THE IMPORTANCE OF WATER TEMPERATURE AND ALGAL ASSEMBLAGE FOR FROG CONSERVATION IN NORTHERN CALIFORNIA RIVERS WITH HYDROELECTRIC PROJECTS
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ACKNOWLEDGEMENTS

The authors would like to thank S. Becerra-Munoz, A. Campbell-Craven, P. Furey, R. Hulme, R. Peek, and A. Smits for field and laboratory assistance. We thank Craig Addley and Placer County Water Agency for sharing data from the upper American River system; Scott McBain and Bill Sears for data from the Tuolumne River (San Francisco Public Utilities Commission); Don Ashton, Jamie Bettaso, Clara Wheeler, Garth Hodgson, and Hart Welsh, for data and float trips on the Trinity River. We appreciate the use of the Angelo Reserve of the University of California Natural Reserve System and thank P. Steel and C. Bode for maintaining the long term hydrologic monitoring of the South Fork Eel River. This research was supported and funded by the Public Interest Energy Research Program of the California Energy Commission.
PREFACE

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The Importance of Water Temperature and Algal Assemblage for Frog Conservation in Northern California Rivers with Hydroelectric Projects is the final report for the The Importance of Water Temperature and Algal Assemblage for Frog Conservation in Northern California Rivers with Hydroelectric project (contract number 500-08-031) conducted by [research entity]. The information from this project contributes to Energy Research and Development Division’s Energy-Related Environmental Research Program.

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Multiple stressors such as altered flow regimes and habitat fragmentation associated with water storage and hydroelectric power generation have contributed to the decline of foothill yellow-legged frogs (*Rana boylii*) in California. One potential mechanism contributing to its decline is the effect of altered thermal conditions on development and survival of early life stages. Thermal conditions measured in six focal watersheds with known *Rana boylii* populations suggest that maximum average temperature during the warmest 30-day period is a useful metric for characterizing the period critical for successful maturation of tadpoles into frogs. Comparisons among sites occupied by frogs and cooler sites further upstream where frogs were sparse or absent demonstrated that thermal conditions for successful reproduction differed between the Coast and the Sierra. Observations averaged over 2009 (a warm, dry year) and 2010 (a cooler, wetter year) indicate that the realized thermal niche for tadpoles was approximately two degrees centigrade higher in the Sierra, and that frog breeding was absent where the warmest 30-day average water temperatures was below 17.6 degrees centigrade (°C) in the Sierra. Laboratory experiments showed that when raised at a mean daily temperature of 19.5 °C, tadpoles reared from eggs collected at Sierran sites exhibited a capacity for higher growth and faster development than those from coastal populations, but not at 16.5 °C. Experiments manipulating food resources along with temperature showed that thermal effects on tadpole digestion, growth and survival were mediated through the quantity and quality of algal food, especially protein content. Algal foods downstream of dams operated for peak power generation were not conducive to tadpole growth. Conservation of *Rana boylii* in rivers with hydroelectric power generation projects will depend on management that maintains sufficiently warm water during the breeding and tadpole rearing seasons. Forecasts of changes in water temperature could be extended to predict consequences for frog populations when alternative flow schedules were being evaluated.

**Key Words:** amphibian declines, Angelo Reserve, climate change, hydropower, thermoregulation

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EXECUTIVE SUMMARY

Introduction
Construction of dams and operation of hydropower projects in many California rivers have resulted in population declines and local extinction for native aquatic species. The foothill yellow-legged frog (*Rana boylii*) exemplifies this phenomenon, having declined dramatically over the last half century. Dam-related stressors include untimely pulsed releases of water that cause mortality to early life stages, altered channel morphology that diminishes habitat suitability, genetic isolation caused by reservoirs as barriers to movement, non-native predators and altered thermal regimes. Temperatures can be either raised or lowered by dam operation compared to the free-flowing condition. In many cases the retention of water behind a dam results in artificially low summer base flow discharges, causing unnaturally warm downstream temperatures. Water is drawn from the dense bottom layer of the upstream reservoir when summer base flows are increased and outfalls are at low elevation on a dam, and the resulting temperatures downstream are colder than they would be naturally.

Project Purpose
The purpose of this project was to evaluate the range of thermal conditions and the consequences for frogs in six watersheds of northern California (three Sierran, two North Coast and one Central Coast) and to make comparisons between paired rivers with and without dams in each watershed.

Project Results
Temperature monitoring and field surveys of frog breeding downstream of dams and in reference rivers without dams showed that reproductive populations were absent when summer maximum 30-day average temperatures (M30DAT) were less than 17.6 degrees centigrade (°C) in the Sierra. Dense Sierran populations occurred when M30DAT was between 20.3°C and 24.2°C when researchers averaged conditions between a dry and wet year (2009 and 2010, respectively). Maximum population abundance occurred at 19.7°C in the Eel watershed, which had a coastal climate. The lower thermal limit of abundant populations among coastal locations was approximately 18.8°C; although a gap in sampled sites with temperature in the 17-18.8°C range existed.

The density of breeding frogs was less than five kilometers (km) where M30DAT was less than 20°C in the 3 focal Sierran watersheds with hydropower projects (Feather, American, Tuolumne). Thermal differences between unregulated and regulated rivers at similar elevations varied among the watersheds, but frog densities were lower when the regulated rivers were cooler when compared to the unregulated rivers. There was uncertainty about the long-term viability of populations in locations where temperatures were at the lower thermal limit of distribution. Small populations where temperatures are relatively low have been reported elsewhere, such as one near Wolf Creek on the Middle Yuba River where M30DAT was 19.3°C and on the McCloud River where 2008 M30DAT was 16.5°C. Small populations may not be sustainable when abiotic, biotic, or anthropogenic stressors such as cooler temperatures and mortality due to untimely flow fluctuations decrease recruitment. Sites in regulated rivers
where breeding density is approximately two clutches/km (such as in the NF Feather River Cresta reach and Alameda Creek below Calaveras Dam) appeared to be in strong demographic decline (Kupferberg et al. 2012). An empirically derived viability threshold needs to be developed by sustained monitoring of low density populations.

In controlled laboratory thermal gradients the average temperatures selected \( T_{sel} \), and middle 50 percent of temperatures \( T_{set} \) occupied by tadpoles were plastic traits, but mirrored the range of summer temperatures at occupied sites. \( T_{sel} \) and \( T_{set} \) varied with the temperature of the rearing environment. \( T_{sel} \) ranged from 18.5°C to 23.1°C, and \( T_{set} \) ranges varied from 16.5-22.2°C to 21.0-25.8°C depending on the stream or laboratory environment where tadpoles were raised. There was some evidence of a counter-gradient response, with tadpoles from cooler environments selecting warmer temperatures. When tadpoles from diverse origins (with dams, without dams, Sierran and Coastal) were reared under common conditions there were no significant differences in temperature preference.

Selecting warm temperatures that are optimal for consumption rate, food conversion efficiency, metabolism, and growth is important across a taxonomically diverse spectrum of vertebrate aquatic ectotherms that are herbivorous, including larval amphibians. The benefits of operating at temperatures near an organism’s thermal preference included many physiological and biochemical processes and these benefits may be greater in rapidly developing stages such as tadpoles, as compared to adults. Tadpoles of \( R. boylii \) are scrapers of periphyton, consuming filamentous algae and diatoms. Researchers investigated the interactive effects of algal food quality and temperature to understand the processes creating the distribution patterns they observed in the rivers with and without hydropower projects.

Survival was highest at 19.9°C when tadpoles were reared in streams that had maximum M30DAT colder (16.06°, 16.9°), warmer (21.8°), or similar (19.9°) to the temperature associated with peak population abundance (19.7°). Growth and development were most rapid when M30DAT equaled 21.8°C. There was a humped shaped response curve with the greatest production (average mass at metamorphosis x survival rate) when M30DAT equaled 19.94°. At the warmest site M30DAT equaled 21.8°C. Metamorphosis began four to five weeks sooner and tadpoles were larger at this site, but because of high mortality during the warmest period the total production was slightly lower. The causes of mortality in the experiment were not identified, but researchers believed it was not due to reaching a critical thermal maximum as tadpoles have been observed to successfully metamorphose from isolated side pools where large diurnal fluctuations in temperature exist and daily maxima can reach 30°C.

Laboratory experiments defined the lower threshold temperature at which development and growth did not occur. Although embryos and pre-metamorphic tadpoles develop and grow at relatively cool temperatures typical in the spring, warmer summer temperatures are required by later developmental stages. In growth chambers, researchers reared tadpoles from the Middle Fork American River on diets of periphyton-covered rocks from: (1) a power-peaking reach of the Middle Fork American where the low food quality invasive diatom \( Didymosphenia geminata \) was dominant; (2) the Rock Creek reach of the North Fork Feather River where native mucilaginous diatoms dominated the flora; and (3) an unregulated stream with high food-

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quality periphyton. Food sources were crossed with temperature, cold (16.3˚C daily mean) or matching tadpole thermal preference (20˚C). Under cold conditions tadpoles gleaned little food and did not grow, even on high food quality algae. At 20˚C tadpoles did not grow on the periphyton from the regulated rivers. They ingested *Didymosphenia* dominated periphyton at a rate similar to tadpoles consuming high food quality periphyton, but 72-hour relative weight gain was 4.3 ± 5.4 percent vs. 30.7 ± 3.4 percent for controls. Weight loss was 21.0 ± 9.2 (cold) and 16.6 ± 5.6 percent (warm) for the hydropower river site dominated by native stalked muclaginous diatoms. These results indicated the importance of food resources to define the realized thermal niche of this species.

Temperature also affected susceptibility to predators and parasites. The smaller size of tadpoles at colder temperatures increased their risk of predation by macroinvertebrates in field experiments. At warm locations non-native predators such as bass and bullfrogs were more likely to be present. Some parasites such as the copepod *Lernaea cyprinacea* became prevalent with warm summer temperatures (Kupferberg et al. 2009b). At the unregulated Clavey River sites where M30DAT was greater than or equal to 24.0˚C copepod infection prevalence was 42 percent compared to only one infected individual in the cooler regulated Rubicon River.

Conservation of *R. boylii* in rivers with hydroelectric power generation projects will depend on operations and flow schedules that maintain sufficiently warm water during the breeding and tadpole rearing seasons. Frogs may be present where summer temperatures do not reach preferred temperatures for prolonged periods but robust populations were limited to river reaches where the average temperature for the warmest thirty-day period of the summer was near 20˚C, which promoted rapid growth and development of larvae. The effects of temperature were mediated by the quality of algal food resources available. In regulated rivers where flows fluctuated daily and temperatures were artificially low, the periphyton that flourished provided poor food for grazing tadpoles. When temperatures were cooler than the optimal range, tadpoles were exposed to increased risk of predation by virtue of their small size.

Researchers made the following recommendations based on the results of this study:

- Promote mixing of cold hypolimnetic water with warmer surface water leaving a reservoir via spillways or higher elevation gates where possible.
- Water management entities need to recognize synergisms among various hydrologic changes, such as power peaking, that lead to growth of unpalatable periphyton, which then have consequences for grazing consumers.
- Gather data on thermal conditions at channel margins where amphibians breed as well the thalweg (deepest part of the channel) during Federal Energy Regulatory Commission (FERC) relicensing and adaptive management.
- Improve long-term monitoring programs for *Rana boylii* in both regulated and unregulated (reference) river systems, especially in the Sierra Nevada to determine minimum viable population size.
Appropriate state and/or federal agencies should elevate the protection status of *Rana boylii* so that it is listed under the California or Federal Endangered Species Act.

**Project Benefits**

This research will be valuable to the adaptive management of regulated rivers in terms of evaluating the relative merits of competing flow proposals. There are often discussions of the effects of proposed flows on the quantitative area of habitat available to native aquatic species during FERC relicensing of hydropower projects. Competing flow proposals will also differ with respect to thermal conditions and the periphyton community assembling downstream of the dam. This research provided a template for assessing these impacts in terms of both habitat quality and habitat quantity. Assessing thermal effects on growth and survival of early life stages of *Rana boylii* could help power plant managers determine if their actions may put populations at further risk or aid in conservation.
CHAPTER 1:
Research Problem Statement

1.1 Consequences of Hydropower Projects for Water Temperature and Frog Conservation

Hydrologic alteration has reduced the abundance and imperiled the status of a wide array of riverine species (Richter et al. 1997; Rosenberg et al. 2000; Bunn and Arthington 2002). *Rana Boylii*, the foothill yellow-legged frog, exemplifies this phenomenon, having declined dramatically over the last half century, especially in Southern California and the Southern Sierra Nevada mountains. It is absent from over 50 percent of known historic sites (Davidson et al. 2002, Lind 2005) and is a California Species of Special Concern (Jennings and Hayes 1994; Jennings 1996; California Department of Fish and Game 2008). Absences from historic localities are more common in close proximity to large dams (Lind 2005). During California’s era of hydroelectric dam construction 30-40 years ago, habitats were destroyed and fragmented as river channels were converted to reservoirs. The populations that persist are small relative to those in unregulated rivers (Kupferberg et al. 2012). Long term population viability in this species is sensitive to the survival of early life stages (Kupferberg et al. 2009a). Here we investigate how the growth and survival early life stages (i.e. embryos and tadpoles) are affected by the thermal environment and how thermal suitability compares between rivers with hydropower projects and unregulated rivers in northern California.

There is uncertainty about how to predict the effects of thermal change on specific organisms (Walther et al. 2002). Niche-based bioclimatic envelope models, built upon correlations between current geographic patterns of presence-absence and an organism’s physiological tolerances, can simulate the future distribution of suitable climate space for a species (Peterson 2001; Hijmans and Graham 2006), but have limitations (Davis et al. 1998; Loiselle et al. 2003). These models often treat species as units and are not based on hypotheses about individual behavioral or community-level responses to climate stressors that can be tested with manipulative experiments. These responses, such as phenological shifts in breeding timing (Both et al. 2009; Bauer et al. 2010) or interactions with food resources (Pelini et al. 2009), can have substantial influence on birth and death rates, and unexpected responses have been shown to reverse scenarios based on climate envelope models alone (Davis et al. 1998). To design appropriate management and conservation programs for frogs and other species in California’s regulated rivers, aquatic ecologists thus need to document how birth and death rates of focal organisms are influenced by water temperature within the context of biotic factors such as spatial heterogeneity in primary productivity and predator distribution.

Temperatures can be either raised or lowered by dam operation compared to the free-flowing condition. In many cases, the retention of water behind a dam results in artificially low summer base flow discharges, causing unnaturally warm downstream temperatures. Alternatively, when summer base flows are increased and outfalls are at low elevation on a dam, water is drawn from the hypolimnion of the upstream reservoir and the resulting temperatures downstream are colder than they would be naturally. Negative effects of cooling on native fish
adapted to warm summer temperatures (Klimley et al. 2010) and other vertebrate ectotherms, such as the Western Pond Turtle (Betasso et al. 2006) have been documented, but the extent of impacts on *R. Boylii* has not been investigated. Although adult frogs spend much time in unregulated tributaries, unaffected by upstream reservoirs, they travel to regulated main-stem channels to deposit eggs in shallow slow habitats near water’s edge. There could be direct effects of cooler temperature by retarding tadpole growth and development and thus causing negative outcomes for two measures of frog fitness, time to and size at metamorphosis. There could also be indirect effects of flow regime decreasing tadpole algal food resources (Wootton et al. 1996, Power et al. 2008) and predators, some of which might be more resistant to temperature variation than tadpoles. Thus, any potential benefit from inundating a greater quantity of habitat should be compared to possible detrimental effects caused by diminished habitat quality with respect to temperature and food availability.

In several hydroelectric projects, water temperature is a primary concern. For example in Butte Creek, one part of PG&E’s DeSabla Project, there is intense pressure from stakeholders to mitigate the effects of warm water on spring run Chinook salmon (American Whitewater 2008, California Sportfishing Alliance 2008). While changing flow regime and reservoir operations to reduce summer temperatures for the purpose of protecting and restoring populations of anadromous fish is a laudable goal, the potential effects on other aquatic vertebrate ectotherm taxa also need to be evaluated as part of the complex balancing of multiple management objectives (i.e. power generation, resource conservation, and recreation). As part of a FERC license application, temperature models are produced to predict thermal conditions across a range of discharges and distances downstream of a dam. To date there have been no methods to interpret these forecasts in a meaningful way for frogs because of two major information gaps. First, the models used are one dimensional, providing information about how a parcel of water released from a reservoir will warm as it moves downstream through the thalweg, or deepest part of the channel. However, tadpoles, like fish fry, are mobile in a second dimension moving laterally between the shallow channel margins and slightly deeper water. The objective of the work presented in section 2 of this report is to evaluate the use of thalweg temperatures as a predictive measure of suitability for frog thermal habitat suitability. The objective of the work presented in sections 3 and 4 is to quantify the relationship between water temperature and factors influencing the recruitment of new frogs to populations of *R. Boylii*. We focused on survival to hatching, survival to metamorphosis, growth, and developmental rate.

### 1.2 *Rana Boylii* Life Cycle and the Importance of Thermal Conditions to Recruitment

The natural history of *R. Boylii* illustrates that this species can provide a model system for field studies examining the interactive effects of water temperature and resource availability. Distribution within drainage networks is correlated with maximum weekly averages of summer water temperature (Welsh et al. 2005) and previous experiments have shown that access to nutritious and diatom rich food sources which are high in protein and lipids (Kupferberg et al. 1994) enhances development and growth of tadpoles of *R. Boylii* (Kupferberg 1997a). In addition to water temperature, flow regime influences periphyton assemblage (Wootton et al. 1996, Power et al. 2008).
1996; Wu et al. 2009), which in turn is a factor determining survival of tadpoles that graze algae (Alvarez and Nicieza 2002). Although our understanding of the feeding ecology of many tadpole species is still limited (Altig et al. 2007; Schiesari et al. 2009), the importance of resources is not surprising given that most of the body of a tadpole is made of organs involved in the gathering and processing of food (Viertel and Richter 1999). Therefore, we hypothesize that effects of temperature on tadpole survival are likely mediated by the thermal sensitivity of developing tadpoles as well as their feeding ecology.

*Rana Boylii* adults spend much of their time in tributaries (both permanent and ephemeral) but travel to mainstem channels to mate at lek sites and deposit eggs in shallow slowly moving water (Kupferberg 1996). To avoid disturbance and optimize feeding by larvae, the timing of reproduction occurs in synchrony with the seasonality of runoff and algal blooms (Power et al. 2008) during the predictable cycle of wet winters and dry summers typical across the range of *R. Boylii*. Specifically, the period of fastest tadpole growth coincides with the bloom of epiphytic nitrogen-fixing diatoms. The distribution of these algal blooms along the stream network is regulated by abiotic and biotic factors that scale with watershed size (Finlay 2004; Power et al. 2008). For example, small tributaries have dense canopies which limit the light available for primary producers whereas broad channels further down the drainage network are broad and sun-lit.

To understand the response of *R. Boylii* to thermal regime change, we integrated investigations conducted across several spatio-temporal scales. We combined studies of short-term individual thermoregulatory behavior, field manipulations of water temperature and food resources from embryonic development through metamorphosis, with censuses of breeding density throughout drainage networks. We assessed whether thermoregulatory behavior by tadpoles could be used to predict the effects of temperature on survival to metamorphosis. Using a metric developed by Hertz et al. (1993), we quantified the thermal quality of the tadpole rearing environment as the difference between water temperature where we raised tadpoles in flow-through enclosures and the temperatures selected by tadpoles in a thermal gradient. We tested the hypothesis that the effects of water temperature were mediated by food quality by rearing tadpoles across a gradient of cool to warm stream temperatures and low to high primary productivity, with or without food supplementation. Finally, we tested whether stream temperatures causing larval mortality were consistent with broad-scale spatial patterns of adult frog population density in free-flowing watersheds and compared the thermal quality of the unregulated reference river systems to that of other branches of the same river regulated by an upstream dam and reservoir.

### 1.3 Project Objectives and Report Organization

The purpose of this project is to investigate the role of water temperature changes caused by upstream dams in contributing to patterns of frog decline associated with river regulation.

Thermal regime effects were examined by: (1) monitoring temperature at paired regulated and unregulated rivers in six watersheds; (2) determining the thermal preferences of tadpoles in the
field and laboratory; (3) manipulating the thermal environment of embryos and tadpoles. The specific objectives are:

Section 2: Temperature Monitoring

- Define the realized thermal niche of *R. Boylii*
- Compare temperatures at precise tadpole locations in channel margins to thalweg temperatures

Section 3 and 4: Interactive Effects of Temperature and Algal Food Resources on Embryos and Tadpoles of *R. Boylii*

- Evaluate the effects of water temperature on digestive assimilation efficiency and food consumption rate
- Evaluate effects of water temperature on growth, survival, and risk of predation
- Evaluate the effects of algal assemblages associated with hydropower on tadpoles as grazers.

Section 5: Conclusions and Recommendations

- Discuss and synthesize conclusions based on sections 2-4.
- Recommend future research and direction for FERC studies with the aim of integrating knowledge of temperature effects with other hydrologic factors known to influence frog populations.
- Address commercialization potential
- Assess benefits to California
CHAPTER 2: Temperature Monitoring and Thermal Preference of *Rana Boylii*

A preliminary step to compiling thermal data on a spatial template was to compile spatial distribution of *R. Boylii* populations in rivers with hydropower projects. Beginning with a database of all FERC licensed projects in California, we assessed if *R. Boylii* had been observed in each project during the course of re-licensing or monitoring surveys and whether the location of the project was within the historic range of the species. We used unpublished reports and contacted the consulting firms or biologists employed by the licensees who conducted surveys when reports were ambiguous about the presence or absence of any life stage of the frogs. For the FERC projects where we found *R. Boylii* data, we developed a map (Fig. 1) depicting the stark contrast between northern and southern parts of the range, with frogs absent in regulated rivers south of the Tuolumne River drainage.

![Figure 1: Presence (Yellow Dots) or Absence (Black Dots) of *Rana Boylii* in California Hydroelectric Projects Licensed by the Federal Energy and Regulatory Commission (FERC).](image)

For a subset of the projects represented in Fig. 1, data were sufficient to make comparisons of population abundance as well as presence / absence. We hypothesized that *R. Boylii* populations would be smaller in rivers with dams given the knowledge that regulated river flow regimes disproportionately affect early life stages and recruitment to the adult population (Kupferberg et al. 2012). Secondarily, we hypothesized that populations from watersheds in which peak seasonal flow is driven primarily by winter rainfall (coastal) would be larger compared to those from watersheds driven by spring snowmelt (Sierran). To evaluate this pattern in California we compiled abundance data for 27 *R. Boylii* populations collected by
ourselves, other researchers, utilities, and government agencies (Table 1). We used the number of egg masses per kilometre as an index of abundance because female ranid frogs lay one discrete clutch of eggs per year and this metric is commonly used for this group of frogs (Crouch and Patton 2002, Petranka et al. 2007). We used data from known breeding locations and required that these data meet minimum criteria for accepted survey methods and timing for *R. Boylii*, e.g. multiple visits per breeding season. Abundance data from unpublished reports (e.g. from recent hydropower relicensing) were not included in our evaluation if survey methodology could not be determined, when methods did not follow accepted standards, or where egg masses were not found. We compared the mean egg mass density (egg masses∙km⁻¹) as an index of adult female population abundance of coastal and Sierran populations in regulated and unregulated rivers with a 2-way ANOVA. Egg mass counts are commonly used as an index of population size for ranid frogs that produce only one clutch per year.

Among the *R. Boylii* populations for which egg mass survey data were available (Table 1), populations occur at 4-5 times lower density in rivers where hydrology is regulated by upstream dams than those in naturally free-flowing rivers (2-way ANOVA of ln(egg masses∙km⁻¹), $F_{1,23}=19.9, P<0.001$). The hypothesis that populations in coastal watersheds with rain-driven hydrographs are more dense than those in the Sierra with snowmelt driven hydrographs was not supported ($F_{1,23}=0.004, P=0.95$). The interaction between flow regime and location was not significant ($F_{1,23}=3.1, P=0.09$).

Table 1: Summary of Average Annual Clutch Density from California Populations of *Rana Boylii* Garnered from Censuses Conducted by the Authors of the Current Study, the Literature, and Utility Reports.

<table>
<thead>
<tr>
<th>Watershed (Upstream Dam or Reach Name*)</th>
<th>Mean # (clutches/km/year)</th>
<th>SE</th>
<th>Length (km)</th>
<th>Years</th>
<th>Data Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulated Coastal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trinity (Lewiston Dam)</td>
<td>0.45</td>
<td>0.17</td>
<td>22.5, 33.3</td>
<td>1991-1994, 2004-2006</td>
<td>Lind 2005; Ashton, Bettaso, and Welsh unpublished data</td>
</tr>
<tr>
<td>Eel (Scott Dam)</td>
<td>2.9</td>
<td>--</td>
<td>12.8</td>
<td>2010</td>
<td>this study</td>
</tr>
<tr>
<td>Alameda (Calaveras Dam)</td>
<td>3.7</td>
<td>1.6</td>
<td>1.9</td>
<td>2003-2010</td>
<td>Bobzein and DiDonato 2007</td>
</tr>
<tr>
<td>Regulated Sierran</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF of NF American (Lake Valley Canal Diversion Dam)</td>
<td>0.5</td>
<td>--</td>
<td>4</td>
<td>2008</td>
<td>Nevada Irrigation District and PG&amp;E, 2010</td>
</tr>
<tr>
<td>MF American (French Meadows Dam)</td>
<td>0.65</td>
<td>--</td>
<td>3.1</td>
<td>2007</td>
<td>Placer County Water Agency 2011</td>
</tr>
<tr>
<td>McCloud (McCloud Dam)</td>
<td>1.23</td>
<td>--</td>
<td>9.73</td>
<td>2008</td>
<td>PG&amp;E 2009</td>
</tr>
<tr>
<td>SF Yuba (Spaulding Dam)</td>
<td>1.9</td>
<td>--</td>
<td>10</td>
<td>2008</td>
<td>Nevada Irrigation District and PG&amp;E, 2010</td>
</tr>
<tr>
<td>Watershed (Upstream Dam or Reach Name*)</td>
<td>Mean # (clutches/km/year)</td>
<td>SE</td>
<td>Length (km)</td>
<td>Years</td>
<td>Data Source</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>--------------------------</td>
<td>----</td>
<td>-------------</td>
<td>-------</td>
<td>-------------</td>
</tr>
<tr>
<td>NF Feather (Cresta Dam)</td>
<td>2.1</td>
<td>0.4</td>
<td>7.6</td>
<td>2002-2010</td>
<td>PG&amp;E 2010</td>
</tr>
<tr>
<td>Butte Creek (Forks of Butte Diversion)</td>
<td>4.1</td>
<td>--</td>
<td>1.9</td>
<td>2006</td>
<td>PG&amp;E 2007</td>
</tr>
<tr>
<td>MF Stanislaus (Sand Bar Dam)</td>
<td>6.2</td>
<td>3.6</td>
<td>1.0</td>
<td>2001-2003</td>
<td>PG&amp;E 2004a</td>
</tr>
<tr>
<td>Pit (Pit 4 Dam)</td>
<td>8</td>
<td>2.7</td>
<td>7</td>
<td>2002-2005</td>
<td>PG&amp;E 2004b, Ellis pers. comm</td>
</tr>
<tr>
<td>Butte Creek (Centerville Dam)</td>
<td>9.1</td>
<td>--</td>
<td>5.9</td>
<td>2006</td>
<td>PG&amp;E 2007</td>
</tr>
<tr>
<td>Rubicon (Hell Hole Dam)</td>
<td>9.2</td>
<td>--</td>
<td>7.9</td>
<td>2007</td>
<td>Placer County Water Agency 2011</td>
</tr>
<tr>
<td>NF Feather (Poe Dam)</td>
<td>10.5</td>
<td>1.7</td>
<td>8.3</td>
<td>2001-2010</td>
<td>PG&amp;E 2010</td>
</tr>
<tr>
<td>MF Yuba (Milton Diversion Dam)</td>
<td>13</td>
<td>--</td>
<td>4</td>
<td>2008</td>
<td>Nevada Irrigation District and PG&amp;E, 2010</td>
</tr>
<tr>
<td>W Br. Feather (Hendricks Head Dam)</td>
<td>15.1</td>
<td>--</td>
<td>3.4</td>
<td>2006</td>
<td>PG&amp;E 2007</td>
</tr>
</tbody>
</table>

Unregulated Coastal

| Coyote (US Coyote Lake)                | 11.2                     | --  | 7.8         | 2004-2005 | Gonsolin 2010 |
| Eel (Ten Mile Ck)                      | 12.3                     | 2.6 | 4           | 1993-2003, 2008-2010 | Kupferberg 1996, and unpub. data |
| Alameda (Camp Ohlone)                  | 21.9                     | 4.3 | 1.6         | 1997-2010 | Bobzein and DiDonato 2007 |
| Trinity (SF Trinity)                   | 69.9                     | 22.5| 15.6, 5.9   | 1992-1994, 2004-2006 | Lind 2005; Ashton, Bettaso, Welsh unpub. data |
| Eel (SF Eel)                           | 105.7                    | 6.5 | 5.2         | 1992-2010 | Kupferberg 1996 |

Unregulated Sierran

| San Joaquin (Jose Ck.)                 | 4.6                      | --  | 1.2         | 1995, 2002 | Lind et al. 2003 |
| Tuolumne (NF Tuolumne)                | 9                        | --  | 0.3         | 2001      | Lind et al. 2003 |
| Yuba (Shady Ck.)                      | 14.4                     | --  | 3.2         | 2003      | Yarnell 2005    |
| Stanislaus (Rose Ck.)                 | 29                       | --  | 0.65        | 2001      | Lind et al. 2003 |
| Yuba (Clear Ck.)                      | 29                       | 9.5 | 0.82        | 1992-1994 | Van Wagner 1996 |

* Abbreviations: BR = Branch, MF = Middle Fork, NF = North Fork, SF = South Fork. Populations are sorted from lowest to highest density within region and flow type.
2.1 The Thermal Niche of Rana Boylii in Focal Watersheds: Alameda, Eel, Feather, American, Trinity, Tuolumne

We present the idea of thermal niche to provide a framework for summarizing the thermal ecology of *R. Boylii* in the regulated rivers where it remains. Hutchinson (1957) differentiated the fundamental niche, the multidimensional environmental space where a species could exist, from the realized niche, the subset of this space where the species actually coexists in a community. For ectothermic aquatic vertebrates, temperature is a resource that defines a central axis of the fundamental niche (Magnuson et al. 1979). The fundamental thermal niche is physiologically bounded, controlled at the extremes by low and high lethal temperatures, and more narrowly by the temperatures above and below which growth and reproduction do not occur (even with optimal rations). A species’ field distribution, however, may, or may not, be consistent with preferred temperatures and performance. The realized thermal niche in rivers is determined by both abiotic and biotic factors such as food resource abundance, predator avoidance, intra- and interspecific competition, and the availability of suitable habitat such as appropriate water depth and velocity (Shrode et al. 1982).

To estimate the thermal niche for *Rana Boylii*, we collected data within six diverse California watersheds where reproductive populations are known in both regulated and unregulated rivers of the watersheds (Table 2, Fig. 2). Each watershed has hydrologic, geomorphic and habitat characteristics typical of *R. Boylii* localities in its respective region, the north coast, the Sierra Nevada, and the central coast. These bioregions correspond with three genetically distinct clades, or branches, in the evolutionary tree developed for *R. Boylii* (Lind 2005). These locations were chosen because they have extant frog populations and gaging stations with historic hydrologic records. Some of the regulated rivers of the pairs also have extensive past monitoring of thermal conditions and frog populations, such as the Trinity River, Ashton et al. 2010, Scheiff and Zedonis 2011). In the American River watershed, the Placer County Water Agency (PCWA) has done extensive frog surveys, temperature monitoring and modeling on the Rubicon, Middle Fork American, North Fork Middle Fork American, and various unregulated tributaries (PCWA 2010, 2011). In the North Fork Feather River Pacific Gas and Electric (Garcia and Associates have monitored frog populations and temperature. We have incorporated those data into our analyses.
Table 2: Characteristics of the Focal River Systems for Thermal Monitoring.

| Watershed       | Reach and Nearest USGS Gage # or CDEC code | Regulation               | Drainage Area
|                 |                                             | (km²)         | Mean Annual Discharge
<table>
<thead>
<tr>
<th></th>
<th></th>
<th>(cms)</th>
<th>Elevation range of thermisters (m)</th>
<th>Hydrology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trinity</td>
<td>Mainstem Trinity 11526400</td>
<td>Lewiston Dam</td>
<td>1,137</td>
<td>33.7</td>
</tr>
<tr>
<td></td>
<td>North Fork Trinity 11526400</td>
<td>none</td>
<td>2933</td>
<td>44.1</td>
</tr>
<tr>
<td>Feather</td>
<td>North Fork Feather</td>
<td>Cresta Dam Poe Dam</td>
<td>4976 5078</td>
<td>22.5</td>
</tr>
<tr>
<td></td>
<td>Spanish Creek SPK, 1140200</td>
<td>none</td>
<td>475</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Middle Fork Feather MER</td>
<td>none</td>
<td>2305</td>
<td>39.1</td>
</tr>
<tr>
<td>Tuolumne</td>
<td>Tuolumne above Early Intake 11276600 Downstream of Hetch Hetchy 11276500</td>
<td>O'Shaugnessy Dam</td>
<td>1253</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>Clavey 11283500</td>
<td>none</td>
<td>373</td>
<td>7.1</td>
</tr>
<tr>
<td>American</td>
<td>Rubicon 11428800</td>
<td>Hellhole Dam</td>
<td>295</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>North Fork of the Middle Fork American 11433260</td>
<td>none</td>
<td>239</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>Middle Fork American 11433360</td>
<td>French Meadows, Hell Hole, Loon Lake and others</td>
<td>1357</td>
<td>33.2</td>
</tr>
<tr>
<td>Eel</td>
<td>Mainstem Eel ELP</td>
<td>Scott Dam</td>
<td>750</td>
<td>520-456</td>
</tr>
<tr>
<td></td>
<td>South Fork Eel 11475500</td>
<td>none</td>
<td>114</td>
<td>4.9</td>
</tr>
<tr>
<td>Alameda</td>
<td>Sunol 11173510</td>
<td>Calaveras Dam</td>
<td>273</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Camp Ohlone 11172945</td>
<td>none</td>
<td>88</td>
<td>0.77</td>
</tr>
</tbody>
</table>

1 Drainage area upstream of the gaging station

The flow and water temperature differences between the paired regulated and unregulated river reaches during spring and summer are distinct. For the two rain driven systems, Eel and Alameda, peak discharges occur in winter with occasional spring spates caused by rainfall. The regulated reaches have periodic late spring and summer pulsed flows that are aseasonal. For the snow melt systems, the NF Feather, the Trinity, and the Early Intake reach of the Tuolumne have large magnitude peak flows during winter and spring in wet years when the dams spill at the peak of snowmelt and when rain on snow events occur. Discharge is steady during the summer and fall, unless pulsed flows are manufactured. The Alameda Creek regulated reach has reduced magnitude base flows, with occasional disproportionately high-magnitude peaks associated with flood spills and plateaus associated with continued releases. Since 2001, due to seismic safety concerns about Calaveras Dam, the maximum allowable reservoir height is 40 percent of capacity. Water is released to maintain that level (SFPUC 2007). By comparison, the unregulated Camp Ohlone reach on Alameda Creek has pulse magnitudes and durations directly coupled to rainfall, and in low precipitation years the creek dries to intermittent pools by late summer.
2.2 Temperature Monitoring

2.2.1 Thermal Transect Methods.
We built housings and placed i-button thermocron thermistors (Fig. 3) along transects to capture the thermal heterogeneity of riverine habitats occupied by *Rana Boylii* tadpoles. In rivers, thermal heterogeneity exists on several scales. Longitudinal variation occurs as water moves downstream through channels of increasing width, as it is warmed primarily by exposure to sun. Laterally there is warming at channel margins where water is shallower and velocities are slower (Fig. 4). Deep pools can have thermal stratification with depth. There is also variation in time, diurnally as well as seasonally. Despite this variation, observations of temperatures across the channel and tadpole thermoregulatory behavior (Section 2.3) reveal that appropriately placed fixed sensors can adequately describe temperatures experienced by tadpoles. Additionally, the results of tadpole rearing experiments (Section 3), reveal that the maximum 30 day average water temperature (M30DAT hereafter) is a useful metric for predicting the growth and survival of tadpoles. Thus, we use this metric to compare the thermal conditions along elevational/longitudinal gradients in the study watersheds.

**Figure 3: Temperature Sensor Flow-Through Housings Made from P.V.C. Pipe, Cabled around Boulders or Anchored in the River Bed with Rebar.**
2.2.2 Results: Realized Thermal Niche

Among the 6 focal watersheds, the realized thermal niche for successful reproduction in *Rana Boylii* appears to differ between the watersheds of the North and Central Coast and watersheds of the Sierra (Fig. 5). For the total set of regulated and unregulated locations where we collected thermal data, averaging observations from 2009 (lower flow / warm year) and 2010 (cooler wetter year with higher flows,) we observed a higher range of M30DAT for occupied sites in the Sierra, 17.6–24.2°C, than in Coast Range / Cascade rivers, 15.7–22.0°C. Populations were mostly concentrated between 200 and 800 m elevation. The densest Sierran populations in the regulated rivers we studied were in reaches where M30DAT were greater than 20°C.

For many of our temperature monitoring locations, the point bar channel margin habitat hydraulically mixed with the main channel flows during most of the summer. As discharge declined, the sensors at breeding sites on the margins where frogs laid their eggs were no longer submerged within 3-4 weeks after oviposition and began recording air temperature. Tadpoles thus occupied the mixed water zone, which would warm little relative to the thalweg. In other cases, the particular geomorphology of a breeding site could create a thermal refuge, or in one case near the mouth of a cool tributary, a thermal trap. For an example of warming in an unmixed backwater used for breeding, see Fig. 7 below. Therefore, maintaining water temperatures conducive to the viability of frog populations downstream of dams will likely be more sensitive to flow release management than local lateral warming, but the site specific offset between thalweg and margin temperatures need to be considered. The highlighted comparisons of thermal regime within the focal watersheds which follow illustrate the site specific nature of the effects of flow regulation on water temperatures affecting frogs.
South Fork Eel (unregulated) vs. Eel (regulated)

We monitored temperatures at mainstem and tributary 7 sites in the SF Eel watershed (2008-2010) and 7 (2009-2010) sites in the Eel River watershed below Scott Dam. We also conducted breeding population censuses by searching for clutches of eggs in 1 km reaches associated with each thermal monitoring site. In Fig. 6 we show variations in summer temperatures at a representative unregulated (SF Eel at Elder confluence) and a regulated site (Eel at Old Mine Road). Note that hypolimnetic releases prevent warming until late-summer / early fall below Scott Dam when flows drop. With respect to lateral variation (Fig. 7) in the Eel where temperatures are cool, there is some warming at the channel margins relative to the thalweg (1-2 °C), but the frogs often breed in isolated side pools which can warm more than 4 °C above the channel margin. The frogs also utilize the tributaries, such as Soda, Benmore, and Bucknell Creeks. The most upstream breeding site at the SF Eel, in Admiral Standley State Park, had the lowest range of temperatures recorded across all coastal and Sierran breeding sites, M30DAT = 15.7 ± 0.1 °C. This site is a very useful reference for cross-river comparisons, because it supports a small breeding population despite unfavorable thermal conditions. Non-linear regression (F2,11= 212.3, P<0.0001) to fit a Gaussian curve function shows that the peak abundance of frogs occurs when the M30DAT = 19.7 °C (Fig. 8).
Figure 6: Variation (Solid Line: Daily Mean; Dotted Lines: Daily Minima and Maxima) in 2009 Summer Temperatures at One Unregulated (SF Eel, Red) and One Regulated (Eel at Old Mine Road, B) Site with Breeding Populations. Light Gray Box Indicates Thermal Conditions Preferred by Eel Tadpoles under Experimental Conditions.

Figure 7: Temperatures at Thalweg vs. *Rana Boylii* Breeding Sites at the Eel River, 11.3 km Downstream of Lake Pillsbury (Scott Dam).
**Rubicon (regulated), North Fork of the Middle Fork American (unregulated)**

We monitored water temperature at 3 breeding populations at 461, 391 and 322 m elevation (1512, 1283 and 1056 ft) along the NFMF American and at 3 breeding populations and 2 additional sites between 363 and 1266 m (1190–4153 ft) along the Rubicon. Breeding started in mid-May for the lower reach of the Rubicon and around mid-May/beginning of June for the NFMF American under very similar thermal conditions (Fig. 9) of daily minima and maxima between 14.2 and 21.6°C (57.6–70.9°F). The breeding populations at the Rubicon River are located at large distances from the Hell Hole reservoir (43.5 and 48.4 km), as temperatures closer to the dam are too cold. The longitudinal pattern in thermal heterogeneity in regulated rivers is exemplified by the data collected from a set of sensor arrays arranged longitudinally from upstream to downstream (Fig. 10, left) showing the warming trends as water travels downstream from its release point at a reservoir, where frogs are absent, to locations with frogs present downstream where tributary flows join the main channel. At similar elevations in a nearby unregulated river, NFMF American water temperatures are somewhat cooler (Fig. 10, right).
North Fork Feather River vs. Middle Fork Feather

In this comparison of regulated and unregulated river reaches, the regulated reaches were warmer (Fig. 11). From June through August 2010, mean daily water temperature in the unregulated Middle Fork Feather River was $1.1\pm0.08^\circ C$ cooler than at the site occupied by frogs in the Cresta reach. Garcia and Associates (2010) reports that at 5 other sites in the Cresta reach there was no lateral warming at locations where frogs have bred in the past due to recent increases in summer base flows which increase near shore velocity and mixing. In the Poe reach there was lateral variation between near shore and thalweg temperature with breeding sites.
averaging 1-2°C cooler than thalweg daily minima and up to 4 °C warmer than the daily maxima.

Figure 11: Variation (Solid = Daily Mean; Dotted = Minima and Maxima) in Temperatures in the Free-Flowing M. Fk. Feather Compared to Reaches of the N. Fk. Feather.

High-elevation populations: Spanish Creek vs. Rubicon at SF Rubicon confluence

These two sites are at the same elevation (1085 m, 3560 ft). Spanish Creek near Quincy is unregulated and supports a breeding population, whereas temperatures at potential breeding habitats at the Rubicon are influenced by releases from the Hell Hole reservoir (Fig. 12). The SF Rubicon is also regulated and we monitored potential but unoccupied breeding habitat near the confluence with the Rubicon (9.9 km downstream of the reservoir). Note that Rubicon temperatures at this elevation remain below preferred range for most of the summer.
Tuolumne (regulated) vs. Clavey (unregulated)

Summer conditions in the Early Intake reach of the Tuolumne River, while consistently cooler than in the Clavey, are within the thermal niche of *R. Boylii* (Fig. 13). However, *R. Boylii* is extremely rare in the 12 mile reach between Hetch Hetchy Reservoir and Early Intake. In three field seasons of searching, 2008, 2009, 2010, a few tadpoles were observed on one occasion in the most downstream mile of the reach in August 2010.

Water at point bar channel margin habitats which would be suitable for egg masses and tadpoles do not appear to hydraulically mix with the main channel flows (SFPUC unpublished data). Temperature suitability is thus a function of summer discharge regime. During July 2009, Tuolumne discharge near the Early Intake gaging station was 90 cfs, and the differences between the coolest occupied Clavey sites and the Tuolumne sites ranged from 0 to 2.5 °C. In late August when test flows of 200 cfs were released from Hetch Hetchy for a habitat mapping study being conducted by the SFPUC, the differences in daily mean temperature were as high as 6 °C cooler than the coolest with Clavey sites we monitored.
At the lower elevation sites in the Clavey River where daily maxima frequently exceeded 24 °C (Fig. 14), we observed high prevalence of the copepod parasite, *Lernaea cyprinacea*. The parasites, also known as anchor worms, often embed near developing limb buds on tadpoles and are visible without magnification where the abdomen and egg-sac of the copepod protrudes from the tadpole’s body. We found that 42 percent of tadpoles were infected with copepods at the monitoring sites between 385 and 432 m but were absent above 714 m elevation.
Figure 14: Water Temperature at *R. Boylii* Breeding Sites in the Summer of 2009 and Prevalence of the Parasitic Copepod, *Lernaea Cyprinacea* in Tadpoles and Metamorphs in Late September.

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<td>22</td>
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Rubicon report by C. Bondi, UC Davis

Clavey (42% prevalence)

Rubicon (1 report, low prevalence)

SF Eel (21% prevalence)

*Alameda Creek: Camp Ohlone (unregulated) vs. downstream of Calaveras Reservoir (regulated)*

Differences in warming were not pronounced between the unregulated reach in Camp Ohlone and the reach below the confluence with Calaveras Creek with respect to daily mean water temperature (Fig. 15). However there was greater diurnal fluctuation in the unregulated reach further upstream. These data will provide an important baseline for future comparisons because the proposed summer flow release schedule after the new Calaveras Dam construction is completed will be significantly greater than present levels and temperatures will likely decrease in the future.
Trinity River: North Fork Trinity (unregulated) vs. downstream of Lewiston Dam (regulated)

In the Trinity River, frog breeding has been monitored by Ashton et al (2010). Breeding populations have not observed in the upper elevations of the reach (dark blue thermographs in Fig. 16) and are most dense downstream (light blue lines) and near the confluences with tributaries, such as the North Fk. Trinity. Lateral warming in shallow near shore habitats was observed in June at the confluence with the North Fork Trinity (dotted blue thermograph in Fig. 16) where there is an extensive delta area. In July after discharge declined, and this area was no longer inundated, lateral warming was not pronounced.
2.2.3 Comparisons to Other Known Populations in Rivers with Hydroelectric Projects

Our observations regarding the lower thermal limit for frog reproduction are consistent with information gleaned from other related hydroelectric project re-licensing proceedings, with two exceptions. In the Yuba watershed, the lower thermal limit may be less than what we observed. In the Middle Yuba River near the confluence with Wolf Ck $M_{30DAT} = 19.3^\circ C$ there is a population of *R. Boylii*, however abundance is known only from 2008 (Nevada Irrigation District and Pacific Gas and Electric Company, 2010). In the Lower McCloud River of northern California, frog breeding occurs in the most downstream 12 km of a 37 km reach below McCloud Reservoir where $M_{30DAT}$ in 2008 was 16.5 (interpolated from Fig. 7, PG&E, Stillwater Sciences 2009).

2.3 Thermal Preference of Tadpoles

Much of the literature on thermal preference in vertebrate ectotherms is based on studies with lizards and fish (Huey 1982, Angilletta 2009). In a review of temperate freshwater fishes, Magnuson et al. (1979) found that the width of the fundamental thermal niche was 4-5°C when characterized by the mean ± one standard deviation of temperature occupied in a laboratory thermal gradient, and that realized thermal niches were similar in central tendency, but narrower, in comparison to fundamental niches. In the previous section, we showed that the realized thermal niche for *R. Boylii* reproduction was 4-6°C in terms of maximum summer time 30-day running averages of water temperature. Our objective here is to assess the precision and extent of thermoregulation of tadpoles in the laboratory and field and compare the range of thermal preference (presumably an indicator of the fundamental thermal niche) to the variation in realized thermal niche we observed in the 6 focal watersheds.
We employ a conceptual model to relate thermal preference measured in controlled laboratory conditions to data collected at thermal transects in the field. To assess the extent to which tadpoles actively thermoregulate and to quantify the thermal suitability of conditions among the various rivers, we calculate the following temperature variables, indices of thermoregulation and thermal suitability developed by Hertz et al. (1993):

**Body temperature (Tb):** temperature of a field-active tadpole. Because core temperature (actual body temperature) is difficult to measure in the field, and because the small size of the tadpole is likely to facilitate quick heat exchange between the tadpole and surrounding water, we consider the temperature near a tadpole as an acceptable surrogate for Tb.

**Set-point temperature (Tset):** temperature selected by tadpoles in a thermal gradient. We define the range of Tset as the central 50 percent of the Tbs selected by tadpoles.

**Operative temperatures (Te):** these are the temperatures that tadpoles encounter in their habitats. For example along most thermal transects, Tes correspond to the temperature data collected at edgewater locations with breeding populations.

**Deviation from Tset in operative temperatures (de):** which indicates how closely the available operative temperatures (Te) match the set-point range. It is calculated by taking the absolute value of the difference between each Te and the closest bound of Tset (i.e., lower or upper quartile). The mean de and standard error is taken across all tadpoles at each location.

**Deviation from Tset in field-active tadpoles (db):** which indicates the average degree to which tadpoles experience Tbs outside the set-point range Tset. It is calculated by taking the absolute value of the difference between each Tb and the closest bound of Tset (i.e., first and third quartile of temperatures in a controlled thermal gradient). The mean db and standard error is taken across all tadpoles at each location.

**Effectiveness of thermoregulation (E):** is defined as E = 1 – (db / de). Negative values occur when tadpoles avoid sites providing Tes within the set-point range. Positive values occur when animals thermoregulate, with higher values indicating more precise thermoregulation. We wrote codes to calculate db, de, and E in the statistical package R.

### 2.3.1 Determination of Tset: Thermal Preference in the Laboratory

Given that tadpoles of *R. Boylili* are active thermoregulators (Brattstrom 1962), we assayed preferred temperatures for this population by observing behavior in a controlled thermal gradient. We conducted three sets of trials. The first used a full sibling set of tadpoles reared in flow through enclosures where water temperature was monitored at the South Fork Eel River as well as wild tadpoles for comparison. The second used wild collected tadpoles representing the other two of the three geographic regions and genetic clades in the species, Alameda Ck (central coast), and NF Feather (Sierra). The third set of trials involved tadpoles that had been reared in an outdoor common garden experiment at the UC Berkeley Richmond Field Station from eggs collected at Alameda Ck, Eel, SF Eel, MF Feather, and NF Feather.
Methods – construction and observation protocols for linear thermal gradient

We tested tadpoles individually in 3 m long, 8 cm wide, 5 cm deep (1 tadpole / 12 l of water), thermal gradients made from metal gutters heated at one end and chilled at the other. We used aquarium airstones to oxygenate the water during acclimation and between trials. In 2009, tadpoles were reared in flow-through enclosures in the SF Eel at known thermal conditions and fed ad libitum supplies of algae. In 2010 we tested tadpoles from the watersheds listed above.

On the days of the trial, tadpoles were transported to the laboratory (15 minute transport time for 2009, several hours for 2010 experiments with wild caught tadpoles from Alameda Creek, and zero for common garden reared individuals). Tadpoles were kept at room temperature for approximately 2 hours and then allowed to adjust to the thermal gradient for 1 hour. In order to account for potential diurnal variation in thermoregulatory behavior (Brattstrom 1962), we conducted all trials between 1300h and 1700h under uniform lighting conditions. Small rocks were provided as cover items along the length of the gradient. We maintained a linear thermal gradient from the cold (11.9 ± 0.1°C, n = 108) to the warm (34.7 ± 0.2°C, n = 108) ends of the gutters throughout the adjustment and experimental periods. We checked gradient linearity periodically during the experiment with a quick-reading IR thermometer. We noted tadpole position along the gutter every minute for thirty minutes (n = 31/ trial). We then calculated the temperature at those positions using the linear regression between distance along the gutter (0 = cold end) and temperature. Although temperature preference might vary during ontogeny for some taxa, especially for pre-metamorphic stages (Hutchison and Dupré 1992), we did not observe a relationship between Gosner (1960) stage and temperatures selected in our trials, similar to other studies (Skelly 2004).

The thermal gradient observations yielded two response variables, the mean selected temperature and the set-point range (T_set) as defined above, the central 50 percent of the temperatures selected by tadpoles in a thermal gradient. We calculated T_set with two approaches. First, for each tadpole we calculated the arithmetic mean of its 31 observed positions in the gradient, and then calculated the arithmetic mean across all 56 tadpoles. Second, by bootstrapping (1000 times) values drawn one at a time from the 31 measurements for each tadpole, we calculated the mean across the 56 tadpoles. We report means ± SE and performed analyses in R (R Development Core Team 2005).

Results: selected temperature, T_set and T_sel of tadpoles in thermal gradient trials

Among the populations we tested, thermal preferenda of tadpoles varied in relation to the locations where tadpoles were collected and their rearing history. In 2009, trials with tadpoles from the South Fk. Eel river showed that central 50 percent of body temperatures, T_set was 16.5 – 22.2°C (n = 56). Mean selected temperature, T_sel = 19.60 ± 0.58°C (19.60 ± 0.03°C with the bootstrapping approach). There was no relationship between Gosner stage and mean selected temperature (when averaging temperatures by tadpole: n = 56, R² = 0.002, p = 0.722; with bootstrapped regression, all regressions p > 0.204). Similarly, there was no relationship between mean selected temperature and body size (n = 56, R² = 0.008, P = 0.521) or biomass (n = 55, R² = 0.007, P = 0.537). The value of T_set we observed, 19.6°C, closely corresponds to the peak of the
curve in Fig. 17, 19.8°C, which relates summer M30DAT to breeding population abundance in the Eel watershed.

$T_{sel}$ appears to be a plastic trait and sensitive to the rearing history. For a larger set of South Fk. Eel individuals ($n=226$), analysis of variance indicated that tadpoles reared in cool stream tributaries that do not naturally have breeding populations (Fox and Elder Creek where M30DATs were 16.1 and 16.9 °C respectively) selected significantly ($F_{4,221}=3.1, P=0.02$) warmer temperatures than tadpoles reared in enclosures in the warmer locations where M30DATs were 19.9 and 21.8 °C (Fig. 17, left). These tadpoles came from two groups of full-siblings (i.e. 2 clutches of eggs) and the variation in $T_{set}$ and $T_{sel}$ is likely due to the rearing differences rather than genetic variation or effect of maternal provisioning of tadpoles via yolk size. For free swimming tadpoles collected from Alameda Creek ($n=40$, Gosner stage range 27-35), thermal preferenda were similar to those observed in the South Fork Eel, $T_{set} = 18.5 – 22.5$, and $T_{sel} = 20.48 \pm 0.6^\circ$C. Sierran tadpoles had higher thermal preferenda, however, similar to the warmer boundaries of the realized thermal niche observed in the field. For individuals collected from the Poe reach of the North Fork Feather River ($n=47$, Gosner stage range 26-34), $T_{set} = 21.0 – 25.8$, and $T_{sel} = 23.1 \pm 0.33^\circ$C. These differences are not observed however among tadpoles with a common thermal history, regardless of source population. (Fig. 17, right). When tadpoles were collected as eggs from Sierran sites (Middle Fork Feather, North Fork Feather) and Coastal sites (South Fork Eel, Mainstem Eel) and reared in an outdoor common garden experiment where M30DAT=17.8 °C (see section 3.4), there were no significant differences in mean values of $T_{sel}$ among source populations ($F_{3,60}=0.80, p=0.50$). $T_{set}$ for the common garden tadpoles was 19.1-21.5°C, $T_{sel}=20.5\pm0.55^\circ$C.

Figure 17: Mean ± SE Selected Temperature ($T_{sel}$) in Thermal Gradients of Tadpoles Reared from a Common Source of Eggs in Different Thermal Environments (Blue=Cold, Red=Warm) and Tadpoles Reared from Eggs Collected at Different Rivers and Reared in a Common Garden Experiment (Green).
2.3.2 Determination of $d_e$: Differences in Thermal Suitability between Occupied Habitats in Regulated and Unregulated Rivers

Overall, coastal sites presented thermal conditions that better matched the set-point range than Sierran sites. We collected data for $T_{set}$ for a coastal population (SF Eel) and data for $T_e$ for 28 breeding sites (but significant temporal gaps occurred at some sites). We have computed $d_e$ values based on the data from summer 2009, and by assuming similar thermal preferences for coastal and Sierran populations. This assumption was verified by measuring $T_{set}$ in Sierran populations. The values are presented by month in Fig 18. Differences were especially noticeable in July and August, which are the two most important months for tadpole development. Sites without breeding populations had large deviations from the set-point range in June and July, but not later in the season. Some sites with breeding populations greatly deviated from the set-point range: the SF Eel at Branscomb (colder), and the lower Rubicon and Clavey (warmer).

Figure 18: Extent to Which Available Operative Temperatures ($T_e$, Water Temperature at Potential or Actual Frog Breeding Sites) Match the Set-Point Range ($T_{set}$, Temperatures Preferred by Tadpoles). The Smaller the Value of $d_e$, the Closer the Match (Black = Coastal Sites, Gray = Sierran, Empty = Sites without Breeding Populations, Error Bars + 1 SE).
2.3.3 Determination of $d_b$ and $E$: Diurnal Movement of Tadpoles and Differences between Thalweg and Occupied Habitats

In the field, *R. boylii* tadpoles are known to actively thermoregulate (Brattstrom 1962), but because they are benthic (bottom dwelling) and are generally confined to shallow near shore environments in large rivers within a narrow range of preferred depths and velocities, selected temperatures are constrained by the degree to which local cross-sectional topography allows shallow water to warm relative to the thalweg.

**Methods**

We explored the behavioral response to edgewater warming by comparing temperatures where tadpoles occurred (observed throughout the day) to temperatures from benthic sensors. We deployed thermisters (n=46) in 1m wide arrays and measured the bottom profile from shore to thalweg or across the entire channel at: three locations in the regulated North Fork Feather River (July 22-28, 2010); three locations in unregulated Alameda Creek (June 22-26, 2010) and one location in the South Fork Eel (July 15-16, 2010). At intervals of 1–2 hr between 8 AM and 8 PM we measured the temperature at the precise locations of the first 20 tadpoles encountered in a 1-m wide band wading away from shore. Because tadpoles scatter when people walk on the banks, observers stood still for 5-10 minutes prior to the start of each census, and moved slowly to prevent altering tadpole locations as they progressed across the meter wide band. Spot measurements of water temperature were made with a sensitive quick read thermometer and locations noted. Tadpoles were captured with dip nets, placed in containers, measured with calipers at the end of each survey and then released nearby. Using the laboratory measurements of thermal preference and the field observations of body and ambient water temperature, we calculated $d_b$, deviation from $T_{set}$ in the field-active tadpoles, and $E$, the effectiveness of thermoregulation ($1 - (d_b / d_a)$) at each site.

**Results – Indices of thermoregulatory behavior**

Among the transect sites in the Poe and Cresta reaches of the North Fork Feather River, there was a large degree of spatial heterogeneity in the indices of thermoregulatory behavior (Table 3). The temperatures at precise tadpole locations mirrored thalweg temperatures, with an offset depending on the degree of edgewater mixing at each site (Fig. 19). Within a narrow band relative to the full channel width, tadpoles moved closer to shore during the warmest mid-day hours to utilize warm patches and were found further from shore in the early morning and late afternoon (Fig. 20). Near the confluence with Flea Valley Creek at the upstream end of the Poe reach, tadpole temperatures deviated more than 2°C outside the thermal preference range, the value of $E$, effectiveness of thermoregulation, was the lowest observed among sites. Concomitantly, tadpoles there were significantly less developed (Fig 21; ANOVA of Gosner stage, $n=326$ tadpoles, $F_{2,323} = 41.8$, $P<0.001$) than the other Feather River sites. ANOVA of body size using Gosner stage as a covariate, however, revealed no significant differences in size at any given developmental stage $F_{2,323}=1.5$, $P=0.23$), indicating that rather than temperature being limiting per se, it is the time constraint of not developing and growing quickly that may be the important factor. In contrast, at the Poe Powerhouse transect which is the furthest point downstream from Poe dam in the reach, values of $T_b \geq 24$C occurred during 6 of the 8 sampling
periods throughout the day, and some tadpoles were approaching from limb emergence (stage 40).

In Alameda Creek, which has a much narrower channel, (Fig. 22), differences between the preferred temperature range, $T_{so}$, and body temperatures, $T_b$, were small, <0.4°C. However, we did observe that tadpoles tracked thermal resources, with individuals clustered in warm patches, especially mid-day (Fig. 23).

In the SF Eel where tadpoles remained in a narrow band near shore (Fig. 24), tadpole temperatures (Fig. 25) were infrequently outside the range of thermal preference and mirrored temperatures at sensors up to 1 km away.

A general conclusion from these fine scale observations of thermoregulatory behavior and temperature data collection are that fixed sensors placed at appropriate depths and velocities can closely match operative tadpole temperatures, but that the effectiveness of tadpole thermoregulatory behavior can be constrained by channel morphology. Although tadpoles make small movements away from shore late in the day and in the early morning to track warm patches, they are limited by depth and velocity. Temperatures at precise tadpole locations in the diverse types of river channels where we made observations generally matched those at fixed sensors located 1-2 m from shore. However, information from near shore sensors is not always available to river managers and most models used by dam operators to forecast water temperature under alternate flow scenarios are one dimensional. That is, they predict thalweg temperature as water moves from the upstream reservoir down the many kilometers of a river reach. If a temperature forecast predicts that summer thalweg conditions will cool and approach the lower thermal limit of the realized thermal niche of *R. Boylii*, determining the presence or absence of a site-specific offset between thalweg and edgewater temperatures may be essential to assess the impact of the change on recruitment of new frogs to the population.

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<th>Table 3: Mean ± SE Indices of Thermoregulatory Behavior by <em>R. Boylii</em> Tadpoles.</th>
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<tr>
<td>South Fk. Eel River</td>
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Figure 19: Tadpole Locations (Green Rectangles) and Thermographs from (Top) Shady Rest Site on the Cresta Reach of the North Fk. Feather; (Middle) Downstream of Poe Dam Near Pulga / Flea Valley Creek Confluence; and (Bottom) Near Poe Powerhouse. Mean ± 1 SD Temperature (Points and Error Bars) at Tadpole Locations.
Figure 20: Change in Tadpole Location throughout the Day, Further from Shore Late in the Day and Early Morning in the NF Feather.

Figure 21: Average Body Size (Not Including Tail) in Relation to Developmental Stage of Tadpoles at the Three North Fork Feather Thermal Transect Sites.
Figure 22: Tadpole Locations (Green Rectangles, Left Column) and Thermographs (Right Column) from Near Shore Habitat and Thalweg at Three Transects across the Unregulated Reach of Alameda Creek. Mean ± 1 SD Temperature (Points and Error Bars) at Tadpole Locations (n=20 Tadpoles per Diurnal Sampling Time). Thermister Arrays (Small Circles, Lower Left Plot) Deployed across Channel.
Figure 23: Two Dimensional Spatial Variation in Temperature (°C, Color Contours) at Alameda Creek Transect 3 (see Fig. 2.21). A Representative Subset of Times of Day When Tadpole Censuses were Conducted (June 25, 2010) with Histograms Denoting Number of Tadpoles (Right Axis) at a Given Cross Sectional Distance, Shows How Tadpoles Track Warm Patches. Temperatures Measured by an Array of 46 Thermisters Deployed across the Channel.
Figure 24: Thermal Transect at the South Fork Eel (a) in which Tadpoles Make Small Scale Movements within the Two Meters Closest to Shore (b).

Figure 25: Thermal Environment Utilized by *R. Boylii* Tadpoles in the SF Eel River (July 15, 2010) Compared to Fixed Sensors on the Benthos or Mid-Column at 3 Monitoring Locations up to 1 km Away from Transect.
CHAPTER 3:
Interactive Effects of Algal Food, Water Temperature, and Predators on Growth and Survival of Larval Rana Boylii.

3.1 Digestive Assimilation Efficiency

3.1.1 Methods of Assimilation Experiment

To assess the effects of temperature and algal type on food digestion by Rana Boylii tadpoles, we conducted a two factor fully crossed design field manipulation, three levels of thermal regimes x two food levels (n=5 replicates per treatment). In late spring, we collected embryos from five different clutches of R. Boylii egg masses in the South Fork Eel River (SF Eel) and reared the tadpoles for approximately eight weeks in situ enclosed by plastic laundry baskets (40 x 60 cm) with one mm fiberglass mesh glued over the openings. On July 31, 2010, we placed 30 of these flow-through enclosures, each housing one tadpole, in three locations (ten per site): 1) Fox Creek (Fox), a cool shaded tributary; 2) the edge of a sunny pool in the SF Eel 150 m downstream of the confluence between Fox and SF Eel; and 3) directly at the confluence. We monitored the thermal conditions of each rearing location using Thermocron iButtons DS1921G (± 1°C accuracy, deployed in 2 enclosures per site) programmed to record temperature every two hours.

We stocked all enclosures with a mix of cobbles from the SF Eel and Fox to completely cover the bottom, and half of the enclosures received supplements (25 g damp mass after spinning 50x revolutions in a salad spinner) of the filamentous green alga Cladophora glomerata covered with a heavy growth of epiphytic diatoms. Rock scrapings and filaments of Cladophora were examined microscopically to qualitatively determine the composition of the periphyton flora. Most taxa were identified to the genus level, some to species (Table 5).

At the start of the experiment, the development range of experimental tadpoles ranged between Gosner stages 31 and 37. We assigned one tadpole per clutch, to each of the 6 treatments. The median stage for each treatment was 34. We weighed each tadpole to the nearest 0.1 g on an OHaus field balance, and measured body length and total length with dial calipers. At the conclusion of the experiment, we euthanized the tadpoles with an over-dose of the anesthetic MS-222, re-weighed and measured them, then froze for later dissection to conduct gut content analyses.

In order to estimate each tadpole’s efficiency in absorbing the organic content of ingested food, we used the formula of Conover (1966) who defined assimilation efficiency, U’ = [(F’-E’) · (1–E’–1 · F’)] · 100. F’ is the ratio of ash-free dry weight of ingested food to dry weight of food algae. E’ is the corresponding ratio for the feces (the mean of five daily samples collected Aug 2- Aug 6.) We removed the intestines and measured the anterior gut length as the distance from the end of the manicotto glandular (which secretes digestive enzymes) to the inflection point of the coiled gut. We measured the posterior gut length as the distance from the inflection to the beginning of the colon (Fig. 26). We added those two lengths to calculate total gut length. The anterior gut
contents were dried and processed to determine the ash free dry weight and percent organic content.

We collected fecal samples once daily beginning 48 hours after tadpoles were placed into their enclosures to allow sufficient time for passage of any food ingested prior to the start of the experiment. We used plastic bulb pipettes to suction up fecal material and a small volume of water. To determine the organic content, samples were filtered onto pre-weighed and pre-ashed 2.5 cm diameter glass microfiber filters (GF/C), and then dried at 60 C for 48 hours, or longer if this is was needed for samples to reach constant weight. Samples were placed in a desiccator to cool to room temperature, and weighed to 0.0001g on a Mettler balance. Samples were incinerated in a muffle furnace held at 500 C for 1 hour. After cooling and re-weighing we calculated the ash free dry weight (AFDW) as

\[
\text{AFDW} = \text{dry weight} - \text{ashed weight}
\]

\[
\% \text{ organic material} = \frac{\text{AFDW}}{(\text{dry weight with filter} - \text{filter weight})}
\]

We also assessed gut morphology by measuring the length of the small and large intestines in relation to tadpole size for comparison of *Rana boylii* to other taxa. To assess the interactive effects of temperature and food type on assimilation we used two way ANOVA’s and linear regression.

**Figure 26: Gut Anatomy of Tadpoles, Reproduced from Pryor and Bjorndahl 2005.**

![Diagram of gut anatomy](image)

Figure 1. The bullfrog tadpole gastrointestinal tract. Gut regions: **M** = manilcucco glandularis; **ASI** = upper third of the anterior small intestine; **ASI** = middle third of the anterior small intestine; **ASI** = lower third of the anterior small intestine; **INF** = infusion region; **PSI** = upper third of the posterior small intestine; **PSI** = middle third of the posterior small intestine; **PSI** = lower third of the posterior small intestine; **C** = colon; and **R** = rectum. Illustration by G. Pryor.
3.1.2 Results and Discussion of Assimilation Experiment

In the SF Eel the mean (± 1 SE) daily water was 3-4 °C warmer than the other two sites (Fox = 15.5 ± 0.05, Confluence = 15.7 ± 0.06, SF Eel = 19.1 ± 0.07). The conditions at the confluence of the tributary and mainstem mirrored the water temperatures in Fox Ck (Fig. 27), but there was an influence of greater insolation on the baskets resulting in a higher daily maximum temperature. Thicker lines in Fig. 27 indicate temperatures measured in enclosures with supplemented floating mats of algae which elevated temperatures in the baskets.

**Figure 27: Thermal Conditions during the Week-Long Assimilation Experiment.**

There were significant differences in the efficiency of digestion among temperature and food supplementation treatments (Table 4). At the warm site it appears that the percent organic content of material in the foregut was significantly lower than the other two rearing sites, especially for those tadpoles with access to *Cladophora* and its epiphyte flora (Fig. 28a, Table 4). This finding indicates that at the warmer temperatures the digestive enzymes secreted by manicotto (beginning of the foregut) are more effective than at the colder sites and that the overall thermal differences in absorption efficiency begin early in the digestive process.
Over the short course (one week) of the assimilation experiment, tadpoles grew (Fig. 29), but we observed no internal anatomical changes in response to diet and temperature manipulations as illustrated by the positive allometry of size and gut length (Fig. 30). Gut length was approximately 11 x body length. Growth was positively correlated with the proportion of ash in the ingested food (Fig. 31). The proportion of ash is an indication of the predominance of diatoms in the tadpoles’ diet because diatoms have a silica frustule, whereas green algae and cyanobacteria do not. *Epithemia*, the diatoms which contain N-fixing endosymbionts, have particularly thick frustules and when these diatoms are rinsed off of *Cladophora* filaments, filtered, and then processed for AFDW in a similar manner to our other algal samples, the percent ash content is 78 percent. Thus, high ash content in the foregut likely indicates prevalence by this group of diatoms which dominated the periphyton flora in the food supplemented treatments (Table 5). Tadpole growth was also positively correlated with digestive efficiency, R=0.75.
Figure 29: Food and Temperature Effects on Growth of *Rana Boylii* Tadpoles over the Course of the One-Week Assimilation Experiment ($F=3.6$, df=2,24, $P=0.04$). Treatment Means Covered by a Horizontal Line are Not Significantly Different from Each Other in *Post Hoc* Comparisons.

![Graph showing growth (% change in weight) against average daily maximum temperature (°C).]

Figure 30: Positive Allometry between Overall Tadpole Size and Gut Length ($F_{1,28}=71.7$, $P<0.001$, $R^2=0.72$). Dark Shading of Symbols Indicates Replicates in Which Tadpole Diets Were Supplemented with Epiphytized *Cladophora Glomerata*.

![Graph showing gut length (mm) against total length (mm).]
Figure 31: Ash Content of Ingesta Positively Correlates with Growth of *Rana Boylii* Tadpoles ($r=0.49, P=0.006$). Dashed Lines Indicate Average Ash Content of Algal Foods.

Table 4: Two-Factor ANOVA’s for A) Organic Content of Ingested Food in the Foregut ($R^2=0.46$); B) Assimilation Efficiency ($R^2=0.91$); and C) Growth of *Rana Boylii* Tadpoles ($R^2=0.68$) When Reared on Low vs. High Quality Algal Foods in Three Different Locations That Varied in Thermal Regime and Canopy Cover.

<table>
<thead>
<tr>
<th>Response Variable, Factors</th>
<th>$F$</th>
<th>df</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Organic content in foregut (Ash Free Dry Mass / Total Dry Mass)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>4.5</td>
<td>1, 23</td>
<td>0.045</td>
</tr>
<tr>
<td>Location (temperature and light)</td>
<td>6.05</td>
<td>2, 23</td>
<td>0.008</td>
</tr>
<tr>
<td>Food x Location</td>
<td>1.37</td>
<td>2, 23</td>
<td>0.27</td>
</tr>
<tr>
<td>B) Assimilation Efficiency (Conover’s Index)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>77.1</td>
<td>1, 24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Location</td>
<td>76.8</td>
<td>2, 24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Food x Location</td>
<td>1.23</td>
<td>2, 24</td>
<td>0.309</td>
</tr>
<tr>
<td>C) Tadpole growth (% change between initial mass and mass 1 week later)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>2.2</td>
<td>1, 24</td>
<td>0.15</td>
</tr>
<tr>
<td>Location</td>
<td>20.5</td>
<td>2, 24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Food x Location</td>
<td>3.6</td>
<td>2, 24</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Table 5: Periphyton Taxa on Cobbles Used in a Mixture to Line Enclosures during Assimilation Experiment. Asterisks Indicate Taxa with Nitrogen Fixing Endosymbionts.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>S. Fk. Eel</th>
<th>Fox Creek</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillariophyta (diatom)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cymbella sp.</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Epithemia sorex*, E. adnata*, E. turgida*</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Gomphoneis sp.</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Gyrosigma sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Navicula sp.</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Nitzia sp.</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Rhicocosphenia sp.</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Rhopalodia* sp.</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria (blue-green algae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calothrix sp.</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Melosira sp.</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Rivularia sp.</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Tolypothrix sp.</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Chlorophyta (green algae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulbochaetae sp.</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Cladophora glomerata</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Oedogonium sp.</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

The overall conclusions of the assimilation experiment are that both temperature and food quality influence digestive efficiency and that the effects are additive. Digestive efficiency was highest, 13.8 percent, when a diet rich in epiphytic diatoms was consumed at warm temperatures and that as digestive efficiency increases so does tadpole growth.

3.2 Manipulation of Thermal Regime and Food Quality, Rearing Expt I

3.2.1 Experiment I Methods

In rearing Expt. I we raised tadpoles from unhatched embryos to metamorphosis in four unregulated streams that naturally differ in thermal regime (Fig. 30). These streams also differ in watershed size, canopy cover, mean annual discharge and primary productivity (Table 1). Although all four support adult and juvenile R. Boylii, only the two largest and warmest streams (Tenmile and SF Eel) are used for breeding. We collected embryos at the gastrula stage (Gosner 10) from two egg masses laid on the night of May 11, 2008. Groups of 30 embryos were separated from each clutch, placed in lidded vials with stream water and carried in backpacks to each of four rearing locations. Time spent in transport was roughly equalized to match the travel time to the most distant rearing location. Embryos were placed in flow-through enclosures constructed from plastic laundry baskets (55 x 38 cm interior dimensions) with 1 mm fiberglass mesh glued over the openings. Water depth was approximately 15-20 cm. Native cobbles with epilithic periphyton from each rearing location (low quality food) were placed in the enclosures sufficient to cover the bottom of each basket. Twelve baskets were deployed in each stream, six from one clutch and six from the other. Clutch had no effect on tadpole
mortality and was henceforth excluded as a factor in our analyses. We randomly chose half of the baskets in each stream to receive food supplementation (high quality food) in which 40 g (damp mass) of the filamentous macroalga *Cladophora glomerata* covered with a heavy growth of epiphytic diatoms (including species of *Epithemia*, *Cocconeis*, and *Gomphonema*) harvested from the SF Eel (C:N ratio = 10.13 ± 0.41).

To mimic the natural decline in density that occurs in the open river as tadpoles disperse and with attrition to predators, we periodically removed groups of tadpoles, creating stepped decreases in density over the course of the experiment (Appendix A). We confirmed that this strategy to avoid crowding and density effects while maintaining verisimilitude was effective by comparing the size of tadpoles in baskets in the SF Eel to free-living tadpoles in the nearby vicinity. We removed tadpoles from enclosures when front limbs emerged (Gosner stage ≥ 42).

The thermal regime was monitored by placing temperature data loggers (Thermocron iButtons DS1921G®, ± 0.5°C accuracy programmed to record every two hours) inside a subset of seven enclosures at each of the four sites. We consider these temperatures to be equivalent to operative temperatures (Te, Hertz et al. 1993) for tadpoles, because the high thermal conductivity of water, small size of tadpoles, and limited habitat heterogeneity inside the experimental enclosures effectively reduced the probability that tadpoles could experience a wider range of temperatures. Temperature sensors remained in place until completion of the experiment, which occurred, depending on the stream, either the day when no individuals remained as tadpoles, or the day when enclosures were removed in anticipation of the fall rains (21 September 2008 for tadpoles in the coldest stream, Fox Creek).

For mortality as the response variable, we calculated the difference between number of tadpoles present at the previous weekly visit (after taking into account tadpoles removed during scheduled reductions) and the number of tadpoles still present in the. We excluded tadpoles that were lost during handling or due to the accidental colonization by a predator (e.g. a dragonfly nymph). We assumed mortality for all tadpoles still alive at the end of the experiment in the coldest stream, because these tadpoles could not have completed metamorphosis before the onset of the first fall floods given their developmental stages and slow growth rates. We measured the size using calipers, assessed developmental stage with a 10x hand lens, and measured damp weight with a field balance to 0.1 g.

We used multiple logistic regression models to explore the relationships among tadpole mortality from hatching to Gosner stage 42, thermal regime, and watershed size. We calculated an index of thermal suitability, de, according to Hertz et al. (1993) who defined de as the difference (absolute value) between Te and Tset. Each de value represents the individual deviation of Te from the closer (upper or lower) bound of Tset. When Te falls within the set-point range, the resulting de takes the value of zero. The mean de represents the thermal quality of a habitat from a tadpole’s perspective. We restricted Te to July (n = 2232 measurements/stream) because this month: (1) is when Gosner stages of tadpoles reared in the field enclosures were similar to those of tadpoles used to measure Tset; and (2) coincides with the period of fastest tadpole growth and greatest availability of algal food (Power et al. 2008). We performed separate analyses for tadpoles reared with low quality and high quality food. We considered
watershed size in our models because it controls the algal productivity (i.e., amount and quality of epilithic diatoms available to tadpoles in the unsupplemented treatment). Additionally, there was large overdispersion when the logistic regression model did not include watershed size.

3.2.2 Results Expt I. Tadpole Growth and Survival a Function of Temperature

Time for eggs to hatch (Fig. 32) was a linear function of mean daily water temperature (R²=0.91, days = (-1.662 x mean daily water temperature) + 39.95). Water temperatures and weekly survival patterns differed among the four experimental streams (Fig. 33, Table 28). Weekly survival rates were generally lower at early stages of development, with the exception of the warmest stream (Tenmile Creek), where maximum daily temperatures in excess of 25°C in July corresponded to a sharp drop in weekly survival when tadpoles had reached advanced developmental stages (Gosner 35–42).

Figure 32: Effect of Temperature on Time to Hatching. Each Point Represents the Mean for Groups of 30 R. Boylii Eggs in 56 Flow-Through Enclosures (n=1680 Eggs).

Table 6: Environmental Characteristics and Presence of Rana Boylii in the Streams Used for Tadpole Rearing at the Angelo Coast Range Reserve, California.

<table>
<thead>
<tr>
<th>Stream</th>
<th>Watershed (km²)</th>
<th>Canopy cover (%)</th>
<th>Chlorophyll a (μg∙cm⁻²)</th>
<th>Mean light intensity b (lux)</th>
<th>Maximum 30 d avg water temperature c (°C)</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fox</td>
<td>2.6</td>
<td>96.4 ± 0.5</td>
<td>0.9</td>
<td>3785 ± 1157</td>
<td>16.06</td>
<td>16.06</td>
<td>16.6</td>
</tr>
<tr>
<td>Elder</td>
<td>17.0</td>
<td>93.0 ± 2.9</td>
<td>1.9</td>
<td>4304 ± 1085</td>
<td>16.9</td>
<td>16.9</td>
<td>16.7</td>
</tr>
<tr>
<td>SF Eel</td>
<td>126.0</td>
<td>48.9 ± 4.0</td>
<td>1.6</td>
<td>11823 ± 753</td>
<td>19.9</td>
<td>19.9</td>
<td>19.7</td>
</tr>
<tr>
<td>Tenmile</td>
<td>169.4</td>
<td>16.7 ± 1.6</td>
<td>2.1</td>
<td>38070 ± 3995</td>
<td>21.8</td>
<td>21.8</td>
<td>22.1</td>
</tr>
</tbody>
</table>

a Data on chlorophyll a from Finlay (2004).
The index of habitat thermal quality, $d_e$, in the experimental enclosures in July 2008 ranged between $0.04 \pm 0.16^\circ C$ (SF Eel) and $0.92 \pm 0.92^\circ C$ (Fox Creek; Fig. 34). Our model that incorporates the mean July $d_e$ values predicts the mortality of tadpoles grazing ambient production of epilithic periphyton, after controlling for watershed size, our proxy for productivity (Table 7). Based on this model, a deterioration of the thermal quality of the habitat by $1^\circ C$ (i.e., a $1^\circ C$ change in the mean July $d_e$) increases the odds of mortality by a factor of 8.40 (confidence intervals 1.13–79.60). The model can also predict how the probability of mortality associated with deviation from preferred temperature will vary depending on local productivity, e.g. in a watershed of 150 km², the size at which channels are broad and sunlit, and frog populations are most abundant, or 60 km², the watershed size with narrow shaded channels and which supports small frog populations. The predicted probability of mortality for these two watershed areas was 70.1 percent (87.9 percent) for a mean July $d_e$ of 0°C, and 87.6 percent (95.5 percent) for a mean July $d_e$ of 0.5°C.
Figure 34: Proportion of Tadpoles Reaching Metamorphosis in Field Enclosures as a Function of the Index of Thermal Quality (Mean July $d_e$; Hertz et al. 1993) and Food Quality (Left) and the Production of Metamorphs as a Function of Mean Temperature during the Warmest 30 Day Period of the Summer (Right).

Table 7: Results of Multiple Logistic Regression Models of (a) Mean July $d_e$ and Watershed Area on the Probability of Occurrence of Tadpole Mortality with Low-Quality Food (Epilithic Diatoms Only) and (b) Mean July $d_e$ and Watershed Area (km$^2$) on the Probability of Occurrence of Tadpole Mortality with High-Quality Food (Epilithic Diatoms + Epiphytized Macroalgae).

<table>
<thead>
<tr>
<th>Effect</th>
<th>$z$</th>
<th>$p$</th>
<th>estimate ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) low-quality food</td>
<td>2.491</td>
<td>0.013*</td>
<td>2.718 ± 1.091</td>
</tr>
<tr>
<td>Intercept</td>
<td>2.014</td>
<td>0.044*</td>
<td>2.128 ± 1.057</td>
</tr>
<tr>
<td>Mean July 2008 $d_e$</td>
<td>-1.567</td>
<td>0.117</td>
<td>-0.012 ± 0.008</td>
</tr>
<tr>
<td>(b) high-quality food</td>
<td>0.640</td>
<td>0.522</td>
<td>0.369 ± 0.577</td>
</tr>
<tr>
<td>intercept</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean July 2008 $d_e$</td>
<td>1.420</td>
<td>0.156</td>
<td>0.962 ± 0.678</td>
</tr>
<tr>
<td>watershed size</td>
<td>0.463</td>
<td>0.644</td>
<td>0.001 ± 0.003</td>
</tr>
</tbody>
</table>

When temperatures are described using the metric of maximum 30 day average temperatures (M30DAT), or July mean temperatures, rather than $d_e$, the results (Fig. can be directly compared to data observed in other streams. At mean temperatures colder (16.06°, 16.9°), warmer (21.83°), or close to the mean preferred temperature (19.94°), production of metamorphs was highest when M30DAT closely matched thermal preference (Figure 34 right). Post-hatching
development (Fig. 35) and growth (Fig. 36), however, were most rapid at the M30AT=21.83°C. Analysis of covariance confirms that there were significant effect of temperature (F=59.9, P<<0.001), algal food quality (F=9.6, P= 0.004) and a significant interaction between temperature and algal quality (F=7.4, P=0.01). For the food supplemented tadpoles, the number of days between hatching and front limb emergence = 192.03-5.547x(M30DAT), SE for intercept =7.99 SE for slope =0.419. When considering total production of metamorphs as a performance for tadpoles receiving supplements of high food quality algae, there is a humped shaped (i.e. parabolic) response curve, with the greatest production of metamorphs at M30AT= 19.94°C (Fig. 34). At the warmest site (M30DAT = 21.83°C) metamorphosis began 4-5 weeks sooner and tadpoles were larger, but because of high mortality during the warmest period, the total production was slightly lower. The causes of mortality in the experiment were not identified, however we believe it was not due to reaching a critical thermal maximum as tadpoles have been observed to successfully metamorphose from isolated sidepools where large diurnal fluctuations in temperature exist, and daily maxima can reach 30°C. When the relationship inferred by the response curve is extended beyond the experimental rearing temperatures, the predicted range of the fundamental thermal niche is 15-24.5°C, broader than the laboratory preferred temperature range.

Figure 35: Time to Onset Front Limb Emergence for Algal Supplemented and Non-Supplemented Tadpoles as a Function of Temperature during the Warmest 30 Day Period of Summer when Algal Production Peaks and Tadpoles Complete Pre-Metamorphic Development.
3.3 Laboratory Growth Chamber Common Garden, Rearing Expt II

To assess whether populations of frogs from different regions vary with respect to thermal optima, we reared tadpoles from the Sierra and the Coast in one laboratory location in a what is known as a “common garden” experiment. Common garden experiments, using eggs collected from populations influenced by dams and populations uninfluenced by dams in both regions, could illustrate whether developmental and growth rates have adapted to the changes in thermal regimes present in some regulated rivers.

3.3.1 Methods

Groups of embryos were removed from 4 to 6 clutches of eggs (depending on availability) from 3 coastal and 4 montane source populations: Alameda Creek downstream of Calaveras Dam (May 21), SF Eel (May 31), Eel downstream of Scott Dam (May 28), Middle Fork American River (June 16), Middle Fork Feather River, North Fork Feather River downstream of Poe dam (June 17) and Cresta dam (June 10, June 17). The remainder of the clutches remained in situ at the source. Eggs were transported in aerated river water in coolers to the UC Berkeley Richmond Field Station within 8 hours of collection and placed into temperature controlled incubators (Shellab diurnal growth chambers equipped with grow lights set to 14.5 hr light, 9.5 hr dark). The warm incubator had a daily mean temperature of 19.5 °C (18° – 22°, min - max) and the cold treatment had a daily mean temperature of 16.6 °C (13° – 19°, min – max). After eggs hatched, 5 tadpoles from each full-sibling group were selected and placed in 1 gallon aquaria with a mixture of river water and de-chlorinated tap water. Each aquarium had an individual airstone.
and was stocked with periphyton covered cobbles that had been collected at the South Fork Eel and Alameda Creek. Water changes with treated tap water occurred 2X/week. Tadpoles were fed *ad libitum* high food quality loose algae collected from the South Fork Eel River and Alameda Creek. On the afternoon of July 8, 2010, the compressor failed in the warm treatment incubator. It overheated, killing most tadpoles, and the experiment was terminated.

The response variables for the experiment are growth and development. Measurements of tadpole stage and size (body and total length measured with calipers to the nearest 0.1 mm) were made at approximately 10 day intervals after hatching and immediately after the compressor failure prior to any decomposition. Due to the differences in breeding phenology, with egg laying at coastal sites earlier in spring than Sierran sites, the dates and developmental stages when embryos were placed in the chamber varied. Simple comparisons of stage, size, or growth rate at the termination of the experiment would therefore only be meaningful within each source population. For comparisons among sources, tadpoles would be of disparate ages and not at equivalent phases in their growth curves, i.e., some periods of development have steeper growth than others. To compare source populations, the primary objective of the experiment, we estimated the date of oviposition for each clutch sampled based on the Gosner stage observed in the field at time of collection and calculated growth rate up to an equivalent age for each replicate, approximately four weeks old. We compared the effects of source (seven levels) and temperature (2 levels) using 2-way analysis of variance, treating both factors as fixed effects. To meet assumptions of normality and homogeneity of variances, we transformed the data by taking the natural logarithm of growth, ln(mm/day).

### 3.3.2 Results

Tadpoles developed more slowly in the cool treatment than the warm treatment (Fig. 37). Tadpoles hatched from embryos between stages 18 and 20. Once tadpoles reached stage 25, the last stage before limb buds are discernible, development slows, as can be seen by flat regions in the curves of Fig. 37. Tadpoles continue to grow during this period however and during the first month of development, we observed significant effects of temperature treatment and source population (Table 8) on body length. Post-hoc comparisons of growth rate (Fig. 38) showed that for every source population tadpoles grew significantly faster at the warmer temperatures. Within the warm rearing temperature treatment, tadpoles from Sierran sources collectively grew significantly faster than those from coastal populations (T=-2.7, *P*=.024). At the cold rearing temperature, the capacity for faster growth by Sierran tadpoles was not evident (T=-2.03, *P*=0.1). Tadpoles from the two regulated reaches of the NF Feather had the highest mean growth rates in the warm treatment, but none of the pair wise comparisons among the Sierran groups were significant.

This result suggests that there may be adaptation for rapid early growth among populations in the Sierra snowmelt rivers where the growing season and period of warm water conditions is shorter compared to coastal rivers and streams. The basis for this capacity may be either genetic or due to maternal effects, such as larger yolk size if Sierran females are larger than coastal females. Similar adaptive differentiation in developmental capacity has been observed in the European common frog, *Rana temporaria*, across a latitudinal gradient from southern Sweden to
northern Finland (Laugen et al. 2003). When reared in a common laboratory environment, tadpoles from northern latitudes where growing season is shorter had the capacity to develop more quickly than tadpoles from southern populations when food and temperature were manipulated. In their 3-factor experiments, age at metamorphosis decreased strongly and linearly as a function of latitude and rearing temperature (14°, 18°, or 22° C), but the effects of food level on development were only observed at the higher rearing temperatures. Laugen et al. (2003) concluded that time constraints rather than temperature per se may be the selective force at work because they observed northern populations to frequently encounter the warm water temperatures used in their experiment.

Table 8: Two Way Analysis of Variance of Tadpole Growth, ln(mm/day), during First Month of Larval Development (R²=0.67, n=70 Replicates of Groups of 5 Tadpoles).

<table>
<thead>
<tr>
<th>Source</th>
<th>Sums of Squares</th>
<th>df</th>
<th>F ratio</th>
<th>P value</th>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Source population</td>
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<td>6</td>
<td>5.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature x Source</td>
<td>0.115</td>
<td>6</td>
<td>0.918</td>
<td>0.489</td>
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<tr>
<td>Error</td>
<td>1.174</td>
<td>56</td>
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</tbody>
</table>

Figure 37: Development of Tadpoles in Growth Chambers at 16.6 °C or 19.5°C Daily Mean Temperature.
3.4 Recirculating Trough Common Garden, Rearing Expt III

3.4.1 EXPT III Methods.
While repairs were being made to the growth chambers, we set up a secondary experiment to create a ‘common garden’ rearing environment to address a question related to the one asked in the previous growth chamber experiment. Are there population level differences in the growth and developmental responses of tadpoles from coastal, Sierran, regulated and unregulated rivers in growth rate when exposed to the same thermal stressor? By capitalizing on the cool and foggy conditions at the Richmond Field Station we were able to create conditions similar to a river with hypolimnetic dam releases with respect to mean daily temperature. However, due to the relatively small volume of water recirculated there were relatively large diurnal fluctuations in temperature. These are similar to the conditions in isolated side pools where tadpoles are sometimes found in regulated rivers that have unsuitable in-channel conditions.

We made 15 replicate (Fig. 37) re-circulating troughs. Each channel functioned as a stream mesocosm for rearing tadpoles. We used 7 foot lengths 6” diameter PVC pipe cut in half and mounted them on saw horses at a slight incline so they would drain into a 250 gallon reservoir. Pond pumps and hoses were used to continuously circulate water back to the top of each trough. Troughs were lined with small river cobbles and were supplemented with loose filamentous green algae. On July 21 2010, each trough was stocked with 10 tadpoles between Gosner stages 25-28, and growth was monitored bi-weekly. Tadpoles came from one of 5 different source populations, with three replicate troughs for each source.
3.4.2 Trough Results

By the end of the experiment on Sept. 30, 2010, most tadpoles still had not reached metamorphosis. The median Gosner stages of tadpoles among all sources ranged from 40 to 41, just prior to front limb emergence, there were no significant differences in damp weight among source populations (Fig. 40). This result affirms the previous growth chamber results that under cold stress, any inherent capacity for faster growth that was observed in Sierran populations was not expressed.

When comparing the growth trajectories in the 2010 trough experiment to the 2008 in situ multi-stream experiment, it is interesting to note that peak body mass occurs at earlier developmental stages under cooler rearing conditions. For the 2010 outdoor troughs, where M30DAT =17.8°C, tadpole peak body mass occurred early, at stage 37. At the two comparably cool rearing locations of Fox and Elder Creek in 2008 (Fig. 36), tadpoles continued to grow until stage 39, and in the warm locations growth continued through stage 41, (although still at a younger age). Most life history models regarding the optimal timing of metamorphosis base predictions on theories that tadpoles optimize growth rates (Wilbur and Collins 1973), balance growth opportunities against predation risk (Werner 1986), or must reach a minimum threshold size (Day and Rowe 2002) before maturity is possible. These models are based on mass rather than age. For tadpoles living in ephemeral or seasonally transient habitats, such as ponds that dry up or rivers that flood, time constraints may be more pressing. *R. Boylii* tadpoles could not survive if they did not metamorphose and leave the active channel before the first fall rains and flooding occurs. Our results that suggest a trade-off between development time and growth are consistent with a more recent model of metamorphosis timing which incorporates time constraints by assuming increasing risk of mortality as the season progresses (Rudolf and Rödel 2007).
3.5 Combined Effects of Predators and Temperature, Rearing EXPT IV

3.5.1 Methods of EXPT IV

Tadpoles were reared in four different thermal environments (Fig. 41) in the presence or absence of invertebrate predators. A freshly deposited clutch of eggs (site 0 in Fig. 41) was divided into three parts on May 15, 2009 and placed in flow-through enclosures in three different streams along a longitudinal gradient in the stream network. The three hatching locations, Fox Creek (site 1), Tenmile Creek (site 4), and the South Fork Eel River (site 3) have distinct thermal regimes, cold, warm, and intermediate respectively (Fig. 42). Hatching occurred at the warmest site (#4) after approximately one week, two weeks at site 3, and three to four weeks in Fox Creek (#1). After hatching in their respective locations subsets of larvae were transported to site number 2 in order to have a range of sizes and stages of tadpoles at one intermediate temperature. Rearing and predation trials were conducted at all four sites.
Figure 41: Locations (1-4) of Reciprocal Transplant EXPT IV, Annual Mean Number of Clutches Deposited at Breeding Sites, and Thermal Environments in the South Fork Eel River Watershed.

Figure 42: Water Temperature at the Four Experimental Locations. Temperatures Are Based on Three I-Button Sensors Placed inside Randomly Selected Enclosures at Each Site. Light Shaded Rectangles Indicate Dates of Predation Trials.
Figure 43: Experimental Design and Number of Replicates in Tadpole Rearing Experiments (n=67 in June, n=48). *For Site 2 with Fewer Replicates, Tadpoles were Randomly Redistributed among Replicates to Equalize Sample Size.

The experimental design is illustrated in Fig. 43. On June 18 a single water scorpion was added to 6 enclosures at each site. Twelve enclosures at each site remained predator free controls. These control tadpole replicates were used for the July reciprocal transplant trials. At the completion of each two week predator trial, surviving experimental tadpoles were released. Density in the June trial was ten individuals per enclosure and in the July trial density was 5 per enclosure which mimics the natural decrease in density during the course of the summer in the open river due to dispersal and attrition to predators (Appendix A). With respect to food resources, tadpoles were supplied with copious amounts of algae (Cladophora glomerata with diatom epiphytes) which were re-stocked at 40 g damp weight every 7 days. Damp weight was achieved by spinning the algae in a salad spinner for 50 rotations. Two response variables were measured, survival and growth. Two-way Analysis of Variance tested the significance of the effects of temperature and predation as well as an interaction between a site’s temperature and the presence of a predator. To meet assumptions of normality and homogeneity of variances, survival data were subjected to the angular transformation. Growth was calculated as a proportional increase, the milligrams gained per day per milligram of weight at the start of the trial. Tadpoles were weighed in the field using an OHaus® portable electronic balance, and a
mean weight for each replicate was calculated. For Site 2 where there was a wider spread of initial starting sizes, we used Analysis of Covariance with predator presence as a fixed factor and the natural log of mean initial tadpole weight as the covariate.

3.5.2 Predation Trial Results

Water scorpions significantly decreased survival (Fig. 45, $F_{1,60}=9.09$, $P=0.004$). There was a significant effect of site, with the warmer sites having higher survival ($F_{3,60}=7.85$, $P<0.001$). The predator by site interaction was not significant ($F_{1,3}=1.73$, $P=0.17$). A difficulty in interpreting the results using this approach is that the mechanism is not clear. To determine if there was more predation at lower temperatures because the tadpoles were small and hence more vulnerable, we examined the trends within Site 2 (Fig. 46) where the mean daily temperature over the course of the predator trial was $18.5 \pm 0.35$ °C. As tadpoles grew their survival in the presence of a predator increased (Fig. 46). There was a significant interaction between presence of a predator and size ($F_{1,13}=6.9$, $P=0.02$). In other words, initial tadpole size determined risk of predation. Larger tadpoles are more likely to survive, either because of an escape in size if they are too large for the predator to handle, or an increase in burst swimming speed (Wilson and Franklin 2000) allowing them to evade the predator. In the absence of a predator, however, survival was independent of size.

**Figure 44:** Survival of Tadpoles in the Presence / Absence of a Predator in Relation to the Mean Daily Water Temperature over Two Weeks. Error bars = 1 SE.
For growth as the response variable (Fig. 46), 2-way ANOVA revealed that there were significant effects of site temperature (F_{3,60}=49.662, p<0.001) and predation (F_{1,60}=6.83, p=0.01), as well as a significant interaction between the presence of a predator and the temperature of the site where the trial occurred (F_{3,60}=3.13, p=0.032). Specifically, when tadpoles were less active in the presence of the nepid predator, growth was diminished at the two intermediate temperatures but not at the coldest and warmest sites.
When tadpoles were ten weeks old and size had diverged as a result of rearing temperature (Fig. 47), the reciprocal transplant trial began with dragonfly nymphs as the predators. Tadpoles that had been reared in the warmest site were more developed (Gosner stages 36-41) than at other sites. At equivalent developmental stages, however, tadpoles were larger when reared at intermediate temperatures (Fig. 47), indicating that the 30-day running average of daily temperature at Tenmile Ck up to that point in time (22.2°C) was above the optimal range for growth.

Dragonfly nymph effects on mortality were a function of tadpole size (i.e. temperature of rearing), whereas the sub-lethal effects on tadpole growth were temperature dependent (Fig. 48). The smallest tadpoles which had been reared in Fox Ck (30 day running average prior to reciprocal transplant = 16.0°C) were vulnerable to dragonfly nymphs with 23.3 percent and 26.7 percent eaten when the mean temperatures of predator exposure were 17.2°C and 23.8°C. Predators caused a significant decrease in growth for tadpoles transplanted to the cool site (F1,15 = 78.2, P<0.001), but not when tadpoles were transplanted to the warm site (F1,14 = 1.7, P=0.27). When the cold reared tadpoles were transplanted to the warm site, they were still at early developmental stages (Gosner 25-28), and hence they grew rapidly compared to the more developed tadpoles transplanted from intermediate temperature sites.

These results affirm our previous findings that growth and survival are greatest at intermediate temperatures and extend our understanding to the interaction between thermal stress (too cold or too warm) and predation risk. Similar to Anderson et al. (2001), who observed the vulnerability of larval Pacific Chorus Frog (Hyla regilla) tadpoles to invertebrate predators was a function of temperature, we found that water temperature can influence predator–prey interactions via indirect effects on prey size. Direct predator effects on tadpole growth varied depending on the temperature of exposure. In the presence of cold stress, predators significantly decreased tadpole growth, but did not have a similar impact under warm conditions (Fig. 48).
Figure 48: Tadpole Growth Rate (mm of Body Length Gained per Day Standardized by Initial Size) in a Cold (Top Panel) vs. Warm Stream (Bottom Panel) as a Function of Rearing History.

17.2 C (daily mean water temperature)

<table>
<thead>
<tr>
<th>Rearing History</th>
<th>Fox Creek</th>
<th>SF Eel upstream</th>
<th>SF Eel downstream</th>
<th>Tenmile Creek</th>
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<td>Control</td>
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<tr>
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23.8 C

<table>
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<th>Rearing History</th>
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<th>SF Eel upstream</th>
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<td></td>
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</tr>
<tr>
<td>Predator</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Rearing temperature: cold
Tadpole development: early rear limb bud
Emergence close to front limb

73
CHAPTER 4:
Diatom Flora Downstream of Dams as Food for Tadpoles: The Perils of Unpalatable Periphyton

Co-authors for section 4:

Amy J. Lind, US Forest Service, USDA Forest Service, Pacific Southwest Research Station, 1731 Research Park Drive, Davis, CA 95618. Present address: Tahoe and Plumas National Forests, 631 Coyote St., Nevada City, CA 95959. E-mail: alind@fs.fed.us

Paula Furey, Department of Biology, Saint Catherine University, St. Paul, Minnesota 55105. E-mail: pcfurey@hotmail.com

Didymosphenia geminata (Lyngb.) M. Schmidt, A. Schmidt, is a single-celled alga that can produce excessive mucopolysaccharide stalks. It is becoming increasingly prevalent in North American rivers. Didymosphenia has long been reported from North America. Since the early 1990’s, however, it has been expanding its range globally and within North America (reviewed in Whitton et al. 1990), forming dense mats with proliferations of stalk material (Kilroy & Bothwell 2011). Excess stalk production is triggered by low phosphorus levels (personal communication Max Bothwell, 2013). The expansion in range in several cases, also appears to be associated with flow regulation, colder water and higher elevation sites (Kumar et al. 2009, Bothwell et al. 2009, Kirkwood et al. 2009). Snowmelt rivers in the Sierra Nevada mountain range of California are thus particularly vulnerable to the expansion of Didymosphenia. Dams releasing cold water from the hypolimnion of the upstream reservoir may extend the lower elevations of where Didymosphenia (Fig. 49f) could establish. These conditions may also be creating conditions favorable to other mucilaginous stalked diatom species such as Cymbella, Encyonema, and Gomphonema (Fig. 49g-i). A hypothetical advantage of mucilaginous stalk production, especially in rivers with daily flow fluctuations (Fig. 50) where a varial zone is wetted and dried repeatedly, is that the mucilaginous stalk material may assist in keeping the algae moist as river stage fluctuates.

We examined the potential consequences of proliferation of mucilaginous stalk producing diatoms on tadpoles of FYLF. We assessed periphyton collected from the focal Sierran watersheds (Feather, American, and Tuolumne) for the presence of Didymosphenia and the prevalence of more nutritious diatom taxa (e.g. Epithemia and Rhopalodia which contain nitrogen fixing endosymbionts, Fig. 49a-c) and Fragilaria which are common in tadpole gut contents Fig. 49d,e). We also assessed the flora from varial zone and continuously wetted zones of regulated rivers where flow fluctuates daily with short term power generation and with more stable flows. In the laboratory, we reared tadpoles on diets of periphyton covered rocks from a regulated river with Didymosphenia, a regulated river with native mucilaginous stalk producing diatoms, and an unregulated stream with high food-quality periphyton.
Figure 49: Micrographs of Diatoms Found in Study Rivers and Tadpole Gut Contents. Scale Bars = 10 Microns in Length. Photo Credits, Paula Furey.

Figure 50: Diurnal Stage Fluctuation in the Middle Fork American River at Site Where Cobbles Collected for Tadpole Growth Assay.

**Figure 49: Diatoms**

- **a**: *Ephippium viride*
- **b**: *Epithemia c. edneta*
- **c**: *Rhopalodia gibba*
- **d**: *Fragilariopsis*
- **e**: *Achnanthes minutissima*
- **f**: *Didymosphenia geminata*
- **g**: *Cymbella*
- **h**: *Gomphonema acuminatum*

**Figure 50: Diurnal Stage Fluctuation**

Graph showing diurnal stage fluctuation in the Middle Fork American River at a site where cobbles were collected for tadpole growth assay. The graph indicates changes in water chemistry or another variable over a period of 92 days.
4.1 Periphyton Sampling and Rearing Expt Methods

4.1.1 Periphyton and Tadpole Collection

During the late summers of 2009 and 2010, we sampled the Clavey and Tuolumne Rivers (Stanislaus National Forest, Mariposa County); Middle Fork American River downstream of Oxbow dam, Rubicon River downstream of Hellhole Dam (El Dorado National Forest, Placer County); Trinity and North Fork Trinity Rivers (Trinity National Forest, Trinity County); Middle Fork Feather River, North Fork Feather River downstream of the Rock Creek Dam and Poe Dam (Lassen National Forest, Plumas County). We sampled 3 cobble bars in each river and randomly selected 3 rocks in the varial zone, and 3 in the thalweg (fully wetted zone). From an area delineated by a 23 x 34 mm slide frame, we scraped algae from the rock surfaces using a toothbrush and pipette until no algae were macroscopically visible (Fig. 51). We combined samples from the 3 rocks in each zone (total of 6 samples per river). We topped off samples with river water to 38 ml and added 2mL of formalin for a total volume of 40mL. Samples were stored under refrigeration until processing. We blended samples for 30 sec. on the low-blend setting of an Osterizer® Blender before subsampling. We preserved two to five mL aliquots in ~2 percent formalin for microscopy to detect Didymosphenia (all rivers) and conduct more focused quantitative counts of algal cells for two rivers of particular interest where power-peak discharges occur in some reaches, the North Fork American and Feather River sites.

At the North Fork and Middle Fork Feather River sites we also collected 2 tadpoles per cobble bar for gut content analysis. Tadpoles were euthanized in a 125 mg/50 mL MS-222 solution and similarly preserved in formalin. We sub-sampled the preserved tadpoles’ diet by dissecting out the gut, partially uncoiling it and excising a 6 mm segment from the inflection region (Fig. 26). We chose this region of the mid-gut because it is readily recognizable and thus standardizes the location of sampling from tadpole to tadpole. The compaction of digested material in this region also decreases the variability in concentration and type of algal cells eaten that is observed when sampling the foregut. The gut contents were placed in an eppendorf tube with 1.2 mL distilled water, macerated with a dissecting needle, and homogenized with a Fisher Vortex Genie for 10 seconds.

Figure 51: Periphyton Sampling Methods.
4.1.2 Periphyton Processing

We removed the formaldehyde solution from the algal subsamples prior to microscope analysis by centrifuging samples at 4000 rev/min for 60 sec (Fisher Accuspin Micro 17) and replacing the supernatant with DI water two times. Cells were re-suspended in distilled water and transferred to counting chambers. To determine algal relative abundance, we counted a minimum of 300 cells/counting unit using a Palmer-Maloney chamber (Wildco Wildlife Supply, Buffalo, NY) observed at 400x using Nikon Optiphot (Nikon Corporation, Japan) and Leica DM LS2 (Leica Microsystems, Germany) photomicroscopes. Where algae were sparse and a 300 cell count could not reasonably be achieved, we counted 100 fields of view. A whole cell was considered a counting unit for all algae except for filamentous cyanobacteria where one 10 μm length was considered a counting unit. We counted Didymosphenia cells present in three entire Palmer-Maloney chambers for each sample in order to better estimate densities of these large diatoms. We identified diatom (Bacillariophyta), green (Chlorophyta), and cyanobacteria algal cells to genus.

4.1.3 Nutritional Analyses and Growth Assay

In conjunction with laboratory nutritional analyses (Table 4.5), we assayed the quality of Didymosphenia and native mucilaginous diatom taxa as food for aquatic consumers, such as tadpoles, using a short-term rearing experiment. We conducted a two-factor trial, with three levels of periphyton source, and two levels of temperature. For tadpoles, we used two-way analyses of variance to assess the main effects of periphyton and temperature as well as the interaction between periphyton and temperature on the response variables, tadpole growth (change in weight / initial weight) and food consumption (mg of fecal dry mass produced in one hour). For periphyton we compared the ash free dry mass (mg /cm²) of scraped from the surfaces of cobbles, total nitrogen, percent protein, percent crude fat, and percent silica. Nutritional analyses were conducted by the UC Davis Analytical Laboratory (http://anlab.ucdavis.edu).

On June 16 2010, we collected embryos from a single clutch of eggs from the Middle Fork American River (39.007558, -120.731403), and reared the full sibling group of tadpoles outdoors in 85 L tubs. The feeding experiment was conducted Aug 26 – 29, 2010 when the median Gosner stage was 34. During the feeding trials, tadpoles were housed individually in 5 L aquaria placed in diurnal illumination incubators with a photoperiod set at 14 hr light, 10 hrs dark. Each aquarium was aerated with an airstone connected to a pump outside the chamber and was stocked with a periphyton covered cobble (median diameter 130-160 mm). We collected cobbles at three locations, the Middle Fork American River where Didymosphenia is dominant (Fig. 50), the NF Feather Rock Creek reach where native mucilaginous diatoms predominate, and an unregulated reach of Alameda Creek where tadpoles are known to have relatively large body sizes (Kupferberg et al. 2011). Alameda Creek periphyton served as the high food quality control treatment.

Diurnal lighted growth chambers (ShelLab Model LI 15) were programmed with two different thermal regimes. One mimicked the cool conditions (14°C night, 18°C day) typical of a
regulated river receiving hypolimnetic releases from an upstream reservoir and the warm near shore conditions (17.9°C night, 21.5°C day) of a typical free flowing river occupied by *R. Boylii*.

**Table 9: Nutritional Analysis of Periphyton Fed to Tadpoles.**

<table>
<thead>
<tr>
<th>Site</th>
<th>Regulated/Unregulated and Algae</th>
<th>Total N (%)</th>
<th>Protein (%)</th>
<th>Crude Fat</th>
<th>Si (%)</th>
<th>AFDM (mg/cm²)</th>
<th>Organic material mean % (SE)</th>
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</thead>
<tbody>
<tr>
<td>Alameda</td>
<td>Unregulated, high food-quality periphyton</td>
<td>1.64</td>
<td>10.3</td>
<td>0.39</td>
<td>8.32</td>
<td>3.2 (1.1)</td>
<td>45.7 (4.1)</td>
</tr>
<tr>
<td>MF American</td>
<td>Regulated, <em>Didymosphenia</em> dominated</td>
<td>0.26</td>
<td>1.6</td>
<td>&lt;0.25</td>
<td>13.51</td>
<td>4.4 (0.7)</td>
<td>10.7 (0.89)</td>
</tr>
<tr>
<td>NF Feather</td>
<td>Regulated, other stalked mucilaginous diatoms</td>
<td>0.86</td>
<td>5.4</td>
<td>&lt;0.25</td>
<td>16.21</td>
<td>8.5 (0.6)</td>
<td>20.6 (1.5)</td>
</tr>
</tbody>
</table>

### 4.2 Results: Periphyton Quality among Sites and Effects on Tadpoles

#### 4.2.1 Periphyton Differences among Sites

*Didymosphenia* was present in 3 of the regulated rivers. It was present at low abundance in the colder upstream reaches of the Trinity, Tuolumne, and Rubicon. It was absent from the unregulated NF Trinity, Clavey, and NFMF American. It was absent in the NF Feather/MF Feather, although in the varial zone of the Rock Creek reach native species of stalked diatoms were prevalent (Fig. 52). In the MF American, the diatoms were mostly comprised of large *Didymosphenia* cells, with many very small *Achnanthidium* (which grow as epiphytes on the *Didymosphenia* stalks) and *Fragillaria*, which are also small in biovolume compared to *Didymosphenia* (Fig. 49). Thus, although there was not numerical dominance by *Didymosphenia*, it was prevalent. In the unregulated MF Feather and regulated Poe reach of the NF Feather, diatom taxa with nitrogen fixing endosymbionts were 2-3 times more common in terms of percentage of total cells counted per sample, and stalked mucus producing taxa were rarely found in epilithic samples. Mucus producing stalked diatoms comprised the smallest portion of the free-living tadpole diets.
4.2.2 Interaction between Temperature and Mucilaginous Stalked Diatoms on Tadpole Food Consumption and Growth

Using production of feces as an index, periphyton source had a significant effect on consumption rate \((F_{2,21} = 13.2, p < 0.001)\). Tadpoles appear to have ingested *Didymosphenia* dominated periphyton at warmer temperatures at a rate similar to tadpoles consuming control periphyton from Alameda (Fig. 53) and consumed 2-3 times more periphyton in the warm treatment compared to the cold treatment \((F_{1,21} = 29.7, p < 0.001)\). Periphyton from the NF Feather was not appreciably consumed regardless of temperature, as indicated by the significant interaction of periphyton source × temperature \((F_{2,21} = 7.9, p = 0.003)\). With respect to growth (Fig. 54), the main effects of temperature and periphyton source were significant (temperature \(F_{1,21} = 35.4, p < 0.001\); Periphyton \(F_{2,21} = 40.1, p < 0.001\)) with tadpoles in the cold treatment maintaining their weight on Alameda Creek periphyton, and losing weight on the other two sources. In the warm treatment, tadpoles did consume the *Didymosphenia* dominated assemblage from the MF American, as confirmed by analysis of the fecal pellets (Fig. 55). However most did not grow when eating *Didymosphenia* with a 72 hr relative weight gain of 4.3 ± 5.4 percent, compared to 30.7 ± 3.4 percent for controls with cobbles from Alameda Creek, where tadpoles ate mostly *Epithemia*. For the NF Feather site dominated by other native stalked diatoms, tadpole weight loss was 21.0 ± 9.2 (cool treatment) and 16.6 ± 5.6 percent (warm), and this response contributed to a significant Periphyton Source x Temperature interaction \((F_{2,21} = 6.5, p = 0.006)\).
Microscopic examination of the fecal pellets collected from the tadpoles eating periphyton from the three different source locations revealed that *Epithemia* dominated the diet in the high food quality treatment in which tadpoles grew. Although cell contents may be gone after passage through the digestive tract, they can still be identified by frustule or cell shape and are an accurate measure of tadpole diet. This result, combined with the previous field rearing experiments highlights the importance of *Epithemia* as an important food resource for tadpoles, and that a diet heavy in *Epithemia* mediates the effects of low temperature on growth.

**Figure 53: Tadpole Consumption (Mg of Feces Produced in 1 Hr) of Periphyton from Source Locations with Few Stalked Diatoms (Alameda Creek), *Didymosphenia* (Middle Fk. American River), or Other Native Stalked Diatoms (Rock Creek Reach of the North Fk. Feather River).**

![Figure 53: Tadpole Consumption](image)

**Figure 54: Tadpole Growth (Percent Change in Damp Body Weight) in Relation to Food Source and Incubation Temperature.**

![Figure 54: Tadpole Growth](image)
Figure 55: Diet Composition (Based on Fecal Pellet Contents) of *R. Boylii* Tadpoles Provided Periphyton Cobbles from Three Source Locations. Percent Composition is Based on Counts of Cells (Top) and Biovolume (Bottom) of Algae Ingested.
CHAPTER 5: Conclusions

5.1 Fundamental and Realized Thermal Niche of *Rana Boylii*.

We have used the idea of thermal niche to provide a framework for summarizing the effects of hydropower projects on the thermal ecology of *R. Boylii* at diverse spatial and temporal scales. We explored the physiologically bounded aspects of the fundamental thermal niche by measuring preferred temperatures and performance components for tadpoles (e.g., digestive efficiency, growth, production) in a laboratory setting to define the fundamental thermal niche. This species’ field distribution, however, may, or may not, be consistent with laboratory measured preferred temperatures and performance curves. To determine the realized thermal niche of *R. Boylii* we collected temperature data in paired regulated and unregulated rivers and compiled population censuses and environmental correlation data collected by various utility companies with hydropower facilities in the Sierra (Entrix for Placer County Water Agency, Garcia and Associates 2003, 2004a 2004b,). We found that high densities of *R. Boylii* breeding populations were found where the water temperatures in the near shore environment were similar to those preferred by tadpoles and which were beneficial to survival, growth, and rapid development in controlled experiments.

Our results of compiled distribution and temperature data show that measurements of individual thermoregulatory preferences of tadpoles can be used to predict recruitment success and distribution of populations within watersheds that encompass a network of rivers with impoundments and diversion reaches and unregulated tributaries. Empirical observations of the spatial distribution of *R. Boylii* breeding (Section 2) were generally consistent with experimental observations (Section 3). Optimal growth and development of larvae occurs at summer maximum 30 day average temperatures (M30DAT) near or above 20°C. In the Sierra, at the upstream extent, M30DAT ≤ 19.3°C appears to limit the presence of relatively abundant frog breeding populations when releases from upstream reservoirs cool the water. This threshold temperature is associated with the frog population in Middle Yuba River near confluence with Wolf Ck., where abundance is known only from 2008. All other Sierran sites with relatively abundant frog breeding populations (>5 clutches/km) and longer term frog monitoring records had warmer summer temperatures, 20.3 - 24.2 °C M30DAT as measured in dry years. Temperatures in wet years are 1-2 °C cooler. In the Eel watershed (coastal climate), the cold limit of abundant populations appears to be lower than the Sierra, perhaps somewhere below 18.8°C (dry year M30DAT). However, a gap in sampled sites with M30DAT = 17-18.8°C in the coastal region exists.

Where M30DAT ≤ 19.3°C in the Sierra, the density of breeding frogs was ≤ 5/km. Although there is uncertainty in determining a minimum viable population size for FYLF, these small populations may not be sustainable when abiotic, biotic, or anthropogenic stressors such as cooler temperatures and mortality due to untimely flow fluctuations decrease recruitment. An empirically derived viability threshold in the absence of anthropogenic stressors could be developed by examining the least dense, yet stable, populations. In the Sierra, there are few
unregulated sites with long term monitoring histories, however, the population in Shady Creek has a density of 5.4 clutches/km, based on six years of data (S. Yarnell, pers. comm.). In regulated rivers with long term monitoring, sites where breeding density is lower (approximately 2 clutches/km) appear to be in strong demographic decline (see NF Feather River Cresta reach and Alameda Creek below Calaveras Dam, Kupferberg et al. 2012).

While the benefits of operating at temperatures near preferred temperatures include many physiological and biochemical processes (Huey 1982), the benefits may be greater in developing organisms, such as tadpoles, and thus the primary thermal impact of cold water temperatures associated with hydropower operations may be on recruitment of juveniles, rather than on adults. Temperature directly influences differentiation and growth rates (Berven 1982; Berven and Gill 1983, Harkey and Semlitsch 1988), which are key processes during ontogeny (Smith-Gill and Berven 1979). Selecting temperatures that are optimal for consumption rates, food conversion efficiency, metabolic and growth rates is important for algal grazing tadpoles specifically (Skelly and Golon 2003) and aquatic ectothermic herbivorous vertebrates in general (Wikelski et al. 1993; Clements et al. 2009). A population viability analysis conducted for *R. Boyl ii* indicates that its populations are very sensitive to early life stage survival (Kupferberg et al. 2009a). This pattern contrasts with population projection models for pond breeding amphibians (Biek et al. 2002; Vonesh and De la Cruz 2002; Govindarajulu et al. 2005) which suggest that the most sensitive vital rates are post-metamorphic juvenile survival, but is consistent with findings for many river fish taxa in which populations depend on survival of early life stages (Strange et al. 1993; Humphries and Lake, 2000; Pusey et al. 2001). By integrating the impact of local productivity, temperature, and predation risk on survival with an index of habitat thermal quality derived from the thermoregulatory behavior, our simple experiments illustrate a mechanistic link between thermal regime change and frog population dynamics.

Both the temperature and algal food quality factors had significant multiplicative effects on food consumption rate, tadpole growth and development. However conversion efficiency was influenced by algal food quality alone, not temperature in our experiments. Increased predation risk was a consequence of the small size of tadpoles reared at sub-optimal temperatures. The sub-lethal effect of predators, stunted tadpole growth, was apparent only under cool conditions. The probable mechanism was that when tadpoles remained still and did not forage to avoid detection at cold temperatures, food consumption declined with detrimental effects on growth, whereas at warm temperatures tadpoles could maintain growth in spite of foraging less. Empirical observations of the spatial distribution of *R. Boyl ii* breeding are generally consistent with the aggregate of experimental observations that optimal growth and development of larvae occurs at summer maximum 30 day average temperatures (M30DAT) near or above 20°C. Under cold conditions across all the laboratory and field experiments we conducted, tadpoles gleaned little food and did not grow appreciably, even on high food quality algae. At the warmer temperature (approx. 20°C daily mean), tadpoles did not grow on periphyton from regulated rivers when the algal community was dominated by mucus producing taxa, despite there being similar or greater biomass of periphyton per unit area on the regulated river rocks. At 20°C mean daily temperature, tadpoles ingested *Didymosphenia*
dominated periphyton at a rate similar to tadpoles consuming control periphyton (high-quality periphyton), but did not grow, with a 72 hr relative weight gain of 4.3 ± 5.4 percent, vs. 30.7 ± 3.4 percent for controls. For the regulated river site dominated by native stalked mucilaginous diatoms, tadpole weight loss was 21.0 ± 9.2 (cold) and 16.6 ± 5.6 percent (warm). These results indicate the importance of biotic factors, such as the composition of the periphyton community, to define the realized niche. Despite the presence of preferred thermal conditions, food resources can be limiting.

For tadpoles of *R. Boylii*, use of a thermal preference metric, such as $T_{se}$, which denotes a range of temperatures rather than a single mean value, provides a conservative estimate for bounding the fundamental thermal niche and evaluating the effects of alternative flow proposals that will alter water temperatures downstream of hydroelectric projects. The wide range of temperatures selected by *R. Boylii* tadpoles in the thermal gradient is not unusual among larval anurans (Hutchinson and Dupre 1992). The mean temperature we measured is consistent with values for other ranid species at similar or more northerly latitudes (Workman and Fisher 1941; Herreid and Kinney 1967; Wollmuth et al. 1987), and is warmer than the temperature selected by tadpoles of the tailed frog (*Ascaphus truei*) that inhabit similar but colder streams (De Vlaming and Bury 1970). Unlike tadpoles of *R. Boylii*, tadpoles of *Ascaphus* are morphologically adapted to overwinter in streams, and although they are similarly limited by low light effects on algal productivity, they can utilize multiple growing seasons to reach metamorphosis (Mallory and Richardson 2005). While conduction and convection are the dominant mechanisms of heat exchange in water, field observations suggest that *R. Boylii* tadpoles actively thermoregulate by moving to warmer, shallow edgewater habitats or by staying near the surface to exchange heat from solar radiation (Brattstrom 1962). Observations of such thermoregulatory behavior support the use of $T_{se}$ for measuring preference.

Acclimation temperature could also influence thermal preference, so we took precautions to minimize any effects of acclimation by minimizing transport and holding times prior to testing tadpoles in the thermal gradient. When acclimation has been investigated directly in other anurans, its effects are species-specific ranging from negligible (De Vlaming and Bury 1970; Hutchinson and Hill 1978; Wollmuth et al. 1987) to counter-gradient responses (Freidenburg and Skelly 2004). Counter-gradient response means that tadpoles reared in colder locations have higher thermal preferenda than tadpoles reared in warmer locations. When we tested tadpoles of *R. Boylii* reared in the colder streams of the SF Eel watershed, we did observe a counter-gradient response which thus makes our approach conservative with regard to assessing the thermal quality of habitats and for predicting thermal effects on frog recruitment.

### 5.2 Conservation Implications

Physiological, behavioral and ecological factors act in concert and knowledge of these interactions will improve the ability to forecast a species’ response to environmental change (Kearney *et al.* 2010). As illustrated by the dependency of larval *R. Boylii* survival on both the thermal and food quality of the habitat, appreciation of these factors is especially relevant for populations of this declining amphibian in rivers with hydroelectric projects. The retention of water behind a dam can result in artificially low summer base flow discharges, causing
unnaturally warm downstream temperatures (Lessard and Hayes 2003). Alternatively, when summer base flow discharge is drawn from the hypolimnion of the upstream reservoir, the resulting temperatures downstream are often colder than they would be naturally (Angilletta et al. 2008; Olden and Naiman 2010). Thus, when river managers and dam operators consider alternate flow proposals, the impacts of anticipated thermal changes should be evaluated within the context of the local algal and diatom flora. For example, spread of the invasive benthic diatom, *Didymosphenia geminata* which covers rock surfaces with mucilaginous extracellular stalks to the exclusion of other more edible algae, appears to be positively correlated with cool summer water temperatures and artificially stable base flows (Kilroy et al. 2008; Kumar et al. 2009). Reduced flow fluctuation and flood frequency also promotes extensive encroachment of riparian vegetation into formerly active channels (Poff and Zimmerman 2010, and references cited therein). Encroachment of the banks by woody species ultimately leads to higher canopy cover and shading of the shallow channel margins inhabited by tadpoles, thus creating poor conditions for periphyton growth. Our experiments also showed that another important indirect effect of reduced tadpole growth in cooler water was the increased risk of predation. Considering the interactive effects of water temperature, algal food quality, and predation will improve management strategies that will preserve populations of these and other threatened ectotherms.

When evaluating alternative flow regimes, river managers can forecast what the implications will be for summer temperatures downstream from the release point at the dam. Using the temperature ranges we have documented for optimal reproductive success of frogs, predictions can be made regarding the number of river kilometres of habitat to be lost or gained under the flow schedules being considered. If summer flows are to be increased to improve habitat for trout or other salmonids, suitable thermal conditions are likely to be pushed further downstream. Loss of suitable thermal habitat at the upstream end of a reach may not be compensated for downstream by cooling temperatures that may be above optimal levels. When there are successive reservoirs and dams along the length of a river, bullfrogs (Fuller 2008) and non-native fish predators (Moyle and Light 1996, Marchetti et al 2004) may be well established at the bottom of a reach in close proximity to the next reservoir. The presence of predatory fish (such as bass) and bullfrogs (which are predators as adults and superior competitors as tadpoles) are correlated with the absence of *R. Boylii* populations (Hayes and Jennings 1988, Kupferberg 1997b, Fuller 2008). Additionally, river managers can not uniformly add a set number of degrees to thalweg temperature models to forecast the extent of warm thermal refugia that may be created along the channel margins under various flow schedules. Lateral warming of edgewater habitats is highly site specific and varies among frog breeding sites within a river depending on the channel morphology.

Our results quantifying the relationship between daily mean temperature and development of embryos and tadpoles of *R. Boylii* are relevant to ramping rate planning. Having an accurate way to forecast development at a given temperature, ramping rates can be adjusted to ensure that breeding sites remain inundated for a sufficiently long period to allow embryos to completely develop and hatch and for recently hatched tadpoles to reach a stage when they are competent swimmers and can follow a receding shoreline. Stranding of egg masses when dam
operations result in rapid cessation of flows is a major source of mortality in regulated rivers (Kupferberg, et al. 2012).

5.3 Commercialization Potential

Commercialization is not applicable to this study.

5.4 Recommendations

Where possible, promote mixing of cold hypolimnetic water with warmer surface water leaving a reservoir via spillways to mitigate the effects of cold temperatures on larval development.

Water management entities need to recognize synergisms among various hydrologic changes as well as indirect effects, such as power peaking leading to growth of unpalatable periphyton which then has consequences for grazing consumers.

Gather data on thermal conditions at channel margins where amphibians breed as well the thalweg during FERC relicensing and adaptive management.

Improve long-term monitoring programs for *R. Boylii* in both regulated and unregulated (reference) river systems, especially in the Sierra Nevada.

Appropriate State and/or Federal agencies should elevate the protection status of *R. Boylii* (e.g., listing under the California or Federal Endangered Species Act). Evidence has accumulated documenting the range-wide decline of *R. Boylii* and has identified genetically unique populations in the Southern Sierra Nevada and Central Coast Ranges of California (Lind 2005). Likely mechanisms for *R. Boylii* population declines are well documented and populations that are extant in regulated streams continue to show evidence of declines and vulnerability to conditions downstream of many dams.

5.5 Benefits to California

This research will be valuable to the adaptive management of regulated rivers in terms of evaluating the relative merits of competing flow proposals. By assessing whether early life stages of the *R. Boylii* life cycle are likely to be affected by the thermal regime attribute under consideration, managers can better predict if their actions may put populations further at risk or aid in their restoration.
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**GLOSSARY**

Analysis of Covariance  A statistical method for analyzing the differences in the means of two or more groups of data, while accounting for variation in one interval-ratio variable.

Epilithic Periphyton/Diatoms Algae that grows on the surface of rocks in a water body.

FERC Federal Energy Regulatory Commission; the government body that licenses hydroelectric projects

*F* Ratio A statistical ratio in analysis of variance (ANOVA); the variation explained by a set of treatments or factors in relation to the unexplained (error) variation

M30DAT The maximum 30 day running average temperature

Ranid Frog Any frog species that is a member of the amphibian genus *Rana* and family *Ranidae*. 
APPENDIX A:
Tadpole Density in Rearing Experiments

As tadpoles developed, we manipulated the density in enclosures for two reasons. First, to mimic declining density patterns in the open river when tadpoles disperse from their oviposition / hatching sites and attrition occurs, we periodically removed tadpoles (Table A.1). Second, to maintain equivalent densities among enclosures in the four study streams, it was necessary to remove tadpoles from enclosures in the warmer sites to match numbers in the coldest streams (Elder and Fox Creeks), where weekly mortality rates were greater. Experimental densities were at the high end of the range of natural densities, but below the maximum density observed, during the course of quadrat surveys conducted along 5.2 km of the SF Eel from 1991-1994, and in 2009. We calculated the average developmental stage in each quadrat with tadpoles present, and grouped quadrats according to the ranges of stages used in the removal schedule for enclosed tadpoles (25–31, 32–35, and 36–42) to calculate the 99 percent confidence interval of density (#/m²). We calculated the 99 percent confidence interval for tadpole body size (mm) at each Gosner stages from 27-45 from individuals observed during quadrat sampling conducted between 1991-1994 and 2008-2009 (n=647 tadpoles) in the same reach of the SF Eel where baskets were placed (Fig. B.1).
Table A.1. Schedule for tadpole removal and comparison to open river densities.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Reduction</th>
<th>Density in open river&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dates</th>
<th>Dates (streams from warmest to coldest)</th>
</tr>
</thead>
<tbody>
<tr>
<td>When tadpoles…</td>
<td>tadpoles/enc 1</td>
<td>/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>99% CI, max</td>
<td>Tenmile</td>
</tr>
<tr>
<td>…first reached stage 25</td>
<td>30  ⇒  20</td>
<td>80</td>
<td>26 May</td>
<td>26 May</td>
</tr>
<tr>
<td>…have reached stage 25 for ~1 week</td>
<td>20  ⇒  15</td>
<td>60</td>
<td>14.9–44.3, 428</td>
<td>1 June</td>
</tr>
<tr>
<td>…first reached stage 32</td>
<td>15  ⇒  10</td>
<td>40</td>
<td>5.4–9.1, 44</td>
<td>23 June</td>
</tr>
<tr>
<td>…first reached stages 35 or 36</td>
<td>10  ⇒  5</td>
<td>20</td>
<td>3.7–6.9, 52</td>
<td>1 July</td>
</tr>
<tr>
<td>…have reached stage 42</td>
<td>5  ⇒  1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≤16</td>
<td>starting 20 July</td>
<td>starting 20 July</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sample sizes = number of quadrats with tadpoles in the Gosner stage ranges: n<sub>25-31</sub>= 94, n<sub>32-35</sub>= 104, n<sub>36-42</sub>= 119.

<sup>b</sup> Individuals were removed as they reached stage 42, so remaining tadpole densities were between 1 and 4 per enclosure.

**Fig. A.1**

![Graph showing tadpole body length and 99% CI in SF Eel compared to open river](image)

A-2
APPENDIX B.  
Temperature Summaries - 2009

Table B.1 Temperature variations, $T_{\text{mean}}$ $(T_{\text{min}}–T_{\text{max}})$, at *Rana Boylii* breeding sites and potential breeding habitats in Coast Range rivers – Summer 2009. Square brackets denote sampling period < 1 month. [May] indicates that temperatures are only for period after egg deposition.

<table>
<thead>
<tr>
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<tr>
<td></td>
<td></td>
<td>$T_{\text{max}}$</td>
<td>$T_{\text{mean}}$ $(T_{\text{min}}–T_{\text{max}})$</td>
<td>$T_{\text{mean}}$ $(T_{\text{min}}–T_{\text{max}})$</td>
<td>$T_{\text{mean}}$ $(T_{\text{min}}–T_{\text{max}})$</td>
</tr>
<tr>
<td>Tenmile (Angelo Coast Range Reserve)</td>
<td>0.7 km upst confl.$^{1}$</td>
<td>382</td>
<td>–</td>
<td>&lt;18 Apr 09</td>
<td>16.5 (11.1–23.6)</td>
</tr>
<tr>
<td></td>
<td>27.7</td>
<td>20.9 (20.9–25.7)</td>
<td></td>
<td></td>
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<tr>
<td>South Fork Eel</td>
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<td></td>
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<tr>
<td></td>
<td>455</td>
<td>–</td>
<td>&lt;3 May 09</td>
<td>12.1 (9.1–15.2)</td>
<td>13.6 (10.7–18.2)</td>
</tr>
<tr>
<td></td>
<td>19.7</td>
<td>14.6 (10.7–18.2)</td>
<td></td>
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<tr>
<td></td>
<td>424</td>
<td>–</td>
<td>~13 Apr 09</td>
<td>12.6 (9.6–16.7)</td>
<td>15.3 (11.7–20.7)</td>
</tr>
<tr>
<td></td>
<td>23.6</td>
<td>18.6 (14.6–22.1)</td>
<td></td>
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<tr>
<td></td>
<td>398</td>
<td>–</td>
<td>~13 Apr 09</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>24.7</td>
<td>18.9 (15.7–22.7)</td>
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<tr>
<td></td>
<td>380</td>
<td>–</td>
<td>~13 Apr 09</td>
<td>[15.8 (11.7–28.2)]</td>
<td>17.4 (11.7–25.7)</td>
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<tr>
<td></td>
<td>29.7</td>
<td>21.0 (14.2–27.2)</td>
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<tr>
<td>Eel River</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Downst. Scott Dam$^{4}$</td>
<td>520</td>
<td>0.9</td>
<td>no breeding</td>
<td>–</td>
<td>13.3 (12.2–14.7)</td>
</tr>
<tr>
<td></td>
<td>17.7</td>
<td>18.2 (15.7–20.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>Date</td>
<td>Egg Masses</td>
<td>Temperature</td>
<td>Record</td>
<td></td>
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<td>--------------------------------</td>
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<td></td>
</tr>
<tr>
<td>Benmore Ck. confl.</td>
<td>511</td>
<td>3.4</td>
<td>?</td>
<td>19.3 (11.7–33.2)</td>
<td></td>
</tr>
<tr>
<td>Old mine road</td>
<td>492</td>
<td>6.7</td>
<td>&gt;6 June 09</td>
<td>14.9 (12.7–18.2)</td>
<td></td>
</tr>
<tr>
<td>Bucknell Ck. confl.</td>
<td>470</td>
<td>11.6</td>
<td>&gt;6 June 09</td>
<td>16.1 (12.6–20.1)</td>
<td></td>
</tr>
<tr>
<td>As above, sidepool</td>
<td>470</td>
<td>11.6</td>
<td>&gt;6 June 09</td>
<td>[20.2 (14.7–24.8)]</td>
<td></td>
</tr>
<tr>
<td>Trout Ck. confl. Isl.</td>
<td>462</td>
<td>12.8</td>
<td>?</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Trout Ck. confl. Edge</td>
<td>462</td>
<td>12.8</td>
<td>?</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Pioneer bridge</td>
<td>456</td>
<td>17.4</td>
<td>&gt;6 June 09</td>
<td>16.9 (13.2–20.7)</td>
<td></td>
</tr>
</tbody>
</table>

**Bucknell Creek (Eel confluence 11.4 km downstream of Scott Dam)**

<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>Egg Masses</th>
<th>Temperature</th>
<th>Record</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upst. Eel confl.</td>
<td>469</td>
<td>–</td>
<td>&gt;6 June 09</td>
<td>[21.1 (17.2–24.7)]</td>
</tr>
</tbody>
</table>

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1. All egg masses scoured on 4 May 2009; no egg masses were laid after this date
2. Record for May starts on 3 May 2009
3. Record for May starts on 15 May 2009
4. Record for June starts on 4 June 2009
5. Data collected only between 14 July and 14 August 2009 (iButtons stolen)
6. Data collected only between 14 July and 10 September 2009
7. Data collected only between 29 July and 11 September 2009
8. Data collected only between 14 and 20 July 2009 (creek dry after 20 July 2009)
Table B.2. Temperature variations at *Rana Boylii* breeding sites and potential breeding habitats in Sierran foothills rivers – Summer 2009. Square brackets denote sampling period < 1 month. [May] indicates that temperatures are only reported for period after egg deposition.

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<tr>
<td><strong>Clavey</strong></td>
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<tr>
<td>USFS Road 1N01</td>
<td>734</td>
<td>734</td>
<td>–</td>
<td>–</td>
<td>22.0</td>
<td>25.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–</td>
<td>(15.7–27.7)</td>
<td>(22.5 (15.7–27.7)]</td>
</tr>
<tr>
<td>At432m²</td>
<td>432</td>
<td>432</td>
<td>&gt;22 June 09</td>
<td>–</td>
<td>22.0</td>
<td>23.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–</td>
<td>(17.2–30.2)</td>
<td>(19.6–27.1)</td>
</tr>
<tr>
<td>At398m³</td>
<td>398</td>
<td>398</td>
<td>&gt;22 June 09</td>
<td>–</td>
<td>25.0</td>
<td>23.5</td>
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<td></td>
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<td></td>
<td>–</td>
<td>(18.1–20.2)</td>
<td>(19.7–27.2)</td>
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<tr>
<td>Upst. Tuol confl.³</td>
<td>385</td>
<td>385</td>
<td>~22 June 09</td>
<td>–</td>
<td>22.3</td>
<td>23.1</td>
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<td>–</td>
<td>(18.2–20.7)</td>
<td>(17.2–25.7)</td>
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<tr>
<td><strong>North Fork Middle Fork American</strong></td>
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<td></td>
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<tr>
<td>At461m⁴</td>
<td>461</td>
<td>461</td>
<td>?</td>
<td>–</td>
<td>17.5</td>
<td>18.2</td>
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<td></td>
<td>–</td>
<td>(15.7–20.2)</td>
<td>(16.2–20.7)</td>
</tr>
<tr>
<td>Mosquito Bridge⁵</td>
<td>391</td>
<td>391</td>
<td>~28 May 09</td>
<td>17.5</td>
<td>17.9</td>
<td>22.3</td>
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<td>(17.2–24.7)</td>
<td>(17.7–25.2)</td>
</tr>
<tr>
<td>Upst. MF Am confl.⁵</td>
<td>322</td>
<td>322</td>
<td>~28 May 09</td>
<td>18.2</td>
<td>18.6</td>
<td>22.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–</td>
<td>(16.2–20.7)</td>
<td>(17.7–24.2)</td>
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<tr>
<td><strong>Rubicon</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Downst. Hell hole</td>
<td>1266</td>
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<td>1.2</td>
<td>no breeding</td>
<td>9.3</td>
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<td>–</td>
<td>(7.2–18.2)</td>
<td>(7.7–27.7)</td>
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<td>Upst. SF Rubicon⁶</td>
<td>1085</td>
<td>1085</td>
<td>12.5</td>
<td>no breeding</td>
<td>[14.6 (12.1–17.2)]</td>
<td>15.8 (13.1–18.2)</td>
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<td>–</td>
<td>15.5 (13.7–18.2)</td>
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<td>11 Pines Rd Bridge</td>
<td>1021</td>
<td>1021</td>
<td>14.7</td>
<td>no breeding</td>
<td>14.3 (11.1–18.1)</td>
<td>17.2 (14.6–20.2)</td>
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<td>–</td>
<td>16.8 (15.2–19.7)</td>
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<td>Temperature</td>
<td>Breeding Status</td>
<td>Notes</td>
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<td>Long Canyon confl. 7 417</td>
<td>16 May 09</td>
<td>17.3 (14.2–20.7)</td>
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<td>18.5 (14.2–24.7)</td>
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<td>Powerhouse 7 363</td>
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1 Record for July starts on 7 July 2009
2 Record for June starts on 22 June 2009
3 Record for July starts on 23 July 2009
4 Record for June starts on 11 June 2009

5 Record for May starts on 28 May 2009

6 Record for June starts on 18 June 2009

7 Record for May starts on 16 May 2009

8 Record for June starts on 18 June 2009

9 Record for July starts on 9 July 2009

10 Record for June starts on 9 June 2009

11 Data collected only between 20 August and 15 September 2009 (reported values are for this period)