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EFFECTS OF WATER TEMPERATURE ON BREEDING PHENOLOGY, GROWTH, AND METAMORPHOSIS OF FOOTHILL YELLOW-LEGGED FROGS (*RANA BOYLII*): A CASE STUDY OF THE REGULATED MAINSTEM AND UNREGULATED TRIBUTARIES OF CALIFORNIA'S TRINITY RIVER

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ABSTRACT

Many riverine organisms are well adapted to seasonally dynamic environments, but extreme changes in flow and thermal regimes can threaten sustainability of their populations in regulated rivers. Altered thermal regimes may limit recruitment to populations by shifting the timing of breeding activities and affecting the growth and development of early life stages. Stream-dwelling anurans such as the foothill yellow-legged frog (*Rana boylii*) in the Trinity River of northern California are model subjects for examining associations between water temperature and the timing of oviposition, hatching, and metamorphosis, and body condition and size of tadpoles and metamorphs. Breeding activity, hatching success, and metamorphosis occurred later, and metamorphs were smaller and leaner along the regulated and colder mainstem relative to six unregulated tributaries of the Trinity River. Persistently depressed summer water temperatures appear to play a seminal role in inhibited tadpole growth on the regulated mainstem and may be a causative factor in the pronounced decline of this population. Environmental flow assessments should account for the influence of the thermal regime on the development of vulnerable embryonic and larval life stages to improve outcomes for declining amphibian populations. Published 2014. This article is a U.S. Government work and is in the public domain in the USA.

KEY WORDS: Anuran development; body condition; water thermal regime; stream anuran

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INTRODUCTION

Human demands for water can stress riverine organisms in regulated systems when their life history requirements are not fully accommodated in management decisions. Conflicts over water appropriations for agricultural or hydroelectric use and the protection of wildlife have become increasingly common. In regulated stream environments, water temperatures are commonly affected by flow management, and the modification of thermal regimes can have ecological implications for freshwater ecosystems; the effects of altered thermal regimes on stream biota are often not recognized and appreciated in the development and application of environmental flow assessments (Olden and Naiman, 2010). Cold water released from the bottom of reservoirs (hypolimnetic releases) may have a cooling effect on the receiving streams, reducing the seasonal thermal variability in streams and delaying or depressing peak spring and summer water temperatures (Olden and Naiman, 2010). Lower water temperatures resulting from cold-water dam releases have been responsible for delayed and shortened spawning periods, reduced growth rates, population declines, and extirpations of native fish species (Olden and Naiman, 2010). Hypolimnetic releases reduced reproductive success, decreased population densities, increased susceptibility to parasites, and reduced body condition of freshwater mussels (Layzer *et al*., 1993; Heinricher and Layzer, 1999; Galbraith and Vaughn, 2009, 2011).

In this study, we examined the influence of water temperature on the breeding phenology and embryonic and larval development of the foothill yellow-legged frog (*Rana boylii* Baird 1854), the only obligate stream-breeding ranid frog in western North America. This species has evolved a life history strategy adapted to dynamic lotic environments; however, frogs may not be able to adapt when the natural sequence or magnitude of physical regimes is altered (e.g. unseasonal high flows, delay in the recession of peak flows, rapid increases, or reductions in water release or changes in natural thermal regimes). *R. boylii* has experienced significant population declines across its range in California and Oregon, and studies have linked altered flow regimes in regulated rivers as a major threat to species' persistence (Lind *et al*., 1996; Lind, 2005; Kupferberg *et al*., 2012).

The geographic range of this species is extensive (Stebbins, 2003), so the timing of optimal stream

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temperatures for breeding varies greatly across its range. *R. boylii* inhabit streams that range from primarily rain- fed (coastal populations) to primarily snow- influenced (e.g. most Sierra Nevada and Klamath-Siskiyou populations). Even within the same watershed, breeding may span a several-week period among tributaries (Hayes *et al*., in press). Earlier breeding may allow for earlier hatching and subsequently more time spent foraging during the tadpole stages prior to metamorphosis. If there is a critical minimum temperature that prompts female frogs to oviposit, then breeding could be delayed in cooler streams, and slower development is predicted at colder water temperatures.

Hypolimnetic releases are documented to have lowered late-spring and summer water temperatures of the mainstem Trinity River (10–20 °C lower; US Fish and Wildlife Service, Hoopa Valley Tribe, 1999). Also, as a consequence of post-dam reduction in the frequency of large floods, a deeper and steeper trapezoidal-shaped channel has formed, resulting in fewer available edgewater habitats where the water can warm quickly in the sun. Without lateral warming in the shallows, tadpoles may be unable to find adequate warm-water refuges during development (Kupferberg *et al*., 2011). Persistently low water temperatures at or below the species' thermal preference may not be mediated through behavioural responses. The main objective of this study was to evaluate the influence of the water temperature regime on the reproductive phenology, including rate of development, growth, and time to and size at metamorphosis, among *R. boylii* populations on the regulated mainstem and unregulated tributaries of the Trinity River.

Water temperature is a major influence in the development and growth of amphibians. However, thermal regime impacts are often not sufficiently incorporated into environmental flow assessments. Research regarding the effects of the management of regulated systems on *R. boylii* is no exception. Studies have focused on the effects of increased stream flows on this species' reproduction and breeding habitat loss (e.g. Lind *et al*., 1996; Lind, 2005; Kupferberg *et al*., 2012; Yarnell *et al*., 2012); few have examined the effects of altered thermal regimes that result from the regulation of flows. Recent research by Kupferberg *et al.* 2011, Catenazzi and Kupferberg (2013), and Furey *et al.* 2014 found that water temperature influenced the distribution of *R. boylii* populations and that water temperature combined with algal food quantity and quality and predation interacted to affect tadpole survival.

METHODS

Study sites

Our study was conducted within the Trinity River watershed (7389 km^2) in Humboldt and Trinity Co., in northern California, USA (Figure 1). We collected data along the mainstem Trinity River and six tributaries: snow-influenced Stuart's Fork and Upper Trinity; snow-influenced and rain-influenced Canyon Creek, North Fork Trinity, and Weaver Creek; and primarily rain-influenced South Fork Trinity (Figure 1). Two dams, Trinity and Lewiston, impound the Trinity River (Figure 1). They were constructed in the early 1960s as part of a federal water management project diverting water to California's Central Valley for irrigation and municipal uses.

Breeding phenology and tadpole development surveys

From 2006 to 2009, we conducted weekly visual encounter surveys (Crump and Scott, 1994) for egg masses at gravel

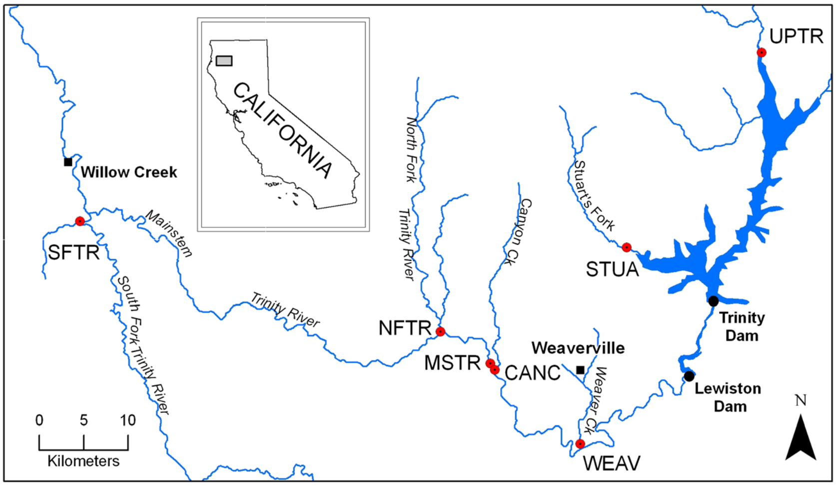

Figure 1. Sites in the Trinity River watershed sampled from 2006 to 2009 for *Rana boylii* breeding phenology and larval development. CANC = Canyon Creek, MSTR = mainstem, NFTR = North Fork, SFTR = South Fork, STUA = Stuart's Fork, UPTR = Upper Fork, WEAV = Weaver Creek. This figure is available in colour online at wileyonlinelibrary.com/journal/rra

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bar habitats where breeding activity had been previously documented. In 2006, we collected data on the mainstem at Valdor Gulch, the North Fork at the gravel bar below California Highway SR-299 bridge, and the South Fork at the pool below the confluence of Madden Creek. In 2007, we re-sampled these sites and collected data at additional sites on the mainstem at Hocker Flat and at Pear Tree Gulch and at another tributary Canyon Creek below the SR-299 bridge. In 2008 and 2009, we added McCartney's Pond (Conner Creek) on the mainstem and the following sites with unregulated streamflow: Weaver Creek near the confluence with the mainstem, Stuart's Fork, and the Upper Trinity above the East Man Road Bridge (the latter two feed into the Trinity Reservoir; Figure 1).

We collected Universal Transverse Mercator locations using a global positioning system unit at each breeding site and conducted a visual encounter survey searching for egg masses along the shoreline and out 2–4 m towards the thalweg. Surveyors zigzagged back and forth in edgewater up to 1 m deep. We identified, marked, and recorded the embryo development stage (Gosner, 1960) of individual egg masses.

After eggs hatched, we scheduled a brief pause before surveying for tadpoles to allow for some growth prior to handling animals to obtain morphometric measurements. We continued to survey breeding sites weekly until tadpoles completed metamorphosis. During each visit, we collected ≥30 tadpoles or metamorphs with dip nets or by hand. Because of low numbers at mainstem sites, we were not able to obtain a sample size of 30 individuals during every survey. We determined the Gosner stage (GS) of each tadpole/metamorph and measured snout–vent length (SVL). In 2009, we also weighed animals (g) with a digital scale. After processing, all tadpoles were released where collected.

Water temperature

In 2008 and 2009, we placed HOBO® (Onset Computer Corporation, Bourne, MA) data loggers set to record temperatures every 2 h at breeding sites on the mainstem and six tributaries, submerged in edgewater to best represent the water temperatures that were experienced by egg masses and tadpoles. In 2008, edgewater temperatures collected with data loggers on the mainstem and the South Fork were found to be unreliable because the locations of equipment frequently dried up, leaving the data loggers stranded. We acquired water temperature data for the mainstem and South Fork from a database maintained by the Trinity River Restoration Program (http://odp.trrp.net/). Water temperatures obtained from the database were in-stream water temperatures, so values may not accurately represent actual temperatures experienced by egg masses and tadpoles. However, to validate the use of these data, we examined the association between in-stream water temperatures obtained for the North Fork and Canyon Creek from the database and edgewater temperatures collected by data loggers from 2008. The measures of water temperature were highly correlated ($r > 0.99$), and daily averages consistently differed by less than 1 °C.

Data analysis

Because we performed weekly surveys, we converted all dates to corresponding Julian weeks. Egg masses were typically observed in early embryonic stages (≤GS-11), and oviposition date was estimated as the week of the date of detection. For egg masses detected in later embryonic stages (≥GS-12), we back estimated the oviposition dates on the basis of GSs; oviposition dates of egg masses detected at GS-12 through GS-17 were estimated as the week prior to the date of detection, egg masses detected at GS-18 through GS-23 were estimated to be 2 weeks old, and those detected at GS-24 through GS-26 were estimated to be 3 weeks old. Estimated dates may be somewhat in error because we could not adjust for site-specific variation in water temperature or other factors that can influence development rates. In 2008, post-hatching was defined as GS-26 because we only measured tadpoles once they reached this stage. In 2009, we measured GS-25 tadpoles and refer to this stage as post-hatching. For each stream, we used daily mean water temperatures to calculate monthly mean water temperatures. We also averaged daily mean water temperatures for the 7 days of each Julian week (7-day average water temperature) and computed a mean 7-day average water temperature to represent the 2008 and 2009 breeding seasons; we averaged the 7-day average water temperatures between 27 May and 11 August (Julian weeks 22 and 32) in 2008 and 9 April and 26 August (Julian weeks 15 and 34) in 2009 for each stream. Water temperature data used for calculation of mean 7-day average water temperatures in 2008 were limited by the dates in which data were available at all sites and did not extend through the entire breeding and tadpole development period as in 2009. Data collection in 2009 allowed us to relate tadpole stage-specific growth and differentiation rates, body condition, and water temperature. We used regression coefficients of the relationship between median GS and time (Julian week) as estimates for rates of differentiation (Smith-Gill and Berven, 1979). We calculated body condition indices (BCI), a fitness proxy (Rohr and Palmer, 2013), using the residuals from a regression of log-transformed mass (1.0 was added to mass values prior to applying the transformation to avoid negative log-values) versus log-transformed length (Bancila *et al.*, 2010) with the data from all sites, to compare differences in the relative condition of tadpoles at post-hatching [here, we defined post-hatching as GS-26 because the weights of individuals at

GS-25 were often too low for precise measurement (recorded as '<0.1 g')] and metamorphic climax (GS-42) among the mainstem and tributaries. We plotted mean SVL and BCI by GS and visually determined the linear section of these growth curves (GS-25–GS-36 for SVL and GS-32–GS-38 for BCI). We used regression coefficients of these relationships as estimates of stage-specific tadpole growth (Smith-Gill and Berven, 1979). One caveat we acknowledge in comparing differences in tadpole size, body condition, and growth trajectories in natural populations is that differential tadpole survival and resulting densities may influence the results. For example, smaller tadpoles are more vulnerable to predators, and removal of these individuals from the population may skew the size or condition of individuals in the population towards larger means (Kupferberg *et al.*, 2011).

We averaged the estimated oviposition dates of egg masses, detection dates of post-hatching stage tadpoles, and detection dates of individuals observed at metamorphic climax to obtain mean oviposition, post-hatching, and metamorphosis dates for each site. We used simple linear regression to examine the relationships between mean oviposition, post-hatching, and metamorphosis dates and mean 7-day average water temperatures and to examine the influence of water temperature on time to metamorphosis (number of weeks between mean post-hatching and metamorphosis dates). We used simple linear regression to determine the explanatory power of mean 7-day average water temperatures on differentiation rates, stage-specific growth, SVL, and BCI of tadpoles and metamorphs. Alpha was set at $p < 0.10$ as is appropriate for exploratory ecological studies (Shrader-Frechette and McCoy, 1993).

RESULTS

Water temperature

Overall, water temperatures were colder during 2008 compared with those during 2009. In both years, South Fork and Weaver Creek were the warmest tributaries. Canyon Creek, North Fork, and Upper Trinity had lower but comparable water temperatures (Table I and Figure 2). Stuart's Fork, which is fed entirely by snowmelt from the Trinity Alps, was generally the coldest stream in the spring (Apr–May); however, the mainstem had the coldest water temperatures during the summer months (Jun–Aug; Table I).

Breeding phenology

Across all streams and all years, oviposition occurred as early as Julian week 15 (early Apr) and as late as Julian week 25 (mid-Jun; Figure 3). Tadpoles reached post-hatching from Julian weeks 19 (mid-May) through 35 (late Aug) and metamorphosis between weeks 28 (mid-Jul) and 40 (early Oct; Figure 3). The average difference between phenological events on the South Fork and mainstem was 7 weeks (range 5–9 weeks, $n = 4$) for oviposition, 6 weeks (range 0–9, $n = 4$) for post-hatching, and 6 weeks (range 4–9, $n = 3$) for metamorphosis.

Water temperatures on mean oviposition dates averaged 10.8 °C (range 10.0–12.0) in 2008 and 12.3 °C (range 10.9–13.4) in 2009. During both years, oviposition, post-hatching, and metamorphosis occurred later on streams with colder mean 7-day average water temperatures (Table II; APPENDIX I). The average time to metamorphosis (from

Table I. Monthly mean edgewater temperatures (°C) and mean 7-day average water temperatures for *Rana boylii* breeding sites sampled on the mainstem and tributaries of the Trinity River, Trinity Co., California

Year/month	Canyon Creek	Mainstem	North Fork	South Fork	Stuart's Fork	Upper Fork	Weaver Creek
2008							
Apr (15–17)	7.5[a]	10.2[a]	7.5[a]	10.3[a]	n.d.	n.d.	n.d.
May (18–22)	9.2[a]	9.2[a]	9.9[a]	14.2[a]	n.d.	n.d.	n.d.
Jun (23–26)	12.8	10.4[a]	13.6	18.2[a]	10.9	13.2	16.3
Jul (27–30)	18.3	11.9[a]	18.2	21.1[a]	16.5	18.6	20.1
Aug (31–32)	19.5	14.8[a]	19.4	22.0[a]	17.3	19.0	20.2
Mean 7-day average (22–32)	15.7	11.6	16.1	19.7	13.9	15.9	18.2
2009							
Apr (15–17)	8.2	10.7	8.4	11.7	6.5	6.9	n.d.
May (18–22)	10.6	10.8	11.1	14.8	8.9	10.0	14.2
Jun (23–26)	14.8	14.6	15.2	19.0	13.0	15.1	18.0
Jul (27–30)	20.0	17.9	19.9	23.4	17.5	19.4	21.2
Aug (31–32)	20.7	16.9	20.7	23.3	18.1	19.9	21.2
Mean 7-day average (15–34)	15.1	14.2	15.2	18.6	13.0	14.5	17.3

The Julian weeks used to calculate monthly and 7-day average means are shown in parentheses. n.d., no data.
[a] Water temperatures that represent in-stream (rather than edgewater) temperatures in 2008.

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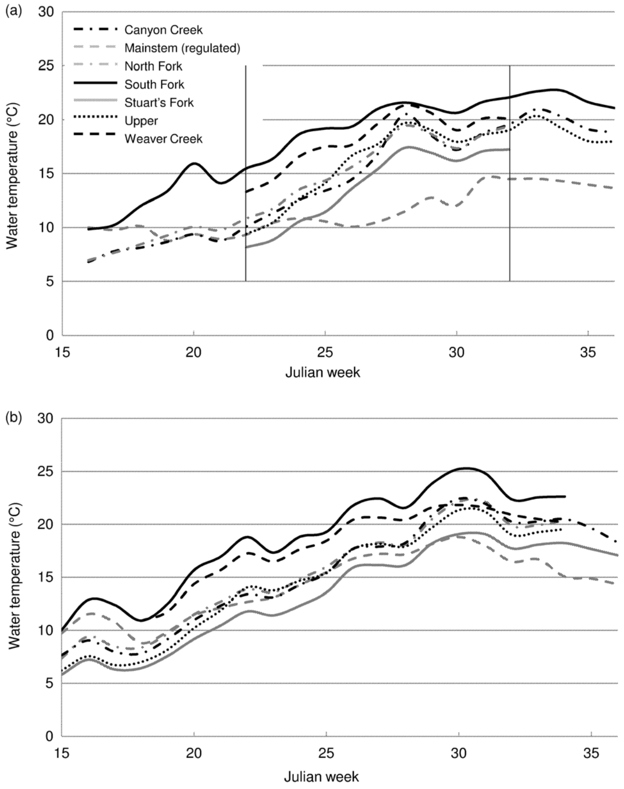

Figure 2. Continuous water temperature on the mainstem and tributaries of the Trinity River, Trinity Co., California in (a) 2008 (vertical lines represent the range of data used to calculate mean 7-day average temperatures) and (b) 2009. All 2008 values for the mainstem and South Fork sites represent in-stream water temperatures; 2008 values prior to Julian week 22 for Canyon Creek and North Fork also represent in-stream temperatures. All other temperatures were measured along edgewater habitats

post-hatching to metamorphosis) was 9 weeks (range 7–11) in 2008 and 8.5 weeks (range 7–10) in 2009. The mean 7-day average water temperature did not explain the variation in time to metamorphosis in either year (Table II).

Differentiation rate

In 2009, differentiation rate was highest for the Stuart's Fork population and lowest for the North Fork population, but the relationship between differentiation rate and mean 7-day average water temperature was not significant ($\beta = -0.07$, $F_{(1,5)} = 1.96$, $p = 0.22$, $R^2 = 0.28$).

Size and body condition index

In 2008, the mean 7-day average water temperature did not explain a significant proportion of variance in tadpole length at GS-26 ($\beta = 0.35$, $F_{(1,5)} = 3.44$, $p = 0.12$, $R^2 = 0.41$), but metamorphs were smaller on tributaries with colder mean 7-day water temperatures ($\beta = 0.50$, $F_{(1,5)} = 5.36$, $p = 0.07$, $R^2 = 0.52$). In 2009, tadpoles were smaller in length at GS-25 on tributaries with colder mean 7-day average water temperatures ($\beta = 0.34$, $F_{(1,5)} = 11.81$, $p = 0.02$, $R^2 = 0.70$), and metamorphs were smaller on tributaries with colder mean 7-day average water temperatures ($\beta = 0.58$, $F_{(1,5)} = 10.87$, $p = 0.02$, $R^2 = 0.69$; Figure 4a). The body condition of GS-26 tadpoles was higher for tributaries with colder mean 7-day average water temperatures ($\beta = -0.01$, $F_{(1,5)} = 5.80$, $p = 0.06$, $R^2 = 0.54$), but water temperature did not explain the variance in the BCIs of metamorphs ($\beta = 0.009$, $F_{(1,5)} = 1.88$, $p = 0.23$, $R^2 = 0.27$; Figure 4b). Mainstem animals were the smallest in size and had the lowest body condition at metamorphosis.

Stage-specific growth

Mean stage-specific growth in SVL (increase in millimetre per GS) was higher for populations on streams with colder mean 7-day average water temperatures ($\beta = -0.06$, $F_{(1,5)} = 7.57$, $p = 0.04$, $R^2 = 0.60$; Figure 5a); at colder sites, tadpoles were longer in length at any given stage (GS-25–GS-36). However, tadpoles at colder sites were shorter at metamorphic climax (Figure 4a, APPENDIX IIa). Individuals on the mainstem, Stuart's Fork, North Fork, and Canyon Creek appeared to stop growing between GS-38 and GS-40, whereas tadpoles from the South Fork, Weaver Creek, and Upper Trinity populations continued to grow to metamorphic climax (APPENDIX IIa). All populations exhibited a peak in BCI prior to metamorphic climax when tadpoles presumably lose mass during the process of metamorphosis (APPENDIX IIb). Peak body condition occurred at earlier GSs on streams with colder mean 7-day average water temperatures ($\beta = 0.49$, $F_{(1,5)} = 9.84$, $p = 0.03$, $R^2 = 0.66$). Mean 7-day average water temperature did not explain a significant proportion of variation in stage-specific BCI growth ($\beta = -0.001$, $F_{(1,5)} = 0.79$, $p = 0.41$, $R^2 = 0.14$; Figure 5b).

DISCUSSION

Our results indicate that water temperatures of streams affected the timing of oviposition and the development of *R. boylii* embryos and tadpoles. As expected, oviposition and metamorphosis generally occurred later, and frogs were smaller at metamorphosis on colder streams. The influence of the thermal regime on size and body condition could have been a function of a direct effect of temperature on these traits (e.g. cold temperature inhibition of growth and development) or an indirect effect of temperature on tadpole feeding rate or quality and/or quantity of food resources. We did not have the data necessary to reveal the relative effects of the mechanisms underlying the observed patterns. Regardless, results suggest that cold-water releases from impoundments can negatively affect the fitness of individuals of this species, with the cooling effect appearing to

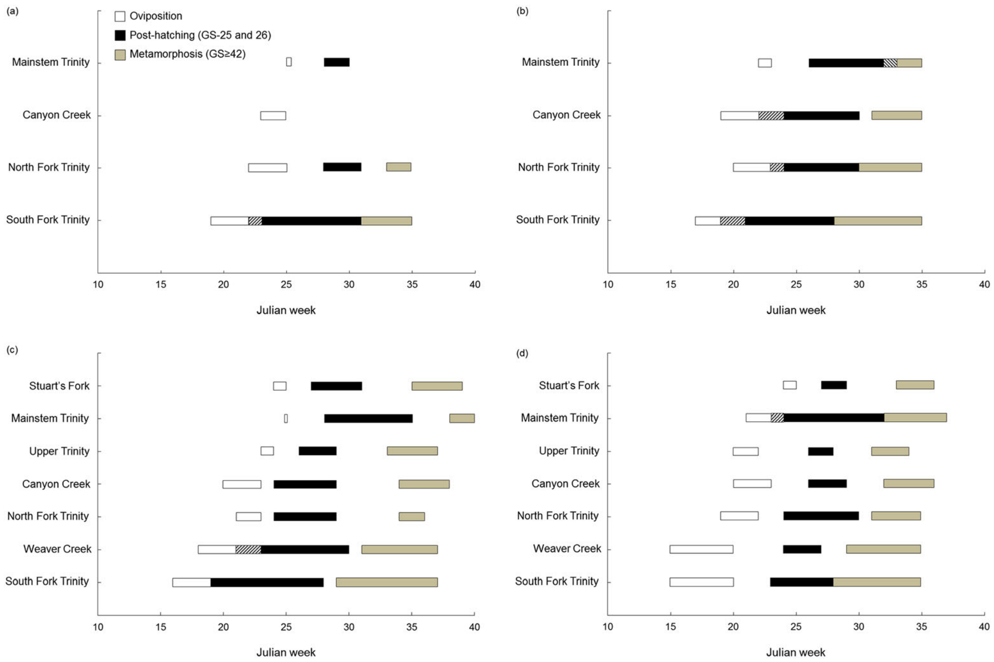

Figure 3. Variation in dates of oviposition, post-hatching, and metamorphosis for *Rana boylii* on the mainstem and tributaries of the Trinity River, Trinity Co., California in (a) 2006, (b) 2007, (c) 2008, and (d) 2009. Diagonally shaded areas represent an overlap in oviposition and post-hatching dates. In 2006, we did not sample post-hatching or metamorphic life stages at Canyon Creek, and mainstem metamorphs were not detected because of mortality of tadpoles that resulted from stranding. This figure is available in colour online at wileyonlinelibrary.com/journal/rra

lower water temperatures below the species' preferred lower thermal limit for optimum growth and development.

Breeding activity occurred later, and individuals were smaller at metamorphosis on the upper section of the regulated mainstem of the Trinity River, where water temperatures were unseasonably low. Previous studies have documented low reproductive output and a small population size along this reach (Lind *et al*., 1996; Wheeler *et al*., 2013). Colder thermal conditions also appeared to have negative effects on individuals from this population. In the spring and summer, hypolimnetic release of water from the reservoir results in low water temperatures in the mainstem and post-dam modifications to the river channel have led to fewer available warm edgewater habitats (gravel and cobble bars) that are important fish spawning, *R. boylii* breeding, and tadpole rearing sites (http://www.trrp.net/background/impacts/). Temperature regimes can have a profound influence on the fitness of ectothermic organisms like *R. boylii* (Angilletta *et al*., 2002; Rohr and Palmer, 2013), and the population persistence of *R. boylii* appears to be particularly affected by the survival of early life stages (Kupferberg *et al*., 2009).

Table II. Linear regressions results for relationships between mean 7-day average water temperature and phenology of breeding and development of *Rana boylii* populations on the mainstem and tributaries of the Trinity River, Trinity Co., California

Year/dependent variable	β	F (df)	p	R^2
2008				
Oviposition date	−0.81	47.36 (1, 5)	0.001	0.90
Post-hatching date	−0.66	18.26 (1, 5)	<0.01	0.79
Metamorphosis date	−0.64	41.86 (1, 5)	<0.005	0.89
Time to metamorphosis	0.03	0.01 (1, 5)	0.91	0.003
2009				
Oviposition date	−1.18	30.51 (1, 5)	<0.005	0.86
Post-hatching date	−0.88	17.39 (1, 5)	<0.01	0.78
Metamorphosis date	−0.61	15.30 (1, 5)	0.01	0.75
Time to metamorphosis	0.27	1.87 (1, 5)	0.23	0.27

Water temperature influences *R. boylii* at multiple spatial and temporal scales and various life stages; it affects breeding population occurrence (Welsh and Hodgson, 2011; Kupferberg *et al*., 2011), and tadpoles exhibit thermal preferences (Kupferberg *et al*., 2011). Breeding populations were more abundant along warmer streams where the realized thermal niche coincided with temperatures preferred by tadpoles, which optimized their growth and development (Kupferberg *et al*., 2011). These authors found that the lower thermal limit (summer maximum 30-day average water temperature) for *R. boylii* population occurrence was 18.8 °C for coastal sites, which is warmer than the water temperatures recorded for the mainstem during the summer

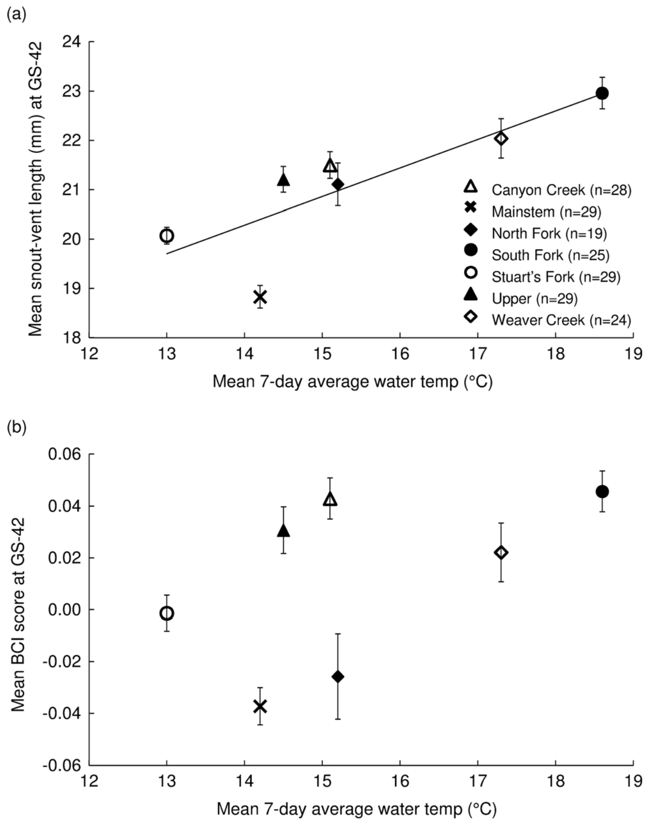

Figure 4. Relationships between *Rana boylii* (a) mean snout-vent length of Gosner stage (GS)-42 metamorphs and mean 7-day average water temperature and (b) mean body condition index (BCI) of GS-42 metamorphs and mean 7-day average water temperature in 2009 for the mainstem and tributaries of the Trinity River, Trinity Co., California

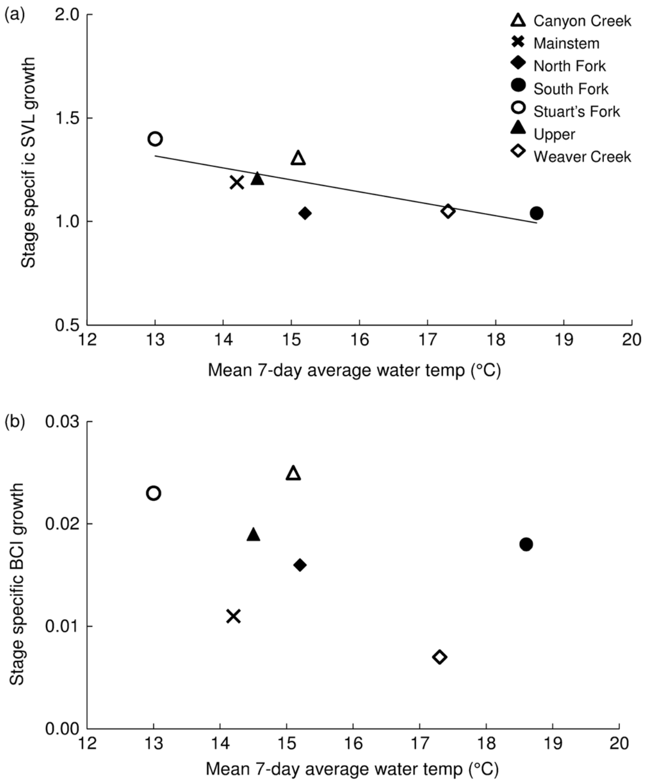

Figure 5. Relationships between *Rana boylii* (a) stage-specific growth in snout–vent length (SVL) and mean 7-day average water temperature and (b) stage-specific growth in body condition index (BCI) and mean 7-day average water temperature in 2009 for the mainstem and tributaries of the Trinity River, Trinity Co., California. Stage-specific growth estimates (regression coefficients of SVL on stage and BCI score on stage) were based on the linear sections of growth trajectories (APPENDIX II)

months. The depressed summer water temperatures may be lower than is required to support a viable population long term and may in part explain the overall small and possibly declining population on the mainstem.

Rana boylii oviposition appears to occur when water temperatures are at least 10 °C (Hayes *et al*., in press). The mainstem reached suitable temperatures for oviposition as early as tributaries, but breeding activity was not observed until much later. The delay in breeding activity on the mainstem was likely attributed to high water levels with high velocity, two factors that have been demonstrated to influence timing of breeding activity in this frog (Kupferberg, 1996a; Wheeler and Welsh, 2008). We observed earlier oviposition, post-hatching, and metamorphosis on warmer streams. These events generally occurred later on the mainstem relative to the tributaries except for Stuart's Fork, a cold-water tributary where the headwaters flow from the snowfields of the Trinity Alps Wilderness.

The mainstem population experienced a lower-than-expected differentiation rate relative to tributary populations. Kupferberg *et al*. (2011) found that *R. boylii* tadpoles preferred habitats with water temperatures that optimized growth and development (summer maximum 30-day average of near or above 20 °C). Monthly average water temperatures for the mainstem Trinity and Stuart's Fork were lower than this optimal temperature during the summer months of July and August, and the water along the mainstem did not warm up to temperatures comparable with other tributaries. Kupferberg *et al*. (2011) found that tadpoles will actively thermoregulate if given the opportunity. In contrast to the mainstem, tadpoles from the snowmelt tributary Stuart's Fork exhibited rapid differentiation and high stage-specific growth; this may be explained by a source effect. Kupferberg *et al*. (2011) also found that Sierran population tadpoles grew faster than tadpoles from coastal populations, and they interpreted these differences as an adaptation of tadpoles from snowmelt-fed rivers to grow more rapidly as a response to a shorter growing season. Kupferberg *et al*. (2011) determined that peak body mass of *R. boylii* tadpoles occurred at earlier stages under colder conditions. We observed a similar pattern in our

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growth trajectories (APPENDIX II), supporting these authors' suggestion that there is a trade-off between development time and growth as a response to an increasing risk of mortality as the season progresses.

Temperature affects size at metamorphosis, but study results including the direction of effects vary, and studies differed in their measure of size, making comparisons difficult (e.g. Smith-Gill and Berven, 1979; Loman, 2002). In this study, metamorphs were larger in length at sites with warmer mean water temperatures. Metamorphs on the mainstem were small in length and had the lowest body condition. Water temperature explained a significant proportion of variance in length at metamorphosis but only explained 27% of the variance in body condition at metamorphosis. Therefore, there were variables we did not examine that were accountable for additional variation in body condition. Reduced body condition of mainstem metamorphs may be influenced by cold-water effects on the quantity and composition of riverine periphyton, the preferred food of *R. boylii* tadpoles (Kupferberg, 1996b, 1997). Food availability affects tadpole growth and time to metamorphosis in other anurans (Wilbur, 1977a, 1977b; Alford and Harris, 1988; Berven and Chadra, 1988; Crespi and Warne, 2013), and tadpoles from cold environments may require more nutrients (Liess *et al*., 2013). Kupferberg (1996b, 1997) found that food quality influenced *R. boylii* mass at metamorphosis and time to metamorphosis and Kupferberg *et al*. (2011) and Furey *et al.* (2014) documented that reaches below dams, with daily fluctuations in flow, contained periphyton assemblages dominated by mucilaginous stalked diatoms, which do not promote *R. boylii* tadpole growth. Also, the feeding rate of tadpoles can decline with temperature (Pandian and Marian, 1985; Warkentin, 1992; Kupferberg *et al*., 2011).

The two coldest streams, Stuart's Fork and the mainstem, presented an informative contrast. Breeding occurred latest on Stuart's Fork, naturally the coldest tributary; but individuals did not exhibit slower development or reduced growth. Stuart's Fork tadpoles were rotund at hatching, developed rapidly, and had high stage-specific growth, and metamorphs were of comparable condition with those on other tributaries. The Stuart's Fork population appeared to have evolved a compensatory response (Mangel and Munch, 2005) that enabled individuals to 'catch up', hatching out and metamorphosing in a similar state as those from warmer tributaries. Compensatory mechanisms are strategies in which development rates are optimized in response to variable environmental conditions (Mangel and Munch, 2005). For example, higher-altitude populations with seasonal time constraints may have the capacity to grow and differentiate rapidly to compensate for a short growing season (Berven *et al*., 1979). Compensatory growth may occur following periods of unfavourable growth conditions (Orizaola *et al*., 2009); the Stuart's Fork and mainstem populations experienced delayed oviposition and cooler water temperatures during development. Water temperatures on Stuart's Fork exceeded those of the mainstem during the period of tadpole development. Failure of the surface edgewater to warm up on the mainstem as the season progresses (as would naturally occur and does so on Stuart's Fork) appears to be a primary factor leading to small size and poor body condition in that population. High stage-specific growth and comparable size and body condition of the Stuart's Fork population may be additionally influenced by the synchrony between the phenology of algal blooms and tadpole development (feeding stage); algal resources may be more abundant when tadpoles begin feeding at colder sites (Catenazzi and Kupferberg, 2013). Catenazzi and Kupferberg (2013) found that food supplementation at lower temperatures sped up the rate of tadpole growth and development. The persistently colder water temperatures of the mainstem may inhibit stream productivity or impose a physiological restriction on the ability of tadpoles to attain the feeding rates necessary for compensatory growth. Better condition at hatching on Stuart's Fork may also be influenced by a maternal effect such as egg yolk volume, which could be an adaptive strategy to mitigate future consequences of late breeding and colder water temperature.

Large size and better condition may increase the probability of overwintering survival and could have carry-over effects such as increased post-metamorphic growth rates, larger size at maturity, and higher reproductive success (Smith, 1987; Berven, 1990; Goater, 1994; Ryser, 1996; Altwegg and Reyer, 2003; Crespi and Warne, 2013). Less fit metamorphs may have delayed reproductive maturity and postponed recruitment into the breeding population (Smith, 1987). Additional experimental study, controlling for confounding variables such as the effect of water temperature on algal quantity and quality, would help to further understand thermal effects on embryonic development, tadpole growth, size, and body condition at metamorphosis of *R. boylii* in the Trinity River watershed. Revealing the relative effects of the suite of potential mechanisms underlying the negative thermal effects on the regulated mainstem population is of certain interest.

CONCLUSIONS

The installation of dams such as those within the Trinity River watershed (Figure 1) has reduced the amount of available breeding habitat for both *R. boylii* (Hayes *et al*., in press) and anadromous fish (http://www.trrp.net/background/impacts/). *R. boylii* breeding habitat has been inundated by the filling of the reservoirs, and anadromous fish can no longer access colder tributaries above the dams for

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spawning. Consequently, the thermal regime of the mainstem is managed to benefit cold-water-adapted salmonids; water is intentionally released from the dam to reduce the water temperatures of the river during the summer months (http://www.trrp.net/background/impacts/). The water in the mainstem is maintained at an unseasonably colder temperature than naturally would occur during the development period of *R. boylii* tadpoles, and the low summer temperatures may be nearing the species' lower thermal limit. Although management of cold-water releases from the dam can benefit fish species during parts of their life history such as spawning and egg development, the effects of cold water on juvenile salmonids that would historically migrate from colder tributaries to the pre-dam warmer mainstem is unknown. Managing to optimize the thermal requirements of salmonids and *R. boylii* may be challenging, especially during a period of extremely high water demand and conflict over appropriations for agriculture and fisheries. Promoting the persistence of *R. boylii* populations along this section of the mainstem may require changes in current management and a multi-species management approach that includes all native species, regardless of cultural and economic significance. Channel restoration efforts along the mainstem have created more edgewater habitat, but because in-stream temperatures are unseasonably cool, adequate lateral warming of edgewater does not occur. Therefore, the addition of breeding habitat alone may not be sufficient to restore and support frog populations. Altered thermal regimes in regulated systems may affect individual fitness, have population-level outcomes, and even have long-term evolutionary consequences (Angilletta *et al*., 2002; Olden and Naiman, 2010), but the impact of flow regimes on downstream water temperatures is often overlooked (Olden and Naiman, 2010). One critical step towards mitigating the effects of cold-water pollution on the biota in this system is obtaining knowledge of the local thermal preferences of salmonids, anurans, and other species on the mainstem Trinity River and integrating this information into environmental flow assessments and management decisions.

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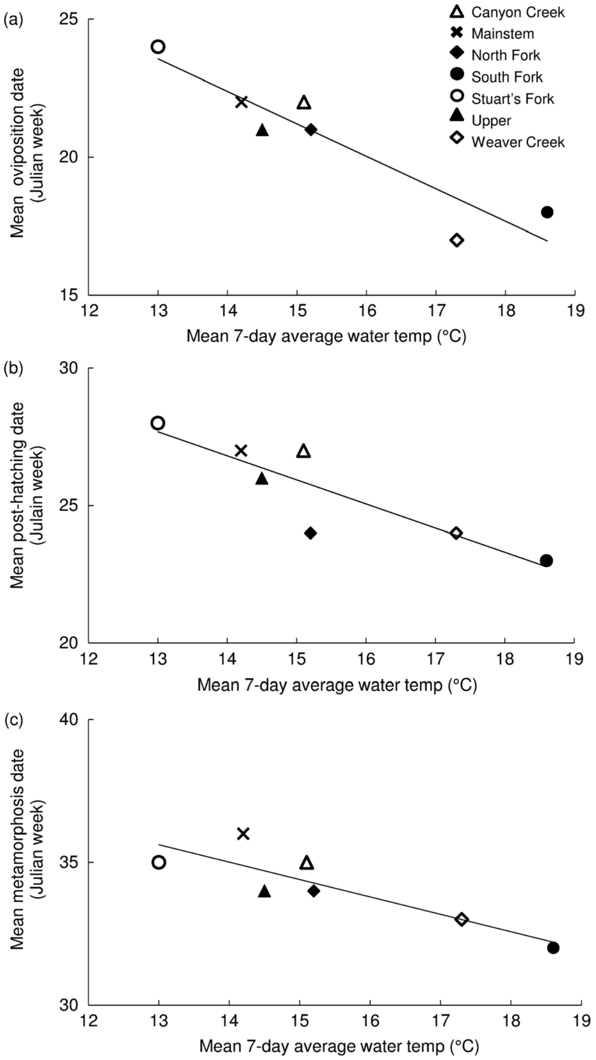
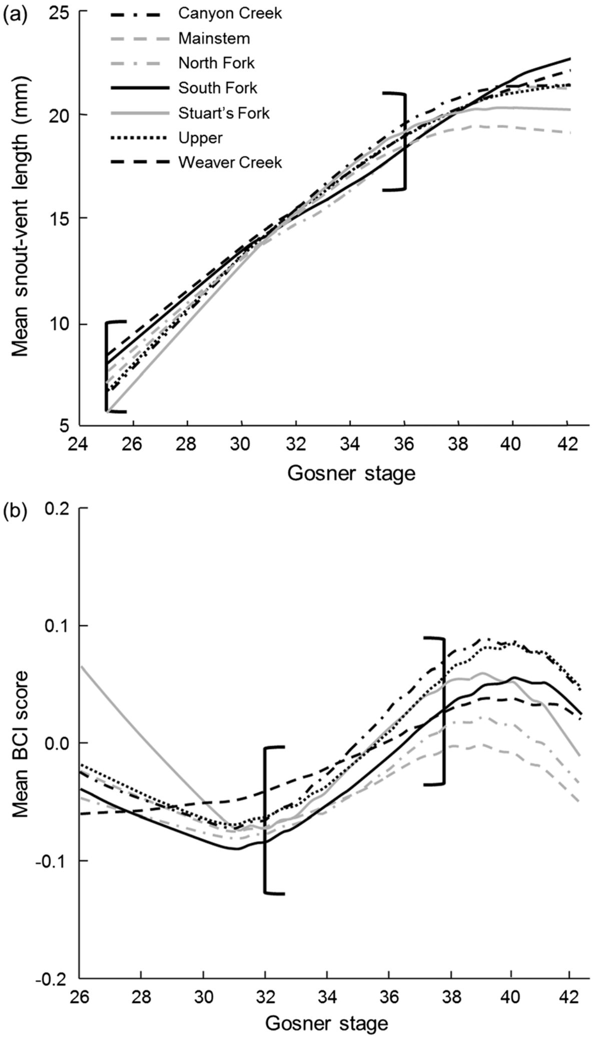
APPENDIX I



Relationships between *Rana boylii* (a) mean oviposition date, (b) mean post-hatching date, and (c) mean metamorphosis date and mean 7-day average water temperature in 2009 for the mainstem and tributaries of the Trinity River, Trinity Co., California

APPENDIX II



Mean *Rana boylii* (a) snout–vent length and (b) body condition index score at increasing Gosner stages for tadpoles and metamorphs in 2009 for the mainstem and tributaries of the Trinity River, Trinity Co., California. Stage-specific growth estimates were based on the linear section (enclosed in brackets) of growth trajectories

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