

Anthropogenic effects on abiotic factors affecting the success of the threatened *Rana boylei*

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ABSTRACT

Freshwater ecosystems disproportionately support a large number of species given the limited availability of suitable habitat. Anthropogenic disturbance in freshwater systems have caused a decline in over 5,700 species globally. Amphibians are especially sensitive to levels of disturbance due to their physiological requirements for homeostasis. Our study set out to investigate how differing levels of human disturbance affect abiotic variables on Foothill yellow-legged frog (*Rana boylei*) in the South Fork Eel River. We studied the effects of these abiotic factors on biotic and reproductive response variables. Measurements were taken on egg mass size, distance to shore, water depth, egg mass density, and developmental stages at two locations with differing amounts of human disturbance. Overall, higher human disturbance resulted in lower condition and reduced size of egg masses. Additionally, less egg masses per kilometer and smaller average egg mass size were observed at the disturbed location. Our findings reinforce the need for restoration efforts to focus on returning watersheds to their natural flow regimes in order to support suitable breeding habitat for *Rana boylei*.

Keywords: freshwater ecology, flow regime, foothill yellow-legged frog, anthropogenic interference

INTRODUCTION

The loss of biodiversity in freshwater ecosystems is a major conservation concern far greater than biodiversity loss seen on terrestrial ecosystems (Dudgeon et al. 2006). Freshwater ecosystems make up less than 1% of the earth's surface yet hosts over 100,000 species worldwide (Dudgeon et al. 2006). Species that rely on freshwater habitats tend to be extremely sensitive to minor changes in the environment due to

the ability of toxins and diseases to diffuse through water (Sodhi et al. 2008). Amphibians are especially responsive to changes in water quality because of their highly permeable skin required for maintaining homeostasis (Llewelyn et al. 2019). Consequently, they act as a bioindicator, with their presence or lack thereof provides information on the overall health of a freshwater system (Hager 1998). Human interference through flow regulation, dams, water diversion, and

habitat modification alter the natural regime in ways that make habitat unsuitable for organisms that specialize in these systems.

These disturbances have led to cascading effects on many freshwater taxa. Amphibians have been particularly hard hit as they have experienced the largest declines in biodiversity to date (Beebee and Griffiths 2005). An IUCN assessment estimated that over a third of amphibians, approximately 5,700 species, have experienced severe population decline with high rates of extinction (Hayes et al. 2016). One species that has had extreme range contractions is the Foothill yellow-legged frog (*Rana boylei*, hereafter referred to as yellow-legged frog). This species has been nearly extirpated from two-thirds of its historical range in California and is now listed as near threatened by the IUCN (Hayes et al. 2016). Similar to most amphibians this species has lost its range due to human interference through introduction of dams and reservoirs. Historically the yellow-legged frog was found all along the South Fork of the Eel River until 1931 when the Benbow dam was constructed and altered the waterway, creating a 123-acre recreational lake and human regulated flow regimes (Hayes et al. 2016). As expected, the level of human disturbance in the form of recreational activities in this stretch of Eel River increased. Yellow-legged frog habitat with favorable abiotic factors were no longer present due to the habitat degradation caused by the dam along the Eel River (Bednarek 2001). Consequently, the identification and protection of watersheds that are key breeding locations for yellow-legged frog populations is crucial to conservation efforts. Furthermore, we wanted to investigate the ability of the

yellow-legged frog to recolonize previously disturbed areas like Benbow State recreation area, since the dam's removal in 2016.

In order to investigate this, we tested the effect of human interference on abiotic factors at two breeding locations with differing levels of human interference. Additionally, we studied the effects of these abiotic factors on biotic and reproductive response variables of the yellow-legged frogs. We predicted the reproductive response variables (hatching, size of egg mass, distance to nearest neighbor, and development stage) would be negatively affected by human interference. Due to public access, the location with a high level of human interference will be detrimental to the size and condition of the egg masses (Levêque et al. 2015). We predicted that biotic response variables (predation, desiccation, scouring) would increase with higher intensity of abiotic factors such as water velocity and water temperature. By investigating the effect of human interference on abiotic factors critical to the reproductive success of the yellow-legged frog, the conservation of this threatened species can be strengthened.

METHODS

2.1 Natural History

This study was conducted at two locations along the South Fork Eel River from May 5 to May 9, 2021. The section of the South Fork Eel River within Angelo Coast Range Reserve has a low level of human interference. Angelo Coast Range Reserve is 7,660 acres of protected land in Mendocino County, California, (39°45'15.4"N, 123°37'53.0"W) and is part of the University of California

Natural Reserve System. The reserve contains four undisturbed watersheds, undeveloped coastal conifer forest, mixed conifer broad-leaf forest, river terrace meadows, and chaparral.

Benbow Lake State Recreation Area has a high level of human interference as it is publicly accessible. The Benbow dam, present from 1937 till 2016 formed a seasonal 123-acre lake. Benbow Lake State Recreation Area is located in Humboldt County, (40°03'57.8"N, 123°47'19.6"W), and includes 4.62 kilometers of the South Fork Eel River. Yellow-legged frog egg masses were relocated to Benbow after the removal of the dam and the species has since reestablished in this area.

2.2 Survey Selection

In order to determine the effects of human interference and abiotic factors on biotic and reproductive response variables, we selected previously documented breeding sites at Angelo Reserve and Benbow to survey egg masses (Kupferberg 1996). We classified breeding sites as areas with at least five egg masses. Within each breeding site a maximum of ten egg masses were selected to survey, additional egg masses were sampled at Benbow.

For each egg mass surveyed, we recorded the following abiotic, biotic, and reproductive response variables. Abiotic variables measured at each egg mass were water depth, distance of egg mass to closest shoreline, and size of the substrate the egg mass was attached to. Biotic variables recorded for each egg mass were predation (if the egg mass was missing pieces or had empty egg shells and tadpoles were absent, predation was present), desiccation (using

the Kupferberg Scale (1: egg mass fully completely exposed to the air, 2: egg partially exposed to air, and 3: egg completely submerged in the water, (Kupferberg 1996), and scouring (using Kupferberg Scale: 1: egg mass completely removed from substrate, 2: egg mass partially removed, 3: egg mass intact (Kupferberg 1996). Reproductive response variables documented at each egg mass included hatching, size of egg mass, egg masses per kilometer, distance to nearest neighbor, and the development stage using the Gosner Staging System (Gosner 1960).

At each breeding site, a transect was run parallel to the shore from the farthest downstream egg mass to the farthest upstream egg mass to quantify the length of the breeding site. The farthest egg mass downstream and upstream were determined by the lack of egg mass within visible range. At the beginning, middle, and end of the breeding site length, canopy cover, distance to nearest vegetation from shoreline, and length of channel width was recorded. Water temperature was measured at each breeding site. Additionally, velocity of the water was measured where egg masses were visually concentrated within a breeding site and the water velocity of the mainstream flow was measured.

2.3 Data Analysis

All statistical analyses were conducted using JMP software version 16.0.0. We used a multiple logistic regression to test the effects of abiotic factors and location on biotic and reproductive response variables.

RESULTS

Overall, we counted 208 egg masses along 1.82 kilometers of the South Fork Eel River within Angelo Reserve and 107 egg masses along 1.37 kilometers of the South Fork Eel River within Benbow (Figs. 1 and 2). Angelo had a greater density of egg masses per kilometer, 114.4 egg masses/km, compared to Benbow, 78.22 egg masses/km. The average water temperature at Angelo was over 2°C lower than the average water temperature at Benbow (18.34°C compared to 20.67°C, respectively) (Table 1). The average channel velocity at Benbow was nearly double the channel velocity at Angelo (62.9 cm/s vs 32.2 cm/s, respectively) (Table 1). The average channel width at Angelo was 34.17 meters and was more than twice as wide as the average channel width observed at Angelo (14.7 meters) (Table 1). The average

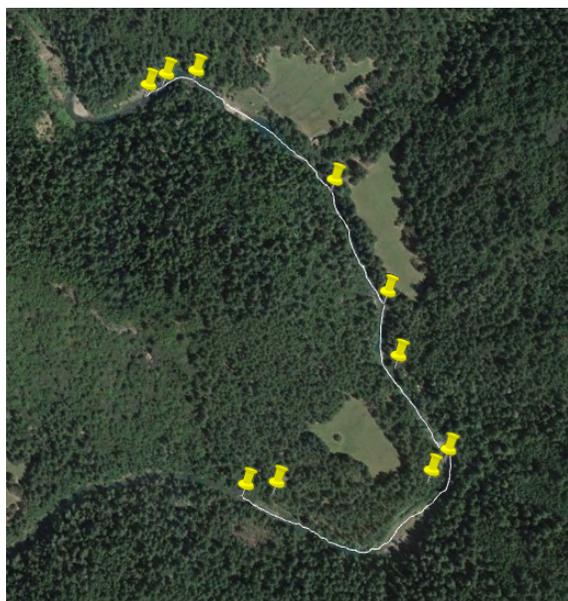


Figure 1. Survey sites at Angelo Coast Range Reserve. Each pin represents yellow-legged frog oviposition sites along the South Fork Eel River. At each site abiotic, biotic, and reproductive variable were collected. A total of 10 sites were surveyed.

substrate size sampled at Benbow was 12.55 cm, 33 percent smaller than the substrate size at Angelo (18.89 cm) (Table 1). The egg masses surveyed at Angelo were 30 percent larger than the egg masses sampled at Benbow (Fig. 3, Table 2). There was no relationship between location and distance to vegetation, canopy cover, breeding site water velocity, water depth, and distance to shore (Table 1).



Figure 2. Survey sites at Benbow State Recreational Park. Each pin represents yellow-legged frog oviposition sites along the South Fork Eel River. At each site abiotic, biotic, and reproductive variable were documented. A total of three sites were surveyed.

Table 1: Summary statistics of location on abiotic factors (N=13 for all factors tested).

	Location	
	F	P
Abiotic Factors		
Distance to Vegetation (m)	1.05	0.25
Canopy Cover (%)	1.74	0.25
Water Temperature (°C)	13.88	0.0024
Channel Velocity (cm/s)	13.7	0.0022
Channel Width (m)	16.75	0.0012
Breeding Site Velocity (cm/s)	1.61	0.23
Water Depth (cm)	0.73	0.4
Substrate Size (cm)	30.84	<0.0001
Distance to Shore (m)	3.6	0.058

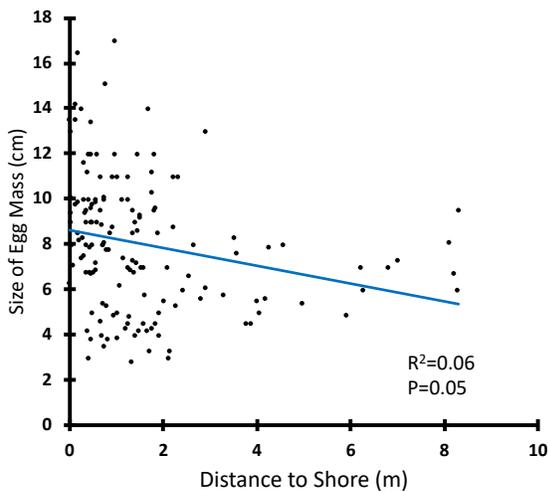


Figure 3. Relationship between the distance to shore and size of egg masses. Each point represents an egg mass. Egg masses that were further away from the shore were smaller in size. the distance to shore (N = 150, F = 4.01, P = 0.05).

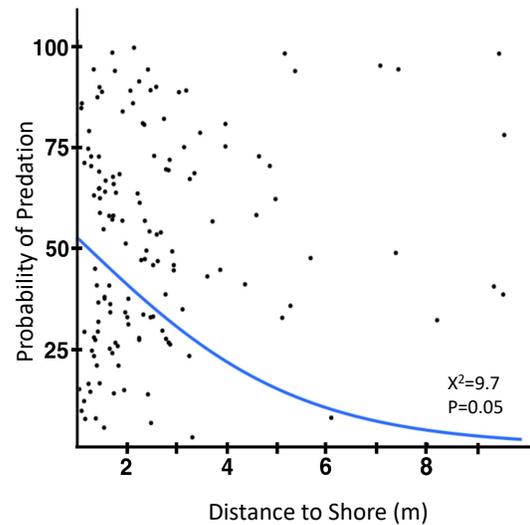


Figure 4. Logistic regression on the effect of distance to shore on the probability of egg mass depredation. The probability of predation decreases as egg masses are found further away from the shoreline (N = 150, F = 3.89, P < 0.05).

Table 2. Summary statistics of egg mass level abiotic factors biotic and reproductive response variables (N = 150 for all factors tested).

	Distance to shore (m)		Water depth (cm)		Substrate size (cm)		Location	
	Test Statistic	P	Test Statistic	P	Test Statistic	P	Test Statistic	P
Biotic Response Variables								
Predation	X ² =3.89	0.05	X ² =2.93	0.19	X ² =0.11	0.09	X ² =1.76	0.75
Desiccation	F=0.77	0.38	F=21.96	<0.0001	F=3.06	0.08	F=7.04	<0.0001
Scouring	F=3.43	0.07	F=22.92	<0.0001	F=0.15	0.69	F=1.28	0.26
Reproductive Response Variables								
Size of Egg Mass (cm)	F=4.01	0.05	F=0.12	0.66	F=0.87	0.35	F=24.12	<0.0001
Distance to Nearest Neighbor (cm)	F=0.22	0.64	F=0.16	0.69	F=0.004	0.95	F=0.14	0.7
Development Stage	F=0.07	0.79	F=7.22	<0.0001	F=1.03	0.31	F=1.58	0.21
Hatching	X ² =8.43	<0.0001	X ² =7.96	<0.0001	X ² =0.77	0.38	X ² =1.88	0.17

Across the 13 breeding sites surveyed at Angelo and Benbow, the likelihood of predation was greater in higher water temperatures and in narrower channels (Table 3). Egg masses oviposited further from shore had lower rates of predation, hatching, and were smaller in size (Fig. 4, Table 2). Egg masses were smaller, had lower rates of hatching, and were less developed in

deeper water (Table 2). Additionally, egg masses in deeper water experienced less desiccation and more severe scouring (Table 2). There was no relationship between distance to vegetation from shoreline, channel velocity, breeding site velocity, and canopy cover with any of the biotic and reproductive response variables (Table 3).

Table 3. Summary statistics of site level abiotic factors on biotic and reproductive response variables (N = 150 for all factors tested).

	Water Temperature (°C)		Channel Water Velocity (cm/s)		Breeding Site Water Velocity (cm/s)		Distance to Vegetation (m)		Canopy Cover (%)		Channel Width (m)	
	F	P	F	P	F	P	F	P	F	P	F	P
Biotic Response Variables												
Average Desiccation	3.24	0.23	4.13	0.41	1.92	0.66	6.91	0.29	4.67	0.20	0.4	0.61
Average Scouring	2.11	0.14	0.35	0.10	0.09	0.22	0.42	0.65	0.87	0.12	1.78	0.67
Average Predation	2.71	0.03	2.18	0.81	1.87	0.42	4.41	0.77	0.17	0.27	4.24	0.02
Reproductive Response Variables												
Average Size of Egg (cm)	0.12	0.81	1.23	0.12	0.01	0.99	0.37	0.30	0.0002	0.91	0.035	0.98
Average Distance to Neighbor (cm)	0.31	0.21	0.4	0.29	3.77	0.08	1.01	0.24	0.16	0.18	0.17	0.17
Average Hatching	0.01	0.79	0.18	0.80	0.71	0.54	0.41	0.55	2.13	0.28	0.23	0.72
Average Developmental Stage	0.004	0.62	0.12	0.71	0.82	0.26	0.07	0.54	0.25	0.45	2.38	0.12

DISCUSSION

We predicted that Angelo Reserve would have a higher density of egg masses per kilometer compared to Benbow due to the absence of human interference. In agreement with our predictions, there was a higher density of egg masses per kilometer and larger egg masses within Angelo Reserve. The lower density and smaller egg size observed in Benbow could be attributed to the ongoing history of disturbance and human activity. The removal of the dam and the return of the unregulated flow regime allowed for the reestablishment of the yellow-legged frog at Benbow (Hayes et al., 2016). However, recreational activities such as boating and kayaking continue to be allowed within Benbow. These activities can alter stream hydrology and lower the water table, impacting the shallow habitats in which reproduction sites occur, harming the development of juvenile yellow-legged Frogs (Kupferberg 1996). In comparison, public access is not allowed at Angelo and may support a more robust population of yellow-legged frogs. In addition to altered flow regimes and human disturbance, the dam had subtle effects, such as potentially changing the geological composition of substrates downstream.

The substrate size the egg masses attach to are an important abiotic factor that impacts the developmental success of the egg mass (Hayes et al. 2016). Egg masses were observed on larger substrate sizes in Angelo compared to Benbow. The difference between the two locations could be attributed to the legacy of the dam. Dammed rivers cannot support the movement of large substrates and results in smaller substrate sizes, rising stream beds, and the destruction of cobble bars and riffles (Hayes et al., 2016). Despite the removal of the dam at Benbow, our results suggest that there continues to be smaller substrates along that stretch of the river. The difference in substrate sizes can have detrimental effects on the egg size and conditions of the yellow-legged frog as cobble bars and riffles provide vital protection from varying water velocities (Kupferberg, 1996). Our results highlight the impact of human interference on abiotic factors that are crucial in the developmental success of the yellow-legged frog. Further, interesting relationships between abiotic factors and biotic and reproductive variables across both locations were seen.

Across both locations, a tradeoff mechanism appears to exist for the successful development of the egg masses. As oviposition moved further from the

shore, the chance of predation and the size of the egg mass decreased. These findings suggest that while being closer to shore is a more beneficial habitat for reproduction and rapid development, the near-shore egg masses were more at risk to environmental conditions and predation. Water depth showed a similar tradeoff mechanism as deeper water allowed for less predation and scouring but smaller egg masses and slower development. Future studies could focus on understanding the tradeoffs between the egg mass oviposition site in regard to water depth and distance to the shore.

Understanding the environmental factors and mechanisms that influence the success of the yellow-legged frog's development is important for conservation efforts regarding the protection of these habitats. The yellow-legged frog has been removed from over two-thirds of its natural range in large part due to human disturbance and alterations of waterways (Hayes et al. 2016). Despite this, the species shows promise following the restoration of the natural flow regime and the reestablishment of the population after the removal of Benbow Dam.

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