April 2025

Contra Costa Livestock Pond eDNA Monitoring

2024 Progress Report

Ben Weise, Agriculture Program Director
Camille Marinier, Livestock Pond Sampling Intern
CONTRA COSTA RESOURCE CONSERVATION DISTRICT

Cover Page Photo Captions: Camille Marinier collects environmental DNA samples from livestock ponds within the Curry Canyon property owned by Save Mount Diablo (top) and Black Diamond Mines Regional Preserve managed by the East Bay Regional Park District (bottom).

This report was written and prepared by Ben Weise, Agriculture Program Director at Contra Costa Resource Conservation District and Camille Marinier, Livestock Pond Sampling Intern, in Fall 2024/Spring 2025. For more information about this report or questions, please contact Contra Costa Resource Conservation District.

www.ccrcd.org
(925) 672-4577
info@ccrcd.org

Ben Weise, Agriculture Program Director
(925) 690-4145
bweise@ccrcd.org

This report is made possible by numerous funders including:

- The California State Coastal Conservancy for their funding of our joint application with Alameda County Resource Conservation District "Water for Wildlife, East Bay Rangeland Pond and Trough Enhancement Project" that partially paid for staff time and equipment on this project.
- The Contra Costa Fish and Wildlife Committee for their funding of our grant "eDNA Monitoring of Restored Livestock Ponds" that partially paid for laboratory processing fees and equipment.
- The East Contra Costa County Habitat Conservation for funding "eDNA Monitoring of Restored Livestock Ponds in Contra Costa County" through the Science and Research Grant Program.

This work, and report, is only possible through their generous funding and we would like to thank them all for contributing to more effective livestock pond management in the future that will come out of this project.

Contra Costa Resource Conservation District would also like to acknowledge this project occurs on the unceded lands of numerous Native American tribes that managed these rangelands and vernal pool systems including the Ohlone, Miwok, Yokuts, and Karkin tribes prior to colonization and forced removal from the landscape. Contra Costa Resource Conservation District acknowledges this violent past and is actively working toward a better future that brings these tribes back into the active management and stewardship of these lands and ponds.

Table of Contents

Photos

Figures

Tables

Photo 1. A Western Bluebird resting in a Blue Oak, observed while collecting eDNA samples. Photo taken by Ben Weise, June 2024.

Photo 2. A California red-legged frog (Rana draytonii) observed while collecting eDNA samples. Photo by Ben Weise, June 2024.

Introduction

History of Contra Costa RCD Voluntary Local Program

Prior to colonization, what is now Contra Costa County and the Central Valley was largely a native grass, Oak Woodland/Shrubland landscape with free-flowing and unconstrained creeks and rivers that formed wetlands and dotted vernal pools across the landscape through the County. As colonization progressed and land was developed, many of these habitats, particularly vernal pools, were paved over and removed, forcing many species to escape to refuge in the upper parts of watersheds that to this day are still fairly undeveloped. Many aquatic and amphibious species found refuge in constructed livestock ponds, largely built between 1930 and 1970 by damming springs or building embankments around ephemeral streams and creeks in the upper rangelands. Since that time and with advent of species protections enshrined in the federal Endangered Species Act and the California equivalent (California Endangered Species Act), it's become significantly more challenging to work on or repair these ponds given the presence of listed species. In response to that, the Alameda County Resource Conservation District in partnership with the USDA's Natural Resource Conservation Service (NRCS) worked together with the California Department of Fish and Wildlife to create a first-of-its-kind Voluntary Local Program agreement that provided a path forward to restore these degrading habitats for the benefit of species and livestock.

Since 2015, Contra Costa Resource Conservation District has managed an identical Voluntary Local Program agreement with the California Department of Fish and Wildlife. Under this agreement, CCRCD acts as a go-between between the California Department of Fish and Wildlife and Ranchers/Landowners interested in restoring livestock pond function in rangelands of Contra Costa County. Ranchers voluntarily enroll in the program with Contra Costa RCD who provides necessary permits to CDFW. By enrolling in the program, Ranchers receive incidental take coverage for listed species (Alameda whipsnake (*Masticophis lateralis euryxanthus*) and California tiger salamander (*Ambystoma californiense*)) under the California Endangered Species Act (CESA) provided they follow and adhere to a suite of best management practices as it relates to the project. By Spring 2024, CCRCD has restored a total of 16 ponds with five planned for restoration in Fall 2024 and an additional three planned for restoration in Fall 2025, averaging just over two livestock pond restorations per year.

eDNA Explained

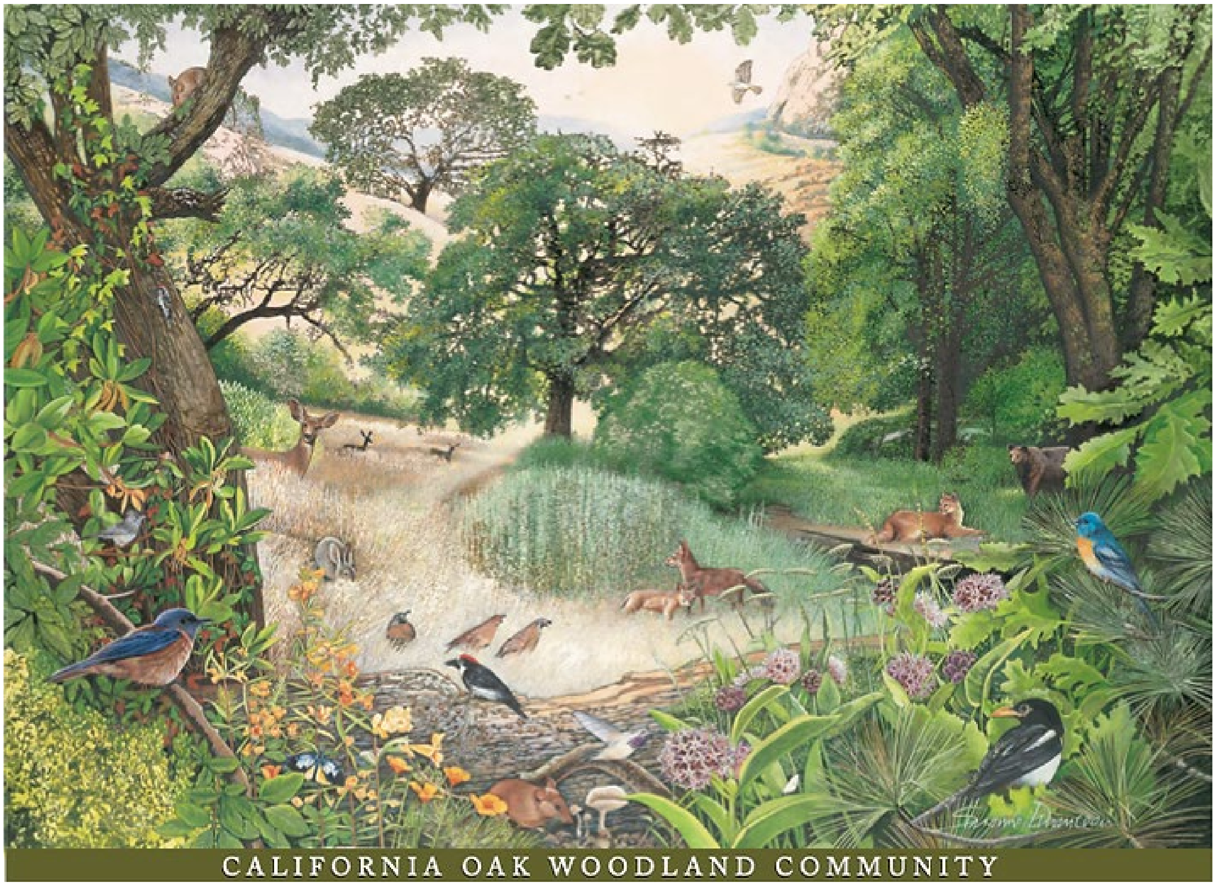

Figure 1. California Oak Woodland Community, painted by Suzanne Duranceau (1996)

Environmental DNA (eDNA) is ambient DNA shed by organisms in a system and can be found in soil, water, and air. It can come from a variety of sources including feathers, saliva, scat, hair, urine, blood, skin cells, mucus, and more. Depending on environmental conditions (wind, weather, heat, etc.) it can exist in the environment for various periods of time depending on the medium, but typically in pond systems lasts anywhere from one to three weeks.

After collecting this DNA from the system through filters or soil samples, the DNA can be extracted in a laboratory setting and referenced against a "primer." Primers at the highest level are referenced sections of DNA sequences that identify higher level taxonomic groups (ex: Animals (animalia), Bacteria, Fungi, Plants (plantae)). Through our partners at eDNA Explorers, 35 (and soon to be 37) different primers are available. Each primer contains a specific genetic sequence shared across all species within the target group (ex: all vertebrates contain the genetic sequence "TAGAACAGGCTCC-TCTAG", all invertebrates contain the genetic sequence "GGWACWGGWTGAACW-GTWTAYCCYCC."). Positive detections against the primer sequence are then amplified (replicated) in the lab and run through a bioinformatics process that further sequences DNA to determine the species or genus of the particular DNA sequence.

Environmental DNA sampling is not a new technology, but one that grew significantly during the 2019 COVID Pandemic. In the early days of the pandemic, COVID testing took time to determine results as the same process described above was utilized to detect the ambient RNA sequence of the coronavirus. As attention and funding shifted to create a rapid-test in as little as 15-minutes, environmental DNA technology received a significant boost and additional uses were explored, including for the detection and monitoring of wildlife presence across a landscape.

Environmental DNA offers significant monitoring power at significantly smaller cost, higher success, and lower impact to wildlife. As described later in this document, environmental DNA can be collected from a shovel full of dirt or a bucket of water, minimizing interactions and potential harassment or take with species. Additionally, it's better suited at detecting an array of wildlife that can be missed through camera traps or seine nets. The entire sampling procedure is fast, taking up to 15 minutes per sample as compared to spending hours seining ponds, or days spent pouring over camera trap photos. However, eDNA sampling is not a silver bullet as it requires the species to have left DNA or interacted with a pond in order to be detected. In combination with other monitoring methods, it has the ability to confirm or even add to observed species within a monitoring effort.

Using eDNA Monitoring to Assess Restoration Success

From the outset of the Voluntary Local Program, Contra Costa RCD and other project partners have wondered whether or not the restoration of these ponds achieved wildlife support goals i.e. does the restoration of these ponds result in recruitment of threatened and endangered species like the California tiger salamander and the California red-legged frog? Previous monitoring efforts by CCRCD Staff have mostly assessed the pond's structural integrity (is it still holding water? Have spillways functioned as designed?) and surrounding landscape (is the landscape still being grazed? Is the landscape being grazed effectively to achieve vegetation targets?). While conducting these prior monitoring efforts, staff conducted informal ocular assessments (looking along pond shorelines and shallows for egg masses, CRLF and CTS larvae and tadpoles, CRLF and CTS adults). CCRCD relied on this informal monitoring efforts as we lack necessary species handling permits.

Given these management goals and monitoring challenges, eDNA monitoring presents a viable solution that does not significantly disrupt the species, provides some answers to questions, and allows CCRCD Staff to assess restoration success. In Spring 2024, CCRCD began its first year of eDNA monitoring and set out to monitor the 24 ponds currently enrolled or soon to be enrolled in the Contra Costa RCD Voluntary

Local Program with a goal of establishing a long-term monitoring effort to regularly visit these ponds, assess species utilization through eDNA, and in the future develop further management activities to increase the usage of these ponds by livestock and wildlife.

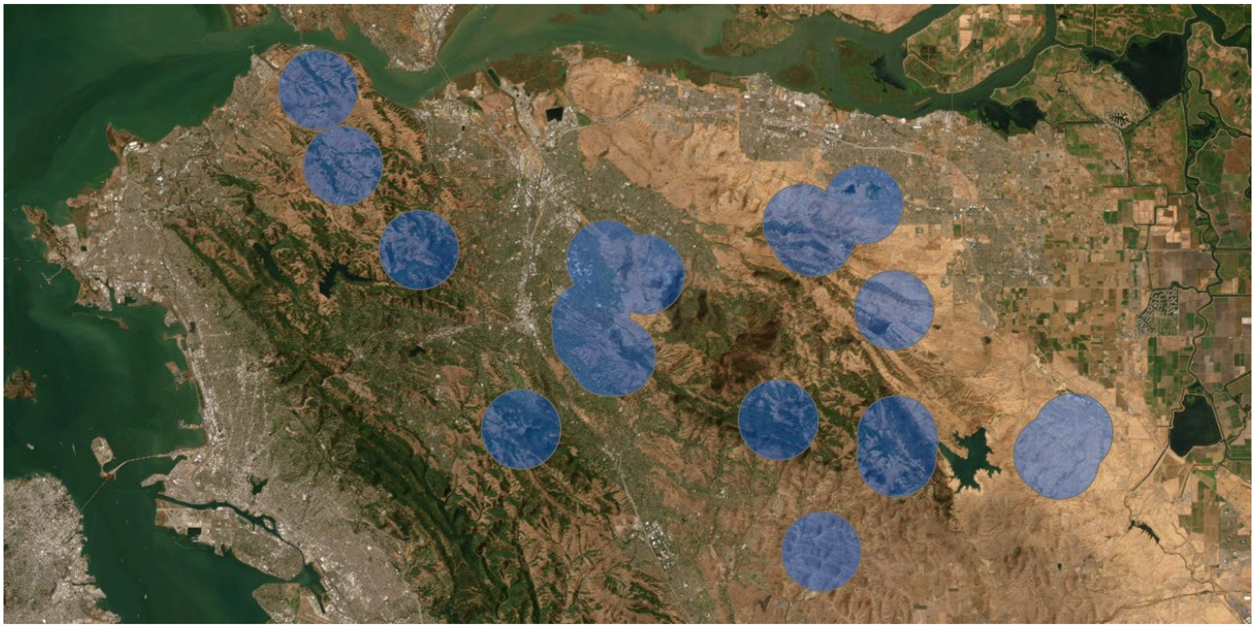

Figure 2. Approximate pond locations (buffered) of sampled ponds within Contra Costa County.

Methodology

Study Design

In Spring 2024, CCRCD had completed the restoration of 16 livestock ponds spread across Contra Costa County with an additional five restorations to occur in Fall 2024 and three in Fall 2025. The ponds themselves served as our dependent variable and the subject of our investigation.

In the first year of this study, CCRCD aimed to collect samples across the ponds, become familiar with the protocol, and identify additional questions and investigations to follow up with in subsequent years. In the following section, CCRCD summarizes some general investigations to occur in future years.

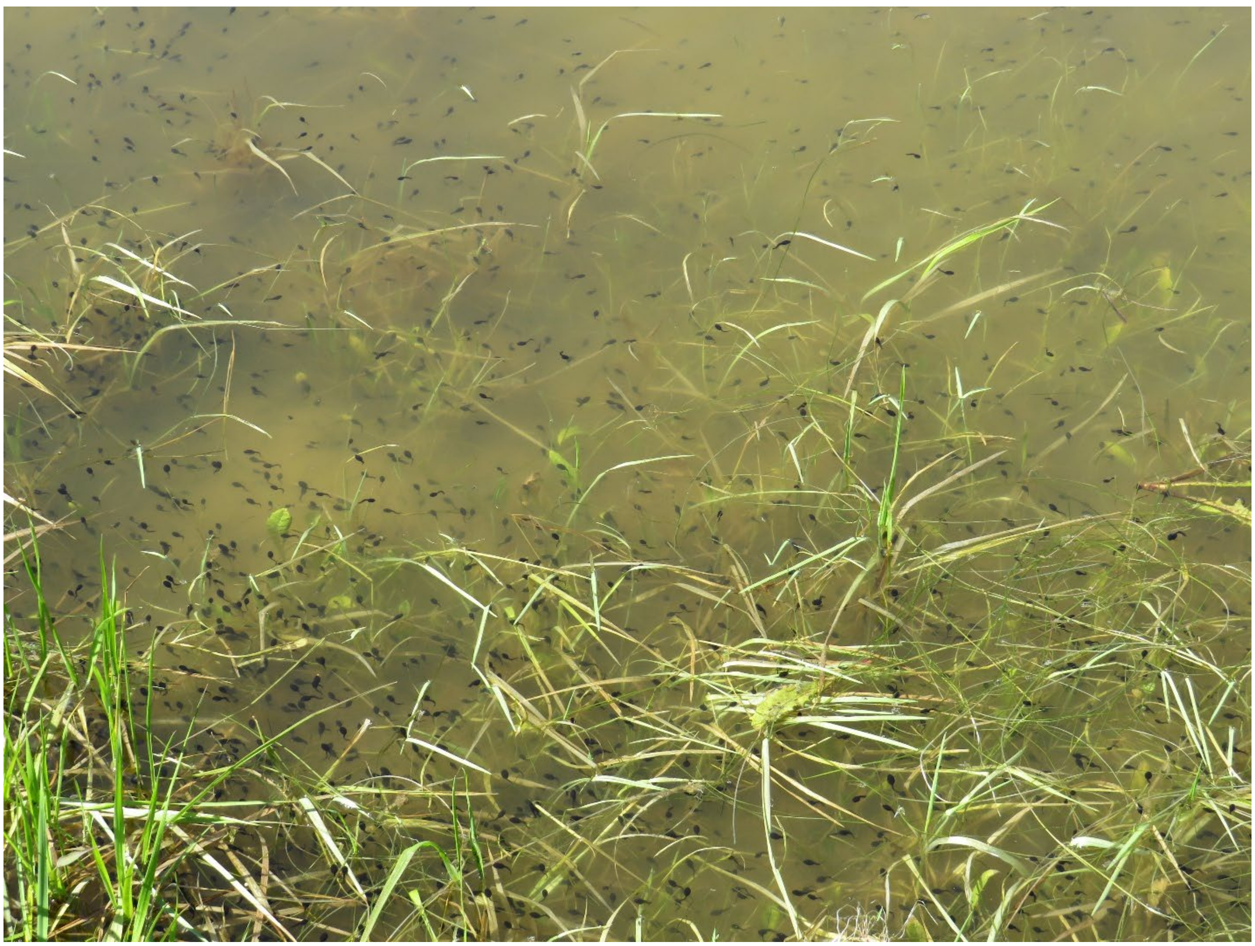

Photo 3. Frog (likely Chorus Frog) tadpoles observed while collecting eDNA samples. Note that despite the high number of individuals, this likely counts as one genus. Photo taken by Lisa Damerel, April 2024.

Species Richness Explainer

Environmental DNA monitoring provides presence/absence data of various detected or undetected species within a given system. Species DNA are either present or not, and no further conclusions can be drawn at this time such as the number of individuals within a species. Given this data output, the primary measure of this study and our monitoring effort is species richness.

Species richness is a relatively simple measure of an ecological community that is simply the number of different species represented. If there are 15 species represented in a sample, the species richness then is 15. This allows us to compare ponds very simply by looking at the total number of species detections within a pond community (ex: Pond A has 4 species, Pond B has 12 species, therefore Pond B has a higher species richness).

Primary Investigation - Species Richness Pre- and Post-Restoration

At the highest level, our investigation and monitoring effort seeks to determine the effectiveness of our restoration efforts. This work is born out of a unique partnership that provides a win-win between ranchers and wildlife regulators that 1) restores livestock pond function for use by livestock and an important source of drinking water in drought years and 2) restores livestock pond function for threatened and endangered species that utilize the pond to breed and rear young California tiger salamander and California red-legged frog. Our first and most important independent variable then is the restored nature of the pond, i.e. whether or not the pond has been restored. In our first year then, we set out to collect eDNA data from 16-restored ponds and 8-soon to be restored ponds. As the program continues, we hope to restore additional ponds and gather more data to be able to establish an average species richness of restored ponds and compare it to a species richness of ponds that are soon to be restored.

Primary Investigation - Species Richness in X Year Post-Restoration

While we won’t be able to do it this year as it is the first year of sampling, we hope to compare species richness over time at the same pond. In other words, we aim to compare the species richness of Pond A in year 0 (Spring/Summer prior to restoration), year 1 (Spring/Summer post restoration), and beyond in additional years.

Table 1. Quantity of ponds grouped by years since or before restoration occurred (negative values are ponds that will be restored in the future)

Years Since Restoration	Number of Ponds
8 (Occurred in 2015)	1
7 (Occurred in 2016)	3
6 (Occurred in 2017)	0
5 (Occurred in 2018)	3
4 (Occurred in 2019)	1
3 (Occurred in 2020)	2
2 (Occurred in 2021)	1
1 (Occurred in 2022)	3
0 (Occurred in 2023)	2
-1 (Planned for 2024)	5
-2 (Planned for 2025)	3

Secondary Investigations

Temporal Analysis of Ponds within One Year

We attempted to sample each pond three times between February/March and June/July to assess pond health and species richness as the seasons change. Livestock ponds are typically engineered and designed to hold water from the first rains in late Fall (November) through ideally the end of Summer (August/September) before going dry for a month or two. This prevents predators and invasive species like American Bullfrogs from establishing in the ponds and protects their prey, California tiger salamanders and California red-legged frogs. In some instances, to-be-restored ponds can go dry as early as April or May in a drought year depending on how late Spring rains go.

Figure 3. The same pond observed at different times of year ranging from April (top left) to May (top right) and June (bottom middle).

Vegetative Communities as a Predictor of Species Assemblage

Using available datasets, CCRCD intends to assess species assemblage as a function of the surrounding vegetative communities (i.e. blue oak woodland, annual grassland, native grassland, scrub/shrub habitat). With the near completion of the East Bay Regional Park District's Fine Fuel Mapper dataset, we expect to have access to high resolution vegetative communities from which to investigate species assemblage near and around these ponds.

Climatic, Geographic, and Pond Structure Predictors of Species Assemblage

Again, using available datasets, CCRCD intends to assess species assemblage as variables change like pond size, pond depth, pond direction, pond elevation, surrounding topography, climate, and more.

While we've listed these secondary investigations here now, we expect in the future to find additional questions and investigations and look forward to submitting those answers in future annual reports as they become available.

Photo 4. A coyote looking on while staff collect a sample from a pond. Photo taken by Ben Weise (June 2024)

Equipment

CCRCD utilized the following equipment over the course of this project.

eDNA Self-Preserving 5 µm Filter Pack Source: Smith-Root Purpose: This is the eDNA Filter where DNA is collected and sent to a laboratory for analysis. The plastic straw attaches on the larger opening and is placed in the water. The eDNA Pump (see below) pulls the water through the filter out the other end. CCRCD selected the 5 µm filter size (the largest available) based on conversations with partners at Alameda County RCD. This particular filter also contains a self-preserving resin that preserves any filtered DNA for later analysis by a laboratory. Quantity: 1 per pond visit in 2024	
eDNA Citizen Scientist Pump Source: Smith-Root Purpose: This battery powered pump pulls water through an eDNA filter into a graduated cylinder to record how much water is collected per sample	
Water Dipper Source: Forestry Suppliers, other Lab Supply Stores Purpose: Collection of water from ponds for eDNA Sampling	
Bucket for Sample Water Holding Source: Hardware Store Purpose: All samples are collected in this bucket and mixed prior to sampling. Quantity: Up to six per day, cleaned and sanitized between uses	

Nitrile Gloves Source: Hardware store, grocery store, etc. Purpose: To prevent contamination of samples with human DNA	
Sterilization and Equipment Cleaning Source: Lab Supply stores Purpose: To prevent cross contamination between samples. Water dippers are cleaned between ponds, Buckets are cleaned at end of the day (multiple buckets are taken into field).	
Other Miscellaneous Supplies ● Boot cleaning and sanitization equipment ● Hip waders ● Paper towels for drying ● Sharpies/Pens for metadata recording ● Gate Keys/Codes ● Permit Paperwork ● Notebook ● Labels	

Photo 5. Jules Mackey (Right, EcoSteward Conservation Technician), Camille Marinier (Middle, Livestock Pond Sampling Intern), and Ben Weise (Left, Agriculture Program Director) process an eDNA sample at a pond.. Photo taken by Eric Akeson, May 2024.

Sampling Protocol

For safety, CCRCD made efforts to collect these samples in pairs. Given safety constraints and remote nature of this work, it is highly recommended to utilize the buddy system. After arriving at the pond, the work was split into two roles, the sample collector and the metadata collector.

Sample Collector

1. Verify that the water dipper, catch cup, bucket, and boots have been cleaned, sanitized and dried.
2. Assess the pond, looking for potential hazards (wet/seep areas, steep slopes, inaccessible areas, ground squirrel burrows, etc.)
3. Using the water dipper, collect between 3-4 gallons of water from the pond and collect in the bucket.
    a. Collect water from all accessible parts of the pond
    b. Avoid disturbing the sediment along the bottom of the pond
    c. Avoid collecting pieces of vegetation, insects, and amphibians in samples.
4. After collecting the sample, run water through the filter until clogged
5. Remove filter from water and allow to air dry while pump runs
6. Place the filter in the bag alongside the metadata label inside the bag and close.
7. Empty bucket and pump back into the pond.
8. When done for the day, place the collected sample in the refrigerator and send it to the lab for analysis.

Metadata Collector

1. Prepare filter and pump for sample processing
2. Record date and time of sampling in notebook
3. While Sample Collector collects water sample, record visible species in area (deer, ducks, birds, amphibians, etc.)
4. Prepare and duplicate sample labels with the following information
    a. Sample Name (PondNameMMDDYYSampleNumber)
        i. Note this was changed in 2025 to a standardized system for easier recording.
    b. Approximate volume of water ran through filter
    c. Park Location - Pond Name
    d. Species Observed
    e. Miscellaneous Notes
5. Enter metadata information in Kobo Form
6. Place the label inside the bag alongside the sample, close the bag, and attach a second label to the outside of the bag.

Results

For this study, CCRCD is utilizing the CO1_Metazoa primer to detect invertebrates and the vert12SS primer to detect vertebrates based on conversations with eDNAExplorers on project goals. In March 2025, we were informed that our partners at eDNAExplorers were using our samples and running them against a potential new primer, Vert16s-U. Depending on the results, future samples may be run against this primer.

Sampling Results

In total, CCRCD sought to sample 24 ponds, three times each for a total of 72 samples. In practice, CCRCD collected 58 samples across all ponds. Sixteen ponds were sampled three total times. Six ponds were sampled two times. Two ponds were sampled just one time. All of the ponds that were sampled once or twice were done so because a) access was an issue in getting to the ponds because of damage to roads OR b) the ponds went dry before second and third samples could be collected (these ponds are slated for restoration in 2024.) All ponds were sampled between February 22, 2024 and July 16, 2024 with the majority of samples collected between March 26th and June 28th, 2024.

Photo 6. Jules Mackey collects water from a livestock pond for sampling. Photo taken by Eric Akeson, May 2024.

The first sample for each pond was collected between February 22, 2024 and June 24, 2024, but the majority of the first samples were collected between March 26 and April 19, 2024. CCRCD began sampling in February 22, 2024 figuring that most of the winter rains had ended. Rains then continued through March until late March when vehicular access was enabled on the sites again. Further, two samples weren't collected until June 24 as access and road conditions prevented sampling. For statistical purposes, these two samples were reclassified into the second sample batch detailed in the next paragraph.

The second sample for each pond was collected between May 6, 2024 and June 26, 2024. By this point in time, most of the road conditions were addressed allowing for a far easier time sampling.

The third sample for each pond was collected between June 17 and July 16, 2024. By this point, most unrestored ponds were nearly completely dried while restored ponds would hold water for another week or so.

The average number of days between the first and the second sample was 56 days. Removing the outlier samples collected in February, the average difference between sample collection dates drops to 40 days. The average number of days between the second and third samples is 38 days. With better planning, CCRCD aims to decrease this difference down to around 30 days between samples dependent on weather and other factors.

Contra Costa Resource Conservation District contracted with eDNA Explorers Inc., a private business that runs eDNA sample processing and advanced tools for analysis, geospatial data integration, and display. CCRCD mailed samples on April 22, 2024, June 21, 2024, and July 17, 2024. Samples were processed by eDNA Explorers and the first batch of results for the first sample shipment was received on August 23rd, 2024. The second and third batch of results were received March 2nd, 2025. A third batch of results is expected in April/May 2025 as laboratory error missed 10 samples. The laboratory analysis part of this process is by far the longest part, requiring processing, cleaning, extraction, amplification, and categorization. Likewise, samples are processed once a requisite number of samples arrives in order to be cost-effective with expensive laboratory equipment. These reasons are the primary reasons for the delay in results in addition to laboratory equipment needing repair.

Available results will be posted on the eDNAexplorer.org. At present, CCRCD is working with the eDNA Explorer team to obscure pond location and results for the protection of special status species and privacy of cooperators and ranchers under the Voluntary Local Program.

Disclaimer

eDNA Monitoring is a relatively new monitoring technology that benefited greatly from the technical and scientific investment in detecting the COVID-19 virus. The same underlying technology (PCR testing) is used to identify and sequence ambient DNA in an environment. It is further built on an international knowledge base of various species across the world and their DNA sequences. As more and more of the “tree of life” is sequenced and found, eDNA monitoring will continue to improve and get more refined and precise. All that to say, there is an error rate in eDNA sequencing, in both the false-positive (a positive detection of a species that wasn’t there) and in missing species that weren’t present. As species continue to grow and evolve and branch into sub, and sub-sub species, genetic sequencing gets trickier and species may be missed. However, eDNA still proves to be a good monitoring technique that when used in combination with other monitoring techniques, can amplify and strengthen monitoring efforts. To better interpret results, CCRCD Staff viewed results through the Genus taxonomic group rather than species. Viewing results at this level reduces some of the error rates and gives a better sense to staff of the wildlife out there.

Lastly, the percentages listed below will likely change with the final update of our results for the 10 missing samples at present. For the latest, please visit ednaexplorers.org.

Organism Diversity

Using the eDNA Explorers interface, the “Organism List” provides a breakdown of the DNA collected in our samples and grouped by the percentage of samples that contain that DNA sequence. The following report was developed by organizing species at the Genus level, with at least 1 detection across any samples, with a confidence level of 1 out of 5. Viewing results at this level allows us to see any and all positive detections in a given sample. While confidence is low (rated 1 out of 5), further insights from CCRCD Staff on pond location, surrounding vegetation type, and other factors help inform the likelihood that the positive detection is accurate. Of the 52 samples processed (including some samples completed by project partners at Alameda County RCD), 78 unique genera were detected.

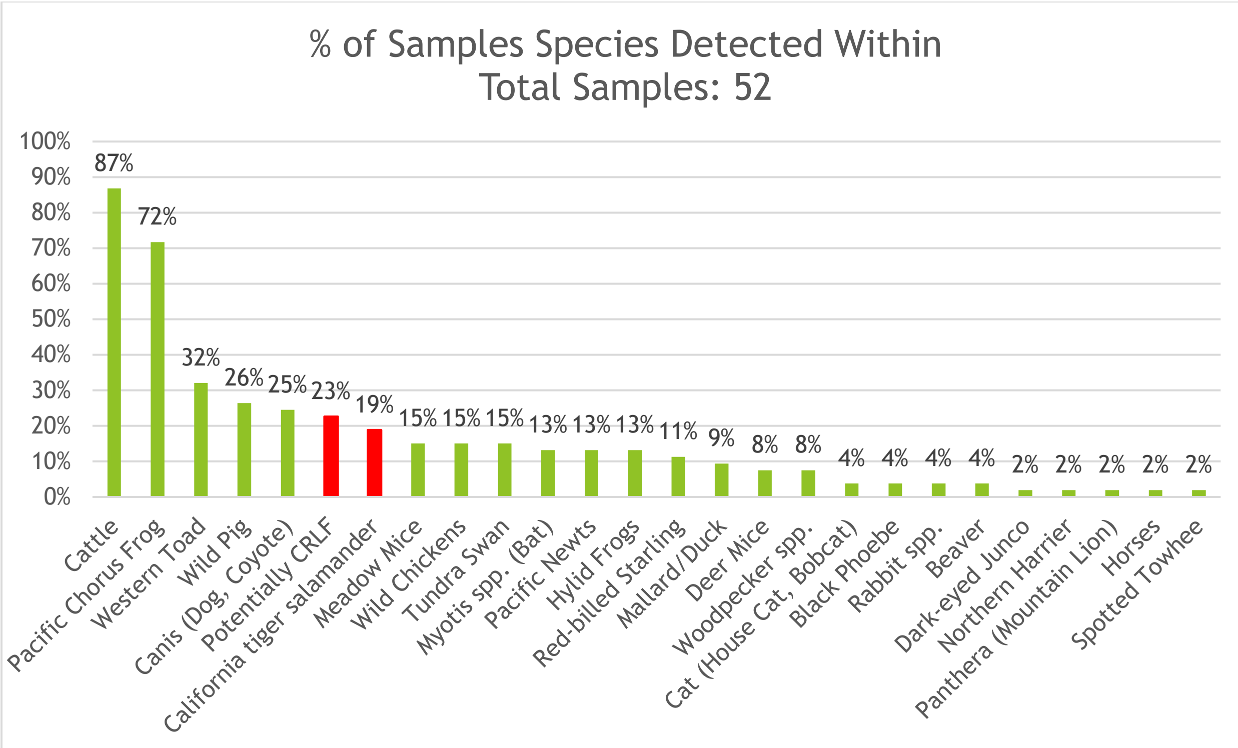

Figure 4. Percent of samples containing individual species DNA

Unsurprisingly, the highest observed DNA sequence across our samples is *Bos spp.*, or cattle, occurring in 87% of our samples (the remaining 13% likely did not have cattle in the area when samples were collected.) Also unsurprisingly, 72% of collected samples had DNA sequences belonging to *Pseudacris,* a Genus containing the commonly named Pacific Chorus Frog that the sampling team observed at nearly every single pond. The third highest observed genus, although there is a significant drop off from the *Pseudacris* genus, is *Anaxyrus* spp. At 32% of samples, more than likely the American Toad. *Canis* and *Sus,* or dogs and pigs tied at 26% of samples. The *Canis* genus includes a wide variety of domesticated dogs that may have interacted with some of these ponds while out on hikes with their owners through these public parks and open spaces, but also includes coyotes. The *Sus* genus likely refers to wild pigs and boars, an invasive species on the rise across the state in open and wild spaces.


Photo 7. One of the many cows that provided DNA in our samples. Photo taken by Eric Akeson, May 2024.

With regard to species of interest to this report (California tiger salamander and California red-legged frog), *Ambystoma* (California tiger salamander) were encountered in 19% of samples, but around 1/4 of all ponds (5). *Ambystoma* typically spend most of their lives underground in ground squirrel burrows, emerging in Winter/Spring to breed before leaving the pond to return to their burrows, which could explain their absence across some samples.

Photo 8. A California tiger salamander (Ambystoma californiense) observed while collecting an eDNA sample. Photo taken by Eric Akeson, May 2024.

Somewhat surprisingly, *Rana* only appeared directly in one pond despite multiple observations by CCRCD Staff in other ponds. Based on that, CCRCD worked with eDNA Explorers and Farley Connelly, Biologist, at Alameda County RCD to determine what may actually be happening. After grouping results by the Family taxonomic level, 25% of samples contained DNA consistent with the *Ranidae* family, but appear further down the taxonomic chain as the genus *Amolops*, *Odorrana, Sanguirana, Sylvirana, and Pristimantis*, all of which are exotic species to South America and Asia. Other exotic frog Genus taxonomic groups include *Dendropsophus* and *Hylid*, both of which are classified higher under the Family taxonomic group *Hylidae* which contains the previously mentioned *Pseudacris* species. All that to say, its highly likely that the mis-labelled genera in the *Hylidae* family are more than likely Pacific Chorus Frog. It gets a little more complicated as the mislabeled genera in the *Ranidae* family are likely California red-legged frog, but could also be the American Bullfrog, an invasive species here in California. CCRCD Staff observed no Bullfrogs while sampling, which leads us to believe the *Ranidae* observations are indeed California red-legged frog, albeit with an asterisk attached. If our assumption was correct, CCRCD Staff would have detected CRLF in 25% of samples and 10 out of 24 ponds.

Staying within the amphibians, 13% of samples contained DNA of the genus *Taricha*, more than likely the native Pacific Newt, which was also encountered a few times while sampling. Elsewhere, other exotic newts also appeared including *Tylotriton* (9%), and *Paramesotriton* (4%). All newts appeared within the same six ponds. In Contra Costa county, there are two newt species, the Sierra Newt (*Taricha sierrae)* and the California Newt (*Taricha torosa*) that have similar ranges in the western part of the county. These exotic newt appearances are more than likely one of these two newt species.

Photo 9. A California Newt crossing a trail enroute to a pond to breed. Photo taken by Eric Akeson, April 2024.

Other genera of interest include *Felis* appearing in two samples across 2 ponds. One of those ponds is within ¼ miles of a suburban housing development suggesting a higher likelihood of an outdoor domesticated cat, but a separate pond with another sample detection is over a mile and a half from the nearest suburban housing development suggesting potentially a wildcat of some type. Additionally, there was one incidence of the *Panthera* genus at a different site, suggesting a detection of a puma or a Mountain Lion (*Puma concolor).* The results also showed the genus *Myotis* or mouse-eared bats appearing in 13% of samples across four ponds. The exact species is unknown, but is more than like the Yuma Myotis (*Myotis yumanensis*) or the California Myotis (*M. californicus*). Which are abundant across Contra Costa county.

Continuing with mammals, a number of small rodents and mammals also appeared in the samples including voles (*Microtus spp.,* 9% of samples), deer mice (*Peromyscus,* 8% of samples), woodrats (*Neotoma,* 6% of samples) cottontail rabbits (*Sylvilagus*, 4% of samples), beaver (*Castor,* 4% of samples)*,* and some exotic species appearing in 4% of samples or less. Interestingly, Deer (*Cervidae*) were not observed in any samples. Contra Costa RCD expected to see mule deer (*Odocoileus hemionus)* and observed some while sampling, but none were present in collected samples.

Lastly for our samples, a number of birds were detected. Swans (*Cygnus*) were detected in 21% of samples. No swans of any species were observed during sampling, but based on known *Cygnus* in the region, it is more than likely a Mute Swan (*Cygnus olor).* The *Gallus* genus was observed in 17% of samples and is more than likely chickens of some type. Following closely behind, Starlings (*Sturnus*) appeared in 11% of samples, while Woodpeckers (*Melanerpes*) appeared in 8% of samples. More than likely, the woodpeckers are Acorn Woodpeckers (*Melanerpes formicivorus*), a species observed in the field while sampling. Similarly, 8% of samples contained the *Anas* genus, which is more than likely Mallards observed while sampling. Other observed genera include *Gampsorhynchus* (8%), *Schoeniparus (*6%), *Pitta* (4%), *Emberiza* (4%), *Zenaida* (4%), *Sayornis* (4%), *Pipilo* (4%), *Helmitheros* (2%), *Junco* (2%), *Aphelocoma* (2%), *Piculus* (2%), *Merganetta* (2%), *Cairina* (2%), *Turdus* (2%), *Tyto* (2%), *Fregata* (2%), *Patagioenas* (2%), *Circus* (2%), and *Chalcophaps* (2%). Some of these are more than likely native species including *Amphelocoma* (Western Scrub Jay, *Aphelocoma californica*), *Tyto* (Barn Owl, *Tyto alba*), *Circus (*Northern Harrier, *Circus hudsonius*), *Sayornis (*Black Phoebe, *Sayornis nigricans*), *Pipilo* (California Towhee, *Pipilo crissalis*). Jumping to the CO1_Metazoa primer, CCRCD detected Tricolored Blackbird (*Agelaius tricolor)* at one site and one sample. CCRCD Staff noted the possibility of species while sampling while the eDNA record confirmed it.

Of note, no reptiles were detected across any samples despite staff observances of what was likely a Garter Snake (*Thamnophis atratus*) at multiple sites. In discussions with eDNA Explorer staff, this likely just means that reptile utilization of the ponds is low and was not captured within our samples (the selected primer should have picked up any ambient reptile DNA.) Western Pond Turtles were not observed at any of the ponds, and because of the shell, may not easily shed DNA into the pond. CCRCD will continue to monitor and investigate reptile absence in these sample results.

Photo 10. An Acorn Woodpecker looking for directions in Diablo Foothills Regional Park, taken by Ben Weise, June 2024.

Other General Observations

After receiving nearly all of the sample data back, CCRCD Staff began cleaning and processing the accumulated data for future analysis. The image below is a snapshot of the entire dataset that will eventually be made public on eDNAexplorer.org once location data is scrubbed from samples sites.

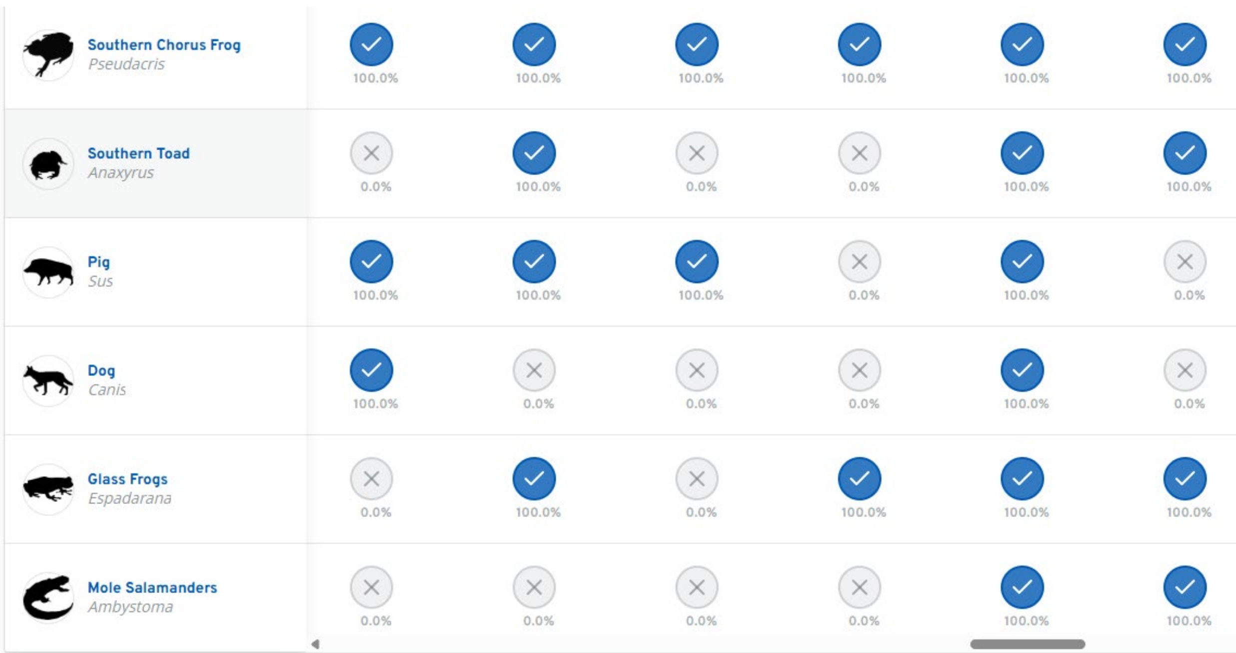

Figure 5. A snapshot of what our results will look like on eDNAExplorers.org once published. Each column indicates one sample.

Note that the number of genera detected DOES NOT indicate the number of individuals within a genera. A single individual and 100 individuals return the same genera presence data. Further, while some of the genera are believed to be incorrect, they still represent some individual strand of DNA collected within the sample which is unique from other collected DNA strains, indicating unique genera. So, while specific species or genera may be incorrect, broader discussions of species richness still hold true.

Species Richness of Pre- and Post-Restored Ponds

One of the first investigations CCRCD performed once data was received and cleaned was comparing the number of genera detected within a sample period across ponds that had been restored prior to sampling or were going to be restored in the near future. In 2024, 16 ponds had been restored while 8 were planned for restoration in 2024 and 2025. These ponds were also compared across the three sample periods.

Table 2. Comparison of average number of detected genera across sample periods and ponds pre- and post-restoration.

Sample Period	Post	Pre
2024-01	6.23	5.85
2024-02	4.43	3
2024-03	4.25	1.5

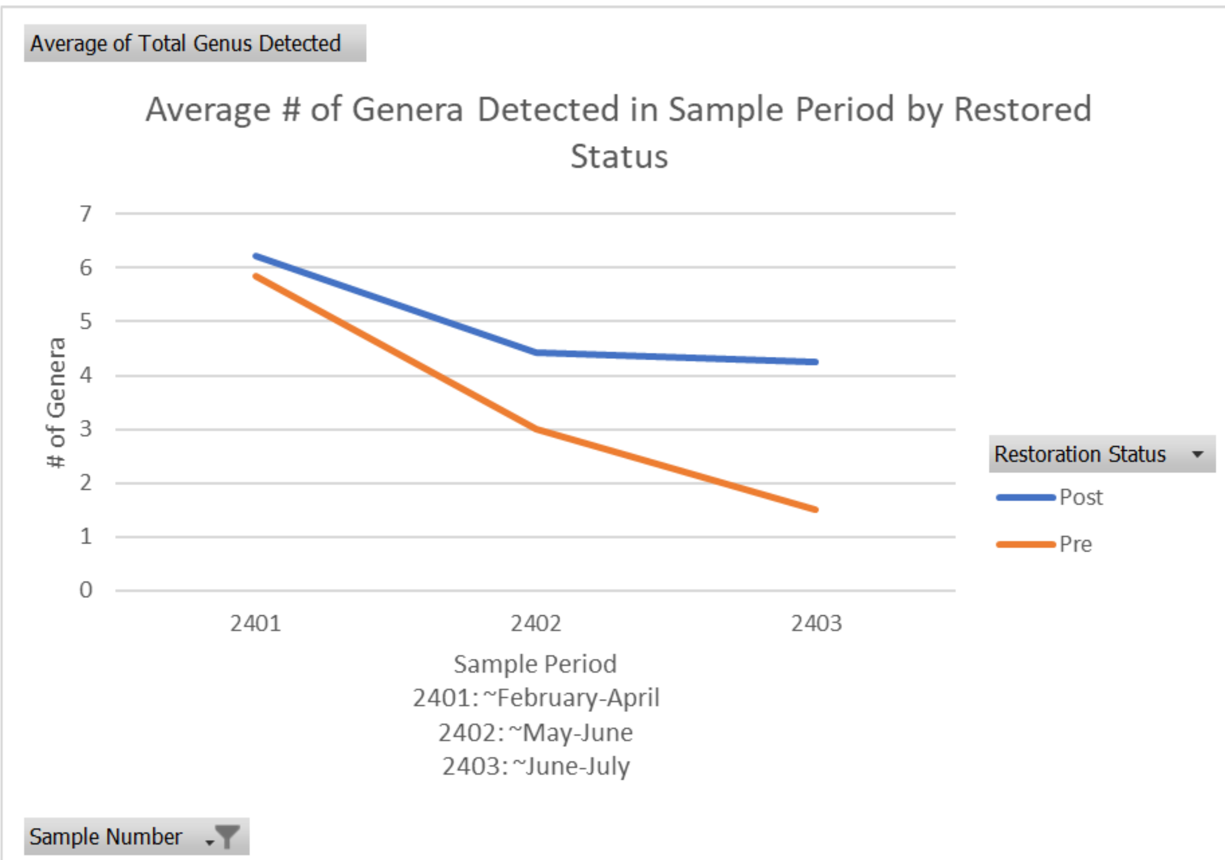

Figure 6. Comparison of average number of detected genera across sample periods and ponds pre- and post-restoration.

Generally speaking, both pre- and post-restored ponds showed similar declines in the number of genera detected over time. This is consistent with observations in the field and knowledge of how these ponds function. As the spring transitions to summer, water availability in the pond decreases as it is consumed by livestock and wildlife, evaporates into the atmosphere, or escapes through the bottom of the pond. As the "waterhole" shrinks, species utilization also declines as livestock and wildlife find other sources of available water. Comparing the two across pre- and post-restoration status shows a higher number of genera observed in post-restored ponds than pre-restored ponds across all three time periods. Post-restored ponds are typically deeper and wider, therefore holding a higher quantity of water. Water quality may also be higher as a result of less active sediment in the ponds. There are a number of potential reasons why restored ponds may be higher, but our initial batch of data suggests a higher species richness in post-restored ponds than pre-restored ponds based on detected genera in samples.

Photo 11. A California Quail observing from a fence post at Fernandez Ranch, taken by Ben Weise, June 2024.

Changes in Species Richness Post-Restoration

CCRCD has also been tracking the restoration of these ponds by year and over time, and should be able to establish a picture of how a pond's species richness changes over time post-restoration. Data presented here is after one year of data collection and will not yield significant insights as to how a pond's species richness changes over time, but can in the future.

In the below table, we've provided a breakdown of the 24 ponds within this study and how recently they were restored through the CCRCD Voluntary Local Program. Ponds are restored under this program between September and October in a given year. A sample collected the following spring is categorized on the table as "0 years since pond restoration occurred". As of 2024, this figure is two as there were two ponds restored in Fall 2023 and sampled for the first timed post-restoration in Spring 2024. Note that one of the ponds restored in Fall 2023 was sampled by Alameda County RCD in May 2023, and would fall under the -1 portion of this table. Eight ponds fall into the "-1 years since pond restoration occurred." This number includes any ponds slated for future restoration that are being monitored in 2024. Of those 8 ponds, 5 were restored in Fall 2024, and thus in Spring 2025, will be recorded in the "Years Since Pond Restoration Occurred". Note that no ponds were restored in 2017.

Table 3. Average number of genera detected in ponds by years before or after restoration

Number of Ponds	Years Since Restoration	Average of Total Genus Detected
8	-1	3.33
2	0	5.73
3	1	2.57
1	2	3.5
2	3	4.25
1	4	7
3	5	6.42
3	7	3.5
1	8	4.66

In this first year, there isn't much to glean yet as the sample size is fairly small. For instance, one might conclude from the data that pond species richness peaks four to five years post restoration given the high increases and decline thereafter. However, that is based on 4 total ponds, three of which are all in the same general area and park. As the ponds continue to age and the years since restoration increase, the data set should improve as all of the ponds in Year 0 will be counted in year 1, all the year 1 Ponds will move to year 2, etc.

Discussion

CCRCD is careful not to draw too many conclusions from our first year of eDNA Monitoring given the inherent uncertainties, the novelty of the technology, and the sample size of our data set. Nonetheless, we remain committed to setting this up as a long-term monitoring program to assess the species richness of these ponds.

First and foremost, we are encouraged by the presence of *Ambystoma californiense* or the California tiger salamander in five of the twenty four ponds that have been restored. Two of those ponds with detections were restored in 2024, and we hope to sample and detect them again in Spring 2025. It also remains encouraging that CTS appeared in the 3 ponds we previously knew them to be in, confirming that our restoration activities did not disturb the species and improved their habitat.

It was also encouraging to see (albeit indirectly) what is more than likely *Rana draytonii*, or California red-legged frog, appearing in nearly half of our restored ponds. This observation is caveated as it could also be American bullfrog, but CCRCD Staff did not observe bullfrogs. Still, we're excited to see the species (CRLF) appearing in our samples and hope that future samples contain the species directly rather than through our best guesses based on Family level taxonomic detections.

At the highest level, it was also encouraging to begin to see some data trends that suggest our livestock pond restorations resulted in an increase in species richness. Pre restored ponds typically had a species richness of 3.61 while post restored ponds typically had a species richness of 4.97. As our data set grows with increased samples and species richness calculations, we hope to see through t-tests a statistically significant difference between our restored ponds species richness and our unrestored pond species richness, indicating that our restorations positively impact the ponds and increases species richness and therefore species utilization.