

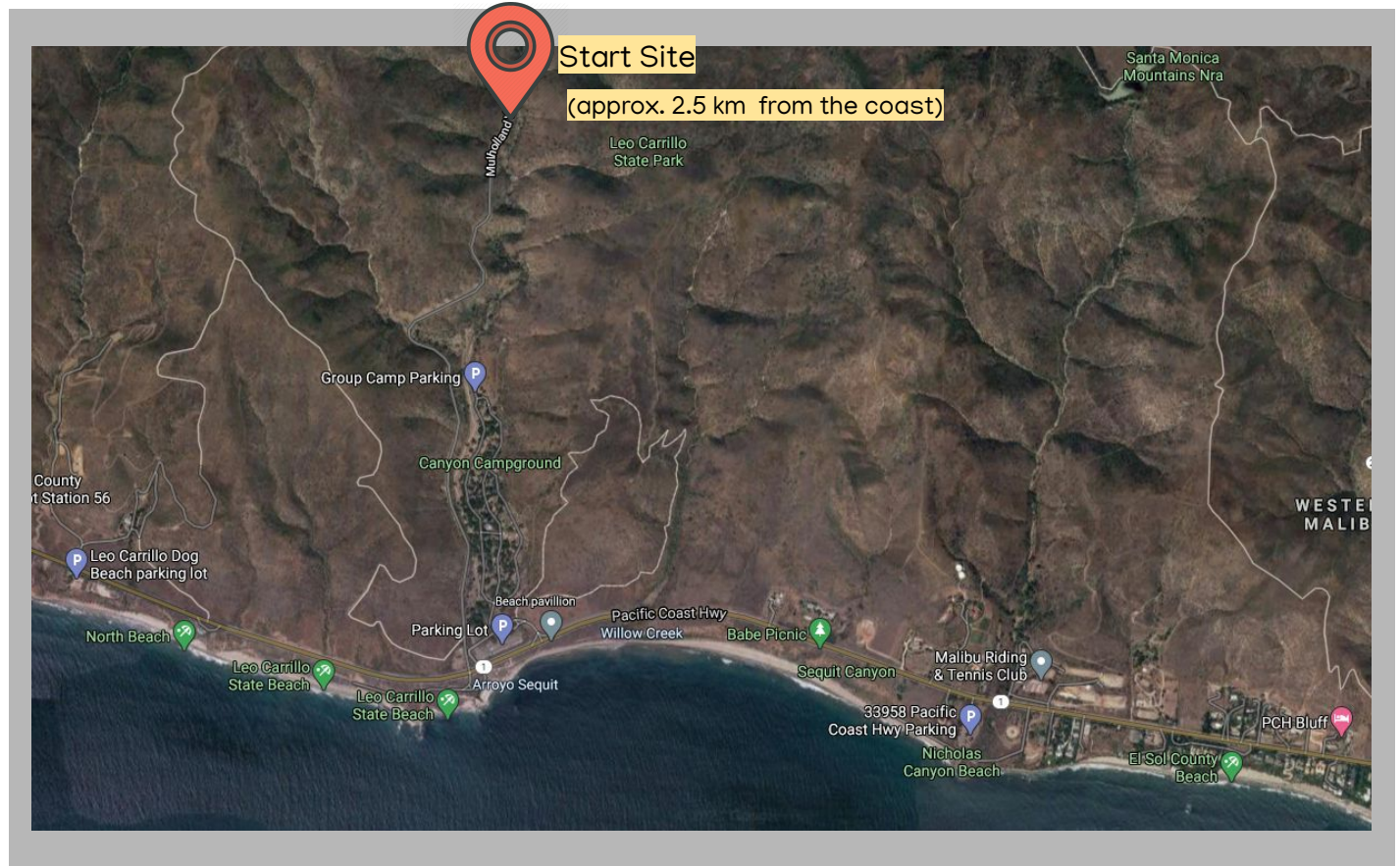
# Decline in Amphibian Health in Local Stream

Elyse Vetter, Elise DeArment, Colton Russell, Audrey Fontes, and Dr. Lee Kats  
Natural Science Division, Pepperdine University, Malibu, CA

## Arroyo Sequit on a Map

Arroyo Sequit is located near Leo Carrillo state Park, approximately 2.5 kilometers from the ocean.

Our indicated start site is at a culvert dividing “Upper” and “Lower” Arroyo.





## Upper Arroyo

(for reference)





## Lower Arroyo

(for reference)





## Culvert dividing Upper and Lower Arroyo

Pictured is the culvert/bridge that divides Arroyo Sequit - cars often drive or park over this culvert.

This point in the stream is also an area in which large changes have occurred over the past few years (as you'll see photos of in later slides).

Additionally, construction has been occurring around this culvert, making it a point of special interest.



## Healthy v. Infected Newts

This past summer, we saw unprecedented quantities of unhealthy newts.

To better demonstrate the poor condition of the newts we were finding, the following slides compare each symptom to that of a healthy newt.

## Infected Newts – Emaciation:

### Example of a Healthy Newt:

The top photo depicts a healthy newt at a standard weight.

When compared to newts at Arroyo, the extent of the emaciation occurring in unhealthy newts is made clear.



### Emaciated Newt at Lower Arroyo:

Emaciation is one of the most common symptoms observed at Arroyo, typically associated with a smooth/slimy texture.

Almost all newts classified as “unhealthy” presented in a similarly emaciated state. Emaciation was often seen in combination with a variety of other symptoms.



## Infected Newts – Eyes:

Example of a Non-Clouded Eye:



Photo of a healthy newt with clear eyes.

Cloudy Eye at Arroyo:



Newt found at Arroyo presenting with cloudy eyes.

Often seen in combination with emaciation.



## Infected Newts – Vents:

### Example of a Non-infected Vent:



Photo of a non-infected vent.

### Infected Vent at Arroyo:



Infected/inflamed vent.

Less common than cloudy eyes or emaciation.

## Infected Newts – Tails:

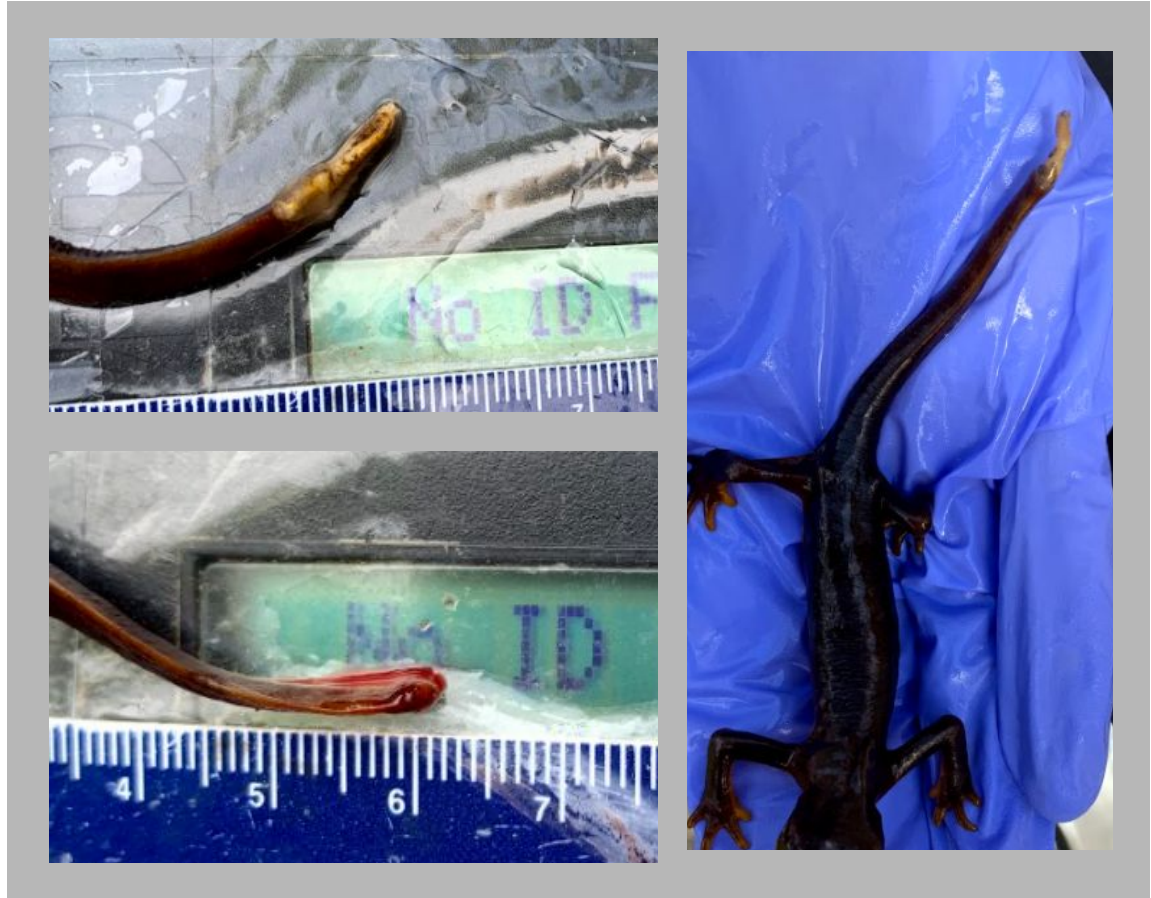
### Healthy Tail:



Large numbers of newts presented with infected, necrotic, or bleeding tails.

Pictured above is a healthy tail, compared to photos on the right portraying unhealthy tails.

## Infection, Necrosis, and Bleeding:





## Deceased Newts

Heightened numbers of deceased newts were also observed.

Pictured are two deceased specimens that were observed and collected during surveys.



## Other Unhealthy Animals

Other amphibians also presented with symptoms.

Pictured is a Pacific tree frog whose right hind leg is infected.





## Pathology Reports & Data

We began correspondence with the National Wildlife Health Center to further investigate our observations.

Additionally, we began looking for trends and patterns in our own data.



NATIONAL WILDLIFE HEALTH CENTER  
6006 Schroeder Road  
Madison, Wisconsin 53711-6223  
608-270-2400 (FAX 608-270-2415)

DIAGNOSTIC SERVICES CASE UPDATE

CASE: 29671 Epi/WID #: 200559

6/26/2020

Legal Inv Num:

FINDINGS TO DATE

Submitter:

Cynthia Hitchcock  
USGS Western Ecological Research Center/Santa Ana  
1801 E Chestnut Ave  
Santa Ana, CA 92701

Date Submitted: 6/2/2020

Specimen description/identification/Location:

ACC	SPECIES	SPECIMEN TYPE	BAND NUMBER	SUBMITTER'S ID	COUNTY	STATE
001	Newt, California	CARCASS			Los Angeles	CA
002	Newt, California	CARCASS			Los Angeles	CA
003	Newt, California	CARCASS			Los Angeles	CA
004	Newt, California	CARCASS			Los Angeles	CA
005	Newt, California	CARCASS			Los Angeles	CA
006	Newt, California	CARCASS			Los Angeles	CA
007	Newt, California	CARCASS			Los Angeles	CA
008	Newt, California	CARCASS			Los Angeles	CA

Summary of Physical Characteristics

ACC	SEX	AGE	WEIGHT	BODY CONDITION	POSTMORTEM STATE
001	Female	Adult	9.5 g	Good	Excellent
002	Male	Adult	8.08g	Emaciated	Excellent
003	Female	Adult	6.1g	Emaciated	Excellent
004			2.75g		Unsuitable for postmortem exam
005	Male	Adult	8.14g	Good	Good
006	Male	Adult	8.67 g	Emaciated	Excellent
007	Female	Adult	11.12g	GoodExcellent	
008			7.88		

**Event History:**

Three California newts were found in Lower Arroyo and collected on 5/29/20. The newts were found in a coastal stream with cobble and boulder; they were emaciated and had cloudy eyes. Affiliates at Pepperdine University have been recording these symptoms in the newts they find during field surveys and reported it to the USGS Western Ecological Research Center. There were fires last year in the region, with subsequent runoff and siltation of many creeks in the area.

The specimens were collected live. Submitter euthanized one newt with MS222 but it took a while and she noticed the solution was old so decided to euthanize the other 2 newts with benzocaine.

## Correspondence with National Wildlife Health Center

External examination noted the following:

- Cloudy eyes
- Necrotic tails

Specimens were tested for the following:

- Chytrid fungus
- Ranavirus
- Parasite identification

Test results:

(-) for Ranavirus

(+) for *Batrachochytrium dendrobatidis*

(-) for *Batrachochytrium salamandrivorans*



## Correspondence with National Wildlife Health Center– Results

### Final Report, 7/22/20

**COMMENTS:** The cause of the morbidity or tail loss in these California Newts for which they were euthanized is not determined. On histopathology, tails were necrotic and infiltrated with myriad bacteria which were most likely secondary invaders. Special stains did not reveal any organisms. Several of the newts had crystals in their renal tubules. These could represent oxalate crystals which can form due to diet. Five of the eight newts had chytridiomycosis. Several newts had pulmonary *Rhabdias* sp., intestinal nematodes and suspect Ichthyophoniasis. The cause of the intestinal lymph vessel dilation was likely parasites. Routine culture yielded no growth in some cases and mixed or environmental bacteria of no significance in others. PCR screens for Ranavirus using pooled kidney, liver and spleen were negative. Virus isolation is pending and results will be sent in a supplemental report.

After a month of correspondence, the final pathology report yielded inconclusive results.

Cause of morbidity and tail loss was not determined.



## Control Group Data

5/26/20	UCC 1	Run	10	-	34° 5' 41.03" N	118° 41' 13.212" W	406.72	N	M	71	-	14.61	-	-	-
5/26/20	UCC 1	Pool	64	020 884 833	34° 5' 33.53" N	118° 38' 51.438" W	411.99	R	M	76	5/4/2009	18.31	-	-	-
5/26/20	UCC 1	Pool	64	-	34° 5' 33.05" N	118° 38' 51.108" W	416.12	N	M	73	-	17.75	-	-	-
5/26/20	UCC 1	Pool	64	602 564 860	34° 5' 32.82" N	118° 38' 51.162" W	415.2	R	M	79	6/12/2019	17.49	-	-	-
5/26/20	UCC 1	Pool	64	000 831 548	34° 5' 33.89" N	118° 38' 50.418" W	418.24	R	M	79	4/23/2004	18.83	-	-	-
5/26/20	UCC 1	Pool	64	053 798 548	-	-	-	R	M	75	-	12.04	-	-	-
5/26/20	UCC 1	Pool	64	-	34° 5' 32.6" N	118° 38' 51.192" W	413.44	N	M	74	-	16.88	-	-	-
5/26/20	UCC 1	Pool	64	-	34° 5' 32.51" N	118° 38' 51.222" W	413.65	N	M	75	-	15.68	-	-	-
5/26/20	UCC 1	Pool	64	-	34° 5' 32.46" N	118° 38' 51.132" W	413.89	N	M	71	-	13.35	-	-	-
5/26/20	UCC 1	Pool	64	-	34° 5' 32.42" N	34° 5' 32.42" N	413.33	N	M	76	-	19.27	-	-	-
5/26/20	UCC 1	Pool	64	-	34° 5' 32.32" N	118° 38' 51.6" W	411.5	N	M	77	-	16.64	-	-	-
5/26/20	UCC 1	Pool	64	-	34° 5' 32.36" N	118° 38' 51.408" W	411.75	N	M	66	-	13.34	-	-	-
5/26/20	UCC 1	Pool	64	-	34° 5' 32.57" N	118° 38' 51.078" W	412.59	N	M	76	-	14.1	-	-	-
5/26/20	UCC 1	Pool	64	016 837 125	34° 5' 32.32" N	118° 38' 51.552" W	414.02	R	M	81	3/14/2009	22.87	-	-	-
5/26/20	UCC 1	Pool	64	-	34° 5' 32.45" N	118° 38' 51.33" W	414.2	N	M	71	-	14.92	-	-	-
5/26/20	UCC 1	Pool	69	600 016 538	34° 5' 32.5" N	118° 38' 51.24" W	413.34	R	M	71	5/9/2018	11.91	-	-	-

5/22/20	Newton 1	Pool	43	-	34° 4' 37.82" N	118° 48' 57.738" W	412.45	N	M	79	-	23.37	-	-	-
5/22/20	Newton 1	Pool	43	-	34° 4' 37.89" N	118° 48' 57.99" W	435.34	N	M	78	-	24.54	-	-	-
5/22/20	Newton 1	Pool	43	-	34° 4' 37.9" N	118° 48' 57.96" W	435.3	N	M	77	-	16.58	-	-	-
5/22/20	Newton 1	Pool	43	-	34° 4' 37.9" N	118° 48' 57.96" W	411.77	N	M	77	-	21.2	-	-	-
5/22/20	Newton 1	Pool	35	-	34° 4' 37.53" N	118° 48' 59.358" W	429.87	N	M	71	-	13.04	-	-	-
5/22/20	Newton 1	Pool	35	-	-	-	-	N	M	73	-	16.3	-	-	-
5/22/20	Newton 1	Pool	26	-	34° 4' 37.35" N	118° 49' 1.308" W	426.19	N	M	72	-	14.6	-	-	-
5/22/20	Newton 1	Pool	40	-	34° 4' 37.53" N	118° 49' 1.2" W	426.37	N	M	80	-	16.7	-	-	-
5/22/20	Newton 1	Pool	40	-	34° 4' 37.49" N	118° 49' 1.29" W	412.04	N	M	59	-	5.2	-	-	-
5/22/20	Newton 1	Pool	40	-	34° 4' 37.47" N	118° 49' 1.512" W	426.11	N	M	66	-	11.1	-	-	-
5/22/20	Newton 1	Pool	40	-	34° 4' 37.1" N	118° 49' 1.56" W	426.34	N	M	75	-	16.8	-	-	-
5/22/20	Newton 1	Pool	40	-	34° 4' 37.42" N	118° 49' 1.482" W	426	N	M	74	-	16.5	-	-	-
5/22/20	Newton 1	Pool	37	-	34° 4' 38.17" N	118° 49' 3.978" W	416.17	N	M	71	-	18.5	-	-	-
5/22/20	Newton 1	Pool	37	-	34° 4' 37.95" N	118° 49' 4.008" W	413.08	N	M	43	-	3.6	-	-	-

In order to put our data into perspective, we calculated the incidence of unhealthy newts in two other streams to serve as stand-in controls:

**Cold Creek May:** 3/92 **3%**  
**Cold Creek June:** 0/34 **0%**  
**Cold Creek July:** 1/7 **14%**

**Newton May:** 0/14 **0%**  
**Newton June:** 0/12 **0%**  
**Newton July:** 0/0 **N/A**

These streams demonstrate a much lower proportion of infected newts than Arroyo.



## Related Research

The included research article asserts the idea that stress can have immunocompromising effects on amphibian populations.

Is stress causing increased susceptibility to the newt population in Arroyo Sequit?

### Research articles

## Salinity stress increases the severity of ranavirus epidemics in amphibian populations

Emily M. Hall†, Jesse L. Brunner, Brandon Hutzenbiler and Erica J. Crespi

Published: 06 May 2020 | <https://doi.org/10.1098/rspb.2020.0062>

### Abstract

The stress-induced susceptibility hypothesis, which predicts chronic stress weakens immune defences, was proposed to explain increasing infectious disease-related mass mortality and population declines. Previous work characterized wetland salinization as a chronic stressor to larval amphibian populations. Thus, we combined field observations with experimental exposures quantifying epidemiological parameters to test the role of salinity stress in the occurrence of ranavirus-associated mass mortality events. Despite ubiquitous pathogen presence (94%), populations exposed to salt runoff had slightly more frequent ranavirus related mass mortality events, more lethal infections, and 117-times greater pathogen environmental DNA. Experimental exposure to chronic elevated salinity (0.8–1.6 g l<sup>-1</sup> Cl<sup>-</sup>) reduced tolerance to infection, causing greater mortality at lower doses. We found a strong negative relationship between splenocyte proliferation and corticosterone in ranavirus-infected larvae at a moderate elevation of salinity, supporting glucocorticoid-mediated immunosuppression, but not at high salinity. Salinity alone reduced proliferation further at similar corticosterone levels and infection intensities. Finally, larvae raised in elevated salinity had 10 times more intense infections and shed five times as much virus with similar viral decay rates, suggesting increased transmission. Our findings illustrate how a small change in habitat quality leads to more lethal infections and potentially greater transmission efficiency, increasing the severity of ranavirus epidemics.

## Potential Immunocompromising Factors

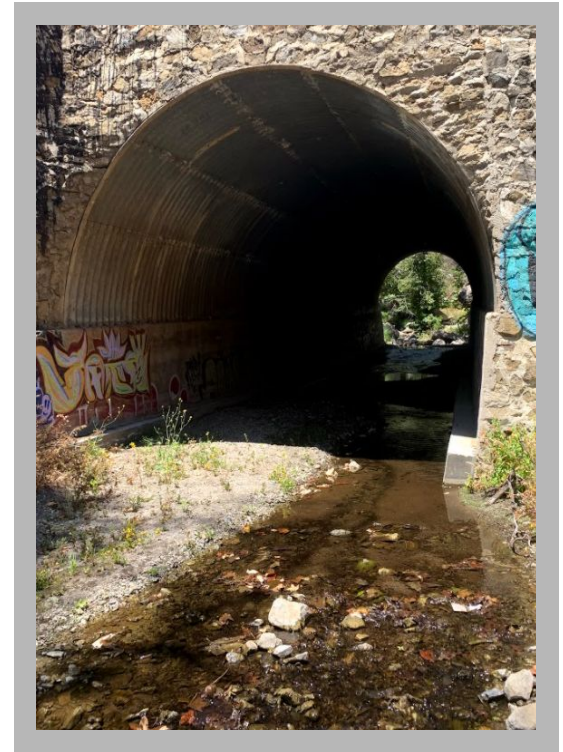
After reviewing our data and relevant research, we began to speculate on potential stressors that may have led to immunocompromising effects on the newt population.

## Pre- & Post- Fire Stream Conditions:

Pre-Fire: May 11, 2018



Post-Fire: July 20, 2020



The left photo depicts what used to be common, deep pools. However, on the right, we see a dramatic decrease in stream depth, post-fire.

We speculate that these stressors (wildfire + habitat alterations) are potential causes of immunocompromising effects on the newt population.



# Residential Ash

August 12, 2018



January 3, 2019



August, 2019



Upstream from our survey sites is a children's camp - Camp Bloomfield - that burned in the fire.

Residential ash from this site may serve as an additional immunocompromising factor.



## Construction

After the Woolsey Fire, large amounts of construction began in and around the stream.

Construction materials, vehicles, and runoff may also be a potential stressor and immunocompromising factor at play.









## Future Directions:

The following are a few directions we're planning on taking our investigation:

1. Comparisons of *B. dendrobatidis* loads to body conditions
2. pH analysis of the soil - evaluation of the contents of residential ash
3. Data collection further upstream - above residential burn sites
4. Analysis of data from years past
5. Experimental work with stress in laboratory settings
6. Evaluation of transmissibility