EFFECTS OF MULTIPLE STRESSORS: HYDROPERIOD, INTRODUCED BULLFROGS, AND FOOD LIMITATION ON NORTHERN RED-LEGGED FROGS (*RANA AURORA*)

By

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Committee Membership

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ABSTRACT

EFFECTS OF MULTIPLE STRESSORS: HYDROPERIOD, INTROUCED BULLFROGS, AND FOOD LIMITATIONON ON NORTHERN RED-LEGGED FROGS (*RANA AURORA*)

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As human activities reach every corner of the globe, climate change, invasive species, habitat destruction, and other stressors causing species' declines no longer act alone. Climate change has the potential to exacerbate (or mitigate) other stressors (e.g. invasive species or pathogens) affecting amphibian populations. I assessed the combined effects of increased pond drying rates (potential impact of climate change), invasive bullfrogs (*Lithobates catesbeianus*) presence, and food availability on northern red-legged frog (*Rana aurora*) survival and body size after metamorphosis by rearing tadpoles under incrementally shortened hydroperiods with and without the presence of invasive bullfrog tadpoles in low and high food environments. To explore the underlying mechanisms driving the impact of bullfrogs on *R. aurora* tadpoles, I had two treatments where bullfrog tadpoles were either separated by a permeable barrier (behavioral cue) or free to move about the tanks (direct competition/predation). To validate the captive experiment, I examined the influence of hydroperiod length on *R. aurora* survival, development, and growth in a field-based mesocosm experiment. I found hydroperiod to have a threshold effect on survival through metamorphosis in the captive experiment.

Once the hydroperiod threshold was met in both the captive and field study, I found no benefit of longer hydroperiods on survival through metamorphosis. Drying rate influenced *R. aurora* developmental rates, but the effects were dependent on life stage and time of season in the field study. Size at metamorphosis was synergistically affected by bullfrog presence and food availability in the captive experiment. Tadpoles emerged as smaller metamorphs when exposed to bullfrogs in a low food environment. In the field experiment, size at metamorphosis was positively affected by longer hydroperiod and later emergence date. Understanding how multiple stressors impact larval growth and survival is an important component for managing and potentially mitigating the interactive effects of climate change and invasive species for amphibian conservation.

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INTRODUCTION

Species face a myriad of stressors including climate change, invasive species, habitat loss, and pollution (Baillie et al. 2004). As humans increase in population size and rapidly convert land, species can be progressively exposed to the effects of multiple stressors simultaneously (Leu et al. 2008). Climate change and invasive species are becoming ubiquitous stressors across ecosystems, communities, populations, and species. As multiple stressors are introduced into an ecosystem, the magnitude and direction of their effects on individual species may change (Bårdsen et al. 2018). In some cases, the effects of multiple stressors are synergistic and lead to local extirpations (Wilkins et al. 2019).

Climate change has been implicated in the decline and extirpation of numerous species and threatens the stability of biological communities around the globe (Urban 2015). One of the most salient features of a changing climate is altered temperature regimes. Global average surface temperatures have increased by 0.1-0.3^{o}C per decade since 1998 and are projected to rise 2.6-4.8^{o}C by 2100 (RCP 8.5 projection, IPCC 2014). Precipitation projections are less certain, however overall warming temperatures will increase evaporation and water vapor capacity in the air causing the severity of storms to worsen and droughts to lengthen (Trenberth 2011). Ectotherms are particularly susceptible as they rely on abiotic factors such as temperature for physiological functions (Paaijmans et al. 2013) and precipitation for reproduction (Ficetola and Maiorano 2016). The stress imposed by climate-induced drying and warming can negatively impact

amphibian populations, but these impacts may be compounded or mitigated by other stressors, such as the introduction of non-native species.

Freshwater ecosystems are vulnerable to climate change (Woodward et al. 2010) and have suffered an increase in biological invasions over the past 50 years (Ricciardi and Macisaac 2010). Aquatic invasive species shifting beyond native habitats can be linked to warmer temperatures (Rahel and Olden 2008). Moreover, a high tolerance to abiotic factors, such as stream drying (Larson et al. 2009) and temperature fluctuations (Leuven et al. 2011), provides a competitive advantage for many invasive species. The ability for aquatic invasive species to establish in novel habitats is attributed to their tolerance of degraded habitats (Riley et al. 2015) and efficient foraging behaviors (Kieseceker et al. 2001, Rehage et al. 2005).

Along with manipulating the foraging behavior of native species, overall food resources can be reduced by invasive species in aquatic ecosystems (Joseph et al. 2011, Kupferberg 1997). Species competing for shared resources can exclude one another, especially in low food environments (Keddy 1989). Conversely, warming temperatures from climate change will likely lead to higher primary production in aquatic systems (Frederick et al. 2006), potentially mediating resource competition. The addition of food availability as a stressor within an ecosystem can change the effects of invasive species on native species. Therefore, evaluating the combined impact of several stressors concurrently is crucial for predicting population viability and for informing management decisions, especially for species at risk of exclusion or extirpation.

The northern red-legged frog (*Rana aurora*), a Species of Special Concern in California (Thomson et al. 2016), is exposed to climate change and invasive species throughout its range. In California, precipitation is predicted to shift between extreme wet and dry conditions (Swain et al. 2018). The effects of increased temperatures and dry periods can lead to shortened hydroperiods (i.e., the duration of water on an area of land). Shortened hydroperiods can result in increased stress in tadpoles and reduce the size of amphibians at metamorphosis (Salice 2012). At the same time, many ephemeral habitats in the western United States have been invaded by American bullfrogs (*Lithobates catesbeianus*, hereafter referred to as bullfrog) where they compete with and prey upon native amphibians (Kiesecker and Blaustein 1998). The presence of bullfrog tadpoles increases resource competition and thereby forces foraging *R. aurora* tadpoles into sub-optimal habitat (Kiesecker et al. 2001). These threats affect many amphibians worldwide including *R. aurora's* closest relative, the endangered California red-legged frog (*Rana draytonii*), making *R. aurora* an excellent model system in which to study the combined effects of shortened hydroperiod, bullfrog presence, and limited food resources.

My thesis aimed to examine how shortened hydroperiod, bullfrog presence, and food limitation influence *R. aurora* size at metamorphosis and survival to metamorphosis. To evaluate the interactions of the abiotic and biotic stressors, I manipulated hydroperiod duration, bullfrog presence/absence, and food availability concurrently. Multiple stressors could interact in three different ways: antagonistically, additively, or synergistically. I hypothesize that the combined effects of shortened hydroperiod and bullfrog presence are either additive or synergistic. For example, if the

interaction is additive, the effect of shortened hydroperiod on *R. aurora* tadpole survival would not be influenced by the addition of bullfrogs (Figure 1). In contrast, if the interaction is synergistic, the effect of shortened hydroperiod would have a stronger negative impact on tadpole survival when bullfrogs are present (Figure 1). The same hypothesis structure can be applied for the combined stressors of bullfrog presence and food availability. If the effects of bullfrog presence on size at metamorphosis is not influenced by the amount of food available, then the effects would be additive. If the effects of bullfrog presence depend on food availability, then the interaction would be synergistic. Understanding the complexity of interactions from multiple stressors can inform management decisions aimed to mitigate the impacts of anthropogenic activities.

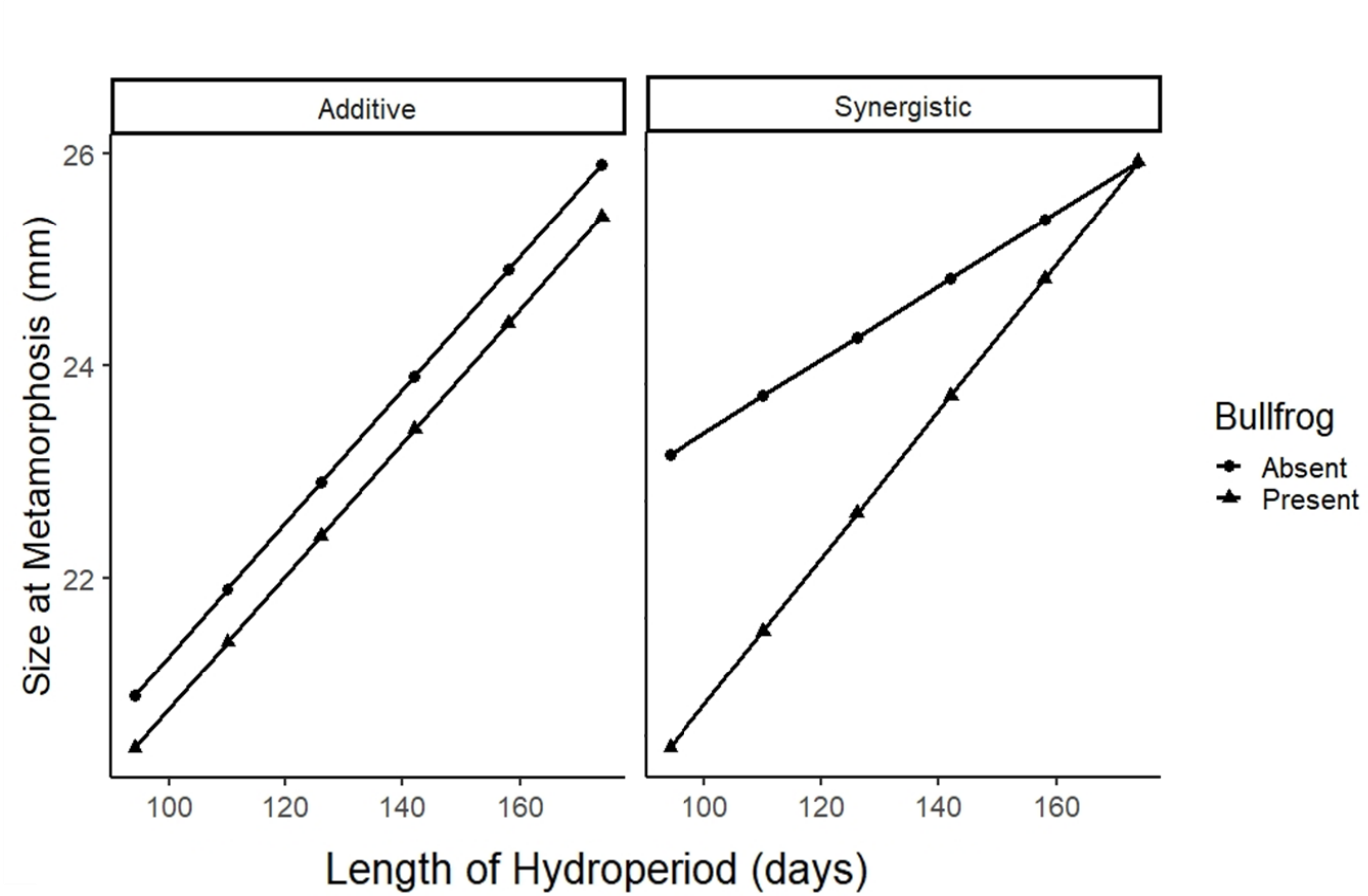

Figure 1. Example of additive (left) and synergistic (right) effects of shortened hydroperiod and bullfrog presence on size at metamorphosis. In the additive example, the relationship between length of hydroperiod and size at metamorphosis is not dependent on the presence or absence of bullfrogs. In the synergistic example, the relationship between length of hydroperiod and size at metamorphosis is dependent on the presence or absence of bullfrogs.

MATERIALS AND METHODS

My thesis was developed in two parts to evaluate the effects of *R. aurora* survival and growth: 1) an experimental manipulation of hydroperiod length, bullfrog presence, and food availability in a series of mesocosms and 2) a field study taking advantage of natural variation in hydroperiod at different pond depths. A captive experiment allows for greater control in manipulating and evaluating multiple stressors compared to a field setting. However, while captive experiments can isolate interacting stressors, they lack the natural complexity that field studies inherently take into account. The field study aimed to compliment the captive experiment by evaluating the response of *R. aurora* tadpoles to varying hydroperiod in a natural population. I did not examine the effects of bullfrog presence on *R. aurora* in the field because bullfrogs had previously been eradicated from the study site.

This project was approved by Humboldt State University's Institutional Animal Use and Care Committee (18/19.W.37-A) for the 2019 captive experiment and field study. All field data obtained during the 2017-2018 seasons were collected by Institute for Wildlife Studies under their California Department of Fish and Wildlife Scientific Collecting Permit (# 5759).

Captive Experiment

Experimental design

I conducted a 6 x 3 multi-factorial experiment to determine how shortened hydroperiods and bullfrog presence affect survival and size at metamorphosis in *R. aurora* tadpoles. The captive experiment was conducted at Institute for Wildlife Studies' property in Humboldt County, California. The experiment was positioned in a large open field on the property that was bordered by coniferous trees. I set up 36 water stock tanks for the experiment. Tanks were split into three bullfrog treatments: 1) *R. aurora* only (control), 2) *R. aurora* and bullfrog tadpoles separated by a permeable divider (signal), or 3) *R. aurora* and bullfrog tadpoles together without divider (direct). This design allowed for the separation of effects due to direct competition and those due to behavioral changes in response to perceived competition or predation risk from a chemical cue. Concurrently, I applied six hydroperiod treatments by changing the hydroperiod length in days (87, 99, 109, 121, 133, and 147 days). Progressively shortened hydroperiods simulated the drying of an ephemeral wetland over the course of the summer season under Mediterranean climatic conditions. To evaluate whether competition for food resources occurred, I added two levels of food quantity, 2 tablets for low food and 4 tablets for high food, which I randomly assigned to all tanks for a total of 18 low food and 18 high food treatments.

I arranged the tanks in a 6 x 6 grid, separated by approximately 2 m, and placed each tank on a leveled wooden pallet. Each tank had a 567 L capacity (approximate dimensions: 99.06 cm x 147.32 cm x 60.96 cm). Bullfrogs in the signal treatment tanks were contained in a permeable hamper (approximate dimensions 35.56 cm x 35.56 cm x 67.31 cm). The permeable 1 mm mesh polyester material of the hamper allowed for the exchange of water, nutrients, and chemical cues between the hamper and tank (Figure 2A). Control and direct bullfrog treatment tanks did not contain hampers. Bullfrogs in the direct treatment could move freely in the tank with *R. aurora* (Figure 2B). *Rana aurora* tadpoles were able to move freely in all tanks. I constructed cylinder refuge shelters from plastic fencing with 2.5 cm square openings and placed one vertically in each tank, extending through the entire water column (Figure 2). The openings allowed *R. aurora* entry into the cylinder while restricting bullfrog tadpole access. I covered each tank with a screened lid to exclude predators and prevent animals from escaping.

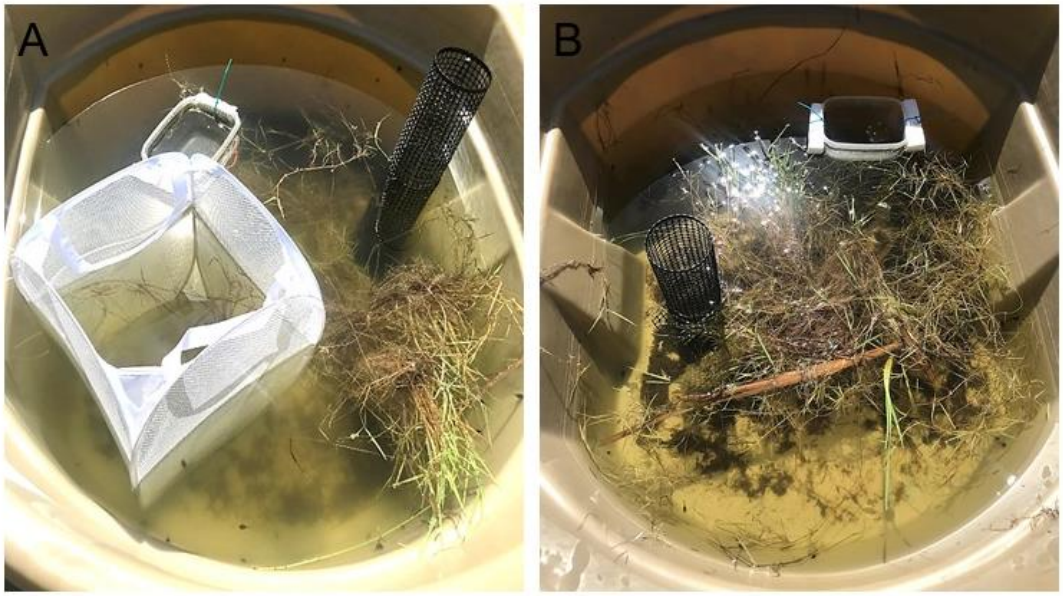

Figure 2. Tank design for captive experiment. Tanks included a floating cage to house embryos until hatching, a refuge shelter to provide intermittent protection, and plant material for food and shelter. A) Signal bullfrog treatment tanks contained permeable hampers to house bullfrogs and separate them from *R. aurora*. B) Control and direct bullfrog treatment tanks did not contain hampers, both bullfrogs and *R. aurora* were able to swim freely throughout the tank.

I standardized the water level in each tank at the beginning of the experiment to 50 cm. The maximum water depth and longest hydroperiod were designed to mimic an ephemeral pool at Humboldt Bay National Wildlife Refuge in 2018. The maximum water depth in 2018 occurred in late January during the breeding season. Due to changing rainfall during the winter, the water depths fluctuated around the maximum water depth for approximately two weeks followed by periodic drawdowns with drying rates increasing further into the season. To shorten the length of the hydroperiod, the start day for drying was 10-14 days earlier for each hydroperiod treatment (Figure 3). A daily water level was calculated for each hydroperiod treatment based on the drying rate curve and the start day of drying. To decrease water levels over the experiment, I drilled holes in millimeter increments into a polyvinyl chloride standpipe fixed to each tank. Each treatment was drawn down every two to five days which fluctuated over the course of the experiment as drying quickens later in the season. Water level was drawn down to a depth of 3 cm and maintained for three days, ending the experiment for that tank.

Prior to introducing *R. aurora* egg masses and bullfrog tadpoles, each tank was passively filled by rainwater during the fall and winter season and supplemented with plant material (dead cattails and grasses) from the same population *R. aurora* eggs were collected for the study to provide refuge and food resources. I performed water quality tests including pH, nitrites, phosphates, chlorine, hardness, ammonia, and alkalinity at initial set up. Water quality parameters influenced by processes from live organisms, including algae growth and amphibian defecation, including ammonia, phosphates, nitrites, and pH were tested at least monthly. These parameters with the addition of

dissolved oxygen and temperature, were monitored closely once water levels were low and the experiment moved into the summer in order to ensure tadpoles were maintained in a healthy environment. All water quality parameters were based on captive amphibian care guidelines (Odum and Zippel 2008, see Appendix A).

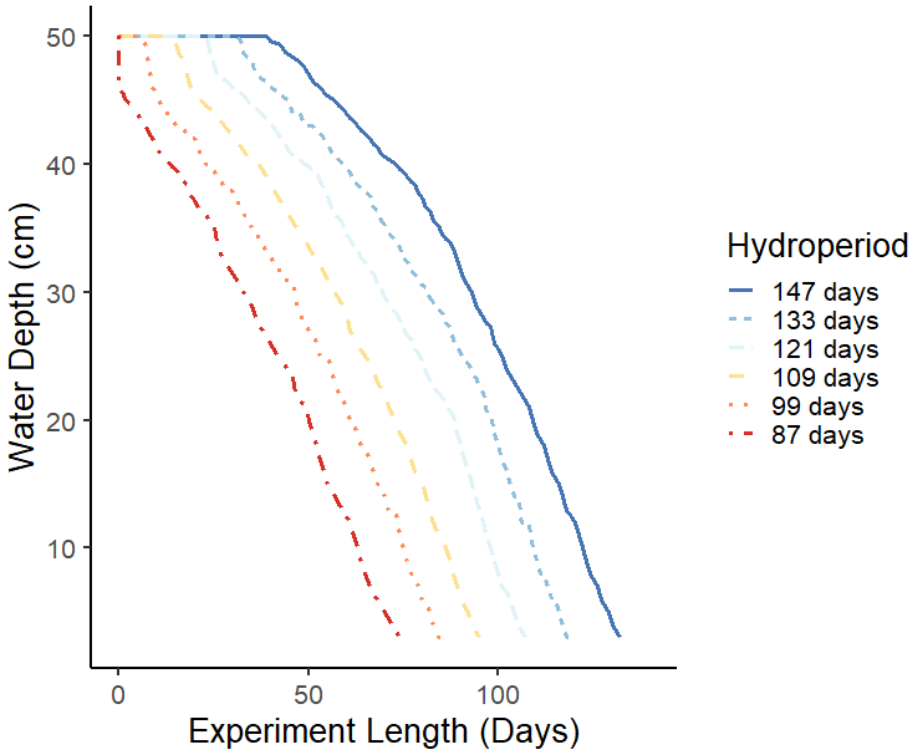

Figure 3. Hydroperiod treatments for the captive experiment. All hydroperiods started a depth at 50 cm and were subject to the same draw down rate. Hydroperiod lengths differed by initiating draw down at different dates.

Data collection

I collected 10 *R. aurora* egg masses, each of which contained approximately 500 to 820 embryos, from the Humboldt Bay National Wildlife Refuge and divided them among the 36 tanks. *Rana aurora* egg masses were added to the tanks on day 1 of the experiment (February 24th, 2019) toward the end of the breeding season at the collection site (last egg mass laid found March 8th, 2019). I placed the designated sample of embryos for each tank in a white tray, took a photograph, and quantified embryo number from still photographs. I placed *R. aurora* eggs into floating cages to cluster single eggs separated during the dividing process. Each of the 12 control tanks housed between 100-170 (122.25 average) *R. aurora* eggs. Each of the 24 signal and direct tanks housed between 80-140 (112 average) *R. aurora eggs.*

Rana aurora embryos began hatching on day 15 of the experiment and all embryos were hatched within the next 10 days. I counted unviable eggs and released *R. aurora* tadpoles into the tank from the floating cages. Tadpoles were left physically undisturbed for approximately 20 days after hatching. I collected bullfrog tadpoles from a private landowner's pond in Mendocino County, California and introduced 10 individuals to each signal hamper and direct tank on day 33 of the experiment. On day 48, I collected a sample of 20 *R. aurora* tadpoles from each tank by dipnet and subsequently weekly thereafter to visually observe body condition. On a weekly visual check in mid-April, I discovered tadpoles in many of the tanks appeared emaciated, therefore I supplemented food for the rest of the experiment to improve body condition. I administered food to all tanks on day 62, and once a week thereafter in a low (2 tablets) or high (4 tablets) food

treatment. Food consisted of algae and spirulina tablets made as catfish food (Aquatic Foods Inc., Fresno, CA).

Once a tadpole completed metamorphosis, I removed the froglet from the tank using a dip net, measured the snout-to-vent length in millimeters, and subsequently removed it from the experiment. For the captive component, I considered fully metamorphed frogs to be any individual with all limbs developed and tail fully absorbed, referred to as metamorphs (corresponds to Gosner stage 45-46, Gosner 1960). This indicated the end of the experiment for the individual. Each individual tank was removed from the experiment once all tadpoles completed metamorphosis or three days after a tank reached three cm, at which time all individuals were counted and removed from the tank. At the end of the experiment, animals were euthanized in tricaine methanesulfonate (MS-222) bath at a minimum concentration of 500 mg/L for at least 1 hour (Ramlochansingh, et al. 2014).

At the end of the experiment, I tallied the number of tadpoles that died before the hydroperiod ended (pre-desiccation mortality), tadpoles that remained in the tank 3 days after the water levels reached three cm (survival prior to desiccation), the number of metamorphs that were removed from each tank during the course of the experiment (survival through metamorphosis), and the snout-to-vent length of each metamorph.

Data analysis

All analyses were performed in R 3.6.1 (R Core Team, 2019). I used Akaike information criterion (AIC) or overdispersed modification of AIC, QAIC, both corrected for small sample size, to compare models for each analysis (Burnham and Anderson

2004) using MuMIn (Bartoń 2013). I used lme4 to estimate parameters from general linear mixed-effect models (Bates et al. 2012). Survival and size at metamorphosis were treated as response variables in the captive experiment. I treated hydroperiod as a continuous variable in all analyses.

Survival

Survival was assessed by two measures: survival through metamorphosis and survival prior to desiccation. Survival prior to desiccation represents tadpoles that survived to the end of the hydroperiod treatment but would have desiccated and died if water was drained completely from the tank. I constructed logistic regression models for survival prior to desiccation and survival through metamorphosis as a function of hydroperiod, bullfrog presence, and food availability. The candidate model set included models with either additive, interactive, or additive and interactive effects. I weighted models by the total number of tadpoles in each tank at the start of the experiment. Due to the common issue of over dispersion in binomial data, I utilized a quasibinomial distribution in both analyses (Warton and Hui 2011).

All tanks (n=6) representing the 121-day treatment experienced a complete die off. This was not caused by direct desiccation, but from extreme water temperatures (35-40 degrees C) when the water depth reached six cm. These tanks were excluded from both the survival through metamorphosis and survival prior to desiccation analyses. All tanks (n=18) from the three shortest hydroperiod treatments (87, 99, and 109 days) reached three cm before any tadpoles completed metamorphosis. All tadpoles from these treatments either died or would have died if the treatment was allowed to draw down

completely. Tanks across all hydroperiod treatments were used in the survival prior to desiccation analysis. In an effort to examine the effects of hydroperiod length on survival through metamorphosis post the three shortest hydroperiod treatments, I utilized 11 tanks from the two longest hydroperiod treatments (133 and 147 days) in the survival through metamorphosis analysis. I was unable to examine interactive effects in this analysis due to the relatively small sample size. I modeled survival prior to desiccation using 29 tanks across hydroperiod treatments of 87, 99, 109, 133, and 147 days.

Size at metamorphosis

To assess differences in *R. aurora* size at metamorphosis, I constructed a series of linear mixed-effects models with snout-to-vent length as the response variable. Models included a combination of hydroperiod length, bullfrog presence, and food availability treatments as main effects, while tank was treated as a random effect. I was unable to analyze interactive effects between hydroperiod and bullfrog treatments due to the small sample size in hydroperiod treatments (11 tanks). To evaluate additive or interactive effects with bullfrog treatments and food availability, I combined the signal and direct bullfrog treatments, because metamorph size did not differ between the signal and direct bullfrog treatments.

Field Study

Study area

In addition to the captive experiment, I utilized a three year in-situ field experiment to evaluate the effects of hydroperiod on survival, development, and size at

metamorphosis in *R. aurora* within a natural ecosystem. Institute for Wildlife Studies provided field data for 2017 and 2018. I collected field data in 2019 to increase sample size. Methods were the same across all three years.

The field study was conducted at the United States Fish and Wildlife Service's Humboldt Bay National Wildlife Refuge (HBNWR) Loleta, California. HBNWR is approximately 405 hectares situated southeast of the South Humboldt Bay State Marine Recreational Management Area and consists of over eight habitat types including salt marshes, freshwater wetlands, and streams (USFWS, 2013). Many of the freshwater wetlands across the HBNWR are occupied by *R. aurora*. The study location within HBNWR, Hookton Slough, contains a robust population of *R. aurora*. The Hookton slough population was the same population from which I collected *R. aurora* egg masses and hydroperiod information for the captive experiment. The field site is a natural ephemeral pool with emergent vegetation consisting of cattails (*Typha spp.*), sedges (*Carex spp.*), and grasses under a primarily open canopy. The hydroperiod at Hookton Slough spans approximately seven to nine months, with the onset of precipitation and flooding beginning in November. Once the rain subsides in late winter, the pond slowly draws down. The rate of drawdown quickens with the progression of the seasons into spring. Typically, the site completely dries by late summer (~August). The cumulative precipitation for the area from November to August in 2017, 2018, and 2019, was 124.6 cm, 93.3 cm, and 106.7 cm, respectively (NOAA, 2019).

Each year of the field-based experiment, enclosures were set up across a depth gradient to simulate varying hydroperiods. Enclosures were constructed out of woven

polypropylene material (approximate dimensions: 4 x 2 meters). The enclosure was buried into the ground and each side was reinforced with sandbags to prevent the escape of tadpoles. The interior of each enclosure included naturally growing emergent vegetation for refuge and food.

I set up two enclosures (2019-E6 and 2019-E7) in 2019 with hydroperiod lengths of 126 days and 139 days, respectively (previous years shown in Table 1). I calculated hydroperiod length as the total number of days from when egg masses were placed in the enclosure until the water level dropped to 10 cm in each enclosure. The hydroperiod treatments within the enclosures were approximately four to five months, slightly shorter than the hydroperiod for the whole pond, which starts when rain begins to fill the pond before the breeding season in early winter. I measured water depth from the center of each enclosure at each weekly site visit. I ended the experiment when the water level dropped to 10 cm in each enclosure to reduce mortality. The degree of drawdown could fluctuate greatly during a week potentially drying completely and desiccating tadpoles.

Data collection

I collected *R. aurora* eggs from the open pond on February 21st, 2019 and distributed 775 eggs to the short hydroperiod enclosure (2019-E6) and 873 eggs to the long hydroperiod enclosure (2019-E7) (previous years shown in Table 1). I monitored eggs and tadpole hatchlings weekly until *R. aurora* tadpoles reached taggable size (35 mm in total length). I collected tadpoles from each enclosure via dipnet and individually marked each tadpole with visible implant elastomer (VIE). I injected a unique color combination VIE into the base of each tadpole's tail following the methods described in

McHarry 2017. Tadpoles were anesthetized prior to marking by placing each tadpole in a water bath containing MS-222 at a concentration of (200 mg/L) following procedures and guidelines outlined in Anholt et al. (1998) and Grant (2008).

For each captured tadpole, I recorded body length, total length, and development stage. Developmental stages were created by grouping numerical stages outlined in Gosner (1960) into six categorical groups (with corresponding approximate Gosner stages): buds (26-30), feet (34-36), back legs (37-39), front buds (40-41), front legs (42-44), and metamorphs (45-46) (Figure 4). After marking, tadpoles were placed in a freshwater bath to recover from anesthesia and then placed in their designated enclosure.

To reduce the influence of density on tadpole survival and growth, I aimed for each enclosure to house 500 or fewer marked tadpoles. To increase the sample size and genetic diversity of tadpoles within each enclosure, I caught tadpoles from the open pond during each marking occasion and divided them evenly between the two enclosures. A total of 53 additional tadpoles were added to each hydroperiod enclosure (2019-E6 and 2019-E7, previous years listed in Table 1).

Once tadpoles completed metamorphosis, I removed the metamorph with a dipnet, measured the snout-to-vent length, and released it to the open pond. For the field component, I considered metamorphs to be any individual with all limbs developed and tail partially or fully absorbed. Due to the height and angle of the enclosure walls, the movement of metamorphs into or out of the enclosures was limited, however metamorphs exiting the enclosure would be more likely to attempt climbing the walls in search of alternative habitat and food resources than metamorphs in the open pond to which had

preferred upland foraging habitat. I assumed any metamorphs captured within the enclosures had developed from tadpoles living in the enclosures. Sixty five of the 118 metamorphs I captured from the two enclosures in 2019 had tadpole tags that were visible on their hind quarters, indicating the individual was placed in the enclosures as a tadpole (previous years shown in Table 1). The number of metamorphs that were tagged as tadpoles was likely higher than 65, because not all metamoprphs that were tagged as tadpoles retain tags in their hind quarters.

The field-based experiment ended for an individual when it completed metamorphosis or for the enclosure once the water depth dropped to 10 cm. Once the enclosure water level was at 10 cm, I opened the enclosure to the open pond to avoid trapping and desiccating tadpoles. At the end of the field season, I obtained tadpole capture histories, development stages, water depths, and snout-to-vent lengths of metamorphs.

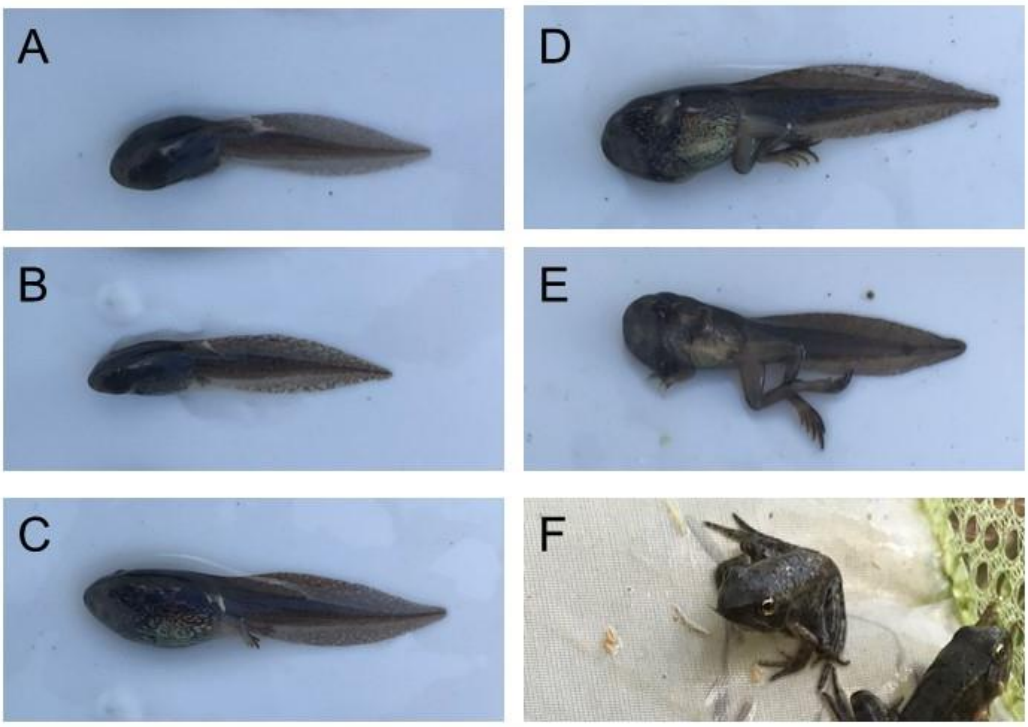

Figure 4. Tadpole developmental stages. A) Buds – back limb buds B) Feet – toes formed on back bud, no bend in leg C) Back legs – joint formed on back legs with feet, D) Front buds – front limb buds developing under skin with back legs E) Front legs – front legs exposed with toes developed and back legs F) Metamorph - individuals with four fully developed limbs, down turn mouths, and absorbed tails.

Table 1. Field component hydroperiod, egg mass, tadpole, and metamorph summary data across years and enclosures. Enclosure ID begins with the year the enclosure was surveyed.

Enclosure ID	Hydroperiod length (days)	Date egg masses added to enclosures	Number of eggs added to enclosure	Number of tadpoles captured inside/outside of enclosure (proportion from inside)	Capture period for tadpole mark recapture study	Number of metamorphs with/without visible tadpole tags (proportion with tags)
2017-E1	173	12/14, 12/22	1760	(100/49) 0.671	4/6-6/12	68/11 (0.861)
2017-E2	173	12/14, 12/22	1957	(53/84) 0.387	4/6-6/12	57/3 (0.95)
2018-E3	125	2/16	686	(20/91) 0.180	4/17-5/28	58/9 (0.866)
2018-E4	125	2/16	727	(67/63) 0.515	4/20-5/28	89/9 (0.908)
2018-E5	136	2/16	561	(84/101) 0.454	4/24-5/28	23/10 (0.697)
2019-E6	126	2/21	775	(74/35) 0.679	5/7-5/23	34/20 (0.630)
2019-E7	139	2/21	873	(61/53) 0.535	5/7-5/23	31/33 (0.48)

Data analysis

All analyses were performed in R 3.6.1 (R Core Team, 2019). I used Akaike information criterion (AICc), corrected for small sample size, to compare models for each analysis (Burnham and Anderson 2004) using MuMIn (Bartoń 2013). I treated survival through metamorphosis and size at metamorphosis as response variables for the field component. Survival through metamorphosis was estimated by a simulation analysis (described in detail below). Similar to the captive experiment, I treated hydroperiod as a continuous variable in all analyses.

Survival

Since it was possible for metamorphs to escape from the enclosures, I could not directly measure the survival to metamorphosis. Therefore, I used a simulation to estimate the percentage of *R. aurora* tadpoles that would complete metamorphosis based on two analyses: 1) daily tadpole survival and 2) transition probability through tadpole development stages.

I estimated daily survival rates for tadpoles marked from 2017-2019 using Cormack-Jolly-Seber (CJS) models (Cormack 1964, Jolly 1965, Seber 1965). Daily survival probability was estimated in MARK (White and Burnham 1999) using RMark (Laake 2013). Cormack-Jolly-Seber models estimate apparent survival probability (ϕ) and capture probability (p). However, because it would be extremely unlikely for tadpoles to leave the enclosures, estimates of apparent survival herein are likely true estimates of survival.

Tadpole enclosures represented group effects in the analysis. Start date varied by enclosures both within and between years. I estimated survival across 24 sampling occasions spanning three years (Table 1). For enclosures that were not surveyed on a particular day, capture probability was set to zero. I fit CJS models using a two-step approach following the procedures outlined in Lebreton et al. 1992. First, I constructed a series of models focused exclusively on capture probability. The candidate model set included models where capture probability was constant, varied by occasion (time), or varied by a capture effort index (number of tadpoles caught in the enclosure on that day). For these models, survival was held constant (i.e., the phi(.) model). The time varying model had the most support. Next, I constructed a second candidate model set where survival varied by enclosure, hydroperiod, year, and occasion (time) and capture probability varied by time across all of these models.

Next, I conducted an ordinal regression analysis to estimate transition probability through tadpole development stages using MASS (Venables and Ripley 2002). Initially, I attempted to estimate transition probabilities using a multi-state survival analysis. Unfortunately, my relatively small sample size prevented this approach.

In the ordinal regression analysis, I only incorporated tadpoles that were recaptured on at least one occasion. I considered tadpole stage growth to be the number of transitions a tadpole made between each recapture occasion. For example, a tadpole beginning at buds and recaptured at feet would equate to one transition, but a tadpole recaptured with front legs would equal four transitions (Figure 4). The ordinal regression candidate set include models consisting of different combinations of the following

variables: 1) starting development stage, including buds, feet, legs, front buds, and front legs; 2) period, which represented the total number of days between each recapture occasion; 3) Julian date, which was defined as the number of days that elapsed from the discovering of the first egg mass of that season to each tadpole's capture date; and 4) change in water depth, which was measured as the change in water level between recaptures of an individual divided by the total number of days between recapture occasions.

I predicted the proportion of tadpoles that completed metamorphosis in each enclosure by simulating outcomes using estimates of 1) tadpole transition probabilities obtained from the best fit ordinal regression model, 2) daily survival rate based on each enclosure's best fit Cormack-Jolly-Seber model, and 3) each enclosure's unique hydroperiod. I began each simulation on the first day a tadpole was marked at the bud stage in the enclosure the simulation was based on. Each simulation had the ability to run the length of the hydroperiod for the particular enclosure it was based on. For example, if a tadpole was first marked on day 50 of a 150-day long hydroperiod in the enclosure, the simulation predicting metamorphosis based on that enclosure could only run for a maximum of 100 days. After 100 days, the simulated tadpole will have died or transitioned through development stages and survived through metamorphosis. At each day in the simulation, a tadpole was determined to have died or survived based on a random draw between 0 and 1. If on a particular iteration, the simulation drew a number greater than the daily survival rate, the tadpole died. If the individual tadpole survived the random draw, it progressed through development stages based on predicted transition

probabilities from the ordinal regression analysis. This continued until the tadpole either died or successfully advanced through the development stages and completed metamorphosis. The simulation advanced through the lives of 10,000 tadpoles. I conducted a separate simulation analysis for each of the seven enclosures based on that enclosure's specific hydroperiod and model estimates from the CJS analysis. Transition probabilities obtained from the ordinal regression was used across all seven enclosures.

Size at metamorphosis

To assess differences in *R. aurora* size at metamorphosis, I constructed a candidate set of linear regression models. Models estimated snout-to-vent length as a function of hydroperiod length in days, emergence date, year, or combinations thereof.

RESULTS

Captive Experiment

For the captive component, a total of 29 tanks were available for analyses. Seven tanks experienced complete die offs, likely caused from extreme high temperatures during three consecutive days in June. Die offs affected all six enclosures representing the 121-day hydroperiod treatment and one enclosure representing the control, high food tank in the 147-day hydroperiod treatment. Die offs occurred when the water level for the 121-day hydroperiod treatment was at 6 cm and 5 days from the end of the experiment. Die offs occurred in the 147-hydroperiod treatment when the water level was at 20 cm and 31 days from the end of the experiment.

Survival

Tadpoles did not complete metamorphosis in hydroperiods of 109 days or shorter indicating a strong effect of shortened hydroperiod on survival through metamorphosis (Figure 5). The longest two hydroperiod treatments (133- and 147-days) produced a total of 399 metamorphs, the majority of metamorphs were produce in the 147-day treatment (Figure 6). The proportion of tadpoles completing metamorphosis from individual tanks within the two longest hydroperiod

treatments can be found in Appendix B. The top two models estimating survival through metamorphosis included a model with food only and the null model, these models were similarly supported ($\Delta QAIC_c < 1$, Table 2). Hydroperiod or bullfrog variables were not present in the four top models ($2.58 \leq \Delta QAIC_c \leq -14.93$, Table 2). The top four logistic regression models estimating survival prior to desiccation performed similarly ($\Delta QAIC_c < 1$, Table 3). I found no support for synergistic effects of hydroperiod and bullfrog presence (Bullfrog * Hydroperiod) on survival prior to desiccation ($\Delta QAIC_c > 4$, Table 3).

Size at metamorphosis

I measured 399 snout-to-vent lengths in metamorphs across all treatments. The best fitting model included bullfrog and food treatments (

Table 4). Metamorphs were smaller in the bullfrog treatments when food availability was low, but not when food availability was high (Figure 7). There was no difference in the effect of bullfrogs between the signal and direct bullfrog treatments. A model that included an interaction between grouped bullfrog treatments and food treatments performed better than the additive model for ungrouped bullfrog treatments and food availability (Figure 5Table 5). Mean metamorph snout-to-vent length in the low food, signal + direct bullfrog treatment was at least 1.7 mm shorter than any of the other treatment combinations (Figure 8).

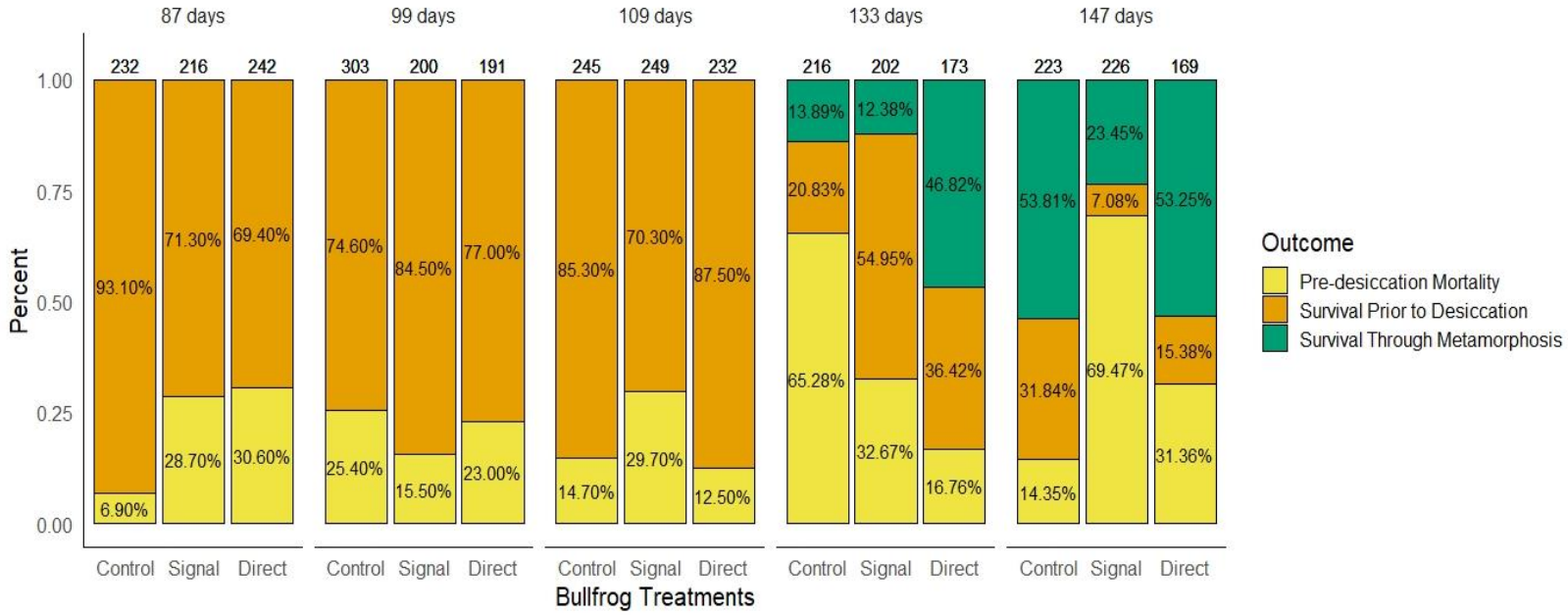

Figure 5. Captive experiment pre-desiccation mortality, survival prior to desiccation, and survival through metamorphosis in hydroperiod and bullfrog treatments. The total number of *R. aurora* tadpoles at the beginning of the experiment for each hydroperiod/bullfrog combination is shown directly above the bar and below the hydroperiod length.

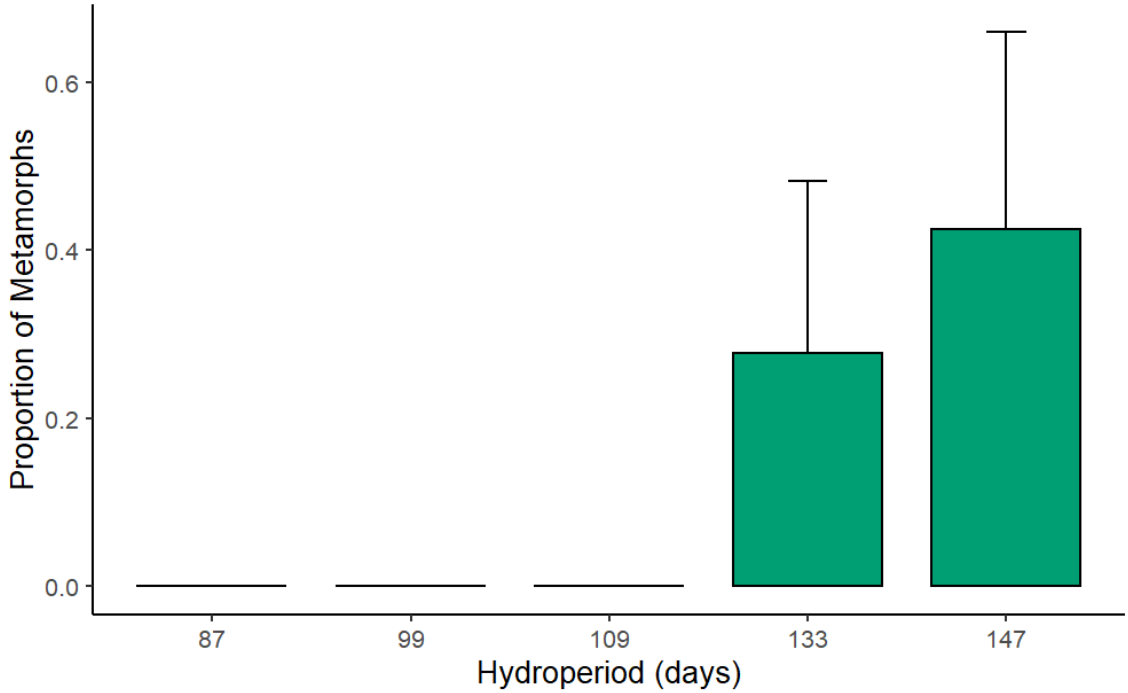

Figure 6. Proportion of metamorphs across the five, captive experiment hydroperiod treatments (mean ±1 SE; n = 163 metamorphs for 133-day treatment and n = 263 metamorphs for 147-day treatment).

Table 2. Captive experiment logistic regression candidate model set for survival through metamorphosis in tanks with hydroperiods ≥133 days. Models are ranked in ascending order. The direction of effect is indicated by a "+" (positive effect) or a "-" (negative effect) and no effect is indicated as a blank.

Bullfrog	Food	Hydroperiod	df	logLik	$QAIC_c$	$\Delta QAIC_c$	weight
	+		2	-139.303	28.4	0.00	0.387
			1	-171.543	28.9	0.46	0.306
+			3	-119.819	31.0	2.58	0.106
		+	2	-158.366	31.0	2.60	0.105
	+	+	3	-128.749	32.2	3.80	0.058
+	+		4	-84.296	33.5	5.08	0.031
+		+	4	-106.600	36.5	8.12	0.007
+	+	+	5	-75.911	43.3	14.93	0.000

Table 3. Captive experiment logistic regression candidate set for survival prior to desiccation across all hydroperiod treatments. Bullfrog * Hydroperiod indicates an interaction model. The direction of effect is indicated by a "+" (positive effect) or a "-" (negative effect) and no effect is indicated as a blank.

Bullfrog	Food	Hydroperiod	Bullfrog * Hydroperiod	df	logLik	$QAIC_c$	$\Delta QAIC_c$	w
		-		2	-332.586	43.4	0.00	0.254
+				3	-314.413	44.1	0.72	0.178
				1	-362.752	44.2	0.81	0.170
+		-		4	-288.384	44.2	0.81	0.170
	+	-		3	-332.159	46.1	2.66	0.067
	+			2	-361.883	46.6	3.21	0.051
+	+			4	-312.882	46.9	3.49	0.044
+	+	-		5	-287.480	47.3	3.92	0.036
+		-	+	6	-261.785	48.0	4.62	0.025
+	+	-	+	7	-261.310	51.8	8.43	0.004

Table 4. Captive experiment mixed-effect candidate model set for size at metamorphosis in tanks with hydroperiods ≥133 days. The direction of effect is indicated by a "+" (positive effect) or a "-" (negative effect) and no effect is indicated as a blank.

Bullfrog	Food	Hydroperiod	df	logLik	AIC_c	ΔAIC_c	weight
+	+		6	-733.973	1480.2	0.00	0.516
+	+	+	7	-733.929	1482.1	1.98	0.191
	+		4	-737.361	1482.8	2.66	0.136
	+	+	5	-737.192	1484.5	4.38	0.058
			3	-739.597	1485.3	5.10	0.040
+			5	-737.943	1486.0	5.88	0.027
		+	4	-739.345	1486.8	6.63	0.019
+		+	6	-737.761	1487.7	7.53	0.012

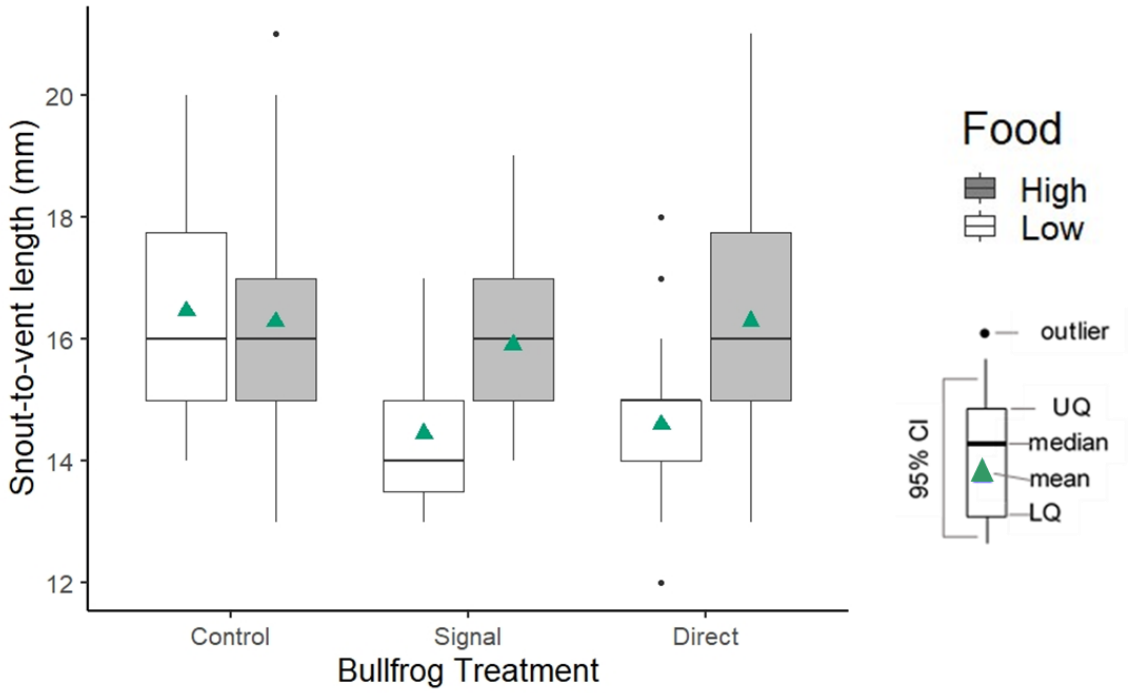

Figure 7. Captive experiment variation in snout-to-vent lengths of metamorphs from the two longest hydroperiod treatments (133-day and 147-day) grouped by bullfrog presence/absence and food level.

Table 5. Captive experiment mixed-effect candidate model set for size at metamorphosis. Bullfrog * Food indicates an interaction model. The direction of effect is indicated by a "+" (positive effect) or a "-" (negative effect) and no effect is indicated as a blank.

Bullfrog	Food	Hydroperiod	Bullfrog * Food	df	logLik	AIC_c	ΔAIC_c	weight
+	+		+	6	-731.967	1476.1	0.00	0.436
+	+	+	+	7	-731.314	1476.9	0.77	0.298
+	+			5	-733.976	1478.1	1.96	0.164
+	+	+		6	-733.930	1480.1	3.93	0.061
	+			4	-737.361	1482.8	6.68	0.016
+				4	-737.998	1484.1	7.95	0.008
	+	+		5	-737.192	1484.5	8.39	0.007
				3	-739.597	1485.3	9.11	0.005
+		+		5	-737.845	1485.8	9.69	0.003
		+		4	-739.345	1486.8	10.64	0.002

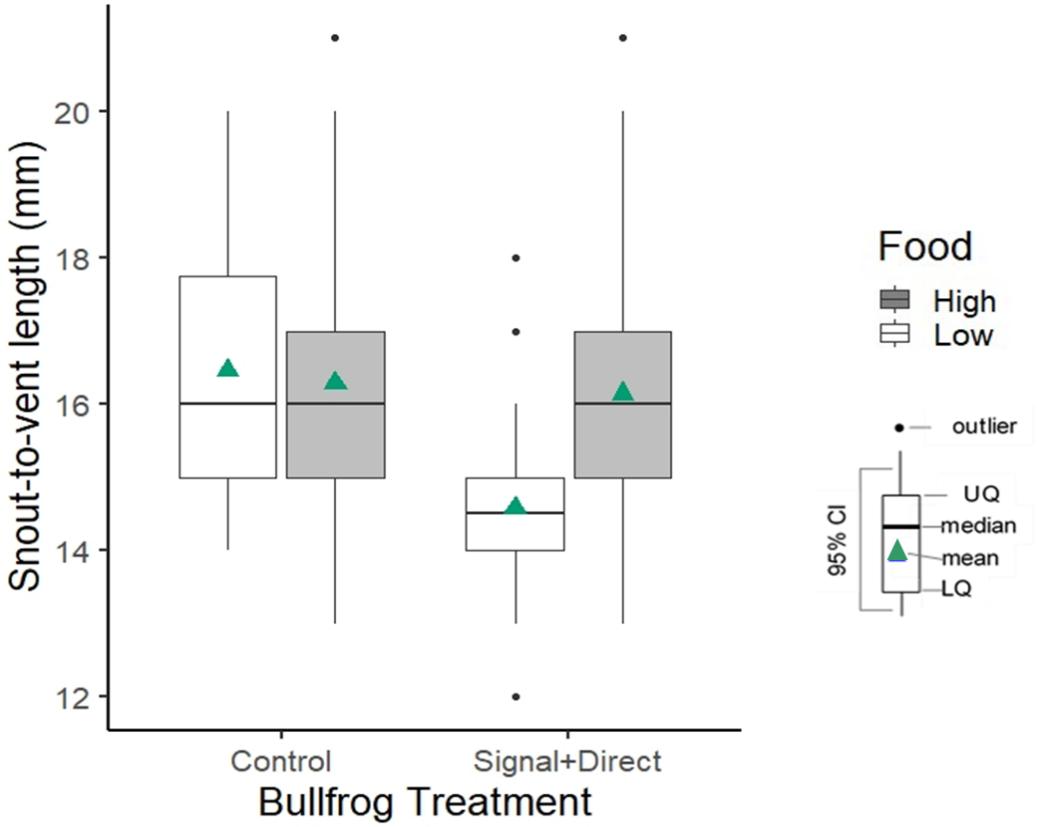

Figure 8. Captive experiment variation in snout-to-vent lengths of metamorphs from the two longest hydroperiod treatments (133-day and 147-day) grouped by bullfrog presence/absence (signal and direct treatments combined) and food level.

Field Study

Survival

I estimated daily survival probability from encounters of 852 *R. aurora* marked tadpoles. Daily survival estimates ranged from 0.89 (95% CI, 0.82-0.94) to 0.99 (95% CI, 0.86-1.0). The top model for tadpole daily survival varied by enclosure for survival probability, but no evidence that those differences were due to hydroperiod, year to year variation, or changes in pond conditions over the course of a season (Table 6, Figure 9). Survival estimates from 2019 had large confidence intervals (Figure 9) due to few (n=3) recapture occasions.

The probability of a tadpole transitioning into the next development stage was positively correlated with the water depth change (Figure 10). In other words, tadpoles transitioned faster in treatments with shortened hydroperiods. On average tadpoles advanced one development stage every 12 days. As the experiment progressed, the rate of drawdown increased. Consequently, the probability of a tadpole transitioning to the next development stage increased as the drawdown increased (Figure 10).

In the simulation, the proportion of tadpoles that survived and completed metamorphosis ranged from 8.2-70.20% (Figure 11). The simulated survival through metamorphosis varied across hydroperiods but shortened hydroperiods did not necessarily correspond to decreased survival through metamorphosis (Figure 11). For each enclosure, the pattern of survival through metamorphosis from the simulation-based

study was similar to the daily survival estimates from the CJS model, despite the simulation study also including transitional probabilities from the ordinal regression.

Size at metamorphosis

I measured 455 metamorph snout-to-vent lengths from seven enclosures across three years. The best fitting model included hydroperiod, emergence date, and year, all of which had a positive effect on size at metamorphosis (Table 7). On average, tadpoles metamorphosed at larger sizes in treatments with longer hydroperiods (Figure 12). However, on average, metamorphs did not increase in size from the 139-day hydroperiod treatment to the longest hydroperiod of 173 days. The 173-day hydroperiod treatment included two enclosures from 2017. Moreover, the 2017 egg laying year was an anomaly in that eggs were laid an average of six weeks earlier than they were in 2018 and 2019. This resulted in the high number of metamorphs emerging early in the 2017 173-day hydroperiod treatment. Despite this, and similar to patterns I observed in the best-fitting model across all hydroperiods, size at metamorphosis was positively related with emergence date (0.13 mm ±0.02 SE) in the 173-day hydroperiod treatment.

Table 6. Field study Cormack-Jolly-Seber candidate model set for survival (ϕ) and recapture (ρ) probabilities across 2017 to 2019.

ϕ	ρ	K	AIC_c	ΔAIC_c	w
enclosure	time	28	5990.8	0.0	0.999
constant	time	22	6015.2	24.5	0.000
year	time	23	6016.0	25.3	0.000
hydroperiod	time	23	6017.3	26.5	0.000
time	time	44	6038.8	48.1	0.000

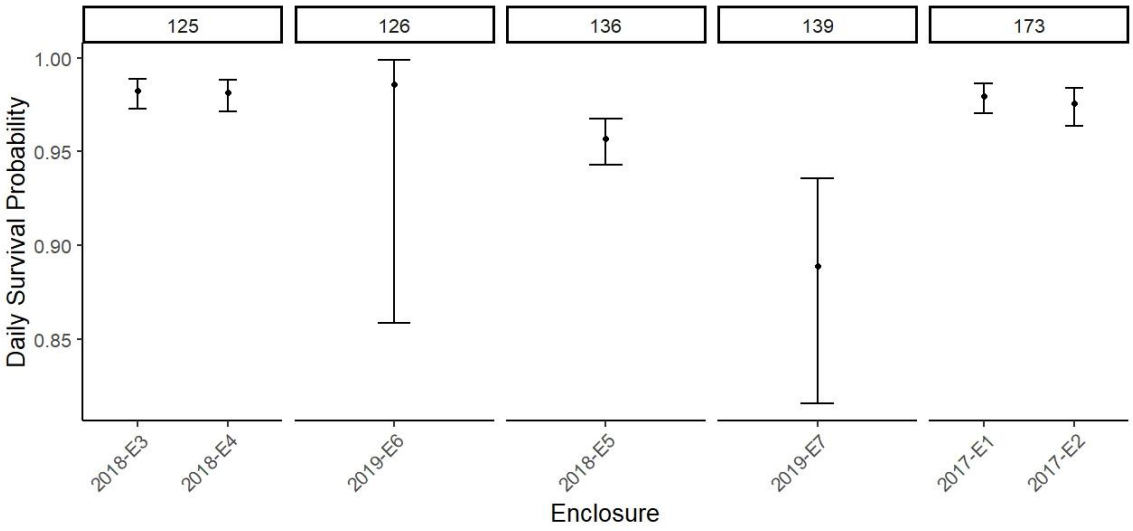

Figure 9. Field study daily tadpole survival estimates and 95% CI based on the ϕ (group) ρ (time) model paneled by hydroperiod from 2017 to 2019.

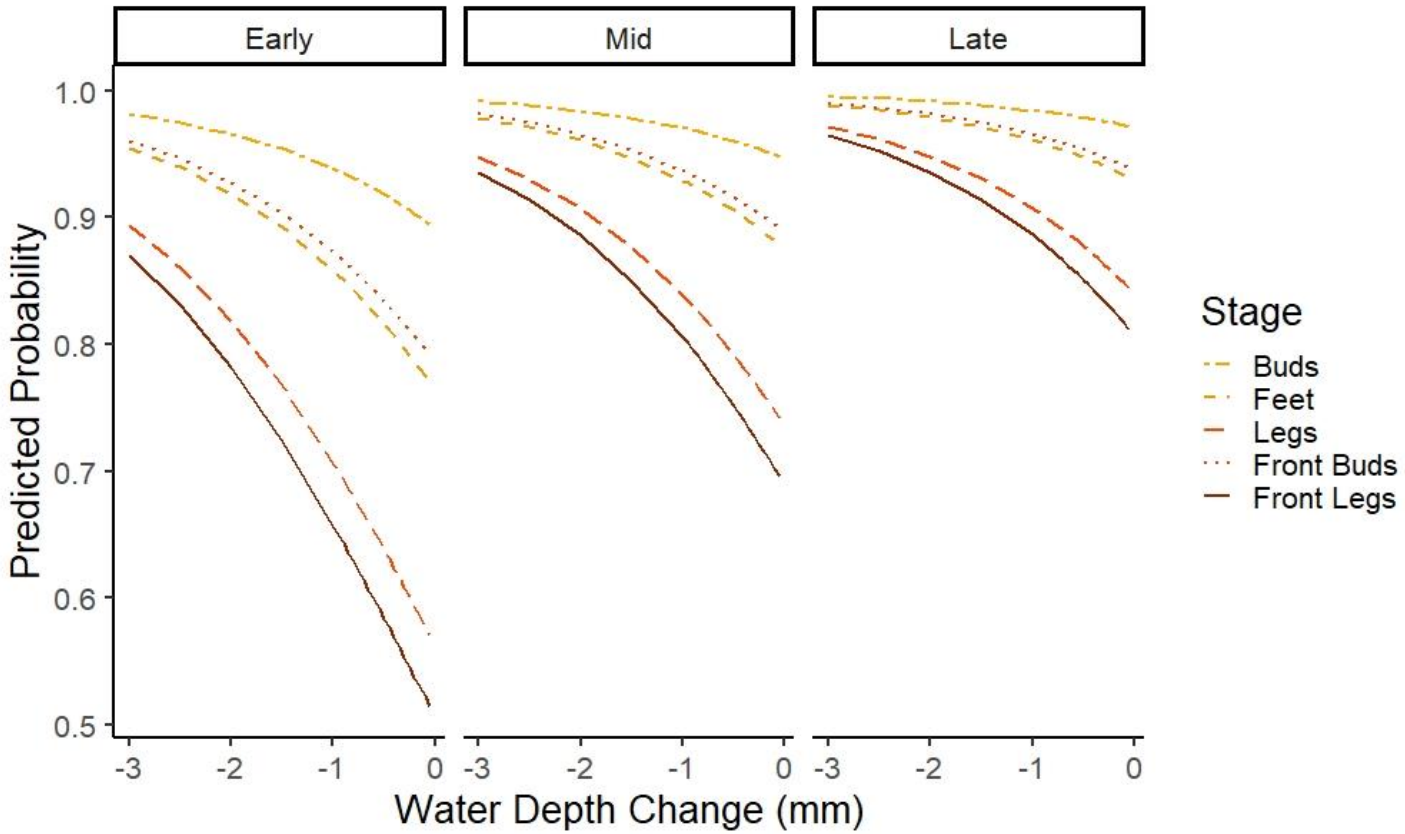

Figure 10. Field study ordinal regression predicted probability estimates. The y axis represents the predicted probability that a tadpole will transition into the subsequent development stage based on water depth changes over a 12-day period. Predicted probabilities are paneled by time of season (early = May, mid = June, late = July). Stage represents a tadpole's development stage upon first capture.

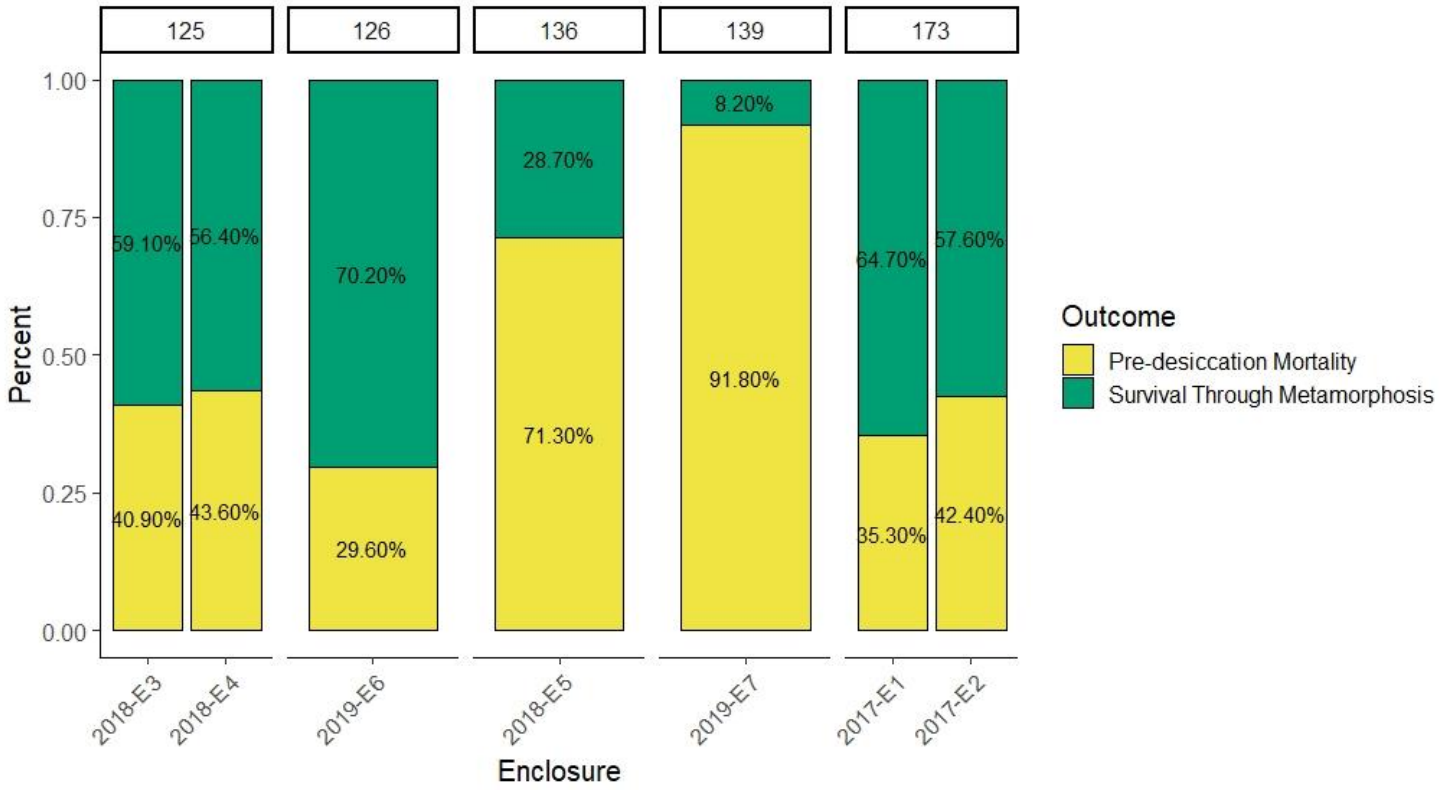

Figure 11. Percentage of mortality and survival outcomes from the simulation-based analysis across 10,000 tadpoles per enclosure. Enclosure ID is shown below each bar and grouped based on hydroperiod length (days) shown above the bar(s).

Table 7. Field study general linear candidate model set for size at metamorphosis. The direction of effect is indicated by a "+" (positive effect) or a "-" (negative effect) and no effect is indicated as a blank.

Hydroperiod	Julian date	Year	df	logLik	AICc	ΔAIC_c	weight
+	+	+	6	-910.097	1832.4	0.00	0.872
+	+		4	-914.068	1836.2	3.84	0.128
	+	+	5	-923.455	1857.0	24.66	0.00
+		+	5	-936.061	1882.3	49.87	0.00
		+	4	-968.128	1944.3	111.96	0.00
+			3	-977.583	1961.2	128.84	0.00
	+		3	-980.746	1967.5	135.16	0.00
			2	-997.712	1999.5	167.07	0.00

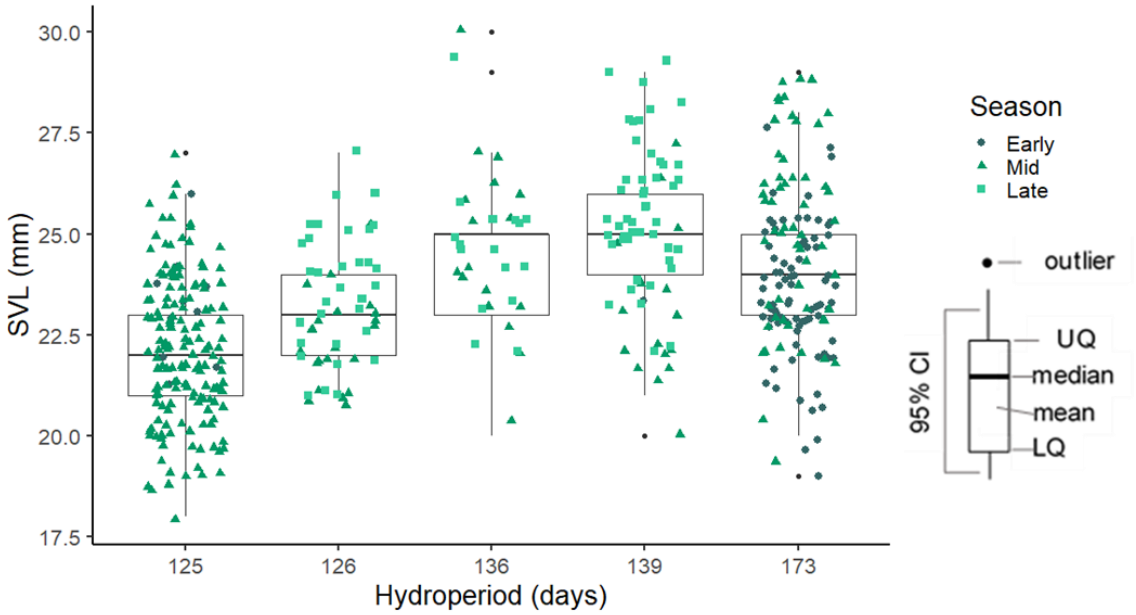

Figure 12. Field study variation in snout-to-vent lengths (SVL) of metamorphs from the five hydroperiod treatments. Emergence date of metamorphs is represented by symbols based on time of season (early = May, mid = June, late = July).

DISCUSSION

Although climate change and invasive species are ubiquitous stressors and are individually well studied, little research exists that quantifies their combined effects simultaneously. My thesis assessed the effects of three stressors on tadpole survival and size at metamorphosis in *R. aurora*, a species of special concern in California: 1) altered hydroperiods (an index of climate change), 2) bullfrog presence/absence (an invasive species), and 3) resource (food) availability.

Hydroperiod had a threshold effect on survival through metamorphosis, whereby once the hydroperiod threshold was met, no additional benefit of a longer hydroperiod was detected. No tadpoles successfully metamorphosed in three of the hydroperiod treatments comprising 18 of the 36 tanks. Moreover, an additional treatment comprising six tanks suffered 100 percent mortality likely due to a combination of heat stress and shallow water depths (six cm). There was no discernable distinction between the two longest hydroperiod treatments or effects of bullfrog presence and food availability on survival through metamorphosis. Similarly, I did not find a strong relationship between hydroperiod, bullfrog presence, and food availability and survival prior to desiccation.

Previous studies have found a linear relationship between hydroperiod and survival through metamorphosis (Brannelly et al. 2019, Boone et al. 2004). These studies may have been restricted to a small range of hydroperiods above the threshold, thus making it impossible to detect a threshold effect. Moreover, in field-based studies examining few water bodies, similar to the field-based component in my thesis, there

exists little opportunity to detect threshold effects because there is not enough variation in hydroperiods. For example, one pond will only have one hydroperiod in a single wet season. To detect threshold effects in hydroperiods, researchers would need many years of hydroperiod data from a single pond or they would need to measure multiple ponds with varying hydroperiods simultaneously. However, utilizing varying hydroperiods from multiple ponds in the field may be difficult to detect threshold effects for amphibians that avoid breeding in shallow water bodies when deeper ponds are present, as is the case with *R. aurora* gravid females (Sendak 2008). Without the captive experiment, I would not have been able to detect a threshold effect in hydroperiod.

The presence of a threshold effect highlights the vulnerability of aquatic systems and the animals that reside within them. As seen here, reducing a hydroperiod by a few days can push tadpoles beyond a tipping point leading to complete mortality of a tadpole population in a given water body. In some areas this could represent an entire generation in a region.

Although tadpoles have the ability to increase their development rate to mitigate the effects of shorter hydroperiods (O'Regan et al. 2014, Denver et al. 1998), tadpoles are still susceptible to other stressors related to reduced hydroperiods. Not only do reduced hydroperiods increase the chance of desiccation, as water bodies get shallower, they lose thermal inertia, leading to increased chances of heat related mortality. This exact phenomenon was observed in six of the 36 tanks in my study.

In the captive experiment, size at metamorphosis was best predicted using an interaction between bullfrog presence and food availability. Metamorphs were smaller in

treatments with both bullfrogs and low food availability than in treatments with either bullfrogs only, low food availability, or no stressor (control). In treatments with only one stressor, metamorphs were similar in size to the control treatment (no stressors). This result is consistent with Reylea and Mills (2001), in which the effect of one stressor was absent until combined with an additional stressor.

Additionally, I found size at metamorphosis in the captive component to be reduced across all treatments compared to the size at metamorphosis in field study (Figure 8 and Figure 12). The differences in body size were likely driven by the disparity in food availability. The variety and amount of food resources available to tadpoles in the field were likely higher than the food provided in the captive experiment. Similarly, in a study measuring *R. aurora* metamorph size at multiple breeding ponds, metamorphs were smaller sizes at sites with less emergent vegetation (IWS unpublished data). Emerging at smaller sizes can be directly linked to reduced adult fitness (Semlitsch et al. 1988). These carryover effects demonstrate how impacts on growth during the larval period can affect population viability.

Controlled laboratory experiments integrated with field studies lead to insights easily lost if each is conducted independently. The increased control in manipulating both abiotic and biotic factors were benefits of this captive experiment. In order to predict the effects of climate change on species without the sole reliance of projected climate models, it is necessary to manipulate abiotic factors outside of current conditions (Fordham 2015). However, captive experiments may have limitations such as lessening the realistic exposure and complexity species experience in natural environments. The

shortcomings in captive experiments can be strengthened by pairing a field study that incorporates factors difficult to transfer to a captive experiment such as multiple species interactions, changing environmental conditions, and food resources.

As we continue to recognize the growing effects humans have on the abiotic environment, it is even more important to understand how these effects impact living organisms. The effects from more than one stressor can be antagonistic, additive, or synergistic with context-dependent responses through variation across taxa and populations (Sih et al. 2014, Koprivinikar 2010, Rohr et al. 2004). Likewise, these effects can produce different responses depending on an animal's life stage (Snigula et al. 2017). Predicting how species with complex life histories will respond to multiple stressors hinges on the development of studies that consider a wide range of future environmental conditions.

LITERATURE CITED

Anholt, B.R., S. Negovetic, and C. Som. 1998. Methods for anaesthetizing and marking larval anurans. Herpetological Review 29:153–154.

Baillie, J. E. M., Hilton-Taylor, C., and S. N. Stuart. 2004. 2004 IUCN red list of threatened species. A global species assessment. IUCN, Gland, Switzerland and Cambridge, UK. xxiv + 191 pp.

Bårdsen, B. J., Hanssen, S. A., and J. O. Bustnes. 2018. Multiple stressors: modeling the effect of pollution, climate, and predation on viability of a sub-arctic marine bird. Ecosphere 9 (7):e02342.10.1002/ecs2.2342

Bartoń, Kamil. 2013. MuMIn: Multi-model inference. R package version 1.10.0.1.

Bates, D., Mächler, M., Bolker, B. M., and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67(1).

Boone, M. D., Little, E. E. and R. D. Semlitsch. 2004. Overwintered bullfrog tadpoles negatively affect salamanders and anurans in native amphibian communities. Copeia 3:683-690.

Brannelly, L. A., Ohmer, M. E. B., Saenz, V., and C. L. Richards-Zawacki. 2019. Functional Ecology 00:1-10.

Burnham, K. P., and D. R. Anderson. 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociological Methods and Research, 33:261–304.

Cormack, R. M. 1964. Estimates of survival from the sighting of marked animals. Biometrics 51:429–438.

Denver, R. J., Mirhadi, N., and M. Phillips. 1998. Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. Ecology 79:1859-1872.

Doak, D. F. and W. F. Morris. 2010. Demographic compensation and tipping points in climate-induced range shifts. Nature 467:959-962.

Ficetola, G. F., and L. Maiorano. 2016. Contrasting effects of temperature and precipitation change on amphibian phenology, abundance and performance. Oecologia 181:683–693.

Fordham, D. A. 2015. Mesocosms reveal ecological surprises from climate change. PLOS Biology, 13(12): e1002323. https://doi.org/10.1371/journal.pbio.100232

Frederick, J. W., Prowse, T. D., Reist, J. D., Hobbie, J. E., Lévesque, L. M. J., and W. F. Vincent. 2006. Climate change effects on aquatic biota, ecosystem structure and function. AMBIO: A Journal of the Human Envrionment 35:359-369.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190.

Grant, E. H. C. 2008. Visual implant elastomer mark retention through metamorphosis in amphibian larvae. Journal of Wildlife Management 72:1247–1252.

IPCC. 2014. Climate change 2014: synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jolly, A. G. M. 1965. Explicit estimates from capture-recapture data with both death and immigration- stochastic model. Biometrika 52:225–247.

Joseph, M. B., Piovia-Scott, J., Lawler, S. P., and K. L. Pope. 2011. Indirect effects of introduced trout on Cascades frogs (*Rana cascadae*) via shared aquatic prey. Freshwater Biology 56:828–838.

Keddy, P. A. 1989. Competition. Netherlands: Springer.

Kiesecker, J. M., and A. R. Blaustein. 1998. Effects of introduced bullfrogs and smallmouth bass on microhabitat use, growth, and survival of native red-legged frogs *(Rana aurora*). Conservation Biology 12:776–787.

Kiesecerk, J. M., Blaustein, A. R., and C. L. Miller. 2001. Potential mechanisms underlying the displacement of native red-legged frogs by introduced bullfrogs. Ecology 82:1964-1970.

Koprivnikar, J. 2010. Interactions of environmental stressors impact survival and development of parasitized larval amphibians. Ecological Applications 20:2263-2272.

Kupferberg, S. J. 1997. Bullfrog (*Rana catesbeiana*) invasion of a California river: the role of larval competition. Ecology 78:1736–1751.

Laake, J. L. 2013. RMark: An R interface for analysis of capture-recapture data with MARK. AFSC Processed Report 2013-01.

Larson, E. R., Magoulick, D. D., Turner, C., and K. H. Laycock. 2009. Disturbance and species displacement: Different tolerances to stream drying and desiccation in a native and an invasive crayfish. Freshwater Biology 54:1899–1908.

Lebreton, J., Kenneth, P. B., Clobert, J., and D. R. Anderson. 1992. Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. Ecological Monographs, 62 (1), 67-118.

Leu, M., Hanser, S. E., and S.T. Knick. 2008. The human footprint in the west: A large-scale analysis of anthropogenic impacts. Ecological Applications 18 (5), 1119–1139.

Leuven, R. S. E. W., Hendriks, A. J., Huijbregts, M. A. J., Lenders, H. J. R., Matthews, J., and G. van der Velde. 2011. Differences in sensitivity of native and exotic fish species to changes in river temperature. Current Zoology, 57:852–862.

McHarry, K. 2017. Influence of canopy cover and climate on early life-stage vital rates for northern red-legged frogs (Rana aurora), and the implications for population growth rates. (Master's Thesis, Humboldt State University, Arcata, California). Retrieved from https://digitalcommons.humboldt.edu/cgi/viewcontent.cgi?article=1102&context=et

NOAA. Climate graphs. 2019. https://www.wrh.noaa.gov/climate/temp_graphs.php

Odum, R. A., and K. C. Zippel. 2008. Amphibian water quality: Approaches to an essential environmental parameter. International Zoo Yearbook 42:40–52.

O'Regan, S. M., Palen, W. J., and S. C. Anderson. 2014. Climate warming mediates negative impacts of rapid pond drying for three amphibian species. Ecology 95:845-855.

Paaijmans, K. P., Heinig, R. L., Seliga, R. A., Blanford, J. I., Blanford, S., Murdock, C. C., and M. B. Thomas 2013. Temperature variation makes ectotherms more sensitive to climate change. Global Change Biology 19:2373–2380.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Rahel, F. J., and J. D. Olden. 2008. Assessing the effects of climate change on aquatic invasive species. Conservation Biology, 22:521–533.

Ramlochansingh, C., Branoner, F., Chagnaud, B. P., and H. Straka. 2014. Efficacy of tricaine methanesulfonate (MS-222) as an anesthetic agent for blocking sensory-motor responses in Xenopus laevis tadpoles. PLoS ONE 9:1–11.

Rehage, J. S., Barnett, B. K., and A. Sih. 2005. Foraging behaviour and invasiveness: Do invasive Gambusia exhibit higher feeding rates and broader diets than their noninvasive relatives? Ecology of Freshwater Fish, 14:352–360.

Relyea, R. A. and N. Mills. 2001. Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). 98:2491-2496.

Ricciardi, A., and H. J. Macisaac. 2010. Impacts of Biological Invasions on Freshwater Ecosystems. Fifty Years of Invasion Ecology: The Legacy of Charles Elton 211–224.

Riley, S. P. D., Busteed, G. T., Kats, L. B., Vandergon, T. L., Lee, L. F. S., Dagit, R. G., Kerby, J. L., Fisher, R. N., and R. M. Sauvajot. 2005. Effects of urbanization on the distribution and abundance of amphibians and invasive species in southern California streams. Conservation Biology 19:1894–1907.

Rohr, J. R., Elskus, A. A., Shepherd, B. S., Crowley, P. H., McCarthy, T. M., Niedzwiecki, J. H., Sager, T., Sih, A., and B. D. Palmer. 2004. Multiple stressors and salamanders: effects of an herbicide, food limitation, and hydroperiod. Ecological Applications 14:1028-1040.

Salice, C. J. 2012. Multiple stressors and amphibians: contributions of adverse health effects and altered hydroperiod to population decline and extinction. Journal of Herpetology, 46:675–681.

Seber, A. G. A. F. 1965. A note on the multiple-recapture census. Biometrika 52:249–259.

Semlitsch, R. D., Scott, D. E. and Pechmann, J. H. K. 1988. Time and size at metamorphosis related to adult fitness in Ambystoma talpoideum. Ecology 69:184-192.

Sendak, C. M. 2008. Spatial ecology and site occupancy of the northern red-legged frog (*Rana aurora*) in a coastal dune environment. (Master's Thesis, Humboldt State University, Arcata, California). Retrieved from http://humboldt-dspace.calstate.edu/bitstream/handle/2148/348/final_sendak.pdf?sequence=1

Sih, A., Bell, A. M., and J. L. Kerby. 2004. Two stressors are far deadlier than one. TRENDS in Ecology and Evolution 19:274-276.

Sniegula, S., Janssens, L., and R. Stoks. 2017. Integrating multiple stressors across life stages and latitudes: combined and delayed effects of an egg heat wave and larval pesticide in a damselfly. Aquatic Toxicology 186:113-122.

Swain, D. L., Langenbrunner, B., Neelin, J. D., and A. Hall. 2018. Increasing precipitation volatility in twenty-first-century California. Nature Climate Change 8:427-433.

Thomson, R. C., A. N. Wright, and H. B. Shaffer. 2016. California amphibian and reptiles species of special concern. California Department of Fish and Wildlife and University of California Press, Oakland, CA.

Trenberth, K. E. 2011. Changes in precipitation with climate change. Climate Research, 47:123–138.

Urban, M. C. 2015. Accelerating extinction risk from climate change. Science 348:571-573.

USFWS. Fish and Wildlife Service. Humboldt Bay National Wildlife Refuge. 2013. https://www.fws.gov/refuge/Humboldt_Bay/wildlife_and_habitat/HumboldtHabitat.html.

Venables, W. N. and B. D. Ripley. 2002. Modern applied statistics with S. Fourth edition. Springer, New York. ISBN 0-387-95457-0.

Warton, D. I. and F. K. C. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3-10.

White, G. C., and K. P. Burnham. 1999. Program mark: Survival estimation from populations of marked animals. Bird Study 46:S120–S139.

Wilkins, L. G. E., Matthews, K. R., Steel, Z. L., Nusslé, S. C., and S. M. Carlson. 2019. Population dynamics of Rana sierrae at Dusy Basin: influence of non-native predators, drought, and restoration potential. Ecosphere 10(11):e02951. 10.1002/ecs2.2951.

Woodward, G., Perkins, D. M., and L. E. Brown. 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. Philosophical Transactions of the Royal Society B: Biological Sciences 365:2093–2106.

APPENDIX

Appendix A: Water quality guidelines used for amphibians in captivity including standard levels and treatment options. (Odum and Zippel, 2008). The temperature range is based on appropriate levels specifically for Rana species (Litch, 1971).

Parameter	Method	Appropriate Level	Treatment Method
Ammonia	Test Strips	<0.2 mg litre^{-1}, N as unionized ammonia	Chemical filtration, water changes
Dissolved Oxygen	DO meter	> 80% Saturation; some species can handle low levels	Aeration, water change
Chlorine	Test Strips	0	Aeration and/or add chemical dechlorinator (e.g. Dechlorinator Plus)
Hardness	Test Strips	<75 mg litre^{-1} (ppm) of $CaCO_3$ for soft water >100 mg litre^{-1} for hard water	Diluting with distilled water
pH	Litmus test strips	6-8	Add pH buffer
Temp	HOBO loggers	< 21^{o} C	Add shading material to tank
Phosphates	Test Strips	10 mg litre^{-1} (EPA limits – but species specific)	Add phosphate sponge
Nitrites	Test Strips	<1.0 mg litre^{-1}	Add plants, water changes

Appendix B. Proportion of tadpoles that completed metamorphosis in the two longest hydroperiod treatments (133-days and 147-days) in the captive experiment.

Tank ID	Bullfrog Treatment	Hydroperiod Treatment (days)	Number of metamorphs/start tadpole count (proportion of metamorphs)
C1	Control	133	0/104 (0.00)
C2	Control	133	29/112 (0.26)
S1	Signal	133	14/103 (0.14)
S2	Signal	133	11/99 (0.11)
D1	Direct	133	28/88 (0.032)
D2	Direct	133	53/85 (0.62)
C3	Control	147	45/100 (0.45)
C4	Control	147	75/123 (0.61)
S3	Signal	147	0/141 (0.00)
S4	Signal	147	53/85 (0.62)
D3	Direct	147	49/86 (0.57)
D4	Direct	147	41/83 (0.49)