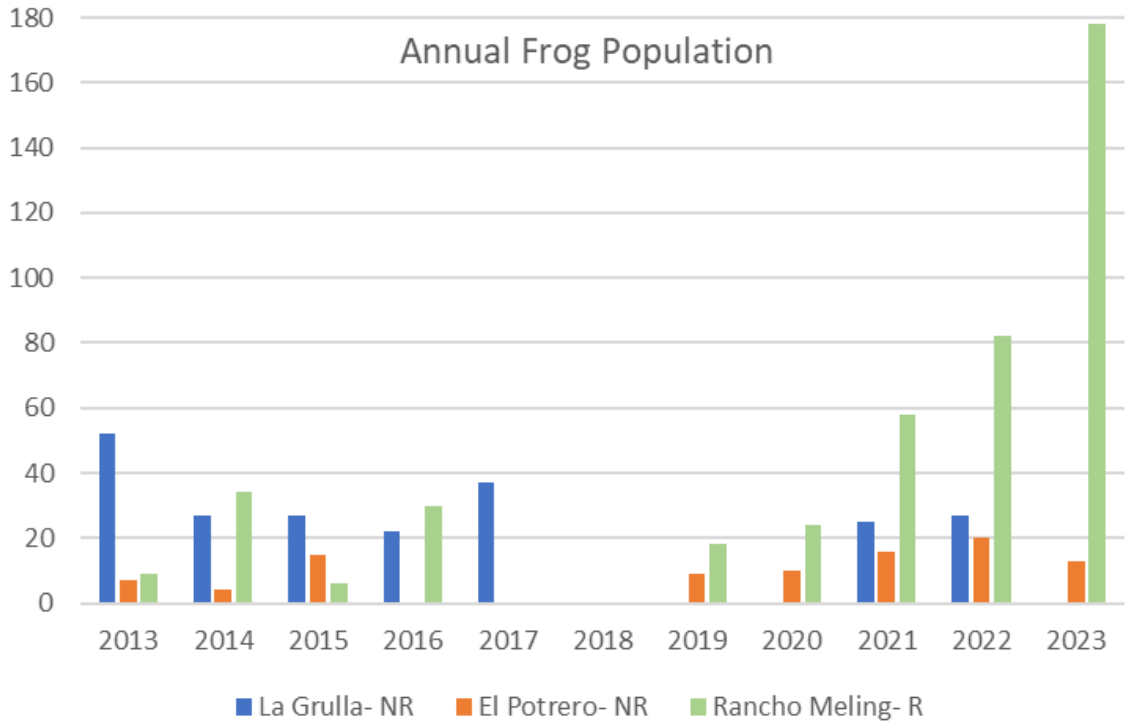

Figure 14. Annual estimated population abundance (number of frogs observed) at survey sites in Baja California, 2013-2023.

Using the multiple linear regression model I tested for a relationship between estimated frog abundance, and year, and whether the relationship differed between sites. Notably, model results showed a strong correlation between frog abundance and year and that this relationship depended on the site (year^2 × site interaction, $p = 0.006$, Table 2, Figure 15). The model results suggest a relatively good fit to the data (adjusted $R^2 = 0.63$).

Table 2. ANOVA table showing model results for the relationship between frog abundance and year at the three survey sites in Baja California, 2013-2023.

Model Parameters	F Value	Degrees of Freedom	Sum of Squares	P Value
Year	8.472	1	4147.5	0.010
$Year^2$	7.412	1	3628.7	0.014
Site	7.124	2	6976.4	0.006
$Year^2 \times site$	7.146	2	6997.0	0.006

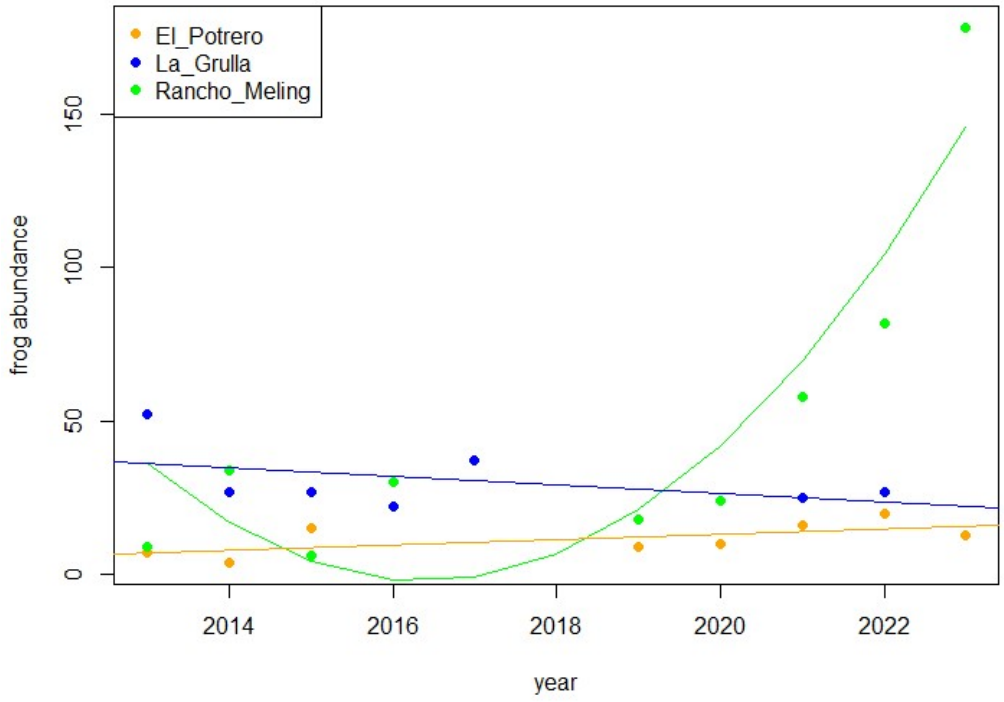

Figure 15. Scatterplot showing frog abundance at three survey locations in Baja California, 2013-2023.

Population – Percentage Change

Estimated annual population percentage change at La Grulla averaged -4% overall during the study period, ranging from -19% to 68% (Figure 16). At El Potrero, the estimated average

percentage change was 36% with increases in 2015 and 2020-2022. At Rancho Meling, the estimated average percentage change was 111% over the study period, with strong increases in estimated population abundance in 2016 and from 2020 to 2023.

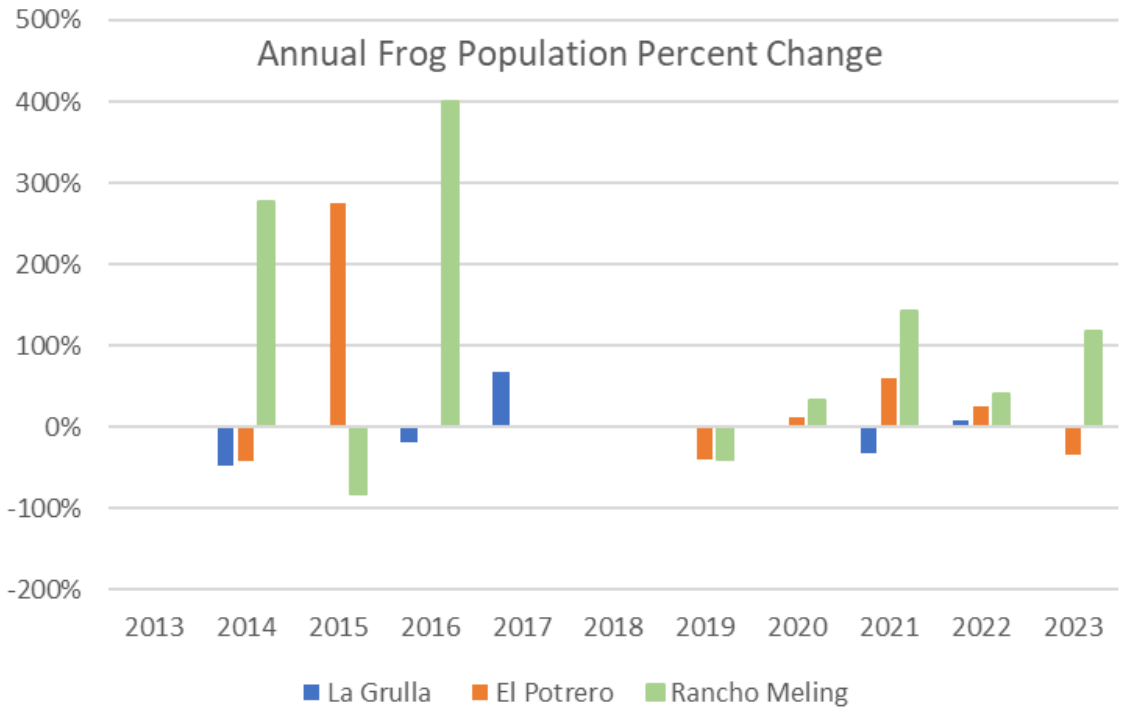

Figure 16. Annual population estimated year-over-year percentage change at survey sites in Baja California, 2013-2023.

Population – Age Classes

The average proportion of individuals varied with age class in 2013-2017 compared to 2019-2023 (Table 3). Pre-restoration years at Rancho Meling (2013-2017) were compared to the post-restoration years at Ranch Meling (2019-2023). Note that for each age class group, the total percentages do not commonly sum to 100% because data were averaged across multiple years of data sets. At La Grulla, there was not a marked difference in age classes when comparing 2013-

2017 to 2019-2023, with adult percentages ranging from 89-85%, juveniles from 15-17%, and a lack of subadult observations. At El Potrero, adult percentages within the population ranged from 68-88%, juveniles from 7-12%, and no subadults were observed. Rancho Meling had the highest percentage of juvenile individuals across the study period, with juveniles ranging from 26% in 2013-2017 to 16% in 2019-2023. Adult percentages at Rancho Meling increased from 54% in 2013-2017 to 75% in 2019-2023.

The raw numbers of juvenile frogs within those populations show additional detail in the lack of juvenile frog recruitment at El Potrero when compared to Rancho Meling. For example, from 2019-2023 at El Potrero, one juvenile was observed from 2019 to 2021 and eight were observed in 2022-2023, totaling only 10 juveniles this five-year period. In comparison, at Rancho Meling, 15 juveniles were observed from 2019 to 2021 and 59 were observed in 2022-2023, totaling 74 juveniles over this five-year period.

Table 3. Average percent of annual frog populations for each age class at survey sites in Baja California, 2013-2017 and 2019-2023.

Year	Age Class	La Grulla- NR	El Potrero- NR	Rancho Meling- R
2013-2017	Adult	89%	68%	54%
	Subadult	0%	0%	0%
	Juvenile	17%	7%	26%
2019-2023	Adult	85%	88%	75%
	Subadult	0%	0%	2%
	Juvenile	15%	12%	16%

Population - Egg Masses

The number of egg masses at Rancho Meling increased, while egg mass counts at El Potrero changed minimally (Figure 17). At Rancho Meling, egg mass counts increased steadily from 20 in 2020, to 35 in 2021, to 43 in 2022, and 192 in 2023. At El Potrero, egg masses increased from 9 to 11 from 2020 to 2022. No egg mass survey data were available for La Grulla.

Figure 17. Annual count, egg masses observed at survey sites in Baja California, 2020-2023.

CHAPTER FOUR

DISCUSSION

Population Abundance and Habitat Restoration

I found that there was a strong statistical correlation between estimated frog population abundance and survey year that depended on study site. Rancho Meling showed the greatest increase in total population abundance across years, the strongest increase in egg mass production, and the greatest increase in year over year population change. Rancho Meling is also the only site where successful restoration and on-going management took place to enhance and maintain habitat for California red-legged frog. Although our statistical approach and observational study design cannot show a direct cause and effect relationship, our analysis suggests that habitat restoration and management at Rancho Meling may have positively influenced the population abundance of California red-legged frog during the study period. Within the context of habitat restoration, there are specific factors that may have influenced the change in estimated population abundance across years at Rancho Meling. To further explore potential influences on the change in estimated abundance at the study sites, two factors to consider are increases in breeding habitat availability and increases in off-channel ponded habitat.

At Rancho Meling, like all sites in the region, California red-legged frogs breeding habitat availability may be the limiting habitat type that mostly strongly controls the population abundance (Peralta-Garcia et al. 2016; North et al. 2022). Through the construction of three pond habitats in 2018, restoration actions at Rancho Meling increased perennial ponded breeding

habitat by approximately 750 m^2. Such an increase in suitable breeding habitat is especially important in a habitat constrained region where habitat for breeding can limit population abundance and growth. Importantly, breeding habitat has not increased at El Portrero and La Gulla over the study period, which may help explain why populations appear to be increasing at Rancho Meling but not at other sites.

A potential result of the increase in breeding habitat availability at Rancho Meling is the recent increase in egg mass production at this location. The ponds at Rancho Meling were restored in 2018 and naturally colonized in 2019 by California red-legged frogs from a stream about 60 m from the ponds. Breeding was observed in the ponds in 2020. Three years later, in 2023, a dramatic increase in egg masses was observed, from 20 egg masses in 2020 to 192 egg masses in 2023. The observed lag in increased egg mass production following colonization fits with the life cycle of the species where sexual maturity is reached at 2-3 years of age (Jennings and Hayes 1984; Jennings and Hayes 1994). Similar lags in egg mass production have also been observed in other Ranid populations. For example, pond-dwelling California red-legged frogs in Hayward, California saw a strong increase in egg mass laying after a two-year delay (Hayes et al. 2016). In addition, stream-dwelling foothill yellow-legged frogs in the South Fork Eel River saw a precipitous drop in egg mass production three years after a mortality event removed a large number of adult females from the population (Riensche et al. 2019). More data on egg mass production following colonization events in restored ponds at restoration sites in Baja California will be helpful to further understand the reproductive cycles and population dynamics of California red-legged frog populations.

The importance of off-channel ponded habitat is critical for aquatic and semi-aquatic species in Mediterranean environments like Baja California as these areas are especially susceptible to weather and climatic conditions such as long periods of drought and flooding, as well as anthropogenic influences like groundwater pumping (Backlin et al. 2015; Peralta-Garcia et al. 2016; Fidenci 2004). In the study area and surrounding region, off-channel ponded habitat (compared to on-channel habitat) is especially important to California red-legged frog, as it provides breeding habitat that is more resilient to stochastic events like flooding or debris flows (Peralta-Garcia 2016; North et al. 2022). For example, in 2020, nine egg masses were observed at El Potrero during the survey season, but two egg masses were lost prior to hatching due to a flooding event in March and April 2020 (North et al. 2022). During high flow events, the newly created and restored off-channel ponds at Rancho Meling are not as susceptible to flooding and provide refugia for California red-legged frog egg masses during these events. This is particularly important, as species such as California red-legged frog that are endemic to localized, high-elevation temperate climates within xeric habitats are particularly susceptible to stochastic events like wildfire, flooding, and sedimentation events (Davis et al. 2015; Wiens et al. 2019). Due to climatic change, we can expect to see an increase in stochastic events such as these in the Sierra de San Pedro Martir and other similar habitats. Consequently, habitats such as those restored at Rancho Meling and other locations with off-channel ponded habitat may provide habitat that is suitable for the species in the face of ongoing climate change, when main-stem aquatic habitats in creeks and streams are not suitable for California red-legged frogs due to increased flooding events and associated debris flows.

In the San Pedro Martir Mountain Range, bullfrogs and fish species are important predators of California red-legged frogs. In particular, San Pedro Martir rainbow trout has negatively impacted California red-legged frog populations where its range has increased due to fish stocking for recreational purposes (Peralta-Garcia et al. 2016). However, San Pedro Martir rainbow trout are rarely found in off-channel ponded habitat due to unsuitable habitat conditions including decreased stream flows, increased temperature, and lower dissolved oxygen (Ruiz-Campos et al. 2022). Because these environmental conditions are all suitable for California red-legged frog in the region, increased off-channel habitat in restored sites can create areas along the streams where San Pedro Martir rainbow trout are absent and predation on California red-legged frog is reduced on the landscape. At Rancho Meling, newly restored off-channel habitat is available to the frogs and this habitat is absent of San Pedro Martir rainbow trout. This may be one important factor that has influenced increased estimated frog abundance at Rancho Meling. Conversely, at El Potrero, San Pedro Martir rainbow trout exist in higher numbers compared to Rancho Meling, and off-channel ponded habitat that lacks trout is minimally available to California red-legged frog (Peralta-Garcia pers. comm). These conditions may be creating increased predation pressure on California red-legged frog egg masses and tadpoles from San Pedro Martir rainbow trout at El Potrero and could negatively influence abundance.

Age Class

One of our study goals was to focus on juvenile recruitment year-over-year to determine if frogs are maturing past the tadpole stage and moving toward the reproductive adult stage. In Ranid frog species across many habitats, juvenile recruitment is seen as an important indicator of

healthy populations. Juvenile recruitment can help to maintain genetic diversity across landscapes as juveniles migrate to new breeding populations and leads to future dispersal of subpopulations within metapopulations (Hayes et al. 2016; USFWS 2022).

Overall, when examining the estimated number of juvenile frogs at sites across recent years, Rancho Meling had the highest estimated juvenile population with 74 juveniles observed from 2019 to 2023. El Potrero had a much lower estimated juvenile population with only 10 individuals during the same period. Adults also made up a higher percentage of the population at El Potrero at 88%, compared to Rancho Meling at 75%. Data were unavailable from La Grulla. A high percentage of adults within the population combined with low juvenile recruitment may be one of the main factors limiting the overall population abundance at El Potrero. There are multiple factors that may be influencing recruitment at this location, such as lack of off-channel habitat, lack of breeding habitat, and increased risk from stochastic events. Notably, ongoing restoration is occurring at El Potrero, and two ponds were built at this location in 2023. Future surveys at this location will help further our understanding of how juvenile recruitment may respond to habitat restoration and how juvenile recruitment responses may influence overall population abundance.

California Red-Legged Frog Translocation in the United States and Baja California

The California red-legged frog is at risk across its range, particularly in California south of the Los Angeles basin, where it was considered extirpated in the early 2000s (Backlin et al. 2018). After identifying translocation as a potential approach to aid the recovery of California red-legged frog in Riverside and San Diego Counties, it was determined by the regulatory

agencies that the donor populations should be sourced from genetic lineages that were closely related to the extirpated populations (North et al. 2022; Fauna del Noroeste 2021). This approach meant that donor populations needed to come from Baja California, as these were the only populations remaining with a similar genetic lineage. Genetic analyses from Fauna del Noroeste, the United States Geological Survey, and their partners indicated that stable populations of California red-legged frog from the Sierra de San Pedro Martir were most suitable for translocation efforts (Fauna del Noroeste 2021; North et al. 2022). Using this knowledge, the project partners sourced egg masses from Rancho Meling and El Potrero in 2020 and 2021, along with a third site (San Rafael) in 2022. The egg masses were successfully translocated to San Diego and Riverside Counties in the United States and adults and sub-adults have been observed since these introductions. Breeding at the translocation sites is anticipated to begin in 2023-2024 (North et al. 2022). Importantly, reintroduction efforts from a close genetic lineage to the United States were only possible due to extant (and growing) populations of California red-legged frog in Baja California. As a result of these increasing populations of California red-legged frog in Baja California, potentially due in part to habitat restoration success, future planning for translocations is currently taking place. In the United States, this includes continuing to translocate egg masses to San Diego and Riverside Counties and exploring future locations where translocation may be suitable. In Baja California, this includes planned translocations of California red-legged frog to locations that do not currently have frogs, but do have suitable habitat and have historically supported populations of California red-legged frog (Peralta-Garcia pers. comm.).

Future Restoration Actions and Recommendations

Due the potential positive impacts of restoration at Rancho Meling and the potential for recovery of the California red-legged frog in Baja California, Fauna del Noroeste and their partners are continuing restoration efforts at multiple locations in Baja California. San Rafael is another mid-elevation site in the Arroyo San Rafael watershed north of Rancho Meling where survey efforts have been recently conducted, and one 500 m^2 pond was built in 2022. At El Potrero, two additional ponds were built in 2023, adding 1,250 m^2 of breeding habitat. Arroyo San Vicente is a mid-elevation site in the Sierra Juarez Mountain Range to the north of Rancho Meling where one pond was built in 2023. Restoration at Arroyo San Vicente makes this a promising site for future translocation, as the California red-legged frog was recorded at this location in 1978 but was thought to have been extirpated sometime in the 2000s.

A large part of Fauna del Noroeste's work is environmental education and engagement with citizen scientists in the region. For example, in 2021, more than 30 volunteers contributed their time to habitat management and vegetation removal at Rancho Meling, which is a critical cornerstone of the habitat management approach at this location (Fauna del Noroeste 2021). The habitat management regime (cattail reed removal) being employed at Rancho Meling and other sites would not be possible without the contribution of local volunteers to assist in these efforts. In addition, other volunteers have contributed time and resources to assist in pond creation, bullfrog control, crayfish removal, and fund-raising drives. The input from community members from across both the United States and Mexico borders has a strong influence on the success of restoration efforts and is a good example of where citizen scientist and local volunteers can contribute towards the recovery of a threatened species and its habitat.

Future studies that focus on the potential effects of habitat restoration for California red-legged frog in Baja California will also help to better define which specific aspects of habitat restoration and management, or other efforts, are most effective for recovery of the species. For example, future studies that assess the type of microhabitat used for breeding (e.g., off-channel ponds vs. on-channel ponds vs. on-channel backwater features) may be an effective way to refine habitat restoration efforts and determine what types of habitats should be targeted for restoration. Additional studies that monitor the effectiveness of predator removal and management at existing California red-legged frog populations is another area of research that would be beneficial to California red-legged frogs in the region. For example, studies that examine the efficacy of bullfrog removal vs. San Pedro Martir rainbow trout removal would help to better inform managers about what type of invasive predator management may be most effective for California red-legged frog restoration. Additionally, surface and groundwater availability during most of the year is critical to California red-legged frogs due to their near-aquatic lifestyle. In Baja California, we know that the species has been extirpated from some locations due to over-drafting of aquifers and lack of water availability (Peralta-Garcia et al. 2016). Targeted studies that provide additional information about the amount of water needed to support California red-legged frog populations in specific regions of northern Baja California may also help to inform selection of restoration sites and guide management efforts. In addition to the research areas listed here there are other factors that may be influencing population abundance of California red-legged frog at these study locations and in Baja California such as grazing practices, soil type, upland habitat availability, and potential impacts from recreation and off-highway vehicles. All of these factors may be influencing California red-legged populations in Baja California and

additional funding and research in these areas could continue to help refine the conservation community's recovery efforts.

Study Limitations

This study produced specific data and information about estimated increases in California red-legged frog abundance at one of the study sites and provided information that explains what may have contributed to these estimated increases, with a focus on habitat restation and management. Additionally, this study provides information about restoration and management strategies, potential predator influence on abundance, and the importance of breeding and off-channel habitat that can be used in the future by researchers and land managers to better inform conservation actions. However, the study was also limited by certain factors that should be considered when interpreting our conclusions.

First, the number of study sites was limited, with only three locations included in the analysis: two non-restored sites and one restored site. There are additional sites where surveys and habitat restoration are occurring within the region for this species, but the data were limited in number of years and locations where it was collected, so it was not used as part of this study. However, current and recent surveys efforts have expanded, and future studies will be able to use these data. Additionally, at El Potrero, new ponds were built in 2023 (after field surveys were conducted), and habitat management similar to activities at Rancho Meling will be on-going at that location if the ponds are successful in holding habitat.

Second, with respect to frog abundance, the survey data represent the highest number of frogs observed in a single surveying event, but does not represent a formal account of the total

estimated population at each location. To formally estimate population size, a capture-mark-recapture approach such as the Lincoln-Petersen index calculation would be needed. This information was available at certain locations in certain years, but was intermittent. Due to its replicability across the survey years and sites, this study used the total frog abundance observed in the highest recorded survey night each year. We recognize that this approach underestimates the total number of frogs at each location and should not be viewed as a true population abundance estimate.

Finally, microhabitat selection is an extremely important aspect of the California red-legged frog life history and influences predation, diet, longevity, breeding, and dispersal (Alvarez and Wilcox 2021; Fellers and Kleeman 2007; Peralta-Garcia et al. 2016). However, microhabitat data available at the time of the study was not available across enough surveys and years to assess in this study. Future studies that assess microhabitat selection may provide additional information on where frogs are breeding and reproducing (specific types of aquatic features), as well as habitat use during dry periods and for dispersal (specific types of riparian and upland areas).

Summary and Conclusions

Restoration efforts for California red-legged frog have been ongoing since approximately 2013 in Baja California and the current status of the species is improving at some locations, in part due to the efforts of the conservation groups involved in this work. This study contributes to the conservation of California red-legged frog in Baja California by providing an assessment of how current habitat restoration and management efforts may be influencing frog recovery, as

well as how the results of current restoration can provide baseline information and context that can inform future restoration efforts.

California red-legged frogs in Baja California still face many threats, such as continued habitat conversion and fragmentation, modified hydrology, pollution, poorly managed livestock grazing, and proliferation of non-native predators across taxa (North et al. 2022; Peralta-Garcia et al. 2022). This study demonstrates that habitat restoration and management projects focused on increasing and improving suitable habitat for California red-legged frogs may be an effective use of conservation funds and could potentially contribute further to the current improvements in population abundance of the species in some locations in both Baja California and the United States.

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