# Effects of Water Temperature on Breeding Phenology, Growth and Timing of Metamorphosis of Foothill Yellow-legged Frogs (*Rana boylii*) on the Mainstem and Selected Tributaries of California's Trinity River - 2004-2009

Final Report to the Trinity River Restoration Program

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**Contents**

## Introduction

The foothill yellow-legged frog (*Rana boylii*) is the only obligate stream-breeding ranid frog in western North America, having evolved a life history strategy adapted to the natural disturbance regimes of dynamic lotic environments. However, when the natural sequence or magnitude of stream system events are altered or excessive (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), the frogs are often incapable of adapting to these perturbations. This can have severe negative consequences for their most vulnerable life stages, the egg masses and larvae (tadpoles). Foothill yellow-legged frogs have experienced significant population declines across their range in California and Oregon. Studies have linked the presence of water impoundments to the species' decline, with ill-advised or uninformed management of dams identified as a major threat to the persistence of the species (Lind et al. 1996, Lind 2005, Kupferberg et al. 2012). Modified flow regimes resulting from such dam management (Leigh et al. 2012) can have both direct and indirect effects on these frogs (Kupferberg et al. 2011). Direct effects resulting from poor timing and magnitude of stream discharges include the scouring or stranding of egg masses and reduced survival of tadpoles (Lind et al. 1996; Kupferberg 1996a, 1996b; Kupferberg et al. 2009; Kupferberg et al. 2011). Numerous studies have examined the effects of stream flow on breeding activity but few studies have examined the effect of changes in water temperatures, commonly altered by flow management (Olden and Naiman 2010), on the life stages of *R. boylii*. Temperature is a critical driver of physiological processes in ectotherms and abrupt and unpredictable changes are a potential threat to survival (Angiletta et al. 2002). Numerous studies have suggested that water temperature influences the onset of *R. boylii* breeding activity (e.g., Fuller and Lind 1992, Kupferberg 1996a, Van Wagner 1996, Wheeler et al. 2006), but the effects of different water temperatures on embryonic development, tadpole growth, and metamorphosis have not until recently been examined (this study; Kupferberg et al. 2013).

Anurans generally exhibit plasticity in larval development (i.e., growth and differentiation) to metamorphosis (Wilbur and Collins 1973; Travis 1984). Environmental factors that can influence these processes include water availability (Merila et al. 2002), water temperature (Loman 2002), food availability (Alford and Harris 1988), intraspecific density, and predator pressure (Wilbur and Collins 1973). In many anuran species colder water temperatures slow embryonic and larval differentiation and this often leads to larger size at metamorphosis

(Harkey and Semlitsch 1988). Larger size at metamorphosis can result in future advantages such as higher reproductive success (e.g., Semlitsch 1987; Semlitsch et al. 1988). Most studies examining plasticity of growth and differentiation in anurans have focused on lentic-breeding species (see Freidenburg and Skelly 2004 and cites therein) where colder water temperature is typically associated with earlier breeding. Studies of the effects of environmental variables such as temperature on growth and differentiation of frogs in lotic environments are lacking.

Foothill yellow-legged frogs appear to oviposit when water temperatures are ≥ 10ºC (Hayes et al. *in press*). The geographic range (latitudinal, longitudinal and altitudinal) of this species is extensive (Stebbins 2003) so the timing in which stream temperatures reach optimal temperatures for breeding varies greatly. Foothill yellow-legged frogs can inhabit streams that are primarily rain fed (coastal populations), primarily snow influenced (e.g., most Sierra Nevada and Klamath-Siskiyou populations), and those that experience both types of precipitation. Even within the same watershed, breeding may occur at different times. Earlier breeding may allow for earlier hatching and subsequently more time spent foraging during the larval stages prior to metamorphosis. If there is a critical minimum temperature that prompts females to oviposit their breeding activity may be delayed in cooler tributaries, with development likely occurring more slowly in the colder water. The strategies used by lentic breeders (Freidenburg and Skelly 2004 and cites therein) may not apply to anurans living in highly variable stream systems.

Water temperatures in the Mainstem Trinity River during the late-spring and summer months have been substantially colder (10-20°C lower) since the construction of the dams compared to pre-dam conditions; water released into the Mainstem Trinity River from the bottom of the Trinity Reservoir is much cooler (hypolimnetic) than the surface water temperature (U.S. Fish and Wildlife Service and Hoopa Valley Tribe 2009). We predicted that frogs would be smaller at metamorphosis in the Mainstem and in colder water tributaries (i.e., higher elevation snow-influenced streams) since breeding likely occurs later and the colder water temperatures would impede growth and differentiation. The objectives of this study were to: 1) examine and compare key aspects of the reproductive cycle of yellow-legged frogs in different Trinity River tributaries, 2) examine and compare the phenology of reproduction, including rates of differentiation, time to metamorphosis, and size at metamorphosis, by tributary, and 3) to examine the differences as they relate to variation in water temperature regime, such that the

influences of the hypolimnetic releases from the reservoir on the Mainstem Trinity could be contextualized and potentially mitigated.

## Methods

*Egg Mass Surveys*

We surveyed for egg masses by floating the Mainstem and South Fork of the Trinity River (Fig. 1) in inflatable kayaks, stopping at suitable breeding habitats; these included open cobble or gravel bars ≥ 10-m in length. We avoided areas that could not be accessed safely (e.g., flows that were too high or swift), unsuitable habitat (e.g., gravel bars on steep riparian berms with no gentle slope to the thalweg), and areas on private property. For the North Fork, we conducted wading surveys by walking stream banks searching all suitable egg mass habitat.

In 2004-2009, we conducted egg mass surveys on the Mainstem Trinity River along approximately 32 river km (20 miles), from Douglas City to the confluence of the North Fork Trinity River. In 2004 and 2005, we surveyed a larger section, from below the Lewiston Dam (approximately 6.4 river km [4 miles] downstream from the dam) to the confluence of the North Fork Trinity River (Fig. 1). In subsequent years, we discontinued surveying the ~25.7 river km (16 river mile) reach from below the dam to Douglas City due to lack of breeding activity. On the South Fork, we initially conducted surveys from PG&E Flats to the confluence of Madden Creek (approximately 5.6 km [3.5 miles]). Due to high numbers of egg mass detections, in later years we surveyed a shorter section from PG&E Flats to Big Slide or from Big Slide to the Madden Creek confluence. We conducted surveys on the North Fork from the bridge just below the confluence of the East Fork of the North Fork down to the confluence with the Mainstem (approximately 1 km [0.6 miles]). Start and end points for each survey were marked on aerial photos and GPS. Surveys were conducted between 0900 and 2000 hours from late-April to mid-June. We determined the total number of unique egg masses for each year and calculated number of egg masses per km (total number of egg masses detected/number of km of river surveyed) in order to compare reproductive efforts among tributaries.

*Breeding Phenology and Larval Development Surveys*

During 2006-2009, we conducted weekly visual encounter surveys (VES; Crump and Scott 1994) for egg masses during the breeding season at gravel bar habitats where breeding activity had been previously documented. In 2006, we collected data at three tributaries of the Trinity River: Mainstem at 25-mph curve, the North Fork at the beach below California Highway SR-299 Bridge, and South Fork at the pool below the confluence of Madden Creek. In 2007 we re-sampled these sites and added a site at Canyon Creek below the SR-299 Bridge. In 2008 and 2009 we expanded this study further by adding the following survey sites: Weaver Creek near the confluence with the Mainstem, Stuart's Fork, and the Upper Trinity River above the East Man Road Bridge (the latter two feed into the Trinity Reservoir); a total of seven sites (Fig. 1).

We collected UTM locations using a GPS unit (e.g., Garmin Extrex) at each breeding site, noting the start time and conducted a VES searching for egg masses along the shoreline and out two to four meters towards the thalweg. Surveyors zigzagged back and forth in edgewater up to one meter deep. We assigned each individual egg mass an identification number and marked it with strip of surveyor flagging attached to a two-ounce fishing weight that was placed in the water underneath the egg mass. Each week we used a new color of flagging to assist in identifying egg masses detected from previous surveys, and recorded the embryo development stage (Gosner 1960) of all egg masses observed. To avoid disturbing or destroying embryos, we did not handle egg masses or pinch off individual embryos to determine Gosner stage, rather we estimated stage based on the shape (e.g., round, bean-shaped) of the outer embryos of each egg mass. We recorded developmental stage and fate of egg masses with each subsequent visit (e.g., hatched-out, scoured or desiccated). Surveys were limited in some years at some locations so scouring and stranding of eggs may have occurred that went undetected.

After egg masses hatched we surveyed breeding sites for tadpoles; we scheduled a brief pause in surveys between those for egg masses and those for tadpole development 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 $\geq$30 animals with dip-nets (tadpoles) or by hand (metamorphs). We collected each entire sample at one time so no tadpoles were sampled twice in a survey. During 2006-2009 we determined the Gosner stage (GS) of each tadpole/metamorph, recording snout-vent length (SVL) and total body length (TL). In 2009, we also weighed animals (g) with a digital scale.

After processing all tadpoles were released where collected. Due to low numbers at Mainstem sites we were not able to obtain a sample size of 30 individuals during every survey.

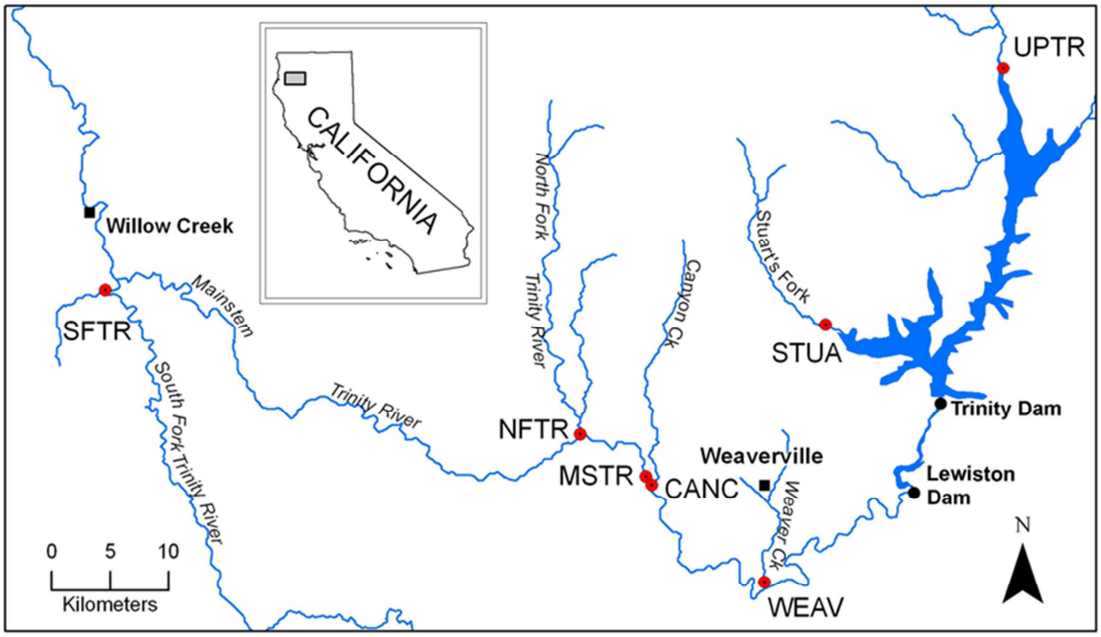



Figure 1. Sites in the Trinity River watershed sampled from 2006 to 2009 for *Rana boylii* breeding phenology and larval development. CANC = Canyon Creek, MSTR = Mainstem, NFTR = North Fork, SFTR = South Fork, STUA = Stuart's Fork, WEAV = Weaver Creek.

*Stream Discharge and Water Temperature*

Stream discharge data during the reproductive seasons of 2006-9 were obtained for the Mainstem, South, North and Upper tributaries of the Trinity River from the Trinity River Restoration Program (http://www.trrp.net/). In 2009 we placed Onset HOBO® data loggers set to record temperatures every two hours at each site to collect continuous water temperature data.

*Data Analysis*

We examined stream discharge and dates of first detection of egg masses, as well as the dates of any egg mass stranding or scouring events observed, at each tributary. We calculated the number of egg masses per kilometer for the Mainstem, South Fork and North Fork tributaries in each year and used ANOVA to test for differences in the mean number of egg masses per km on each tributary. Because data were collected on a weekly basis dates were converted to Julian

week for analyses. We back estimated initiation dates of oviposition based on Gosner stage when egg masses were encountered at later stages of development. We used ANOVA (PROC MIXED; Littell et al. 1996) to test for tributary and year effects on the timing of initial detection of oviposition, hatching (GS-25; tadpoles at this Gosner stage have no visible gills, fully developed opercula, are free-swimming, and feeding), and metamorphic climax (GS-42) for the seven tributaries. We used Tukey-Kramer tests to examine multiple comparisons.

In 2009 the collection of mass and length data for the tadpoles, and continuous water temperatures, allowed us to examine body condition as it related to stream temperature. For each tributary, we averaged mean daily water temperatures for the seven days of each Julian week to calculate weekly average water temperatures. Seasonal average water temperatures were computed by taking the average of the weekly averages between 09 April and 26 August (Julian weeks 15 and 34) for each tributary. For analyses using mass data, we omitted GS-25 tadpoles because the weights of these individuals were often too low for precise measurement (recorded as "< 0.1 g"). We plotted Gosner stage over time (Julian week) to examine differences in tadpole differentiation by tributary and used the regression coefficients of this relationship to estimate and compare 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) vs. log-transformed length (Bancila et al. 2010), to compare the relative condition of tadpoles by tributary. We visually determined the linear section of growth curves (mean SVL and BCI by Gosner stage) and used these data (Gosner stages 25-36 for SVL and stages 32-38 for BCI) to examine the relationship between size and Gosner stage by tributary. We used the regression coefficients of these relationships as estimates of stage-specific tadpole growth rates (Smith-Gill and Berven 1979).

We used linear regression to examine the relationships between oviposition, hatching, and metamorphosis onset dates and water temperatures during each week, and season (using seasonal average, minimum, and maximum water temperatures). One-way ANOVA was used to test for differences in average weekly water temperatures during tadpole development (between first week of hatching and first week of metamorphosis) by tributary. We used linear regression to examine the influence of seasonal water temperatures (average, minimum and maximum) on time to hatching, (number of weeks between initial oviposition and average hatching dates) and

time to metamorphosis (number of weeks between average hatching and metamorphosis dates). We used stepwise regression to explore the relative importance of water temperature and phenology variables to predict differentiation and growth rates, and also to examine the influence of these variables in predicting size (SVL and BCI) of tadpoles (GS-25) and metamorphs (GS-42). Linear regression was used to determine the predictive power of selected variables on differentiation rate, growth rate, and size, and to examine the relationships between differentiation and growth rates with time to metamorphosis. Tributary differences in SVLs and BCIs of metamorphs at GS-42 were tested with one-way ANOVA and Tukey-Kramer. Statistical analysis was conducted in SAS (SAS Institute Inc. 2011), Program R (R Development Core Team 2008), or NCSS (Hintz 2003). Alpha was set at $P < 0.10$ as is appropriate for exploratory (as opposed to confirmatory) ecological studies (Shrader-Frechette and McCoy 1993).

## Results

*Egg Mass Surveys*

We detected egg masses in all six years of float surveys on the Mainstem and South Forks of the Trinity River, and in all four years of wading surveys on the North Fork. The six-year average (four-year average for North Fork) number of egg masses per kilometer was significantly different among the tributaries ($F_{2,16} = 19.52$, $p < 0.0001$; MSTR < NFTR, SFTR; NFTR < SFTR). In all years we observed very few egg masses on the Mainstem (Table 1) despite survey efforts on the Mainstem being greater relative to other tributaries because egg mass detections were so rare. We conducted multiple surveys on the Mainstem in both 2008 and 2009 in order to detect a sufficient number of egg masses to estimate reproductive output. For the South Fork and North Fork reaches, shorter distances were surveyed and stream sections were surveyed only once each breeding season. The reproductive output estimates for South and North Forks are likely even higher than reported because egg masses laid after the surveys would have gone undetected. Therefore, the differences between the reproductive output on the Mainstem relative to the other tributaries is conservative and differences are likely greater than reported. See Appendix A for a summary of survey dates, search effort, and egg mass counts.

We observed scouring or stranding events, sometimes both, every year we monitored (2006-9; Table 2 and Fig. 2). Mortality of egg masses from stranding or scouring occurred on all

Table 1. Date of last survey when new *Rana boylii* egg masses [EMs] were detected, kilometers surveyed, number of EMs detected, water year designation (based on total inflow into the Trinity Reservoir) for the Trinity River, Trinity Co., California. Water year is given as predicted/actual when different. The Mainstem experienced modified flows based on the predicted water year; whereas, South and North Forks experienced the actual water year type flows. Boldface values represent the number of EMs/kilometer for each tributary surveyed. Data includes EMs detected along reaches during float and wading surveys.

| | | Mainstem | | | | South Fork | | | | North Fork | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Water Year | Date | Km | No. EMs | EM/Km | Date | Km | No. EMs | EM/Km | Date | Km | No. EMs | EM/Km |
| 2004 | Wet | 17-Jun-04 | 24.17 | 26 | **1.08** | 9-Jun-04 | 1.81 | 62 | **34.25** | 26-May-04 | 1.18 | 69 | **58.47** |
| 2005 | Wet/Normal | 28-Jun-05 | 24.96 | 12 | **0.48** | 15-Jun-05 | 7.72 | 806 | **104.4** | 23-Jun-05 | 1.61 | 72 | **44.72** |
| 2006 | Extremely Wet | 8-Jun-06 | 12.96 | 10 | **0.77** | 14-Jun-06 | 3.56 | 291 | **81.74** | 28-Jun-06 | 1.27 | 58 | **45.67** |
| 2007 | Dry | 18-Jun-07 | 7.76 | 7 | **0.9** | 23-May-07 | 3.08 | 510 | **165.58** | | No Survey | | |
| 2008 | Dry/Normal | 2-Jun-08 | 21.1 | 19 | **0.9** | 22-May-08 | 6.5 | 810 | **124.62** | 6-Jun-08 | 1.35 | 53 | **39.26** |
| 2009 | Dry | 25-Jun-09 | 28.4 | 50 | **1.76** | 29-May-09 | 2.9 | 441 | **152.07** | 9-Jun-09 | 1.405 | 117 | **83.27** |

Table 2. Summary of stranding and scouring events of *Rana boylii* EMs and the proportion of EMs lost to both types of events combined by year for tributaries of the Trinity River, Trinity Co., California. Stranding events were observed in all years. Scouring events were only observed in 2009. Data represent EMs detected at sites surveyed during the breeding phenology study and do not include float survey data.

| | 2006 | | | 2007 | | | 2008 | | | 2009 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tributary | Total | Stranded | Proportional loss | Total | Stranded | Proportional loss | Total | Stranded | Proportional loss | Total | Stranded | Scouring | Proportional loss |
| Canyon Creek | 41 | 0 | 0.00 | 29 | 0 | 0.00 | 41 | 0 | 0.00 | 26 | 9 | 0 | 0.35 |
| Mainstem | 2 | 0 | 0.00 | 7 | 2 | 0.29 | 3 | 0 | 0.00 | 22 | 4 | 0 | 0.18 |
| North Fork | 6 | 3 | 0.50 | 24 | 1 | 0.04 | 11 | 0 | 0.00 | 47 | 0 | 0 | 0.00 |
| South Fork | 44 | 13 | 0.30 | 30 | 10 | 0.33 | 30 | 0 | 0.00 | 126 | 15 | 24 | 0.31 |
| Stuart's Fork | - | - | - | - | - | - | 4 | 0 | 0.00 | 8 | 0 | 0 | 0.00 |
| Upper | - | - | - | - | - | - | 21 | 2 | 0.10 | 27 | 0 | 0 | 0.00 |
| Weaver Creek | - | - | - | - | - | - | 28 | 0 | 0.00 | 35 | 0 | 13 | 0.37 |

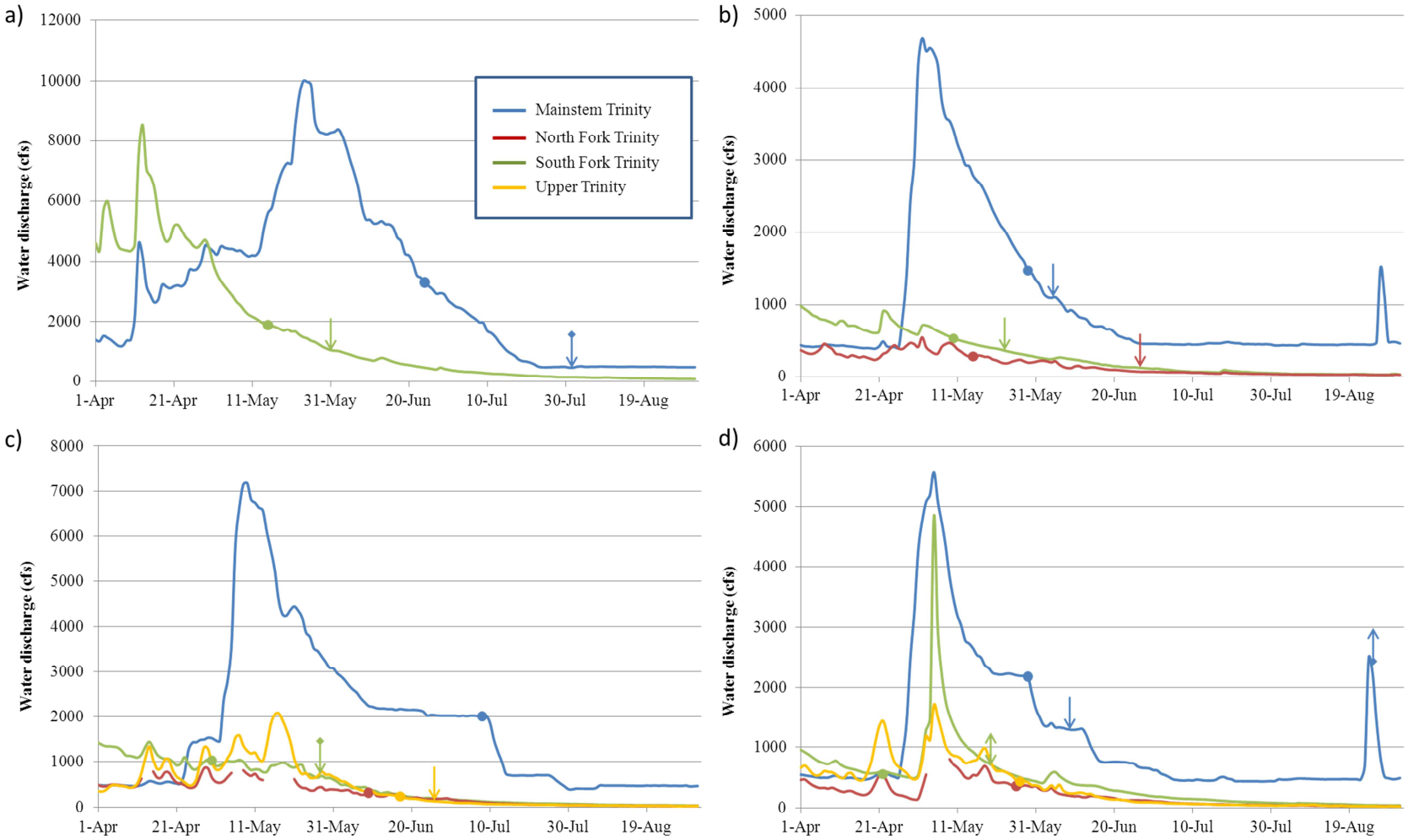



Figure 2. Water discharge data (USGS database) for tributaries of the Trinity River, Trinity Co., California in years a) 2006, b) 2007, c) 2008, and d) 2009. Solid circles represent the dates of initial detection of *Rana boylii* EMs. ↓ indicates stranding of EMs, ↑ indicates scouring of EMs, and ↕ indicates both stranding and scouring of EMs were observed. Capped arrows ( ↕ ) indicate stranding and scouring of tadpoles.

tributaries, except Stuart's Fork, during at least one year. Stranding was observed in all years and scouring occurred in 2009. During the 2008 float surveys on the Mainstem, 6 of 8 breeding sites surveyed between 04 June and 14 July were desiccated, with 13 of 19 (68%) egg masses lost to stranding (not included in Table 2). In 2009, 16 of 50 (32%) egg masses detected on the Mainstem during float surveys became stranded. We also observed tadpole mortality due to desiccation of rearing habitats. Stranding of tadpoles occurred on 31 July 2006 on the Mainstem, resulting in 100% mortality of individuals from the only two egg masses detected during site visits. Stranding of tadpoles also occurred on 25 June 2007 on Canyon Creek and 27 May 2008 on South Fork (Fig. 2). Stranding and scouring did not appear to be predictable based on water year type (i.e., dry, normal, wet). Metamorphs were displaced on the Mainstem during the late summer (August) in 2009 as a result of increased water released for Hoopa Tribal ceremonies; however, we have no information on whether or how this influenced cohort survival.

*Breeding Phenology*

Across all tributaries and all years we detected egg masses as early as April and as late as the beginning of July (Fig. 3). Hatching (GS-25) occurred in May through July, and metamorphic climax (GS-42) was observed between July and September (Fig. 3). There was an average difference of 6 weeks between the first egg masses detected on the South Fork and Mainstem (range 3-10 weeks, n = 4). The average difference between hatching on the South Fork and Mainstem was 5.5 weeks (range 0-9, n = 4). There was a 6 week average difference between start of metamorphosis on the South Fork and Mainstem (range 4-10, n = 3). There were differences among sites in the timing of the first detections of oviposition, hatching, and metamorphosis, and differences in the timing of detected oviposition and hatching by year (Table 3). Oviposition, hatching, and metamorphosis were generally observed earlier on the South Fork and Weaver Creek than on the other tributaries, and later on the Mainstem and Stuart's Fork (Table 3). Oviposition was observed earlier in 2009 than 2006 and metamorphosis was observed earlier in 2007 than 2006 and 2008 (Table 3).

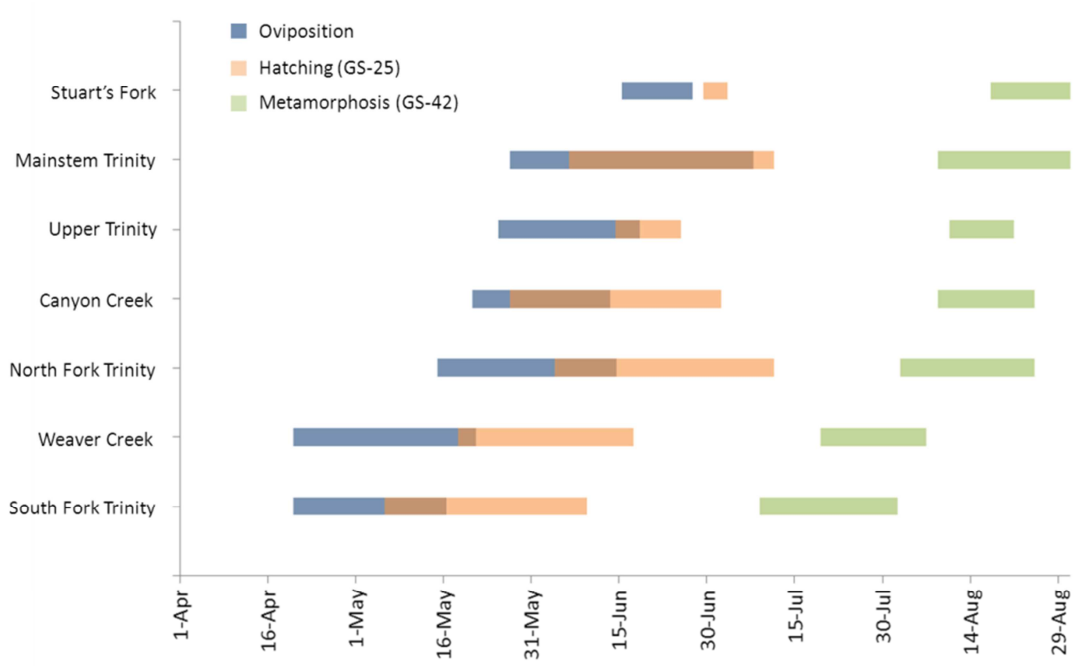



Figure 3. Annual variation in initial dates for detection of oviposition, hatching, and metamorphosis in *Rana boylii* on seven tributaries of the Trinity River, Trinity Co., California. Areas in brown represent an overlap in oviposition and hatching dates. South Fork Trinity, North Fork Trinity, Mainstem Trinity and Canyon Creek were sampled from 2006 to 2009. Weaver Creek, Upper Trinity, and Stuart's Fork were sampled in 2008 and 2009.

Table 3. Tests (ANOVA) of the date (by Julian week) of first observation for specific *Rana boylii* life stages among seven tributaries of the Trinity River, Trinity Co., California, from 2006-9. Tukey-Kramer comparisons are listed by increasing means. Groups separated by commas are not significantly different and results separated by semi-colons are independent of one another. CANC = Canyon Creek, MSTR = Mainstem, NFTR = North Fork, SFTR = South Fork, STUA = Stuart's Fork, WEAV = Weaver Creek.

| Life stage | Factor | *F* | DF | p | Tukey-Kramer |
|---|---|---|---|---|---|
| Oviposition | Trib | 7.89 | 6,12 | 0.001 | SFTR, WEAV < MSTR, STUA |
| | Year | 4.65 | 3,12 | 0.022 | 2009 < 2006 |
| Hatching | Trib | 5.19 | 6,11 | 0.009 | SFTR < MSTR, STUA |
| (GS-25) | Year | 2.60 | 3,11 | 0.105 | |
| Metamorphosis | Trib | 8.61 | 6,10 | 0.002 | SFTR < NFTR, CANC, STUA, MSTR; WEAV < MSTR |
| (GS-42) | Year | 7.44 | 3,10 | 0.007 | 2007 < 2008, 2006 |

*Water Temperature*

Seasonal water temperatures for 2009 are summarized in Table 4. South Fork and Weaver Creek were the warmest tributaries. Canyon Creek, Mainstem, North Fork, and Upper Trinity tributaries had comparable water temperatures. Stuart's Fork, which is fed entirely by snowmelt from the Trinity Alps, was generally the coolest tributary; however, the Mainstem had the coldest maximum temperature.

Table 4. Average, minimum and maximum weekly water temperatures (°C) for breeding sites sampled on tributaries of the Trinity River, Trinity Co., California between 09 April (Julian week 15) and 26 August (Julian week 34), 2009.

| | Canyon Cr. | Mainstem | North Fork | South Fork | Stuart's Fork | Upper | Weaver Cr. |
|---|---|---|---|---|---|---|---|
| Average | 14.9 | 14.2 | 15.2 | 18.6 | 13.0 | 14.5 | 17.3 |
| Minimum | 7.4 | 8.8 | 7.4 | 10.0 | 5.8 | 6.2 | 10.0 |
| Maximum | 22.1 | 18.8 | 22.2 | 25.2 | 19.1 | 21.4 | 21.8 |

In 2009, the average water temperature of tributaries during the first week of egg mass detection was 10.7 °C (range 9.7-12.1). There was a significant association between the first observation of oviposition (first week of egg mass detection) and water temperature ($R^2 = 0.46$, $p = 0.09$; Fig. 4), and between oviposition date and seasonal average water temperature ($R^2 = 0.92$, $p < 0.001$; Fig. 5a). There was no association between first observed hatching date and water temperature ($R^2 = 0.15$, $p = 0.39$; Fig. 4), but hatching date was associated with seasonal minimum water temperature ($R^2 = 0.72$, $p = 0.02$; Fig. 5b). There was a significant relationship between the first week of metamorphosis and water temperature ($R^2 = 0.67$, $p = 0.02$; Fig. 4), and seasonal average water temperature ($R^2 = 0.69$, $p = 0.02$, Fig. 5c); with metamorphosis occurring earlier on warmer water tributaries (South Fork and Weaver Creek) and later on colder tributaries (Stuart's Fork and Mainstem). The average water temperature between the respective weeks of initial detection of hatching (GS-25) and initial detection of metamorphic climax (GS-42), was lower for the Mainstem than all other tributaries ($F_{6, 57} = 5.57$, $P < 0.001$) except Stuart's Fork.

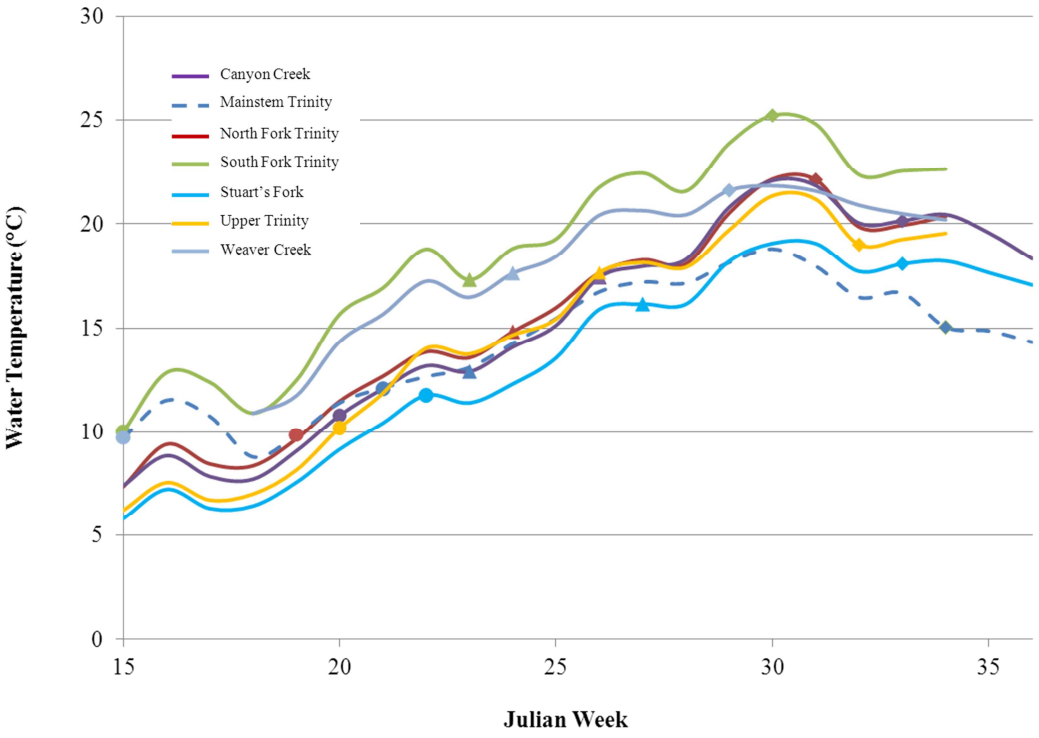



Figure 4. Water temperature and dates (by Julian week) of first detections of *Rana boylii* oviposition (circles), hatching (triangles), and metamorphic climax (diamonds), on tributaries of the Trinity River, Trinity Co., California in 2009.

The average time to hatching (incubation period) was five weeks; however, Stuart's Fork had two weeks between the detection of initial oviposition and average week of hatching, while Weaver Creek had eight weeks between these events. Seasonal minimum water temperature explained 64% of variation in time to hatching ($p < 0.03$). Egg masses on tributaries with warmer minimum water temperatures had longer incubation periods. The average time to metamorphosis (from hatching to metamorphic climax) was eight and a half weeks; Stuart's Fork tadpoles metamorphosed seven weeks after hatching, whereas metamorphosis of North Fork tadpoles took 10 weeks. However, seasonal minimum water temperature did not explain the variation in time to metamorphosis ($R^2 = 0.37$, $p = 0.15$).

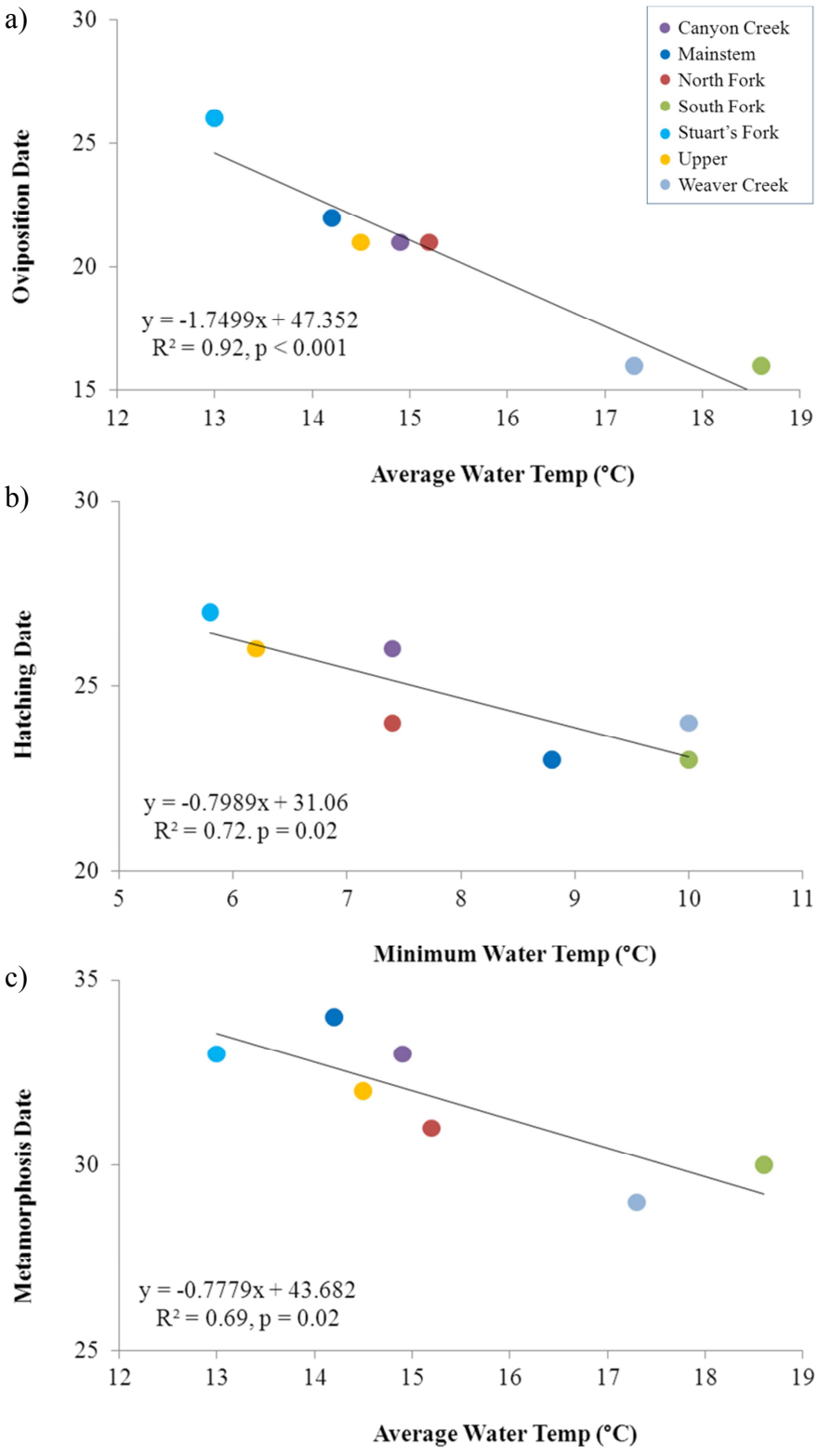



Figure 5. Relationships between *Rana boylii* a) oviposition date and seasonal average water temperature, b) hatching date and seasonal minimum water temperature, and c) metamorphosis date and seasonal average water temperature for tributaries of the Trinity River, Trinity Co., California in 2009. Dates are presented in Julian weeks.

*Differentiation Rate*

In 2009, metamorphic climax (GS-42) was attained earliest on the South Fork and latest on the Mainstem (Fig. 6). Differentiation rate averaged 1.89 and was highest for the Stuart's Fork population and lowest for the North Fork population (Fig. 6). Hatching date was a significant predictor of differentiation rate ($R^2 = 0.75$, $p = 0.01$); hatching date was negatively correlated with minimum water temperature ($r_p = -0.85$, $p = 0.02$). Differentiation rate was higher for tributaries where later hatching occurred (Fig. 7). Differentiation rate was a significant predictor of time to metamorphosis ($R^2 = 0.85$, $p < 0.005$).

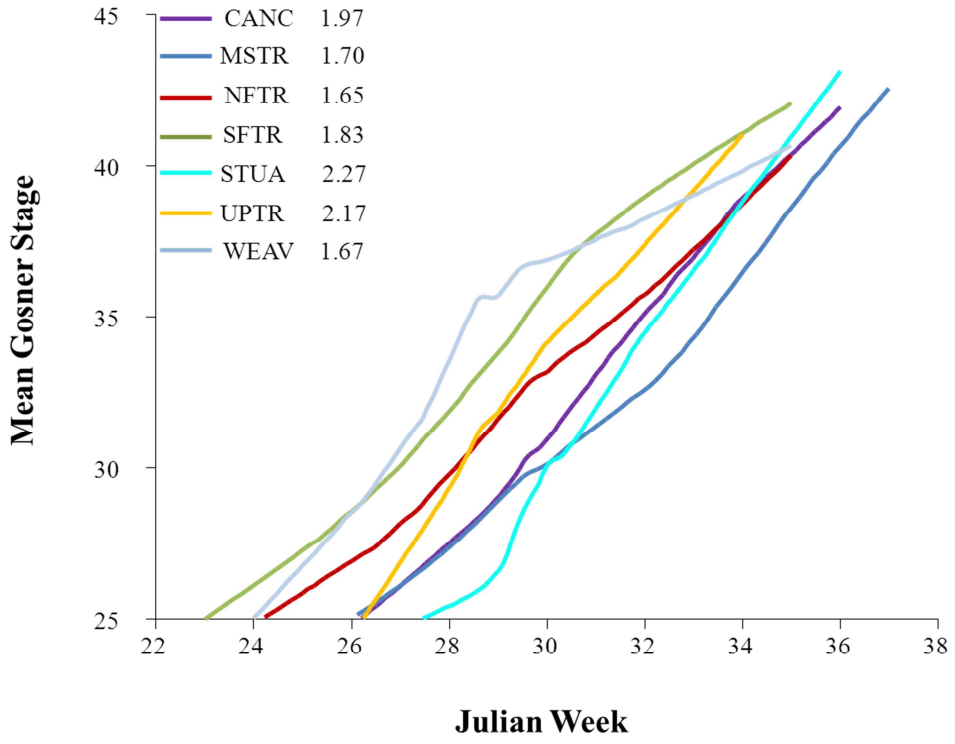



Figure 6. Mean Gosner stage over time (differentiation rates) for *Rana boylii* tadpoles and metamorphs on tributaries of the Trinity River, Trinity Co., California in 2009. Values on the figure are the regression coefficients of the relationships between Gosner stage and Julian week for each tributary and represent differentiation rate estimates. CANC = Canyon Creek, MSTR = Mainstem, NFTR = North Fork, SFTR = South Fork, STUA = Stuart's Fork, UPTR = Upper Trinity, WEAV = Weaver Creek.

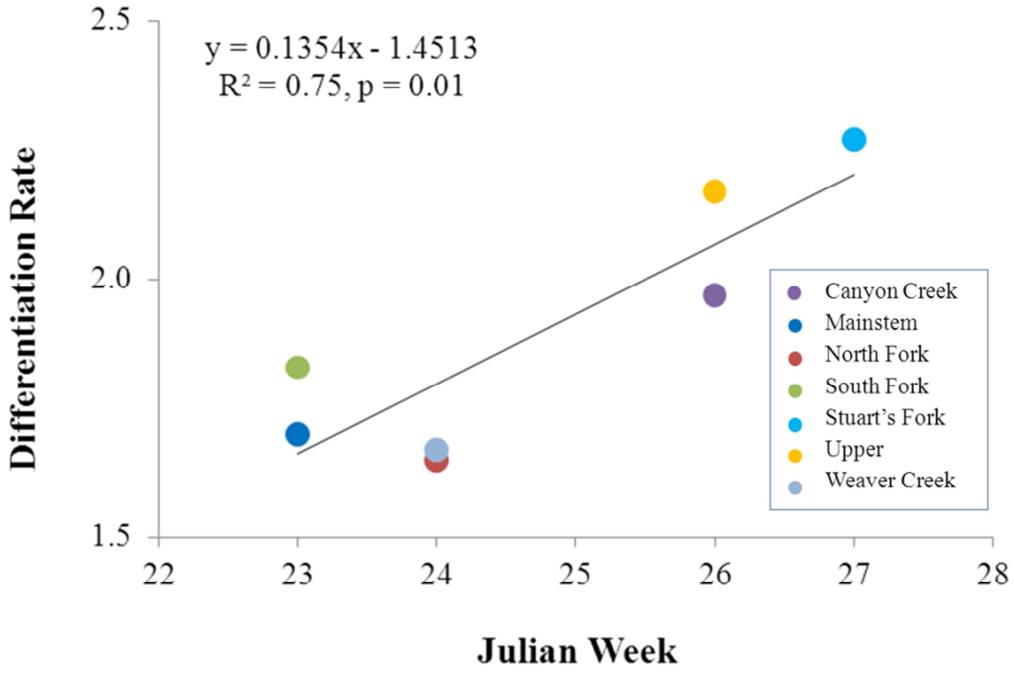
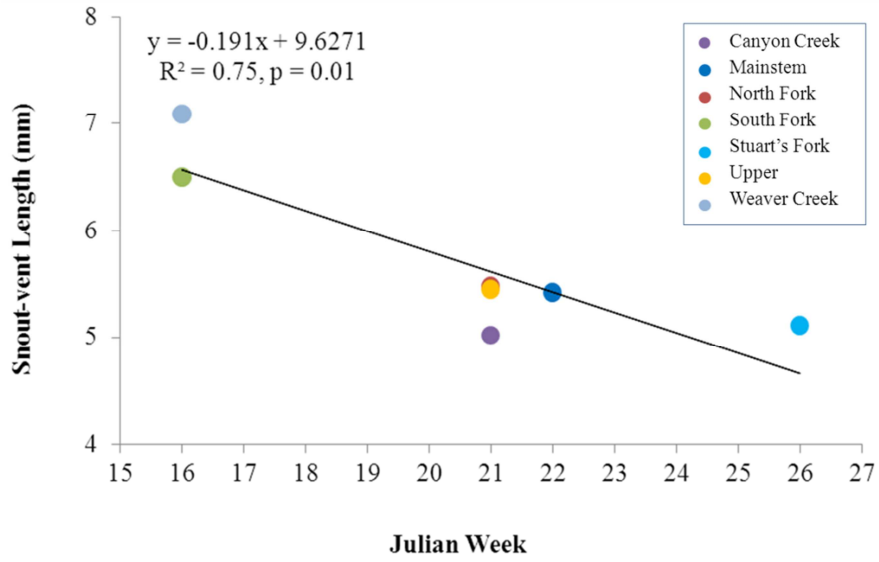



Figure 7. Relationship between *Rana boylii* differentiation rate and hatching date (Julian week) for breeding sites on tributaries of the Trinity River, Trinity Co., California in 2009.

*Size and Body Condition Index (BCI)*

In 2009, South Fork and Weaver Creek tadpoles had greater SVLs than those captured on other tributaries ($F_{6,677}$ = 32.76, P < 0.0001). Oviposition date explained 75% of variance in tadpole SVL (p = 0.01); oviposition date was negatively correlated with seasonal average water temperature ($r_p$ = -0.96, p < 0.001). Tadpoles were smaller in SVL at hatching on tributaries where oviposition started later (Fig. 8a). South Fork metamorphs had the greatest SVLs and Mainstem metamorphs had the lowest ($F_{6,176}$ = 22.02, P < 0.0001; Fig. 9a). Seasonal maximum water temperature explained 86% of variation in metamorph SVLs (p < 0.005); metamorphs were larger in SVL on tributaries with warmer maximum water temperatures. Stuart's Fork tadpoles had higher BCI scores than all other tributaries, with Weaver Creek tadpoles having the lowest BCI scores ($F_{6,440}$ = 26.57, P < 0.0001). Oviposition date was a significant predictor of BCI at hatching ($R^2$ = 0.69, p = 0.02); mean BCI score of tadpoles was higher for tributaries where oviposition occurred later (Fig. 8b). South Fork metamorphs also had the greatest BCI scores and Mainstem metamorphs had the lowest ($F_{6,176}$ = 12.61, P < 0.0001; Fig. 9b). Time to metamorphosis was not a significant predictor of size (SVL and BCI) at metamorphosis ($R^2$ = 0.04, p = 0.67; $R^2$ = 0.11, p = 0.47, respectively).

a)




b)

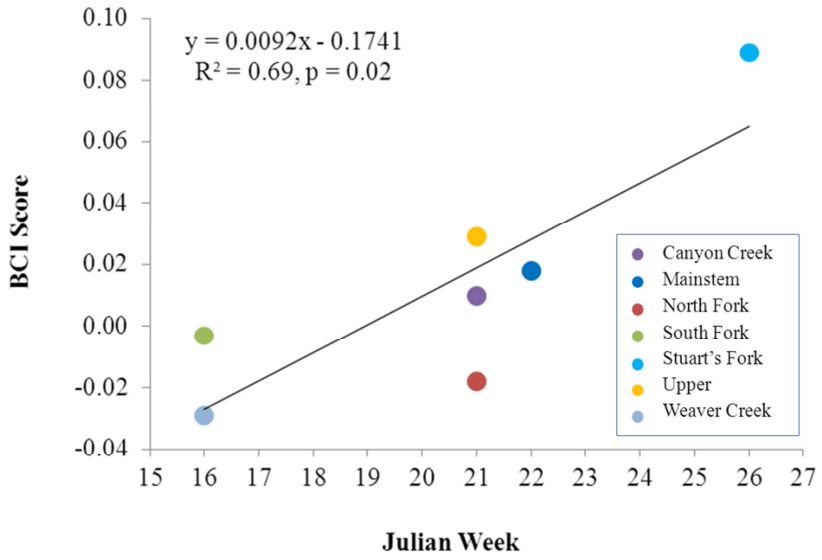



Figure 8. Relationships between date (Julian Week) of first detected *Rana boylii* oviposition and average (a) snout-to-vent length of GS-25 tadpoles and (b) BCI scores of GS-26 tadpoles, in 2009 on tributaries of the Trinity River, Trinity Co., California.

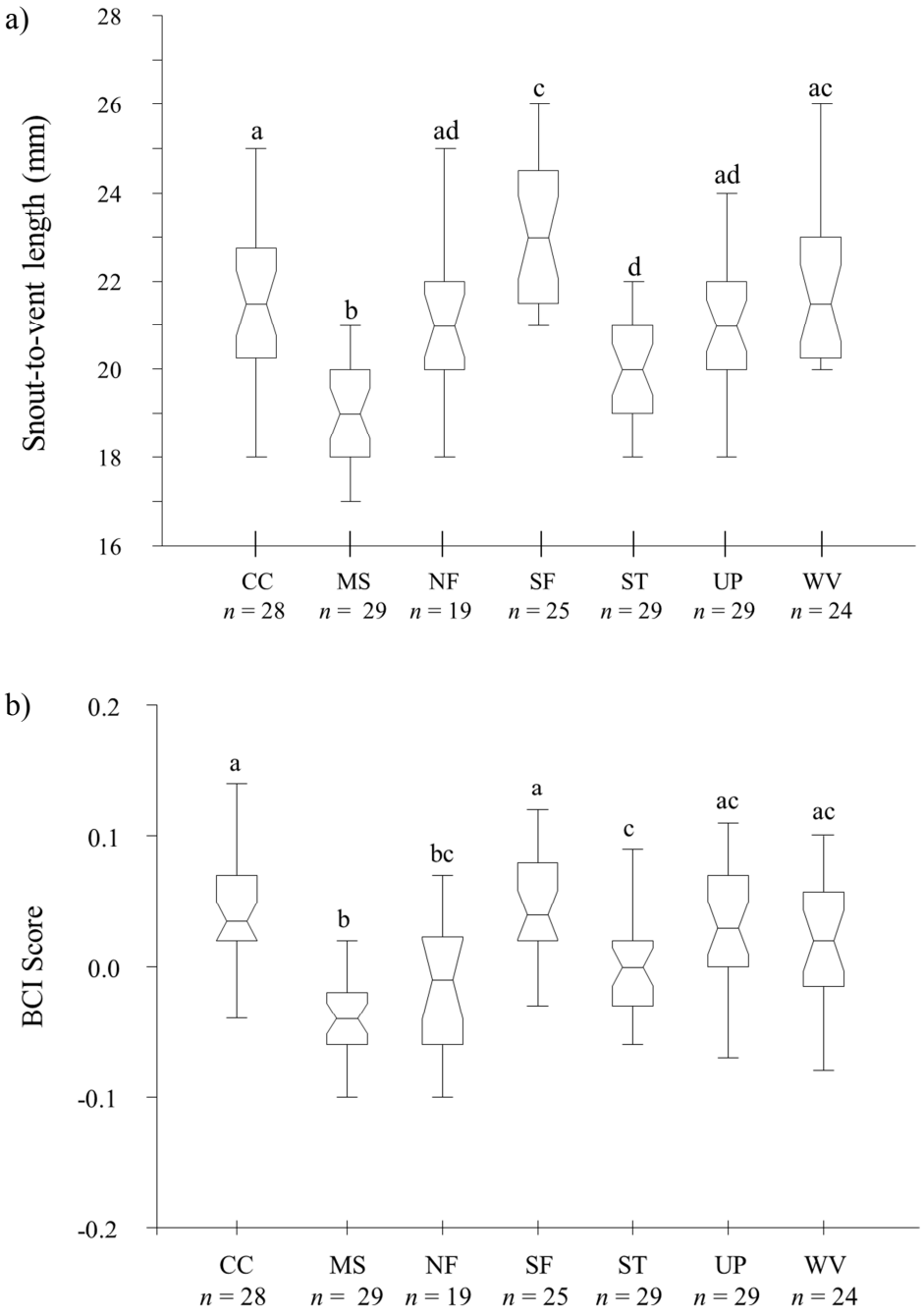



Figure 9. Boxplots of GS-42 metamorph *Rana boylii* (a) snout-to-vent lengths and (b) BCI scores and number of individuals sampled (*n*) on tributaries of the Trinity River, Trinity Co., California in 2009. The center line represents the median value. The top and bottom of the box represent the 25$^{th}$ and 75$^{th}$ percentiles, the notched area represents the 95% confidence interval, and whiskers represent the upper and lower adjacent values, set at 1.5 times the interquartile range. Different letters above the boxes represent significance ($p < 0.10$) in Tukey-Kramer multiple comparisons among tributaries. CC = Canyon Creek, MS = Mainstem, NF = North Fork, SF = South Fork, ST = Stuart's Fork, UP = Upper Trinity, WV = Weaver Creek.

*Growth Rate*

Growth rates of populations on the different tributaries varied (Figs. 10a and b). Individuals on the Mainstem, Stuart's Fork, North Fork and Canyon Creek appeared to stop growing between 38-40 mm in length, whereas tadpoles from the South Fork, Weaver Creek, and Upper Trinity populations continued to grow even after metamorphic climax (Fig. 10a). Average stage-specific SVL growth rate was 1.18 and was highest for Stuart's Fork population and lowest for North Fork and South Fork populations (Fig. 10a). Growth rate in SVL was related to differentiation rate ($R^2 = 0.64$, $p = 0.03$) and variation in hatching date accounted for 67% of the variation in SVL growth rate ($p = 0.02$); growth in SVL was more rapid for later hatching populations. SVL growth rate was a significant predictor of time to metamorphosis ($R^2 = 0.80$, $p < 0.01$). All populations exhibited a peak in BCI prior to metamorphic climax (at ca. stage 40), when tadpoles presumably lose mass during the process of metamorphosis (Fig. 10b). Average stage-specific growth in BCI was 0.017 and was highest for Canyon Creek and lowest for Weaver Creek (Fig. 10b). BCI growth rate was also related to differentiation rate ($R^2 = 0.54$, $p = 0.06$). Variation in hatching date accounted for 50% of the variation in BCI growth rate ($p = 0.08$); hatching date was negatively correlated with minimum water temperature ($r_p = -0.85$, $p = 0.02$). Growth rates in BCI were higher for populations from tributaries with later occurring hatching. BCI growth rate was not associated with time to metamorphosis ($R^2 = 0.38$, $p = 0.14$).

## Discussion

The effects of altered stream flows on foothill yellow-legged frog populations have been extensive and population declines have been, at least in part, attributed to the modification of natural flow regimes and management of the timing of water releases from water impoundments that are physiologically and developmentally challenging for these frogs (Lind 2005; Kupferberg et al. 2012). Stream flow influences the timing of breeding activity (Kupferberg 1996a; Wheeler and Welsh 2008) and affects the survival of egg masses and tadpoles (Lind et al. 1996; Kupferberg et al. 2012). Altered water temperatures are an additional consequence of impoundments on streams below dams. Temperature regimes can have a profound influence on the physiology and ultimately the survival and absolute fitness (i.e. a population's capacity for replacing itself) of ectothermic organisms like the foothill yellow-legged frog (Angilletta et al. 2002; Rohr and Palmer 2013). In the spring and summer, lower water temperatures of the

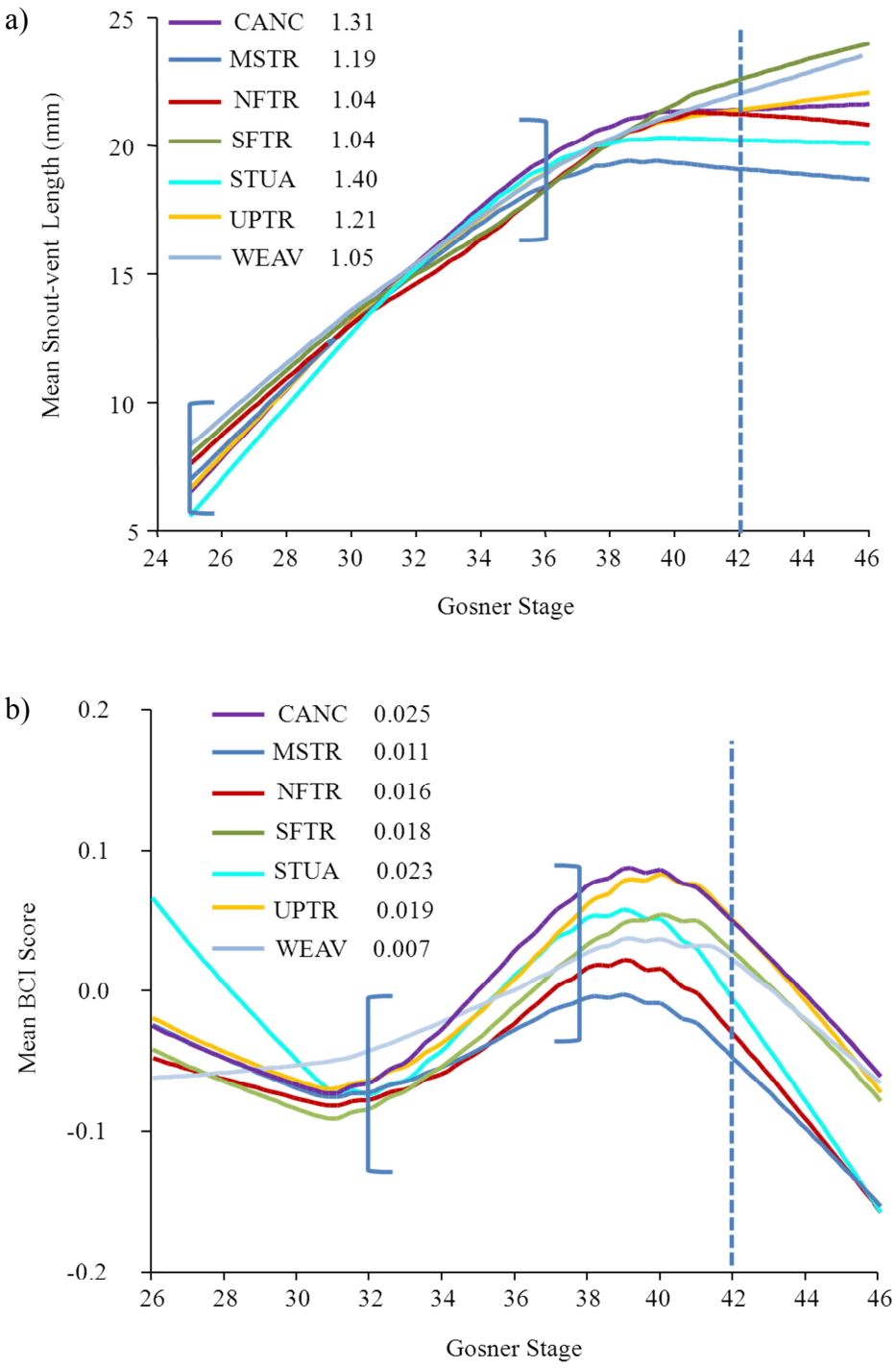



Figure 10. Mean a) SVL and b) BCI score at increasing Gosner stages for tadpoles and metamorphs on tributaries of the Trinity River, Trinity Co., California in 2009. Vertical dashed line indicates metamorphic climax (GS-42). Values in the legend are the regression coefficients of the relationships between size and Gosner stage for each tributary and represent stage-specific growth rate estimates for the linear section (enclosed in brackets) of growth trajectories. CANC = Canyon Creek, MSTR = Mainstem, NFTR = North Fork, SFTR = South Fork, STUA = Stuart's Fork, UPTR = Upper Trinity, WEAV = Weaver Creek.

Mainstem are likely a result of water released from the bottom of the reservoir (hypolimnetic release) that is often substantially colder than the surface water; and this could be confounded by the modified channel geometry, a consequence of post-dam reduction in the frequency of large floods, which causes a deeper and steeper trapezoidal shaped channel with less shallow edgewater. Either or both of these processes may result in water temperatures generally colder than most other nearby tributaries during periods of tadpole growth and metamorphosis. In a recent study (Kupferberg et al. 2013), the lower thermal limit (measured as summer maximum 30 day average water temperature) for foothill yellow-legged frog population occurrence was 17.6°C for Sierran sites and 18.8°C for Coastal sites. In 2009, the maximum weekly water temperature during our study period for the Mainstem was 18.8°C, a temperature that appears to be near or at the lower thermal limit for population viability.

Foothill yellow-legged frogs appear to use water temperature as one of their cues for initiation of breeding activity. Over multiple sites throughout their range, foothill yellow-legged frog oviposition occurred when water temperatures were at least 10°C (Hayes et al. *in press*). In 2009, the average water temperature at breeding sites on the first date of egg mass detection was 10.7°C, and start of breeding activity (i.e., week of first detected egg mass) was positively correlated with increasing water temperature. In 2009, the Mainstem reached suitable temperatures for oviposition (~10°C) as early as other tributaries; however, breeding activity wasn't observed until much later. The delay in breeding activity on the Mainstem in 2009 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). The onset of oviposition, hatching, and metamorphosis were earlier on warmer tributaries. These events occurred later on the Mainstem relative to all other tributaries except for Stuart's Fork. Stuart's Fork is a cold water tributary where the headwaters flow from the snowfields of the Trinity Alps Wilderness, with the low water temperatures likely the cause of delayed breeding on this fork.

The time to hatching (incubation period) was truncated on colder tributaries, particularly on Stuart's Fork. The short time to hatching for Stuart's Fork egg masses suggests that differentiation during embryonic development occurred more quickly for this population relative to the others. This was contrary to what might be expected since warmer water temperatures typically accelerate the rate of development (Berven et al. 1979, Smith-Gill and Berven 1979, Sanuy et al. 2008). Onset of oviposition was later for this tributary so rapid embryonic

development may have been a result of later season warmer water temperatures along shallow stream margins, the microhabitat where egg masses are typically deposited. Edgewater temperature on the date of oviposition for Stuart's Fork (11.8°C) was warmer than all other tributaries, except the Mainstem (12.1°C). However, embryonic development rate can also be influenced by other environmental and by evolutionary (genetic) factors (Williamson and Bull 1989, Gillooly and Dodson 2000, Laugen et al. 2003, Ireland et al. 2007). Rapid embryonic development did not have negative consequences on body condition of tadpoles at hatching. Tadpoles from the Stuart's Fork had the highest BCI scores, whereas tadpoles from tributaries with earlier onset of oviposition and longer incubation periods, South Fork and Weaver (the warmest tributaries), had low BCI scores at hatching. Larger tadpole size in colder environments is common, but this relationship is usually the outcome of slow differentiation coupled with a prolonged period of development (Berven et al. 1979, Berven 1982). High BCI scores of tadpoles that hatched at Stuart's Fork may also be evidence that females of this population generally produced larger embryos. In common frogs (*Rana temporaria*), egg size was directly correlated with tadpole size at hatching (Laugen et al. 2003). Williamson and Bull (1989) found that in the Australian frog, *Ranidella signifera*, clutches with larger eggs had more rapid development and hatched into larger tadpoles. However, this phenotypic trait can be influenced by environmental factors, such as incubation temperature (Williamson and Bull 1989, Gillooly and Dodson 2000), as well as parental effects (Berven 1982, Williamson and Bull 1989, Parichy and Kaplan 1995, Laugen et al. 2003). Body size and morphology at hatching may influence the survival of developing tadpoles. Small foothill yellow-legged frog tadpoles were more susceptible to predation by macroinvertebrates which may be due to ease of handling by predators or a reduced ability of smaller tadpoles to evade predators (Kupferberg et al. 2013). Greater body size and longer tail lengths enhanced the locomotor performance of oriental fire-bellied toad (*Bombina orientalis*) hatchlings, reducing the probability of predation (Parichy and Kaplan 1995).

The onset of hatching appeared to have a strong influence on differentiation and growth rates of tadpoles. Tadpoles from colder tributaries with later hatching dates, such as Stuart's Fork, differentiated and grew more rapidly compared to those from tributaries where hatching occurred earlier. This is consistent with studies that have demonstrated that later developing larvae develop more rapidly as an adaptation to a shorter growing season (Berven et al. 1979, Beattie 1987, Loman 2009). However, other studies have found that tadpole development rates

are slower and time to metamorphosis is prolonged in colder environments (Berven et al. 1979, Smith-Gill and Berven 1979, Sanuy et al. 2008), and genetic influences and environmental variation may also have counteracting effects (Berven et al. 1979). Kupferberg et al. (2013) found that foothill yellow-legged frog tadpoles experienced higher growth and development rates at the warmest site (21.8°C); here metamorphosis occurred earlier and tadpoles attained larger sizes. Contrary to Kupferberg et al. (2013), our results suggested that tadpole development rate was more rapid at colder sites; however, metamorphs attained larger sizes on the warmest tributaries in both studies. Larval development can also be influenced by other environmental and genetic factors (Laugen et al. 2003). Lower than average differentiation rate and BCI growth rate may indicate that the Mainstem population is experiencing negative fitness consequences as a result of atypical thermal conditions. The edgewater along the Mainstem did not warm up to temperatures comparable to other tributaries during critical summer months when tadpoles were growing and undergoing metamorphosis.

Numerous studies have investigated the influence of temperature on size at metamorphosis but results, including the direction of effects, varied (Smith-Gill and Berven 1979). However, studies differed in their measure of size (i.e., snout-vent length, volume, various body condition indices). For example, Smith-Gill and Berven (1979) found that lower temperatures resulted in larger (volume, measured by volumetric displacement) larvae at cooler temperatures. In contrast, Loman (2002) found that metamorphs reared in warmer ponds were larger (body length) than those in cooler ponds. Water temperature at particular phases of development and interactions between these temperatures (e.g. incubation and post-hatching temperatures) can also have distinct effects on phenotypic traits of tadpoles (Watkins and Vraspir 2006). Snout-vent length alone, while a reasonable metric for comparing relative growth rates among tributary populations, lacks the volumetric component of growth provided by incorporating mass, and is thus not suitable for comparing relative condition, whereas BCI is a more accurate index of the relative health of individuals and a more appropriate fitness analog (Rohr and Palmer 2013). Our discussion regarding the differences in metamorph size hereafter focuses on this metric. While Stuart's Fork tadpoles had the highest BCI scores, metamorphs on the South Fork had the highest average BCI for that life stage. Metamorphs on the Mainstem had the lowest BCI scores. Size at metamorphosis was best explained by the phenology of oviposition and hatching. Longer incubation time, which was associated with warmer water temperature, resulted in metamorphs with better body condition. Traits that relate to fitness of

later life stages (e.g., late-stage tadpoles) may depend on conditions such as temperature during the incubation period (Watkins and Vraspir 2006). Size at metamorphosis may have future life stage consequences for individuals and populations; size may influence post-metamorphic survival and reproductive success of individuals, and populations with smaller metamorphs may have delayed reproductive maturity where an additional year of growth may be required for individuals to recruit into the breeding population (Smith 1987).

Breeding occurred late on Stuart's Fork, naturally the coldest tributary, but individuals did not suffer inhibited differentiation or growth. The incubation period of egg masses on Stuart's Fork was short, tadpoles had the highest BCIs after hatching and differentiated rapidly, and metamorphs had BCI scores that were comparable to 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 at similar body condition as those from warmer tributaries. Compensatory mechanisms are strategies in which growth and differentiation 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 unfavorable growth conditions (Orizaola et al. 2009); the Stuart's Fork and Mainstem populations experienced delayed oviposition and cooler water temperatures during embryonic and larval development. Water temperatures on Stuart's Fork in 2009 exceeded those of the Mainstem during the period of larval development. Failure of the surface edgewater to warm up on the Mainstem as the season progresses (as would naturally occur) may be the factor leading to low body condition in that population.

We suspect that the low body condition of Mainstem metamorphs may also be influenced by cold-water effects on the quantity and composition of riverine periphyton, the preferred food of larval foothill yellow-legged frogs (Kupferberg 1996b). Food availability influences tadpole growth and time to metamorphosis in other species (Wilbur 1977a, b; Alford and Harris 1988, Berven and Chadra 1988, Crespi and Warne 2013). The interactive effects between temperature and algal food quality have recently been examined in laboratory experiments (Kupferberg et al 2013), but periphyton composition and food abundance on the Mainstem and tributaries have not yet been examined. In a series of experiments, Kupferberg (1996b and 1997) found that food quality influenced metamorphosis in foothill yellow-legged frogs and tadpoles selected food

types (filamentous green alga with associated diatoms) higher in protein that promoted growth and development. Kupferberg et al. (2013) documented that reaches below dams, with daily fluctuations in flow, contained periphyton assemblages dominated by mucilaginous stalked diatoms (e.g., *Didymosphenia geminate*) which does not promote foothill yellow-legged frog tadpole growth. Water temperature may also interact with feeding and have an indirectly influence on size and body condition. Kupferberg et al. (2013) found that foothill yellow-legged tadpoles reared in cold conditions had low feeding rates. This result was consistent with other studies that examined the variation in tadpole feeding rates in relation to water temperature (Pandian and Marian 1985, Warkentin 1992). The relatively rapid BCI growth rate for the Stuart's Fork may be a result of increased food consumption and changed resource allocation toward body structure, as proposed by Gurney et al. (2003) in an energy-budget model explaining compensatory growth. The persistently colder water temperatures of the Mainstem may impose a physiological restriction on the ability of tadpoles to attain the feeding rates necessary for compensatory growth.

Regardless of the mechanism(s), animals from the Mainstem were smaller at metamorphosis than other tributaries which likely have later negative consequences for their survival and fitness. For example, smaller foothill yellow-legged frog tadpoles at colder temperatures were more susceptible to predation by macroinvertebrates (Kupferberg et al. 2013). In wood frogs (*Rana sylvatica*), reduced mass and body condition at metamorphosis affected future post-metamorphic responses to stressors (Crespi and Warne 2013). Larger larvae may result in better performing adults (Pough and Kamel 1984, Berven and Chadra 1988, Newman and Dunham 1994, Wells 2007), and large size at metamorphosis and early metamorphosis may increase the probability of immediate (i.e., overwintering) survival and could have forthcoming carry-over effects such as increased post-metamorphic growth rates, larger size at maturity, earlier maturity, and higher reproductive success (Smith 1987, Berven 1990, Goater 1994, Ryser 1996, Altwegg and Reyer 2003). While the case we have made as to why Mainstem metamorphs are smaller is correlative, the data are compelling that their smaller size is related to lower tadpole growth prior to metamorphosis. Modifying temperature can change the relationship between growth and differentiation rates (Bizer 1978, Smith-Gill and Berven 1979, Pandian and Marian 1985). The mechanisms that change rates of tadpole growth and development are extremely complex (Denver 1997) and contradictory results among studies suggests that these processes are likely species- and site-specific. In laboratory experiments, foothill yellow-legged

frog tadpoles hatched from eggs collected at Sierran sites were capable of higher growth rates and more rapid development compared to those from coastal populations (Kupferberg et al. 2013). Additional experimental study would help to further understand temperature effects on embryonic development, tadpole growth and size at metamorphosis of foothill yellow-legged frogs in the Trinity River watershed. There may also be a genetic component that explains variation in development patterns (Berven et al. 1979) or small size may be a population-level characteristic. It is also possible that Mainstem frogs are smaller overall; smaller adult size may indicate impaired growth, a potential long-term consequence of stressors such as altered flow and thermal regimes. In a study of western pond turtles (also an ectothermic species) on the Trinity River, Ashton et al. (2011) found that turtles on the Mainstem were significantly smaller than turtles on the South Fork. Their data indicated that this is a relatively recent phenomenon, possibly linked to the change in temperature regime that has resulted since the installation of the dams (Ashton et al. 2011). It would be of interest to compare foothill yellow-legged adults to determine if frogs from the Mainstem population are smaller than those found on other tributaries.

## Conclusions

The foothill yellow-legged frog population on the Mainstem is extremely small and multiple factors, primarily related to unnatural flow and temperature regimes (Olden and Naiman 2010), are negatively impacting this population. Negative effects of the flow regime on frogs in this river system previously documented include delayed breeding activity and stranding or scouring of egg masses (Lind et al. 1996). Our results provide information regarding differences in populations suggesting that reduced water temperature related to the management of the Mainstem Trinity River is an additional stressor impacting frog population survival and fitness. We suspect that low water temperatures may contribute to delays in breeding activity and persistent cold water temperatures result in decreased larval growth and smaller-sized metamorphs on the Mainstem. Smaller size at metamorphosis likely has profound effects on both individual survival and population fitness. Our data indicate that late breeding activity, loss of egg masses, and inhibited larval growth, in addition to extremely low annual reproductive output, have all contributed to a greatly reduced population size on the Mainstem, factors that will likely continue to suppress the Mainstem population of these frogs and could lead to local extirpation. It would be of interest to know if this cold water regime is having the same negative effects on

the growth and development of juvenile salmonids, species that need to achieve considerable mass prior to out-migration, the relative status of which has profound implications for their likelihood of returning to breed. Slower growth and delayed development due to cold-water dam releases has been observed in other fish species (Clarkson and Childs 2000).

## Management Implications

The Trinity River Restoration Program has strategically modified the release of flows and restored riverine habitat to improve conditions for fish and wildlife species. Specific to foothill yellow-legged frogs, the program's efforts have enhanced breeding habitat through: 1) the regulation of flow releases to mimic naturally occurring winter and spring floods that maintain channel diversity, and 2) "bank feathering" restoration projects that create river bar habitat through riparian re-vegetation and gravel augmentation. In 1994, foothill yellow-legged frogs responded positively to bank restoration by using several of the recently restored sites for reproduction (Lind et al. 1996). However, we did not see a difference in reproductive output on the Mainstem between our study and Lind et al. (1996); the six-year average number of egg masses per kilometer from 2004-2009 was 0.98 and the four-year average number of egg masses per kilometer from 1991-1994 was 1.0 (Lind et al. 1996). Lind et al. (1996) found that the timing of high-flows negatively impacted the Mainstem population. The timing of pulse flows is currently based on average pre-dam flow schedules without any adjustment for annual differences in conditions and consequently the risk of egg mass mortality from scouring or stranding can be potentially high. We found that breeding phenology varied by year and onset of oviposition was related to water temperature. As suggested by Lind et al. (1996), an appropriate management strategy for foothill yellow-legged frogs would be to "base real-time changes in flow releases on current environmental conditions." We also found that timing of breeding, which has been linked to water stage and velocity (Kupferberg 1996a, Wheeler and Welsh 2008), affected size at metamorphosis and late oviposition appeared to have negative consequences on Mainstem tadpoles that were likely intensified by cold-inhibited growth during metamorphosis. Since anadromous fish can no longer access colder tributaries above the dams, the thermal regime of the Mainstem is managed to benefit these cold-water-adapted salmonids; water is intentionally released from the dam to reduce the water temperatures of the river during the summer months. The water in the Mainstem is maintained at an unseasonably colder temperature than naturally would occur during the development period of foothill yellow-legged frog larvae

and the unseasonably low maximum summer temperatures may be nearing the species' lower thermal limit. Managing to optimize the thermal requirements of both salmonids and foothill yellow-legged frogs may be especially challenging and promoting the persistence of foothill yellow-legged frog populations along this section of the Mainstem may require changes in current management. Although the focus of the TRRP mission is to maintain and restore anadromous fish populations and their habitat, a multi-species management approach for all native species, whether culturally and economically significant or not, would be a more enlightened conservation goal for participating agencies (Palmer 2009, Palmer et al. 2010). Continued progress towards creating conditions that mimic historic river flows and geomorphology and applied adaptive management, will improve fisheries and habitat for other native species. Long-term monitoring efforts will provide information required to establish the response of foothill yellow-legged frogs to these restoration efforts and offer insight to understanding growth trends of other aquatic vertebrates in managed systems.

The number of egg masses per kilometer is the longest-term parameter monitored by the Trinity River Restoration Program and was outlined in the Integrated Assessment Plan (TRRP 2009) as a Performance Measure (PM) that could be tracked over time if data were collected every year. This would involve minimal survey effort for the species but would still meet program goals of assessing population changes that could be linked to various flow scenarios. Differences in this metric between tributaries warrant continued monitoring. Specifically, we found that the reproductive output on the Mainstem was extremely low relative to the South Fork and North Fork. We recommend that this PM be implemented by the program every year, if possible, and further suggest that the program apply more focused efforts on monitoring and documenting the fate of egg masses (i.e., record the occurrence and frequency of stranding and scouring) at Mainstem breeding sites, in order to relate survival of this life stage to flow management and temperature conditions. Additional study of tadpole development rate and size at metamorphosis may clarify the mechanisms responsible for differences between tributaries and how these differences are influenced by flow and temperature. This would require additional resources and it is uncertain whether the information gathered would result in practically applicable management strategies for the species. However, future research in this watershed focused on egg mass and tadpole life stages could provide the program with a better understanding of the impacts of managed flow and reduced temperatures on developmental and growth endpoints of the foothill yellow-legged frog.

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Appendix 1. Dates, kilometers of river surveyed, and number of *Rana boylii* egg masses detected during 2004-2009 float surveys on the Mainstem, South Fork and North Fork of the Trinity River, Trinity Co., California. The number of EMs represents a count of new egg masses found on a given survey date. For the Mainstem, values in the "stranded" column represent the number of egg masses that were found desiccated during each survey.

| | Mainstem | | | | South Fork | | | North Fork | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Year | Date(s) | Km | EMs | Stranded | Date | Km | EMs | Date | Km | EMs |
| 2004 | 14-17Jun | 24.17 | 26 | 0 | 9-Jun | 1.81 | 62 | 26-May | 1.18 | 69 |
| 2005 | 9-Jun | 10.36 | 10 | 0 | 10-Jun | 4.79 | 480 | 23-Jun | 1.61 | 72 |
| | 21-Jun | 7.56 | 2 | 0 | 15-Jun | 2.93 | 326 | | | |
| | 28-Jun | 7.04 | 0 | 0 | | | | | | |
| 2006 | 7-8Jun | 10.36 | 10 | 0 | 5-Jun | 3.32 | 198 | 28-Jun | 1.27 | 58 |
| | 29-Jun | 12.96 | 0 | 3 | 14-Jun | 3.56 | 93 | | | |
| 2007 | 24-May | 7.62 | 0 | 0 | 23-May | 3.08 | 510 | No Survey | | |
| | 18-Jun | 7.76 | 7 | 0 | | | | | | |
| 2008 | 4-Jun | 21.19 | 1 | 0 | 22-May | 6.5 | 810 | 6-Jun | 1.35 | 53 |
| | 18-Jun | 21.19 | 9 | 2 | | | | | | |
| | 2-Jul | 21.19 | 9 | 6 | | | | | | |
| | 17-Jul | 21.19 | 0 | 5 | | | | | | |
| 2009 | 27-29May | 38.68 | 4 | 0 | 29-May | 2.9 | 441 | 9-Jun | 1.41 | 117 |
| | 8-12June | 38.68 | 26 | 2 | | | | | | |
| | 22-25June | 38.68 | 18 | 12 | | | | | | |
| | 1-2July | 28.4 | 2 | 1 | | | | | | |