Figure 1 eDNA sampling in the Alameda Creek, Photo by Stewart DesMeules

Exploring Presence of Freshwater Mussels in the Alameda Creek Watershed

ALAMEDA COUNTY
RESOURCE CONSERVATION DISTRICT

Drew Engstrom, Stream Restoration Specialist

Stephanie Lew, Project Assistant

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Introduction

Freshwater mussels play a critical role in riverine ecosystems. They improve habitat conditions for fish, serve as an energy-rich food source for predators, increase biodiversity of aquatic communities, and clean water through filtration[1]. However, they are considered one of the most imperiled species in North America. Their populations have declined dramatically due to habitat degradation and destruction as well as changes in water quality and quantity due to water capture and use[1]. Approximately 75% of native freshwater mussels in North America are listed as endangered, threatened, or a species of special concern[2].

Historically, three species have been found in Alameda County, all within Alameda Creek: the western ridged mussel, the California floater, and the western pearlshell. These species have declined 43%, 33%, and 17% respectively within their range[1], which extends throughout much of the western United States. Although, these three species were historically recorded in the Alameda Creek Watershed, most of the records date back to the 1890s. In the last two decades, the California Natural Diversity Database (CNDDB) shows only two records of the California floater species found in Alameda County, one in 2009 and one in 2014. Currently, very little information exists about the location, abundance, or health of the freshwater mussel populations in Alameda County.

The Alameda County Resource Conservation District (ACRCD) received a $5,000 grant the Alameda County Fish and Game (ACFG) Propagation Fund through the Alameda County Fish and Game Commission to perform a pilot investigation into the presence of freshwater mussels in the Alameda Creek Watershed. We performed visual surveys and eDNA sampling to determine the presence of freshwater mussel species.

Methods - Environmental DNA sampling

WRA, Inc. (a biological consulting firm) Senior Wildlife Biologist, Rob Schell and Fisheries Biologist Stewart DesMeules led the process of Environmental DNA (eDNA) collection. A total of 16 sites along Alameda Creek and Arroyo de la Laguna were sampled, 200-400 linear feet separated each transect location (see maps in Appendix A). Beginning downstream and working upstream (to avoid possible sample contamination), samples were taken strategically at each location, selecting sites with concentrated, quickly moving flows to maximize eDNA detection possibility. Depending on the turbidity of the water, a range of 600-1000mL of water was pumped through each filter. Samples were sent to Genidaqs for eDNA testing. All 16 transects were tested for presence of the California floater, this species was prioritized because of the recent historical sightings and higher potential for detection, the focus on the floater also factored into the limited laboratory analysis budget. Conversely, western ridged shell and the western pearlshell species have not been observed since the 19th century, therefore, eDNA testing was conducted at the four primary upstream and downstream transects featuring the highest detection potential. GPS coordinates, site conditions, and photos were taken at each

[1] Blevins, E., McMullen, L., Jepson, S., Blackburn, M., Code, A., Hoffman Black, S. (2018). Conserving the Gems of Our Waters: Best Management Practices for Protecting Native Western Freshwater Mussels During Aquatic and Riparian Restoration, Construction, and Land Management Projects and Activities. *The Xerces Society.*

[2] Mazzacano, C.S., and Blackburn, M. (2015). Native Freshwater Mussels in the Pacific Northwest: Stewardship & Environmental Education or Community-based Organizations. *The Xerces Society.*

location. Refer to the Genidaqs eDNA laboratory analysis results for a summary of results (see Appendix C).

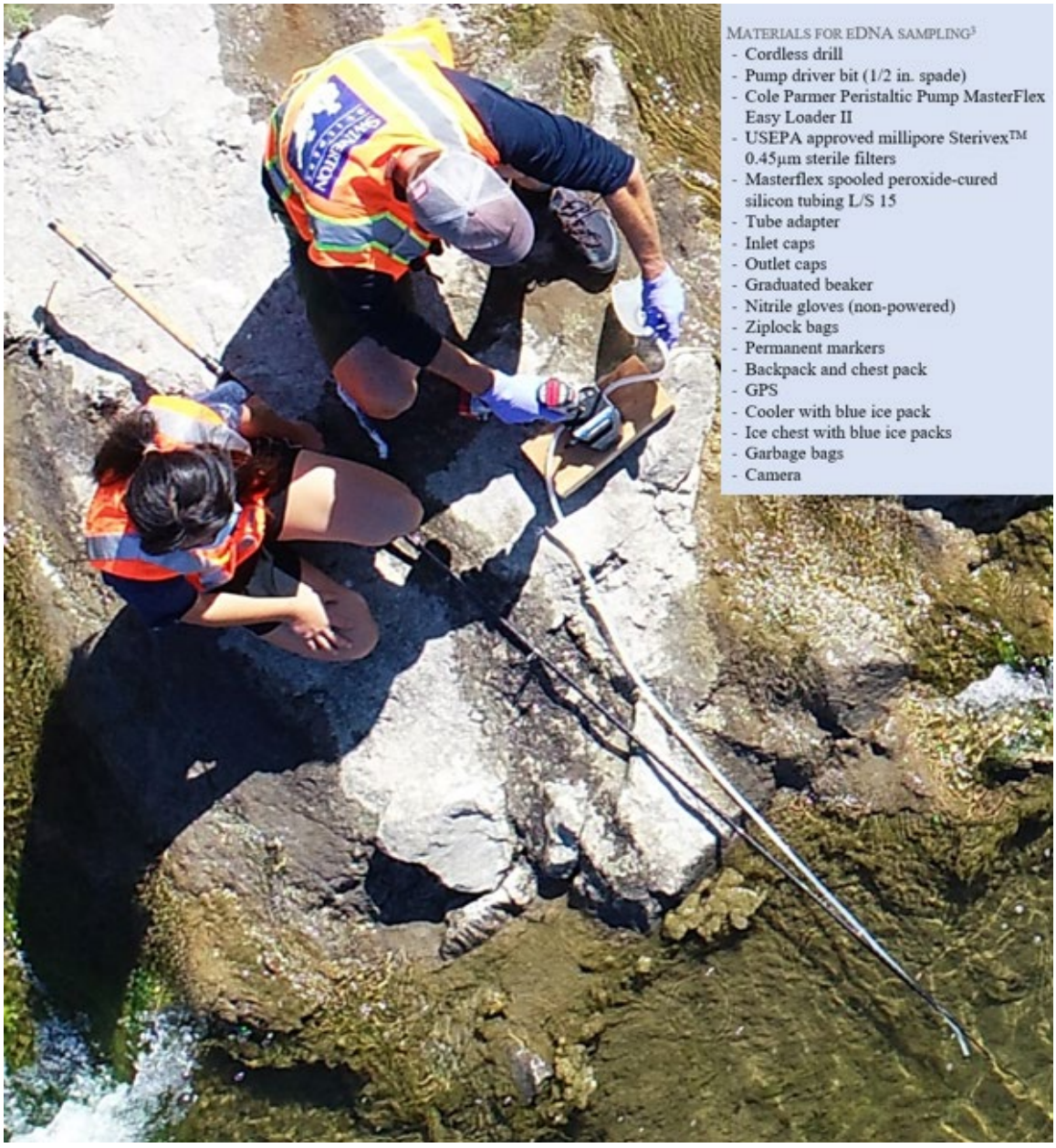


Figure 2 - Stephanie Lew (ACRCD) and Rob Schell (WRA) sampling for eDNA

Methods - Visual surveys

Visual surveys were performed on December 12, 2020 at four preselected locations, two sections along Alameda Creek and two sections along Arroyo de la Laguna (see Appendices A and B for maps and photos). Surveyors from the ACRCD performed visual observations along safely accessible stream sections with waders and viewing scopes. Due to the sheer size of the creek, locations presenting suitable freshwater mussel habitat were prioritized. In addition to in-stream

visual surveys, monitoring for mussel valves (shells) and remnants along the shorelines occurred along all eDNA sampling and visual survey sites. GPS coordinates and photos were taken at each location.

Figure 3 Drew Enstrom (ACRCD) performing visual surveys with an underwater viewing scope

Results

Despite the thorough implementation of the methods described above, eDNA sampling generated negative results showing no presence of the three species. Unfortunately, visual observations produced no signs of freshwater mussels as well. While results from this limited study may be disappointing, it indicates the need for further study and future repopulation efforts.

Work Plan Changes

COVID-19 resulted in many changes to our workplans. Due to the Shelter in Place Order and the need to develop safety protocols for ACRCD staff, we were not able to perform eDNA sampling at its optimal time, which, is in the spring when the mussels release glochidia. Furthermore, the initial visual survey expert had become unavailable for personal reasons. After further consultation with other mussel experts and a new partnership with WRA, Inc. the focus shifted to expanded eDNA analysis (WRA biologists have substantial

experience with performing eDNA sampling in a variety of freshwater conditions). The additional focus on eDNA was determined to provide the most data of the largest stream reaches. The pandemic also eliminated the possibility for volunteer and group events, reducing capacity for a large-scale visual observation system.

Additionally, closures of East Bay Regional Parks District's Lake Del Valle eliminated the option for sampling and surveys at the reservoir and narrowing down the search area to the Fremont, Sunol, and Niles Canyon regions.

Next Steps

The various workplan modifications and budget constraints likely factored into the lack of species presence findings. Combined with the general rarity of mussels within the highly disturbed Alameda Creek Watershed system it leaves us wondering, "are they still there?". The next steps start with continued outreach and education to environmental groups and freshwater hobbyists that frequent Alameda County streams and reservoirs. The ACRCD provided instruction to the Alameda County Fisheries Workgroup about monitoring for freshwater mussel glochidia during any native fish monitoring efforts including population analysis. Also, education about freshwater mussel's ecological role and appearance to the public and particularly to on-the-ground field personnel (e.g., EBRPD rangers) and hobbyists (e.g., boaters and fishermen) will help cast a broader net for future observations. Further scientific analysis including eDNA sampling during the breeding season and expanding to other stream corridors is recommended. The ACRCD will continue looking for future grant opportunities (federal, state, county, and private) to continue building on these studies to help confirm whether native mussels have been extirpated from the Alameda County freshwater systems.

While there is still hope for native freshwater mussel presence in Alameda County, it is also prudent to prepare for the possibility that the species may be extirpated from the region. If this is the case it may be necessary to consider re-introduction of mussels into the Alameda Creek Watershed. This would coincide with continued improvements to water quality and stream systems along with the return of anadromous fish to Alameda Creek and its tributaries. As mentioned in the initial grant proposal, water quality and native fish (hosts for mussel glochidia) are vital for freshwater mussel health and reproduction patterns. Therefore, further monitoring of fish populations, bio invertebrates, and water quality along with implementing corrective actions will be an essential component of population recovery.

Freshwater mussel reintroduction will likely be a complex process that may include consultation with ecological and biological experts to further understand habitat requirements and scientific survey processes. This will also involve consultation with state and federal resource agencies prior to implementation. As mentioned above, prior to the re-introduction of any freshwater species, habitat requirements must be assessed and suitable for native freshwater mussels. A robust breeding population of native mussels will provide long-lasting benefits to the hydrologic ecosystems within the Alameda Creek Watershed and the region.

Appendix A: Maps

Sampling locations along the Alameda Creek.

*Locations with a * indicate that all three species were sampled for. Otherwise, only the California floater was tested for.*

Yellow pins mark eDNA sample areas and red pins are visual survey locations.

Sampling locations along the Arroyo de la Laguna Creek.

*Locations with a * indicate that all three species were sampled for. Otherwise, only the California floater was tested for.*

Yellow pins mark eDNA sample areas and red pins are visual survey locations.

Sampling Location Dataset:

Table 1: AC- Alameda Creek, ADL - Arroyo de la Laguna, VS - Visual Survey

SITE ID	LONG	LAT	DATE
AC 01*	-121.9856086	37.57141939	9/22/2020
AC 02	-121.9820975	37.57236091	9/22/2020
AC 03	-121.9790711	37.57218878	9/22/2020
AC 04	-121.9754006	37.57154242	9/22/2020
AC 06*	-121.9725023	37.57295059	9/22/2020
AC 07	-121.9698123	37.57516987	9/22/2020
AC 08	-121.968643	37.57860838	9/22/2020
AC 09	-121.9670456	37.57939808	9/22/2020
AC 10*	-121.9647978	37.58068114	9/22/2020
ADL 11*	-121.876233	37.60813294	9/22/2020
ADL 12	-121.8797084	37.61314629	9/22/2020
ADL 14	-121.8819797	37.61604975	9/22/2020
ADL 16	-121.8819741	37.61814377	9/22/2020
ADL 17	-121.8822848	37.61969015	9/22/2020
ADL 18	-121.8826442	37.6253882	9/22/2020
ADL 20	-121.8832009	37.62747611	9/22/2020
VS L1	-121.9683333	37.57805556	12/2/2020
VS L2	-121.8797084	37.61314629	12/2/2020
VS L3	-121.8803621	37.61399229	12/2/2020
VS L4	-121.8819797	37.61604975	12/2/2020

Appendix B: Photos

Visual sampling location 1

Visual sampling location 2

Visual sampling location 3

Visual sampling location 4

Appendix C Genidaqs eDNA Laboratory Results

CRAMER FISH SCIENCES
An Employee Owned Consulting Company

GENIDAQS℠
Aquatic Species Sampling Simplified

Molecular Diagnostic Report

Report Status: Final
Report No: 10/1/2020
Contract No:

Client

Name: Alameda County Resource Conservation District
Address: 3585 Greenville Rd. Suite #2
Livermore, CA 94550

Contact: Stephanie Lew
916-947-9443
stephanie.lew@acrcd.org

Service Description

Sample type:
Sterivex
Analysis type:
Quantitative PCR
Received date:
9/28/2020
Reporting date:
10/1/2020

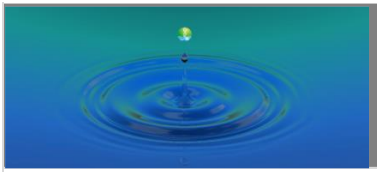
Location Description

Molecular Diagnostics

Samples	Laboratory Tests			Supplemental Sample Information
Identifier	W. floater	O. floater	W. ridged	Comments
20200922AC01	ND	ND	ND	
20200922AC02	ND			
20200922AC03	ND			
20200922AC04	ND			
20200922AC06	ND	ND	ND	
20200922AC07	ND			
20200922AC08	ND			
20200922AC09	ND			
20200922AC10	ND	ND	ND	
20200922ADL11	ND	ND	ND	
20200922ADL12	ND			
20200922ADL14	ND			
20200922ADL16	ND			
20200922ADL17	ND			
20200922ADL18	ND			

Molecular Diagnostics

Samples	Laboratory Tests			Supplemental Sample Information
Identifier	W. floater	O. floater	W. ridged	Comments
20200922ADL20	ND			
Field CT 09.22.2020	ND	ND	ND	
Extraction CT 09.30.2020	ND	ND	ND	
Pos CT	(+)	(+)	(+)	
NTC	ND	ND	ND	



Supplemental
Descriptions of procedures, methods, laboratory tests

Methods General

1 Field sample collections procedures followed Blankenship and Schumer (2017) and Bergman et al. (2016).
2 Total DNA was isolated from each eDNA sample (filter) following Bergman et al. (2016).
3 DNA templates were interrogated for the presence of target species mitochondrial DNA (barcodes) using quantitative PCR.
4 If any technical replicate tests positive for target DNA, then sample is considered (+), otherwise sample is non-detected (ND).
5 No template controls (NTC) are added to each plate to ensure plate to plate consistency.

Laboratory test (assay)

Code	Common Name	Species Name	Publication
W. floater	Winged Floater (Assay b and c)	Anodonta nuttalliana	Rodgers, T.W., Dysthe, J.C., Tait, C., Franklin, T.W., Schwartz, M.K., Mock, K.E. (2020). Detection of 4 imperiled western North American freshwater mussel species from environmental DNA with multiplex qPCR assays. BioRxiv. https://doi.org/10.1101/2020.03.27.012088
O. floater	Oregon Floater	Anodonta oregonensis	Rodgers, T.W., Dysthe, J.C., Tait, C., Franklin, T.W., Schwartz, M.K., Mock, K.E. (2020). Detection of 4 imperiled western North American freshwater mussel species from environmental DNA with multiplex qPCR assays. BioRxiv. https://doi.org/10.1101/2020.03.27.012088
W. ridged	Western Ridged Mussel	Gonidea angulata	Rodgers, T.W., Dysthe, J.C., Tait, C., Franklin, T.W., Schwartz, M.K., Mock, K.E. (2020). Detection of 4 imperiled western North American freshwater mussel species from environmental DNA with multiplex qPCR assays. BioRxiv. https://doi.org/10.1101/2020.03.27.012088

References

Bergman PS, Schumer G, Blankenship S, Campbell E. Detection of Adult Green Sturgeon Using Environmental DNA Analysis. PLOS ONE. 2016 Apr 20;114:e0153500.

Blankenship, S. and G. Schumer. 2017. Field collection procedure for aquatic environmental DNA sample collection and analysis. Cramer Fish Sciences - Genidaqs, West Sacramento, CA. 9p.