1 *Title of article:*

2 Flow modification associated with reduced genetic health of a river-breeding frog, *Rana*
3 *boylii*

4 *Authors' names:*

5 Ryan A. Peek[1†], Sean M. O'Rourke[2], Michael R. Miller[2]

6 [1] Center for Watershed Sciences, University of California, Davis, CA 95616, USA

7 [2] Department of Animal Science, University of California, Davis, CA 95616, USA

8 [†] E-mail: rapeek@ucdavis.edu

9

**Abstract**

River regulation or flow modification—the hydrological alteration of flow by dams and diversions—has been implicated as a cause of fundamental change to downstream aquatic ecosystems. Flow modification changes the patterns and functionality of the natural flow regime, and has the potential to restrict population connectivity and gene flow in river-dependent organisms. Since population connectivity and the maintenance of genetic diversity are fundamental drivers of long-term persistence, understanding the extent flow modification impacts these critical attributes of genetic health is an important goal for long-term conservation. Foothill yellow-legged frogs (*Rana boylii*) were historically abundant throughout many western rivers but have declined since the onset of regulation. However, the extent to which *R. boylii* populations in rivers with altered flow regimes are maintaining connectivity and genetic diversity is unknown. Here we use genetic methods to investigate the impacts of flow alteration on *R. boylii* to explore their potential for long-term persistence under continued flow modification. We found *R. boylii* in rivers with flow modification showed striking patterns of isolation and trajectories of genetic diversity loss relative to unregulated rivers. For example, flow modification explained the greatest amount of variance in population genetic differentiation compared with other covariates including geographic distance. Importantly, patterns of connectivity and genetic diversity loss were observed regardless of flow alteration level but were most prominent in locations with the greatest flow modification intensity. Although our results do not bode well for long-term persistence of *R. boylii* populations under current flow regulation regimes, they do

32 highlight the power of genetic monitoring for assessing population health in aquatic

33 organisms.

34

***Key words:***

Flow alteration; frogs; hydrologic connectivity; hydropower; population genetics; *Rana boylii*; river management; seasonality; Sierra Nevada.

## Introduction

River regulation, or the hydrological alteration of flow by dams and diversions, impacts the seasonal and interannual flow variability within a watershed. This flow modification changes the natural flow regime and dramatically alters geomorphic and hydrologic connectivity of watersheds (Poff et al. 2007), which may restrict population connectivity (Schick and Lindley 2007, Shaw et al. 2016) and gene flow (Deiner et al. 2007, Cazé et al. 2016, Thompson et al. 2019). River regulation can change flow frequency, magnitude, duration, timing, and rate of change, which can have significant impacts on aquatic organisms and ecological processes (Poff et al. 2007, Yarnell et al. 2010). Flow modification associated with hydropower generation has been implicated as a cause of fundamental changes to downstream aquatic ecosystems (Power et al. 1996, Bunn and Arthington 2002, Moyle et al. 2011). The hydrological regimes of over half of the world's largest rivers have been altered by large dams (Nilsson et al. 2005) and only recently has the extent of flow alteration and the associated ecosystem-level impacts been acknowledged (Pringle 2001, Dudgeon et al. 2006, Murchie et al. 2008).

Rivers simultaneously connect and carve the landscapes through which they flow. Rivers provide corridors of connectivity for riparian and aquatic organisms such as fish, amphibians, and macroinvertebrates (Wiens 2002, Pringle 2003), while also acting as physical barriers on the landscape for many terrestrial organisms (Voelker et al. 2013, Cazé et al. 2016). In Mediterranean climates, rivers have strong seasonal patterns associated with cold, wet winters and warm, dry summers. Native aquatic organisms have evolved life histories well adapted to these natural flow patterns, which are both

predictable and seasonal (Yarnell et al. 2010, Tonkin et al. 2017). Thus, changes to temporal and spatial abiotic river processes caused by river regulation can have a substantial impact on biotic communities. The negative effects of flow alteration on migration and loss of spawning habitat (Lind et al. 1996, Fuller et al. 2011, Kupferberg et al. 2012, Rolls and Bond 2017), reductions in population abundances and diversity (Lind et al. 1996, Zhong and Power 1996, Vörösmarty et al. 2010, Fuller et al. 2011, Scribner et al. 2016, Sabo et al. 2017, Guzy et al. 2018), and fragmentation (Zhong and Power 1996, Vörösmarty et al. 2010, Werth et al. 2014, Scribner et al. 2016, Sabo et al. 2017, Guzy et al. 2018) have been well documented. However, most rivers have not been regulated for long periods (e.g., typically less than 100 years) compared to the time aquatic organisms had to adapt to pre-anthropogenic flow conditions. In flow-altered rivers that organisms still occupy, it remains unknown whether populations can persist long-term with continued flow modification. In other words, while some species may have persisted since flow regulation began in a system (e.g., typically four to five decades), this does not necessarily mean these populations will persist into the future under current altered flow regimes. Thus, exploring the potential for long-term persistence of populations under different altered flow regimes is a crucial component for guiding conservation efforts, yet it remains a significant gap.

One tool that can help address this gap is the integration of genetics and hydrology to better assess the impact of flow alteration on aquatic organisms (Scribner et al. 2016). Although aquatic organisms are often difficult to count and monitor by conventional methods, genetic monitoring can be a powerful tool to assess population health by revealing factors such as genetic fragmentation and population declines. It is

widely recognized that reductions in population connectivity can increase inbreeding, leading to a potential "extinction vortex" (Gilpin and Soule 1986), yet there is limited understanding of how flow alteration may impair the processes crucial for maintenance of genetic variation and thus adaptive capacity. In addition, there is a pressing need for more effective and flexible watershed management tools, particularly in relation to monitoring aquatic populations, and assessment of environmental flows (Grantham et al. 2010, Yarnell et al. 2020). Thus, population genetics could be a powerful tool to understand the influence of different flow regimes on population health and this information could facilitate improved flow management to better protect aquatic populations.

The river-breeding foothill yellow-legged frog (*Rana boylii*) historically occurred in lower and mid-elevation streams and rivers from Southern Oregon to northern Baja California west of the Sierra-Cascade crest (Stebbins 2003). *Rana boylii* are intimately linked with river hydrology because they have evolved to spawn in synchrony with natural flow cues associated with seasonal spring snowmelt or rain recession periods (Kupferberg 1996, Yarnell et al. 2010, 2016, Bondi et al. 2013). However, population declines have been documented across the former range of this species, particularly in southern California and the Sierra Nevada where it has been extirpated from approximately 50 percent of its historical range (Jennings and Hayes 1994, Davidson et al. 2002). In California, particularly in the Sierra Nevada, flow alteration may be a significant environmental stressor (Lind et al. 1996, Kupferberg et al. 2012). Regulated river reaches typically alter flows by augmenting or diverting winter and spring runoff, thereby disrupting natural flow regimes and reducing or eliminating flow cues.

Aseasonal flow fluctuation from flow alteration can scour (i.e., detach from substrate) or desiccate *R. boylii* egg masses, and the loss of clutches may have a significant demographic impact because only one egg mass is laid per year. In many regulated rivers in the Sierra Nevada, *R. boylii* populations are now restricted to small unregulated tributaries flowing into the regulated mainstem (Peek 2018).

Here, we investigate the impacts of flow modification on genetic health of *R. boylii* populations across three different flow regimes. Given that population connectivity and genetic diversity are known to be play critical roles in long-term species persistence, our primary questions were:

1. How has flow alteration impacted seasonality and predictability of flow patterns in regulated river reaches?
2. Do populations of *R. boylii* in flow altered river reaches have less genetic diversity and decreased measures of genetic connectivity than populations in unregulated river reaches?
3. Are metrics of connectivity and diversity in *R. boylii* associated with different levels of flow alteration?

Our goal was to assess the genetic health of *R. boylii* under different flow regimes to better inform the potential for long-term persistence with flow alteration. Addressing this issue will help to inform management and conservation efforts for *R. boylii*, through evaluation and prioritization of conservation actions for populations with decreased genetic health. In addition, our approach demonstrates the potential utility of genetics

for future conservation monitoring and assessment efforts in aquatic species in altered systems.

## Material And Methods

To begin investigating the impact of river regulation on *R. boylii*, we collected frog tissue and buccal samples from 30 locations in six rivers representing three different flow alteration levels associated with hydropower generation (Table 1). The three flow regimes assessed were: 1) hydropeaking, where flows are pulsed on most days from late spring through fall to provide electricity during peak-use hours and for recreational whitewater rafting; 2) bypass, which diverts river flows from an upstream portion of the basin to the downstream power generation facilities; and 3) unregulated, a largely natural flow regime where no upstream controls exist to regulate flows (Figure 1A). Flow data were obtained for each river reach using proximal USGS gaging stations (Data S1). We analyzed *R. boylii* samples from sites in three major watersheds (Yuba, Bear, and American) in the northern Sierra Nevada of California (Figure 1A; Table 1, Data S2). The six study rivers share a similar Mediterranean climate, underlying geology, watershed aspect (west-slope), stream morphology (riffle-pool), and vegetative communities, but differ in the intensity of flow alteration (Steel et al. 2017). Although flow alteration occurs in all three of the study watersheds, both the North Yuba and North Fork (NF) American are unregulated whereas the Middle Fork (MF) American is the only river that has a hydropeaking flow regime (Figure 1B, Figure 1C).

### *Assessment of hydrologic impacts of river regulation on patterns of seasonality*

149 To evaluate how flow alteration has impacted seasonal flow patterns in
150 regulated river reaches, we used a wavelet analysis to derive metrics of predictability
151 and Colwell's information-theoretic metrics to define seasonality following methods
152 outlined in Tonkin et al. (2017). Seasonality—how much the environment varies during
153 the course of a single year—was measured using Colwell's M/P metric, which is the
154 contingency (M) divided by within season predictability (P) (Colwell 1974). Predictability
155 was measured using the standardized Morlet wavelet power at the 12-month time
156 interval across the entire data series (Torrence and Compo 1998). Daily average flow
157 data from all available gages within the study area were used to compare the inter-
158 annual (wavelet) and intra-annual (Colwell's indices) flow patterns in each of the study
159 reaches (Data S1, Data S3). In regulated reaches where data was available, metrics
160 were estimated from the full period of record prior to initiation of flow alteration (pre-
161 regulation) as well as from current altered flow regimes.

162 ***Sample collection and DNA extraction***

163 To evaluate genetic diversity and connectivity in *R. boylii* populations, a total of
164 345 tadpole tail clips, buccal swabs, and tissue samples were compiled. The samples
165 were collected between 1992 and 2017, from watersheds across the west slope of the
166 Sierra Nevada (Data S2). Field sampling was conducted following Heyer et al. (1994),
167 under CDFW SCP Permit #0006881, with IACUC protocol #19327. Individual post-
168 metamorphic frogs were buccal-swabbed following established protocols (Goldberg et
169 al. 2003, Pidancier et al. 2003, Broquet et al. 2007). Each post-metamorphic individual
170 was comprehensively swabbed underneath tongue and inside of both cheeks for

171 approximately 30 sec to one minute. Swabs were air dried for approximately five
172 minutes and placed in 1.5 mL microcentrifuge tubes while in the field or placed in lysis
173 buffer (Goldberg et al., 2003). Dried samples were stored in the laboratory at -80°C until
174 DNA extraction. Where possible, tail clips from tadpole larvae were collected, and
175 tadpoles greater than 15 mm total length were targeted (Parris et al., 2010; Wilbur &
176 Semlitsch, 1990). One clip was taken per individual tadpole and dried on Whatman filter
177 paper (grade 1) or placed in 95% ethanol and stored at room temperature. DNA was
178 extracted from samples in ethanol and lysis buffer using Qiagen DNeasy Blood & Tissue
179 kits following manufacturer protocol and stored at -20°C. DNA was extracted from dried
180 buccal swabs and tail clips using an Ampure magnetic bead-based protocol (Ali et al.,
181 2016) and stored at -20°C.

182 ***RAD Sequencing and RAD-Capture (Rapture) bait design***

183 To produce a genomic resource for frog species with large genome sizes, we
184 interrogated a significant fraction of the *R. boylii* genome using RAD sequencing with
185 SbfI (Miller et al. 2007, Baird et al. 2008, Ali et al. 2016). Paired-end Illumina sequence
186 data were generated using 24 *R. boylii* individuals (Data S4) and de novo locus
187 discovery and contig extension were carried out as previously described (Miller et al.
188 2012, Sağlam et al. 2016, Peek et al. 2019) using the alignment program Novoalign and
189 the PRICE assembler (Ruby et al. 2013). This resulted in a set of 77,544 RAD contigs
190 ranging from 300 to 800 bp which served as a de novo partial reference assembly for all
191 subsequent downstream analyses (Data S5). We next removed loci with five or more
192 SNPs to reduce potential paralogs and chimeras, and randomly selected 10,000 loci

193 from the remaining subset. Of these 10,000 loci, 8,533 were successfully designed into
194 120 bp RAD capture baits by Arbor Biosciences (Data S6).

195 ***Rapture Sequencing and Genomic Analysis***

196 A total of 345 individual frog samples were prepared for sequencing following
197 the RAD Capture (Rapture) methods outlined in Ali et al. (2016) and detailed in Peek et
198 al. (2019). We generated RAD libraries from the samples, quantified the libraries using a
199 Fragment Analyzer (Agilent Technologies, Santa Clara, CA) and pooled them, performed
200 capture on the pooled library with the 120 bp baits described above, and sequenced the
201 resulting Rapture library with paired-end Illumina sequencing.

202 The mean number of filtered alignments across all 345 samples was 324,928,
203 and sequence coverage depth was measured at the mid-point (i.e., base-pair 60) of
204 each 120 bp Rapture bait locus using the depth function in SAMtools (Li et al. 2009, Li
205 2011). For downstream analysis, we selected individuals that had greater than 100,000
206 alignments (n=277), which provided sufficient data to investigate population genetic
207 attributes at broad and fine geographic scales (Table 2, Data S2). *Rana boylii* are cryptic,
208 and often occur in low densities within the study area. Thus, we retained a minimum of
209 three individuals per site, and the mean number of samples per site was approximately
210 nine (Table 2). With genomic data, population genetic parameters can be accurately
211 estimated from even low sample numbers (Hotaling et al. 2018), and genomic analyses
212 in non-model organism often use fewer loci than analyzed here (Narum et al. 2013).
213 Thus, the sequence data we obtained should be appropriate for population genetic
214 analyses across our study area.

215 The probabilistic framework implemented in Analysis of Next Generation
216 Sequencing Data (ANGSD) (Korneliussen et al. 2014) was used for all population
217 genetic analyses as it does not require calling genotypes and is suitable for low-
218 coverage sequencing data (Korneliussen et al. 2013, Fumagalli et al. 2013). ANGSD
219 analyses were conducted following details from Peek et al. (2019).

220 ***Principal component analysis to assess population structure and connectivity***

221 To assess *R. boylii* population structure across the collection locations, we used
222 ANGSD (Korneliussen et al. 2014) to perform principal component analysis (PCA).
223 Settings used in ANGSD for PCA to identify polymorphic sites for PCA included a
224 SNP_pval of $1x10^{-6}$, inferring major and minor alleles (doMajorMinor 1), estimating allele
225 frequencies (doMaf 2) (Kim et al. 2011), retaining SNPs with a minor allele frequency of
226 at least 0.05 (minMaf), genotype posterior probabilities were calculated with a uniform
227 prior (doPost 2), and the doIBS 1 and doCov 1 options were used to generate PCA data.
228 Principal components (PC) summarizing population structure were derived from classic
229 eigenvalue decomposition and were visualized using the {ggplot2} package (Wickham
230 2016) in R (R Core Team 2017).

231 ***Genetic differentiation and diversity estimates***

232 To investigate patterns of genetic diversity and the overall trajectory (stable,
233 increasing, or decreasing) of diversity in a population, we summarized patterns of
234 genetic variation using two estimators of θ (4Nμ): Tajima's θ ($\theta_\pi$) is based on the
235 average number of pairwise differences (Tajima 1983), and Watterson's θ ($\theta_S$) is based

236 on the number of segregating sites (Watterson 1975). Estimates of θ were made using
237 the empirical Bayes method in ANGSD (Korneliussen et al. 2014) with the site frequency
238 spectrum (SFS) to calculate each statistic for each site, which were then averaged to
239 obtain a single value for each statistic (Korneliussen et al. 2013). These estimators are
240 influenced by the demographic history of a population and provide information on the
241 trajectory of changes in genetic diversity (Tajima 1989, Wakeley 2009). When genetic
242 diversity has been stable, these estimates should be equal; when genetic diversity has
243 been decreasing, $\theta_\pi > \theta_S$; and when genetic diversity has been increasing, $\theta_\pi < \theta_S$. Scaled
244 $\Delta\theta$ ($[\theta_\pi - \theta_S] / ([\theta_\pi + \theta_S] / 2)$) was used to assess trajectories of genetic diversity in each
245 population.

246 To assess how patterns of genetic differentiation and connectivity are
247 associated with flow alteration in the study, we calculated pairwise $F_{ST}$ (Wright 1943)
248 between all collection locations within a river for all six rivers. Genome-wide $F_{ST}$
249 between location pairs was estimated by first calculating a site frequency spectrum
250 (SFS) for each population (doSaf) (Nielsen et al. 2012) with ANGSD. The two-
251 dimensional SFS and global $F_{ST}$ between each population pair were then estimated
252 using realSFS (Korneliussen et al. 2014). We then plotted the scaled pairwise $F_{ST}$ ($F_{ST}$ /
253 $[1-F_{ST}]$) (Rousset 1997) for each location against the river distance (the distance along
254 the river network from each collection location to every other location within that study
255 river). We calculated the river distances (distance along river network) between
256 locations within watersheds using the {riverdist} package in R (Tyers 2017). These
257 values were plotted and a generalized linear model was fitted (Scaled $F_{ST}$ ~ River
258 Distance) in R (R Core Team 2017). Furthermore, each location was assigned flow

alteration type based on either the regulation type at the mainstem sample location or at the nearest mainstem; for pairwise comparisons the type was assigned based on whichever was greatest (i.e., hydropeaking is of greater intensity than bypass).

***Boosted regression tree modeling of variance in $F_{ST}$***

We used boosted regression tree (BRT) models with the R packages {gbm} (Ridgeway 2015) and {dismo} (Hijmans et al. 2017) to assess the relative influence of flow alteration type as compared to other environmental covariates. Boosted regression trees (BRT) are suitable frameworks for large and complex ecological datasets because they do not assume normality, nor linear relationships between predictor and response variables and they ignore non-informative predictor variables (Graham et al. 2008, Steel et al. 2017). BRTs use iterative boosting algorithms to combine simple decision trees to improve model performance (De'ath 2007) and provide a robust alternative to many traditional statistical methods (Phillips et al. 2006, Guisan et al. 2007). BRTs assess the relative impact of modeled variables by calculating the number of times a variable is selected for splitting a tree across all folds of the cross validation. Following Steel *et al.* 2017, estimates of relative influence for each predictor variable were used to evaluate the relative contribution a variable had in predicting the response. To evaluate the relative influence of covariates on $F_{ST}$, models were trained using river distance (km), elevation (m), upstream drainage area ($km^2$), Strahler stream order, and number of samples per location. Stream segment data on elevation, length, slope, stream order, and drainage area were derived from NHD Plus attributes (U.S. Geological Survey, National Hydrography Dataset, Digital data, accessed, August 2017 at

http://nhd.usgs.gov/data.html). In addition, scaled Δθ was included to assess the effect of genetic diversity trajectory on $F_{ST}$ across regulation types.

Model training and fitting were conducted following methods previously described in Steel et al. (2017). To reduce overfitting, the learning rate (also known as the shrinking rate) was set to 0.001. Stochastic gradient boosting was utilized to reduce prediction error (De'ath 2007) and the fraction of training data sampled to build each tree was 0.75, within the range as recommended by (Brown et al. 2012). Tree complexity was set to three to allow for second and third order interaction effects. The minimum number of observations required in the final nodes of each tree was three. A ten-fold cross-validation technique allowed us to determine the number of trees at which prediction error was minimized using the cross-validation deviance. Model performance was evaluated using the minimum estimated cross-validation deviance which maximized the estimated deviance explained.

295 **Results**

296 ***Flow alteration reduces the strength and predictability of seasonality patterns***

297 Flow patterns between flow-altered and unregulated river reaches could be
298 clearly differentiated by flow regime (hydropeaking, bypass, unregulated). Wavelet
299 analysis and Colwell's M/P metric indicated flow alteration reduced seasonality and
300 predictability of flow patterns in regulated (hydropeaking and bypass) river reaches
301 (Figure 1B, 1C, 2). These patterns were distinctly different before and after flow
302 alteration began in regulated study reaches (Figure 2, Data S3). In all flow-altered
303 reaches, seasonality was reduced compared to pre-regulation flow patterns, and
304 predictability of these flow patterns was also reduced in flow-altered data (Figure 2).

305 ***Anomalous genetic pattern in highly regulated reach of Middle Fork American***
306 ***watershed***

307 To assess *R. boylii* population structure across the collection locations, we used
308 principal component analysis to provide a dimensionless comparison of all samples.
309 The first two principal components revealed four main groups corresponding to the
310 Yuba, Bear, North Fork (NF) American, and Middle Fork (MF) American samples (Figure
311 3A). Unlike the Yuba watershed where all rivers clustered as one group, the two rivers
312 within the American watershed (the NF American and MF American) were separated by
313 both PC1 and PC2. Although the NF American watershed clustered closely with the
314 adjacent Bear watershed, the MF American showed a surprisingly high degree of
315 genetic differentiation from other locations (Figure 3A). These data suggest that there

316 is less genetic differentiation between the NF American and the Bear watersheds, than
317 between the NF and MF American watersheds. We conclude that measurements of
318 overall genetic differentiation in *R. boylii* from our study area largely conform to
319 watershed and geographic expectations, with the exception of the American watershed,
320 which shows a high degree of genetic differentiation between the North (unregulated)
321 and Middle (hydropeaking) Forks.

322 To further investigate patterns of genetic variation within the American
323 Watershed, we performed two PCAs, one on samples from the NF American, and the
324 other on samples from the MF American. The PCA of the NF American showed minimal
325 differentiation among locations, with different study sites blending together and weak
326 patterns of population structure (Figure 3B). In contrast, PCA of the MF American
327 showed strong differentiation between sites (Figures 3C, 3D). The MF American PCA
328 completely resolved all sites, with the first component (PC1) strongly differentiating the
329 samples in the hydropeaking reach from all other sites in the MF American. This pattern
330 may be due to the differential river regulation between the two rivers; the NF American
331 is unregulated and has weak PCA differentiation, whereas the MF American has a
332 higher level of river regulation and all sites form distinct genetic clusters, indicative of
333 reduced gene flow among sites within the MF American.

334 ***Flow regime is the strongest predictor of genetic isolation with R. boylii in the Northern***
335 ***Sierra***

336 To explore the relationship between the flow alteration type and genetic
337 differentiation across the entire study area, we plotted scaled $F_{ST}$ against river distance

338 between sampling sites for each flow alteration type assessed. While there was a clear
339 relationship between scaled $F_{ST}$ and river distance (as shown by the slope of regression
340 lines in Figure 4A), there was a striking pattern of elevated $F_{ST}$ by flow alteration type
341 (Figure 4A, Data S7). Even the bypass flow alteration type showed a distinct pattern of
342 elevated $F_{ST}$. For instance, sites in hydropeaking flow regimes separated by less than
343 10km had scaled $F_{ST}$ values comparable to unregulated locations separated by river
344 distances over 50 km. Hydropeaking was the most extreme pattern of the three flow
345 alteration types and showed highly elevated $F_{ST}$ values compared with the unregulated
346 and bypass flow types. The centroid $F_{ST}$ of each flow type increased with increasing
347 flow alteration intensity from unregulated (centroid $F_{ST}$=0.144, se=0.007) to bypass
348 (centroid $F_{ST}$=0.218, se=0.012) to hydropeaking (centroid $F_{ST}$=0.284, se=0.017). The
349 centroid $F_{ST}$ for hydropeaking was nearly double compared with unregulated centroid
350 $F_{ST}$. Furthermore, the centroid of the river distance between site pairs was comparable
351 between flow alteration types (unregulated=20.9 km, bypass=21.4 km,
352 hydropeaking=21.6 km), further supporting patterns of genetic differentiation are
353 associated with flow alteration and not solely isolation by distance. This suggests a
354 greater degree of isolation within sites in reaches with altered flow regimes compared
355 with *R. boylii* populations in unregulated reaches, as larger $F_{ST}$ values represent
356 reductions in heterozygosity due to population subdivision (Slatkin 1987). We conclude
357 *R. boylii* in rivers with altered flow regimes show patterns of greater population isolation
358 and elevated $F_{ST}$, (i.e., reduced heterozygosity) compared to populations in unregulated
359 locations.

360 To investigate the relative influence of flow alteration compared to other
361 covariates such as river distance on genetic differentiation (i.e. $F_{ST}$), we used boosted
362 regression tree (BRT) modeling. Covariates included flow regime alteration type, river
363 distance, watershed variables derived from National hydrology data (NHD), topographic
364 data, and genetic diversity trajectory (i.e., scaled $\Delta\theta$). We found flow alteration
365 explained the greatest amount of variance in scaled $F_{ST}$ (Figure 4B). Thus, flow
366 alteration has a larger relative influence on genetic differentiation than river distance
367 between sampling locations in altered flow reaches. We conclude there is a pattern of
368 elevated $F_{ST}$ between populations in reaches with altered flow.

369 ***Flow alteration is strongly correlated with decreasing genetic diversity in R. boylii***

370 To investigate the association between flow and genetic diversity trajectory(
371 whether genetic diversity is stable, increasing, or decreasing), we summarized patterns
372 of genetic variation using two estimators of $\theta$ ($4N\mu$): Tajima's $\theta$ ($\theta_\pi$) and Watterson's $\theta$
373 ($\theta_S$). We found zero populations sampled within flow altered watersheds had evidence
374 of increasing genetic diversity (i.e., a $\theta_\pi - \theta_S$ and scaled $\Delta\theta$ ($[\theta_\pi - \theta_S]$ / mean $\theta$)) that was
375 less than zero) (Figure 5A). The altered flow locations showed a clear trajectory of
376 genetic diversity loss (Figure 5A, 5B). Three of the four hydropeaking locations had the
377 highest values of scaled $\Delta\theta$, and the global mean was significantly different from other
378 flow alteration types. Although some tributary populations within unregulated
379 watersheds also showed signs of genetic diversity loss, the mean genetic diversity
380 trajectory at unregulated locations was largely stable (Figure 5B). This indicates many
381 populations in the northern Sierra Nevada which are already limited in number, are

losing genetic variation, and flow alteration is exacerbating these patterns. We conclude there is evidence of recent genetic diversity loss across populations in the flow altered river systems, regardless of alteration type.

## Discussion

Although massive parallel sequencing (MPS) technologies have the potential to facilitate collection of high-quality genetic data in virtually any species, a number of challenges still remain for many species including low quality or non-existent reference genomes, large/complex/repetitive genomes, and high cost of processing/sequencing in studies with many samples. Amphibians are particularly challenging as many species have very large genome sizes (Nunziata et al. 2017) for example, there are less than 10 frog reference genome assemblies available as of 2020 (Hellsten et al. 2010, Sun et al. 2015, Session et al. 2016, Hammond et al. 2017, Edwards et al. 2018, Rogers et al. 2018, Seidl et al. 2019) despite over 7,000 recorded frog species (AmphibiaWeb 2020). Our results demonstrate that Rapture (Ali et al. 2016) is a suitable method to rapidly and inexpensively discover a large number of loci in a frog species with a complex genome. In this study, we used new RAD sequencing and RAD capture (Rapture) methods (Ali et al. 2016) to generate high-quality genomic data suitable for discovering and genotyping many single nucleotide polymorphisms (SNPs) in *R. boylii*. Based on this dataset, we were able to successfully characterize patterns of genetic variation within *R. boylii* as well as design a set of RAD capture baits that can be used as a genetic monitoring resource for *R. boylii* (and likely other ranid species). This highlights that the collection

403 of genetic information, even from large numbers of samples or in complex genomes, is
404 no longer a limitation with current genomic methods such as RAD and Rapture.

405 Demographic connectivity is widely recognized as a fundamental driver of long-
406 term population persistence (Fahrig and Merriam 1985, Taylor et al. 1993). Populations
407 must adapt over time and connectivity is a major way to transfer genetic information.
408 For example, previous studies have shown that adaptation can occur by spreading
409 specific alleles across large geographic distances (Miller et al. 2012, Prince et al. 2017).
410 In many regulated river reaches in the Sierra Nevada, *R. boylii* now occur in isolated
411 locations, breeding in tributaries rather than mainstem habitats. However, since these
412 frogs have the potential to move long distances (*R. boylii* have been observed moving
413 over 1 km per day (Bourque 2008)), the extent to which current population connectivity
414 has been lost due to river regulation was not understood. Examining pairwise $F_{ST}$,
415 revealed a major decrease in connectivity in populations in regulated systems, even with
416 low intensity flow alteration (i.e., bypass reaches). The primary factor influencing
417 genetic differentiation among these rivers is hydrologic alteration (Figure 3B). Thus,
418 despite being able to move long distances, *R. boylii* have not been able to maintain
419 population connectivity in rivers with altered flow regimes. This demonstrates that even
420 in species that can move relatively long distances and pass potential physical barriers
421 (e.g., infrastructure such as dams, canals, and reservoirs likely do not represent barriers
422 to movement of *R. boylii*) loss of connectivity may still occur and can be revealed with
423 genetic analysis.

424 Genetic diversity is also a critical component for long-term population
425 persistence because it is closely related to the evolutionary capacity for adaptation to
426 environmental changes (Lande and Shannon 1996, Frankham 2002, Hoffmann and Sgrò
427 2011, Ishiyama et al. 2015). In some cases, isolated populations can maintain genetic
428 diversity when they are sufficiently sized (Whiteley et al. 2010), however, species of
429 conservation concern typically have small and/or declining populations and thus may
430 be susceptible to genetic diversity loss (Frankham 2002, Krohn et al. 2018). Throughout
431 the Sierra Nevada, *R. boylii* have largely disappeared from regulated mainstem rivers,
432 but the extent to which existing populations have been able to maintain genetic diversity
433 is unclear. Strikingly, our analysis revealed that every single population within the
434 regulated watersheds exhibits a trajectory of genetic diversity loss. Thus, genomic
435 analysis of molecular variation provides a powerful lens to discover and assess
436 trajectories of genetic diversity.

437 Our analyses, using metrics that serve as a reasonable proxy for genetic health,
438 does not bode well for the long-term persistence of *R. boylii* populations in regulated
439 rivers in the Sierra Nevada. Many of these *R. boylii* populations are already losing
440 genetic diversity and the effects of inbreeding will likely exacerbate their problems given
441 their small size and reduced connectivity. *Rana boylii* have evolved in river systems with
442 consistent hydrologic seasonality and predictability, despite inter-annual variation. Flow
443 regulation has altered patterns of hydrologic seasonality and predictability in many
444 watersheds (Kupferberg et al. 2012). Long-term population persistence may still be
445 possible if conservation efforts utilize methods that promote or maintain genetic health
446 and increase population connectivity. For example, simulations by Botero et al. (2015)

447 demonstrated adaptation persisted in modeled populations through large
448 environmental changes—if phenotypic strategies were appropriate before and after the
449 change—but modeled populations declined rapidly when the current strategy was a
450 mismatch to the current environment. Thus, *R. boylii* conservation efforts should focus
451 on river reaches where flow management may provide opportunities to more closely
452 mimic local natural flow regimes and thus improve hydrologic connectivity.

453 Detecting evolutionary responses to within- and among-year changes in an
454 ecological or hydrological context has previously been difficult. However, utilizing
455 genetic data can fill these gaps and provide a highly informative process for identifying
456 the impacts of anthropogenic and environmental change on the process of adaptation
457 (Kahilainen et al. 2014, Botero et al. 2015). We demonstrate that an aquatic species that
458 has adapted to local hydrology patterns shows poor genetic health (i.e., clear patterns
459 of decreased connectivity and trajectories of genetic diversity loss). Our results
460 highlight the impact of river regulation on aquatic organisms and their potential for long
461 term persistence. In the future, similar genetic approaches could be used in many other
462 contexts to explore the impacts of river regulation on aquatic organisms. Taken
463 together, our results demonstrate that genetic monitoring can be a powerful tool for
464 assessment of population health and should be a critical component of conservation
465 management in aquatic organisms.

466

**Acknowledgements**

This research builds on work from Amy Lind, Sarah Kupferberg, Jennifer Dever, and Sarah Yarnell. Many thanks to all of them for insight, and to many who helped collect/prepare samples: Corey Luna, Kristen Hein Strohm, Rick Wachs, and Isaac Chellman. This research could not have been conducted without access to specimens from Brad Shaffer at the Krebb Museum, and field samples from Sarah Mussulman, Caren Goldberg, and Mallory Bedwell. The authors have no conflicts of interest to declare.

## Literature Cited


Ali, O. A., S. M. O'Rourke, S. J. Amish, M. H. Meek, G. Luikart, C. Jeffres, and M. R. Miller. 2016. RAD Capture (Rapture): Flexible and Efficient Sequence-Based Genotyping. Genetics 202:389–400.

AmphibiaWeb. 2020. Species numbers. https://amphibiaweb.org/amphibian/speciesnums.html.

Baird, N. A., P. D. Etter, T. S. Atwood, M. C. Currey, A. L. Shiver, Z. A. Lewis, E. U. Selker, W. A. Cresko, and E. A. Johnson. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3:e3376.

Bondi, C. A., S. M. Yarnell, and A. J. Lind. 2013. Transferability of habitat suitability criteria for a stream breeding frog (*Rana boylii*) in the Sierra Nevada, California. Conservation and Biology 8:88–103.

Botero, C. A., F. J. Weissing, J. Wright, and D. R. Rubenstein. 2015. Evolutionary tipping points in the capacity to adapt to environmental change. Proceedings of the National Academy of Sciences of the United States of America 112:184–189.

Bourque, R. M. 2008, December. Spatial ecology of an inland population of the Foothill yellow-legged frog (*Rana boylii*) in Tehama County, California. MSc, Humboldt State University, USA.

Broquet, T., L. Berset-Braendli, G. Emaresi, and L. Fumagalli. 2007. Buccal swabs allow efficient and reliable microsatellite genotyping in amphibians. Conservation Genetics 8:509–511.

Brown, L. R., J. T. May, A. C. Rehn, P. R. Ode, I. R. Waite, and J. G. Kennen. 2012. Predicting biological condition in southern California streams. Landscape and Urban Planning 108:17–27.

Bunn, S. E., and A. H. Arthington. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental Management 30:492–507.

Cazé, A. L. R., G. Mäder, T. S. Nunes, L. P. Queiroz, G. de Oliveira, J. A. F. Diniz-Filho, S. L. Bonatto, and L. B. Freitas. 2016. Could refuge theory and rivers acting as barriers explain the genetic variability distribution in the Atlantic Forest? Molecular Phylogenetics and Evolution 101:242–251.

Colwell, R. K. 1974. Predictability, Constancy, and Contingency of Periodic Phenomena. Ecology 55:1148–1153.

Davidson, C., H. B. Shaffer, and M. R. Jennings. 2002. Spatial Tests of the Pesticide Drift, Habitat Destruction, UV-B, and Climate-Change Hypotheses for California Amphibian Declines. Conservation Biology 16:1588–1601.

De'ath, G. 2007. Boosted trees for ecological modeling and prediction. Ecology 88:243–251.

Deiner, K., J. C. Garza, R. Coey, and D. J. Girman. 2007. Population structure and genetic diversity of trout (*Oncorhynchus mykiss*) above and below natural and man-made barriers in the Russian River, California. Conservation Genetics 8:437–454.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z.-I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A.-H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews of the Cambridge Philosophical Society 81:163–182.

Edwards, R. J., D. E. Tuipulotu, T. G. Amos, D. O'Meally, M. F. Richardson, T. L. Russell, M. Vallinoto, M. Carneiro, N. Ferrand, M. R. Wilkins, F. Sequeira, L. A. Rollins, E. C. Holmes, R. Shine, and P. A. White. 2018. Draft genome assembly of the invasive cane toad, *Rhinella marina*. GigaScience 7.

Fahrig, L., and G. Merriam. 1985. Habitat Patch Connectivity and Population Survival. Ecology 66:1762–1768.

Frankham, R. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK ; New York.

Fuller, T. E., K. L. Pope, D. T. Ashton, and H. H. Welsh. 2011. Linking the Distribution of an Invasive Amphibian (*Rana catesbeiana*) to Habitat Conditions in a Managed River System in Northern California. Restoration Ecology 19:204–213.

533 Fumagalli, M., F. G. Vieira, T. S. Korneliussen, T. Linderoth, E. Huerta-Sánchez, A.
534 Albrechtsen, and R. Nielsen. 2013. Quantifying population genetic differentiation
535 from next-generation sequencing data. Genetics 195:979–992.

536 Gilpin, M., and M. Soule. 1986. Minimum Viable Populations: Processes of Species
537 Extinction, Conservation Biology. Sunderland, Massachusetts: Sin. Assoc.:19–34.

538 Goldberg, C. S., M. E. Kaplan, and C. R. Schwalbe. 2003. From the frog's mouth: buccal
539 swabs for collection of DNA from amphibians. Herpetological Review 34:220–
540 221.

541 Graham, C. H., J. Elith, R. J. Hijmans, A. Guisan, A. Townsend Peterson, B. A. Loiselle,
542 and The Nceas Predicting Species Distributions Working Group. 2008. The
543 influence of spatial errors in species occurrence data used in distribution
544 models. The Journal of Applied Ecology 45:239–247.

545 Grantham, T. E., A. M. Merenlender, and V. H. Resh. 2010. Climatic influences and
546 anthropogenic stressors: an integrated framework for streamflow management
547 in Mediterranean-climate California, USA. Freshwater Biology 55:188–204.

548 Guisan, A., N. E. Zimmermann, J. Elith, C. H. Graham, S. Phillips, and A. T. Peterson.
549 2007. What Matters for Predicting the Occurrences of Trees: Techniques, Data, or
550 Species' Characteristics? Ecological Monographs 77:615–630.

551 Guzy, J. C., E. A. Eskew, B. J. Halstead, and S. J. Price. 2018. Influence of damming on
552 anuran species richness in riparian areas: A test of the serial discontinuity
553 concept. Ecology and Evolution 8:2268-2279.

554 Hammond, S. A., R. L. Warren, B. P. Vandervalk, E. Kucuk, H. Khan, E. A. Gibb, P. Pandoh,
555 H. Kirk, Y. Zhao, M. Jones, A. J. Mungall, R. Coope, S. Pleasance, R. A. Moore, R.
556 A. Holt, J. M. Round, S. Ohora, B. V. Walle, N. Veldhoen, C. C. Helbing, and I. Birol.
557 2017. The North American bullfrog draft genome provides insight into hormonal
558 regulation of long noncoding RNA. Nature Communications 8:1433.

559 Hellsten, U., R. M. Harland, M. J. Gilchrist, D. Hendrix, J. Jurka, V. Kapitonov, I.
560 Ovcharenko, N. H. Putnam, S. Shu, L. Taher, I. L. Blitz, B. Blumberg, D. S.
561 Dichmann, I. Dubchak, E. Amaya, J. C. Detter, R. Fletcher, D. S. Gerhard, D.
562 Goodstein, T. Graves, I. V. Grigoriev, J. Grimwood, T. Kawashima, E. Lindquist, S.
563 M. Lucas, P. E. Mead, T. Mitros, H. Ogino, Y. Ohta, A. V. Poliakov, N. Pollet, J.
564 Robert, A. Salamov, A. K. Sater, J. Schmutz, A. Terry, P. D. Vize, W. C. Warren, D.
565 Wells, A. Wills, R. K. Wilson, L. B. Zimmerman, A. M. Zorn, R. Grainger, T.
566 Grammer, M. K. Khokha, P. M. Richardson, and D. S. Rokhsar. 2010. The genome
567 of the Western clawed frog *Xenopus tropicalis*. Science 328:633–636.

568 Heyer, W. R., M. A. Donnelly, R. W. McDiarmid, L.-A. C. Hayek, and M. S. Foster. 1994.
569 Measuring and monitoring biological diversity. Standard methods for
570 amphibians. Smithsonian Institution Press, Washington DC.

Hijmans, R. J., S. Phillips, J. Leathwick, and J. Elith. 2017. dismo: Species Distribution Modeling. R package.

Hoffmann, A. A., and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. Nature 470:479–485.

Hotaling, S., C. C. Muhlfeld, J. J. Giersch, O. A. Ali, S. Jordan, M. R. Miller, G. Luikart, and D. W. Weisrock. 2018. Demographic modelling reveals a history of divergence with gene flow for a glacially tied stonefly in a changing post-Pleistocene landscape. Journal of Biogeography 45:304–317.

Ishiyama, N., I. Koizumi, T. Yuta, and F. Nakamura. 2015. Differential effects of spatial network structure and scale on population size and genetic diversity of the ninespine stickleback in a remnant wetland system. Freshwater Biology 60:733–744.

Jennings, M. R., and M. P. Hayes. 1994. Amphibian and reptile species of special concern in California. California Department of Fish and Game, Rancho Cordova.

Kahilainen, A., M. Puurtinen, and J. S. Kotiaho. 2014. Conservation implications of species–genetic diversity correlations. Global Ecology and Conservation 2:315–323.

Kim, S. Y., K. E. Lohmueller, A. Albrechtsen, Y. Li, T. Korneliussen, G. Tian, N. Grarup, T. Jiang, G. Andersen, D. Witte, T. Jorgensen, T. Hansen, O. Pedersen, J. Wang, and

590 R. Nielsen. 2011. Estimation of allele frequency and association mapping using
591 next-generation sequencing data. BMC Bioinformatics 12:231.

592 Korneliussen, T. S., A. Albrechtsen, and R. Nielsen. 2014. ANGSD: Analysis of Next
593 Generation Sequencing Data. BMC Bioinformatics 15:356.

594 Korneliussen, T. S., I. Moltke, A. Albrechtsen, and R. Nielsen. 2013. Calculation of
595 Tajima's D and other neutrality test statistics from low depth next-generation
596 sequencing data. BMC Bioinformatics 14:289.

597 Krohn, A. R., C. J. Conroy, R. Pesapane, K. Bi, J. E. Foley, and E. B. Rosenblum. 2018.
598 Conservation genomics of desert dwelling California voles (*Microtus californicus*)
599 and implications for management of endangered Amargosa voles (*Microtus*
600 *californicus scirpensis*). Conservation Genetics 19:383–395.

601 Kupferberg, S. J. 1996. Hydrologic and geomorphic factors affecting conservation of a
602 river-breeding frog (*Rana boylii*). Ecological Applications 6:1332–1344.

603 Kupferberg, S. J., W. J. Palen, A. J. Lind, S. Bobzien, A. Catenazzi, J. Drennan, and M. E.
604 Power. 2012. Effects of flow regimes altered by dams on survival, population
605 declines, and range-wide losses of California river-breeding frogs. Conservation
606 Biology 26:513–524.

607 Lande, R., and S. Shannon. 1996. The role of genetic variation in adaptation and
608 population persistence in a changing environment. Evolution 50:434–437.

609 Li, H. 2011. A statistical framework for SNP calling, mutation discovery, association
610 mapping and population genetical parameter estimation from sequencing data.
611 Bioinformatics 27:2987–2993.

612 Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis,
613 and R. Durbin. 2009. The Sequence Alignment/Map format and SAMtools.
614 Bioinformatics 25:2078–2079.

615 Lind, A. J., H. H. Welsh Jr, and R. A. Wilson. 1996. The effects of a dam on breeding
616 habitat and egg survival of the foothill yellow-legged frog (*Rana boylii*) in
617 Northwestern Calfifornia. Herpetological Review 27:62–66.

618 Miller, M. R., J. P. Brunelli, P. A. Wheeler, S. Liu, C. E. Rexroad 3rd, Y. Palti, C. Q. Doe, and
619 G. H. Thorgaard. 2012. A conserved haplotype controls parallel adaptation in
620 geographically distant salmonid populations. Molecular Ecology 21:237–249.

621 Miller, M. R., J. P. Dunham, A. Amores, W. A. Cresko, and E. A. Johnson. 2007. Rapid and
622 cost-effective polymorphism identification and genotyping using restriction site
623 associated DNA (RAD) markers. Genome Research 17:240–248.

624 Moyle, P. B., J. V. E. Katz, and R. M. Quiñones. 2011. Rapid decline of California's native
625 inland fishes: A status assessment. Biological Conservation 144:2414–2423.

626 Murchie, K. J., K. P. E. Hair, C. E. Pullen, T. D. Redpath, H. R. Stephens, and S. J. Cooke.
627 2008. Fish response to modified flow regimes in regulated rivers: research

628 methods, effects and opportunities. River Research and Applications 24:197–
629 217.

630 Narum, S. R., C. A. Buerkle, J. W. Davey, M. R. Miller, and P. A. Hohenlohe. 2013.
631 Genotyping-by-sequencing in ecological and conservation genomics. Molecular
632 Ecology 22:2841–2847.

633 Nielsen, R., T. Korneliussen, A. Albrechtsen, Y. Li, and J. Wang. 2012. SNP calling,
634 genotype calling, and sample allele frequency estimation from New-Generation
635 Sequencing data. PloS One 7:e37558.

636 Nilsson, C., C. A. Reidy, M. Dynesius, and C. Revenga. 2005. Fragmentation and flow
637 regulation of the world's large river systems. Science 308:405–408.

638 Nunziata, S. O., S. L. Lance, D. E. Scott, E. M. Lemmon, and D. W. Weisrock. 2017.
639 Genomic data detect corresponding signatures of population size change on an
640 ecological time scale in two salamander species. Molecular Ecology 26:1060–
641 1074.

642 Peek, R. A. 2018. Population Genetics of a Sentinel Stream-breeding Frog (*Rana boylii*).
643 Ecology, University of California, Davis.

644 Peek, R. A., M. Bedwell, S. M. O'Rourke, C. Goldberg, G. M. Wengert, and M. R. Miller.
645 2019. Hybridization between two parapatric ranid frog species in the northern
646 Sierra Nevada, California, USA. Molecular Ecology 28:4636–4647.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190:231–259.

Pidancier, N., C. Miquel, and C. Miaud. 2003. Buccal swabs as a non-destructive tissue sampling method for DNA analysis in amphibians. The Herpetological Journal 13:175–178.

Poff, N. L., J. D. Olden, D. M. Merritt, and D. M. Pepin. 2007. Homogenization of regional river dynamics by dams and global biodiversity implications. Proceedings of the National Academy of Sciences of the United States of America 104:5732–5737.

Power, M. E., W. E. Dietrich, and J. C. Finlay. 1996. Dams and Downstream Aquatic Biodiversity: Potential Food Web Consequences of Hydrologic and Geomorphic Change. Environmental Management 20:887–895.

Prince, D. J., S. M. O'Rourke, T. Q. Thompson, O. A. Ali, H. S. Lyman, I. K. Saglam, T. J. Hotaling, A. P. Spidle, and M. R. Miller. 2017. The evolutionary basis of premature migration in Pacific salmon highlights the utility of genomics for informing conservation. Science Advances 3:e1603198.

Pringle, C. 2003. What is hydrologic connectivity and why is it ecologically important? Hydrological Processes 17:2685–2689.

Pringle, C. M. 2001. Hydrologic Connectivity and the Management of Biological Reserves: A Global Perspective. Ecological Applications 11:981–998.

R Core Team. 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ridgeway, G. 2015. gbm: Generalized Boosted Regression Models. http://CRAN.R-project.org/package-gbm.

Rogers, R. L., L. Zhou, C. Chu, R. Márquez, A. Corl, T. Linderoth, L. Freeborn, M. D. MacManes, Z. Xiong, J. Zheng, C. Guo, X. Xun, M. R. Kronforst, K. Summers, Y. Wu, H. Yang, C. L. Richards-Zawacki, G. Zhang, and R. Nielsen. 2018. Genomic Takeover by Transposable Elements in the Strawberry Poison Frog. Molecular Biology and Evolution 35:2913–2927.

Rolls, R. J., and N. R. Bond. 2017. Environmental and Ecological Effects of Flow Alteration in Surface Water Ecosystems. Pages 65–82 *in* Water for the Environment. Elsevier.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219–1228.

Ruby, J. G., P. Bellare, and J. L. Derisi. 2013. PRICE: software for the targeted assembly of components of (Meta) genomic sequence data. G3 3:865–880.

Sabo, J. L., A. Ruhi, G. W. Holtgrieve, V. Elliott, M. E. Arias, P. B. Ngor, T. A. Räsänen, and S. Nam. 2017. Designing river flows to improve food security futures in the Lower Mekong Basin. Science 358.

Sağlam, İ. K., J. Baumsteiger, M. J. Smith, J. Linares-Casenave, A. L. Nichols, S. M. O'Rourke, and M. R. Miller. 2016. Phylogenetics support an ancient common origin of two scientific icons: Devils Hole and Devils Hole pupfish. Molecular Ecology 25:3962–3973.

Schick, R. S., and S. T. Lindley. 2007. Directed connectivity among fish populations in a riverine network. The Journal of Applied Ecology 44:1116–1126.

Scribner, K. T., W. H. Lowe, E. Landguth, G. Luikart, D. M. Infante, G. E. Whelan, and C. C. Muhlfeld. 2016. Applications of Genetic Data to Improve Management and Conservation of River Fishes and Their Habitats. Fisheries 41:174–188.

Seidl, F., N. A. Levis, R. Schell, D. W. Pfennig, K. S. Pfennig, and I. M. Ehrenreich. 2019. Genome of Spea multiplicata, a Rapidly Developing, Phenotypically Plastic, and Desert-Adapted Spadefoot Toad. G3 9:3909–3919.

Session, A. M., Y. Uno, T. Kwon, J. A. Chapman, A. Toyoda, S. Takahashi, A. Fukui, A. Hikosaka, A. Suzuki, M. Kondo, S. J. van Heeringen, I. Quigley, S. Heinz, H. Ogino, H. Ochi, U. Hellsten, J. B. Lyons, O. Simakov, N. Putnam, J. Stites, Y. Kuroki, T. Tanaka, T. Michiue, M. Watanabe, O. Bogdanovic, R. Lister, G. Georgiou, S. S. Paranjpe, I. van Kruijsbergen, S. Shu, J. Carlson, T. Kinoshita, Y. Ohta, S. Mawaribuchi, J. Jenkins, J. Grimwood, J. Schmutz, T. Mitros, S. V. Mozaffari, Y. Suzuki, Y. Haramoto, T. S. Yamamoto, C. Takagi, R. Heald, K. Miller, C. Haudenschild, J. Kitzman, T. Nakayama, Y. Izutsu, J. Robert, J. Fortriede, K. Burns, V. Lotay, K. Karimi, Y. Yasuoka, D. S. Dichmann, M. F. Flajnik, D. W.

Houston, J. Shendure, L. DuPasquier, P. D. Vize, A. M. Zorn, M. Ito, E. M. Marcotte, J. B. Wallingford, Y. Ito, M. Asashima, N. Ueno, Y. Matsuda, G. J. Veenstra, A. Fujiyama, R. M. Harland, M. Taira, and D. S. Rokhsar. 2016. Genome evolution in the allotetraploid frog *Xenopus laevis*. Nature 538:336–343.

Shaw, E. A., E. Lange, J. D. Shucksmith, and D. N. Lerner. 2016. Importance of partial barriers and temporal variation in flow when modelling connectivity in fragmented river systems. Ecological Engineering 91:515–528.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

Stebbins, R. C. 2003. A Field Guide to Western Reptiles and Amphibians. 3rd edition. Houghton Mifflin Harcourt, Boston.

Steel, A. E., R. A. Peek, R. A. Lusardi, and S. M. Yarnell. 2017. Associating metrics of hydrologic variability with benthic macroinvertebrate communities in regulated and unregulated snowmelt-dominated rivers. Freshwater Biology 63:844-858.

Sun, Y.-B., Z.-J. Xiong, X.-Y. Xiang, S.-P. Liu, W.-W. Zhou, X.-L. Tu, L. Zhong, L. Wang, D.-D. Wu, B.-L. Zhang, C.-L. Zhu, M.-M. Yang, H.-M. Chen, F. Li, L. Zhou, S.-H. Feng, C. Huang, G.-J. Zhang, D. Irwin, D. M. Hillis, R. W. Murphy, H.-M. Yang, J. Che, J. Wang, and Y.-P. Zhang. 2015. Whole-genome sequence of the Tibetan frog Nanorana parkeri and the comparative evolution of tetrapod genomes. Proceedings of the National Academy of Sciences of the United States of America 112:E1257-62.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105:437–460.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Taylor, P. D., L. Fahrig, K. Henein, and G. Merriam. 1993. Connectivity Is a Vital Element of Landscape Structure. Oikos 68:571–573.

Thompson, T. Q., M. R. Bellinger, S. M. O'Rourke, D. J. Prince, A. E. Stevenson, A. T. Rodrigues, M. R. Sloat, C. F. Speller, D. Y. Yang, V. L. Butler, M. A. Banks, and M. R. Miller. 2019. Anthropogenic habitat alteration leads to rapid loss of adaptive variation and restoration potential in wild salmon populations. Proceedings of the National Academy of Sciences of the United States of America 116:177–186.

Tonkin, J. D., M. T. Bogan, N. Bonada, B. Rios-Touma, and D. A. Lytle. 2017. Seasonality and predictability shape temporal species diversity. Ecology 98:1201–1216.

Torrence, C., and G. P. Compo. 1998. A Practical Guide to Wavelet Analysis. Bulletin of the American Meteorological Society 79:61–78.

Tyers, M. 2017. riverdist: River Network Distance Computation and Applications.

Voelker, G., B. D. Marks, C. Kahindo, U. A'genonga, F. Bapeamoni, L. E. Duffie, J. W. Huntley, E. Mulotwa, S. A. Rosenbaum, and J. E. Light. 2013. River barriers and cryptic biodiversity in an evolutionary museum. Ecology and Evolution 3:536–545.

Vörösmarty, C. J., P. B. McIntyre, M. O. Gessner, D. Dudgeon, A. Prusevich, P. Green, S. Glidden, S. E. Bunn, C. A. Sullivan, C. R. Liermann, and P. M. Davies. 2010. Global threats to human water security and river biodiversity. Nature 467:555–561.

Wakeley, J. 2009. Coalescent Theory: An Introduction. W. H. Freeman.

Watterson, G. A. 1975. On the number of segregating sites in genetical models without recombination. Theoretical Population Biology 7:256–276.

Werth, S., M. Schödl, and C. Scheidegger. 2014. Dams and canyons disrupt gene flow among populations of a threatened riparian plant. Freshwater Biology 59:2502–2515.

Whiteley, A. R., K. Hastings, J. K. Wenburg, C. A. Frissell, J. C. Martin, and F. W. Allendorf. 2010. Genetic variation and effective population size in isolated populations of coastal cutthroat trout. Conservation Genetics 11:1929–1943.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Wiens, J. A. 2002. Riverine landscapes: taking landscape ecology into the water. Freshwater Biology 47:501–515.

Wright, S. 1943. Isolation by Distance. Genetics 28:114–138.

Yarnell, S. M., E. D. Stein, J. A. Webb, T. Grantham, R. A. Lusardi, J. Zimmerman, R. A. Peek, B. A. Lane, J. Howard, and S. Sandoval-Solis. 2020. A functional flows

approach to selecting ecologically relevant flow metrics for environmental flow applications. River Research and Applications 9:12.

Yarnell, S. M., J. H. Viers, and J. F. Mount. 2010. Ecology and Management of the Spring Snowmelt Recession. Bioscience 60:114–127.

Yarnell, S., R. Peek, G. Epke, and A. Lind. 2016. Management of the Spring Snowmelt Recession in Regulated Systems. Journal of the American Water Resources Association 52:723–736.

Zhong, Y., and G. Power. 1996. Environmental impacts of hydroelectric projects on fish resources in China. Regulated Rivers: Research & Management 12:81–98.

***Data Archiving Statement:*** Should the manuscript be accepted, the data supporting the results will be archived in an appropriate public repository such as Dryad, and the data DOI will be appended to the end of the article.

Table 1. Locality information for population genomic analysis of *R. boylii* in the Yuba, Bear, and American Watersheds.

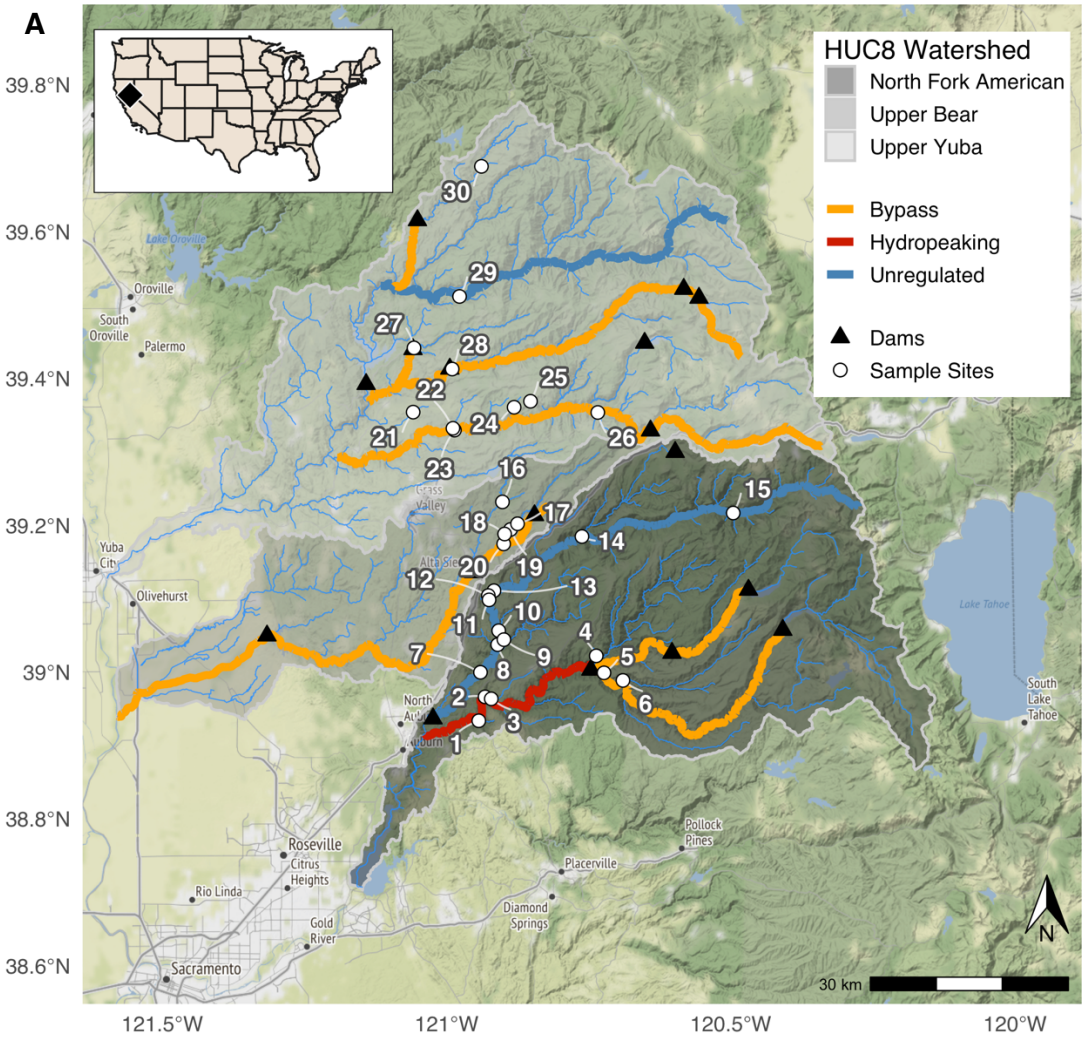
| Locality | Site ID | River | Watershed | Regulation Type | Lat. (WGS84) | Long. (WGS84) | Elev. (m) | NHD† Stream Order | NHD Total Drainage Area (sq. km) |
|---|---|---|---|---|---|---|---|---|---|
| American Canyon | 1 | MF American | American | Hydropeaking | 38.93396 | -120.94356 | 240 | 2 | 9 |
| Gas Canyon | 2 | MF American | American | Hydropeaking | 38.96651 | -120.93252 | 241 | 1 | 13 |
| Todd Creek | 3 | MF American | American | Hydropeaking | 38.96385 | -120.92158 | 367 | 2 | 10 |
| NFMFA-Skunk Canyon | 4 | MF American | American | Hydropeaking | 39.02237 | -120.73693 | 521 | 2 | 6 |
| Rubicon-US Powerhouse | 5 | MF American | American | Bypass | 38.99928 | -120.72333 | 360 | 5 | 816 |
| Rubicon-Long Canyon | 6 | MF American | American | Bypass | 38.98887 | -120.68996 | 415 | 5 | 806 |
| Ponderosa Bridge | 7 | NF American | American | Unregulated | 38.99994 | -120.9406 | 240 | 5 | 857 |
| Bunch Canyon | 8 | NF American | American | Unregulated | 39.03762 | -120.91028 | 286 | 3 | 27 |
| Shirttail Creek | 9 | NF American | American | Unregulated | 39.04446 | -120.89937 | 525 | 4 | 141 |
| Indian Creek | 10 | NF American | American | Unregulated | 39.05665 | -120.90854 | 296 | 2 | 24 |
| Slaughter Ravine | 11 | NF American | American | Unregulated | 39.09865 | -120.92548 | 356 | 2 | 6 |
| Robbers Ravine | 12 | NF American | American | Unregulated | 39.10451 | -120.92672 | 400 | 1 | 4 |
| Iowa Hill Mainstem | 13 | NF American | American | Unregulated | 39.11115 | -120.91684 | 386 | 4 | 605 |
| Euchre Bar | 14 | NF American | American | Unregulated | 39.18492 | -120.76204 | 579 | 4 | 508 |
| Sailor Canyon | 15 | NF American | American | Unregulated | 39.21694 | -120.49595 | 1005 | 3 | 166 |
| Greenhorn Creek | 16 | Bear | Bear | Bypass | 39.23206 | -120.90189 | 820 | 2 | 11 |
| Steep Hollow Creek DS | 17 | Bear | Bear | Bypass | 39.20231 | -120.87537 | 736 | 2 | 52 |
| Hawkins Ravine | 18 | Bear | Bear | Bypass | 39.18833 | -120.8981 | 706 | 2 | 4 |
| Steep Hollow Creek US | 19 | Bear | Bear | Bypass | 39.19444 | -120.88778 | 704 | 2 | 45 |
| Chicago Powerhouse | 20 | Bear | Bear | Bypass | 39.17484 | -120.89978 | 665 | 4 | 136 |
| Shady Creek | 21 | South Yuba | Yuba | Bypass | 39.35433 | -121.059 | 675 | 2 | 15 |
| Spring Creek | 22 | South Yuba | Yuba | Bypass | 39.33233 | -120.989 | 595 | 3 | 24 |
| Rock Creek | 23 | South Yuba | Yuba | Bypass | 39.32983 | -120.98634 | 593 | 4 | 710 |
| Missouri Canyon | 24 | South Yuba | Yuba | Bypass | 39.36096 | -120.88138 | 1094 | 2 | 5 |
| Logan Creek | 25 | South Yuba | Yuba | Bypass | 39.36914 | -120.85258 | 1201 | 1 | 5 |
| US Canyon Creek | 26 | South Yuba | Yuba | Bypass | 39.35387 | -120.73419 | 889 | 4 | 365 |
| Oregon Creek | 27 | Middle Yuba | Yuba | Bypass | 39.44188 | -121.05753 | 620 | 4 | 375 |

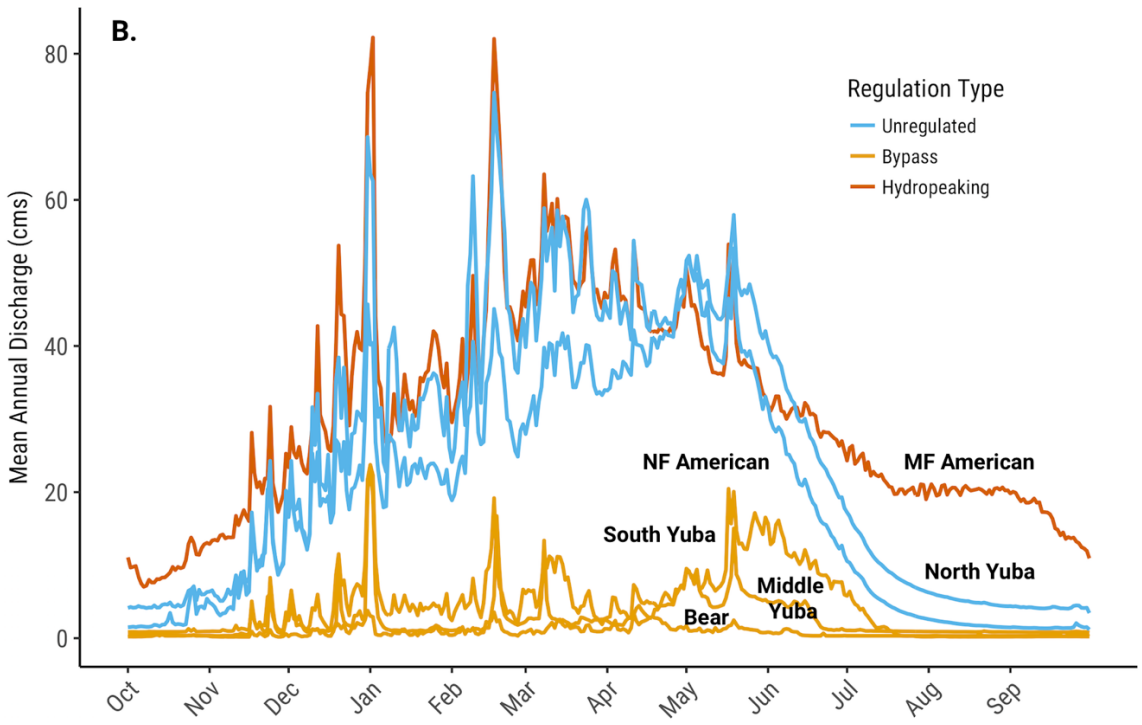
| Locality | Site ID | River | Watershed | Regulation Type | Lat. (WGS84) | Long. (WGS84) | Elev. (m) | NHD† Stream Order | NHD Total Drainage Area (sq. km) |
|---|---|---|---|---|---|---|---|---|---|
| US Our House Dam | 28 | Middle Yuba | Yuba | Bypass | 39.41305 | -120.99035 | 624 | 4 | 375 |
| Rocky Rest Mainstem | 29 | North Yuba | Yuba | Unregulated | 39.51191 | -120.97738 | 704 | 5 | 669 |
| Slate Creek | 30 | North Yuba | Yuba | Bypass | 39.689128 | -120.93889 | 1330 | 3 | 58.7 |

†NHD refers to the National Hydrography Dataset by USGS (U.S. Geological Survey, National Hydrography Dataset, Digital data, accessed, August 2017).

785 Table 2. Sampling information for population genomic analysis of *R. boylii*, the number
786 of individuals (n) is given for the total number sequenced per location and the number
787 of individuals that were retained after filtering (see methods) across the 8,533 baits.

| Locality | Site ID | n initial | n retained |
|---|---|---|---|
| American Canyon | 1 | 16 | 6 |
| Gas Canyon | 2 | 6 | 6 |
| Todd Creek | 3 | 11 | 9 |
| NFMFA-Skunk Canyon | 4 | 18 | 18 |
| Rubicon-US Powerhouse | 5 | 11 | 11 |
| Rubicon-Long Canyon | 6 | 9 | 8 |
| Ponderosa Bridge | 7 | 5 | 5 |
| Bunch Canyon | 8 | 15 | 14 |
| Shirttail Creek | 9 | 16 | 15 |
| Indian Creek | 10 | 12 | 11 |
| Slaughter Ravine | 11 | 8 | 8 |
| Robbers Ravine | 12 | 30 | 11 |
| Iowa Hill Mainstem | 13 | 36 | 30 |
| Euchre Bar | 14 | 13 | 11 |
| Sailor Canyon | 15 | 8 | 5 |
| Greenhorn Creek | 16 | 15 | 6 |
| Steep Hollow Creek DS | 17 | 13 | 12 |
| Hawkins Ravine | 18 | 3 | 3 |
| Steep Hollow Creek US | 19 | 7 | 6 |
| Chicago Powerhouse | 20 | 6 | 6 |
| Shady Creek | 21 | 14 | 12 |
| Spring Creek | 22 | 4 | 4 |
| Rock Creek | 23 | 3 | 3 |
| Missouri Canyon | 24 | 8 | 6 |
| Logan Creek | 25 | 5 | 4 |
| US Canyon Creek | 26 | 6 | 6 |
| Oregon Creek | 27 | 15 | 13 |
| US Our House Dam | 28 | 13 | 12 |
| Rocky Rest Mainstem | 29 | 15 | 12 |
| Slate Creek | 30 | 4 | 4 |


A
HUC8 Watershed
North Fork American
Upper Bear
Upper Yuba
Bypass
Hydropeaking
Unregulated
Dams
Sample Sites
39.8°N
39.6°N
39.4°N
39.2°N
39°N
38.8°N
38.6°N
121.5°W
121°W
120.5°W
120°W
30 km
N
Lake Oroville
Oroville
South Oroville
Palermo
Yuba City
Olivehurst
Grass Valley
Alta Sierra
North Auburn
Auburn
Roseville
Citrus Heights
Rio Linda
Gold River
Sacramento
Placerville
Pollock Pines
Diamond Springs
Lake Tahoe
South Lake Tahoe


B.
Mean Annual Discharge (cms)
80
60
40
20
0
Regulation Type
Unregulated
Bypass
Hydropeaking
NF American
MF American
South Yuba
North Yuba
Middle Yuba
Bear
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep

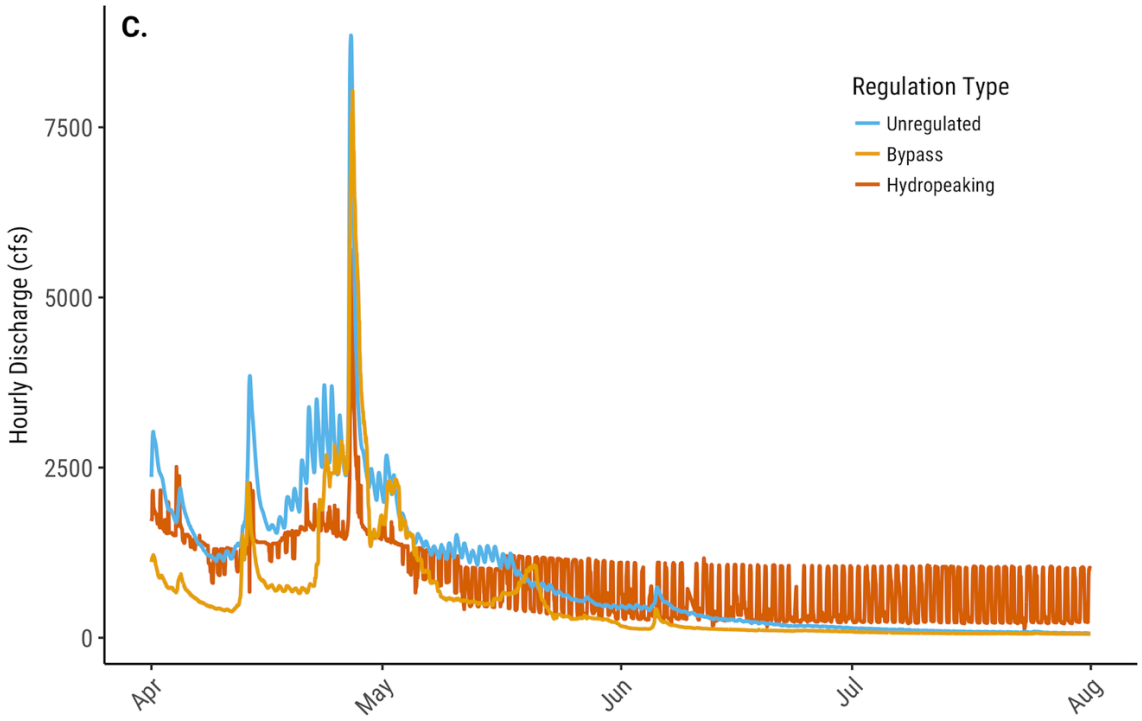

C.
Hourly Discharge (cfs)
7500
5000
2500
0
Regulation Type
Unregulated
Bypass
Hydropeaking
Apr
May
Jun
Jul
Aug

Figure 1. Sampling locations and flow characteristics. A) Map of sampling locations across three watersheds. B) Comparison of annual mean daily discharge from 1981–2016 for three flow types. C) Comparison of hourly discharge in three different flow regimes in April through July 2012, Bypass (South Yuba), Hydropeaking (Middle Fork American), and Unregulated (North Fork American). See Table S1 for USGS gaging station information.

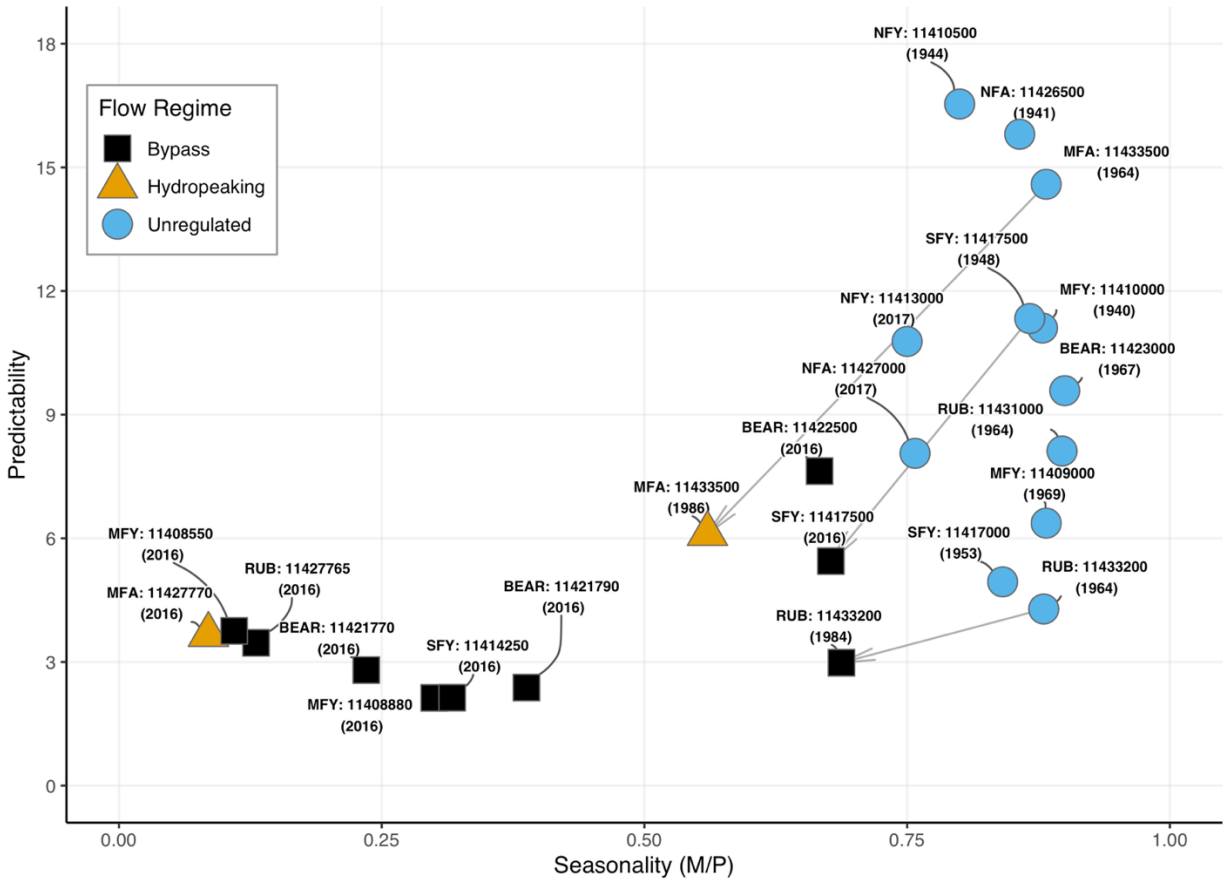



Figure 2. A biplot of seasonality (Colwell's M/P [contingency/predictability] versus predictability (average wavelet power at the 12-month time period following Tonkin et al. (2017). Each point is labeled with the river acronym and USGS gage ID, followed by the end year of the flow dataset used (See Table S3). Data from the same gage but from two time periods (before flow alteration and with contemporary alteration) are connected by arrows.

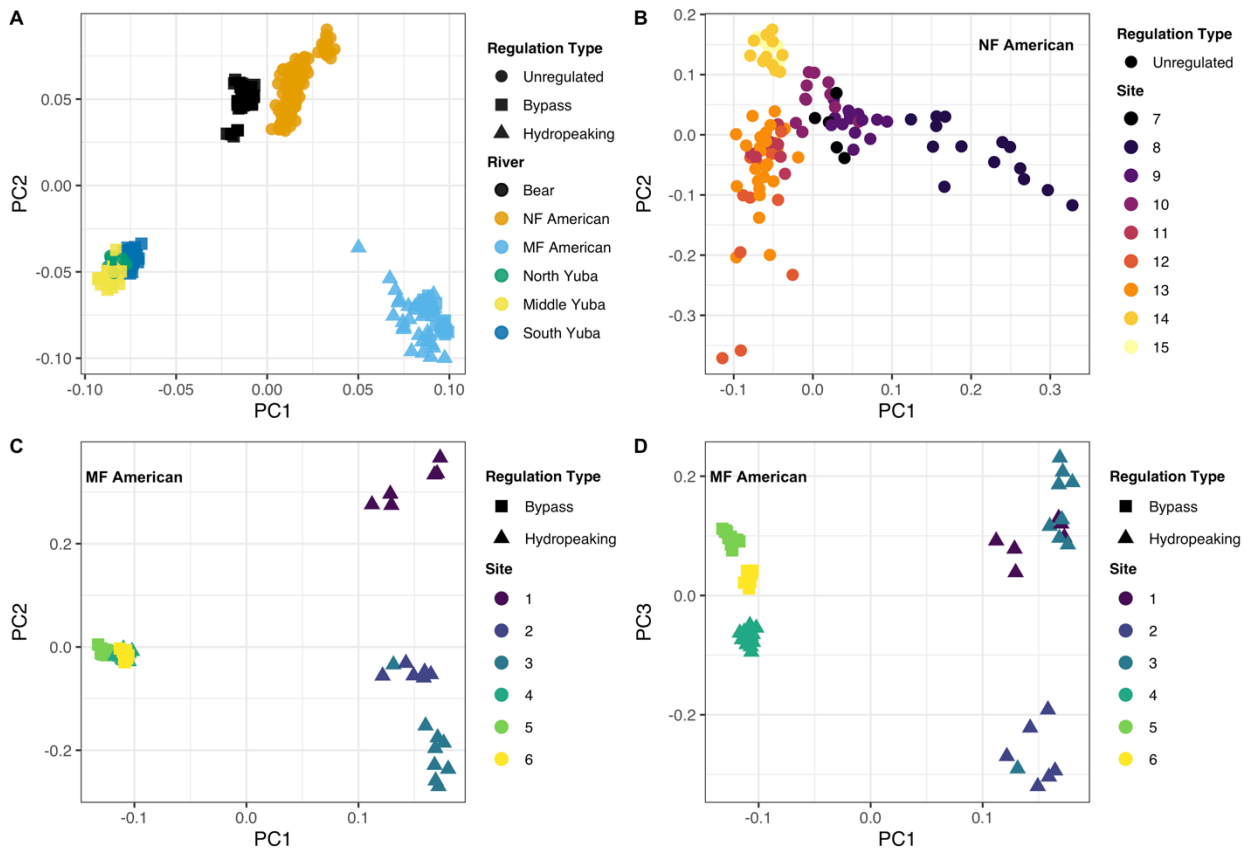



Figure 3. Principal component analysis of Rapture sequencing data. A) Northern Sierra Nevada watersheds; B) Unregulated NF American; C) and D) Hydropeaking MF American Reach.

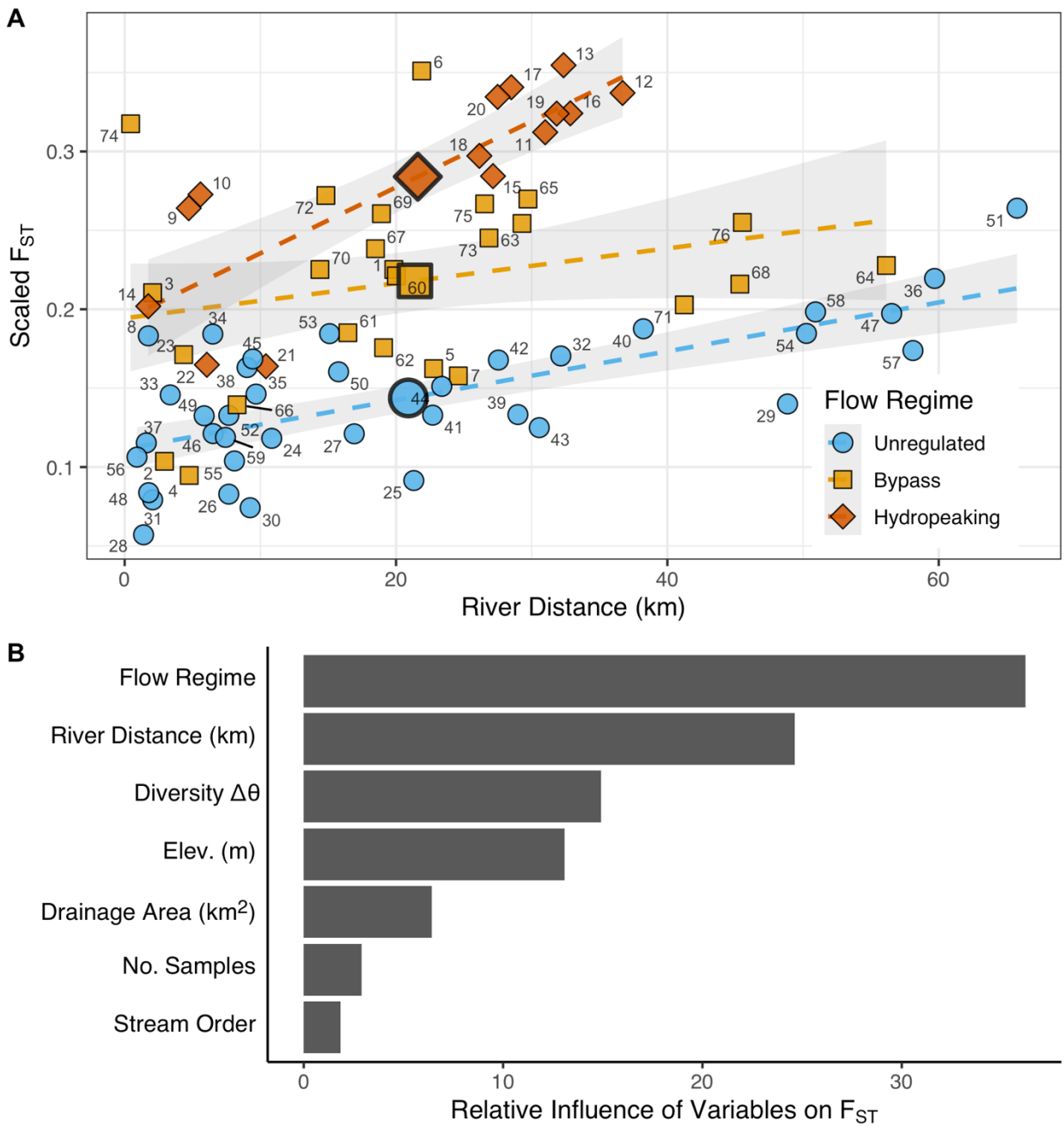



Figure 4. Relationship between river distance and genetic differentiation in *R. boylii*. A) pairwise scaled $F_{ST}$ vs. river distance (km) for each location pair, centroid of each flow alteration type delineated as large point with black outline. Numbers on points

819 correspond to “sitepairID” in Data S7; B) Relative influence of covariates on scaled $F_{ST}$

820 from boosted regression tree models.

821

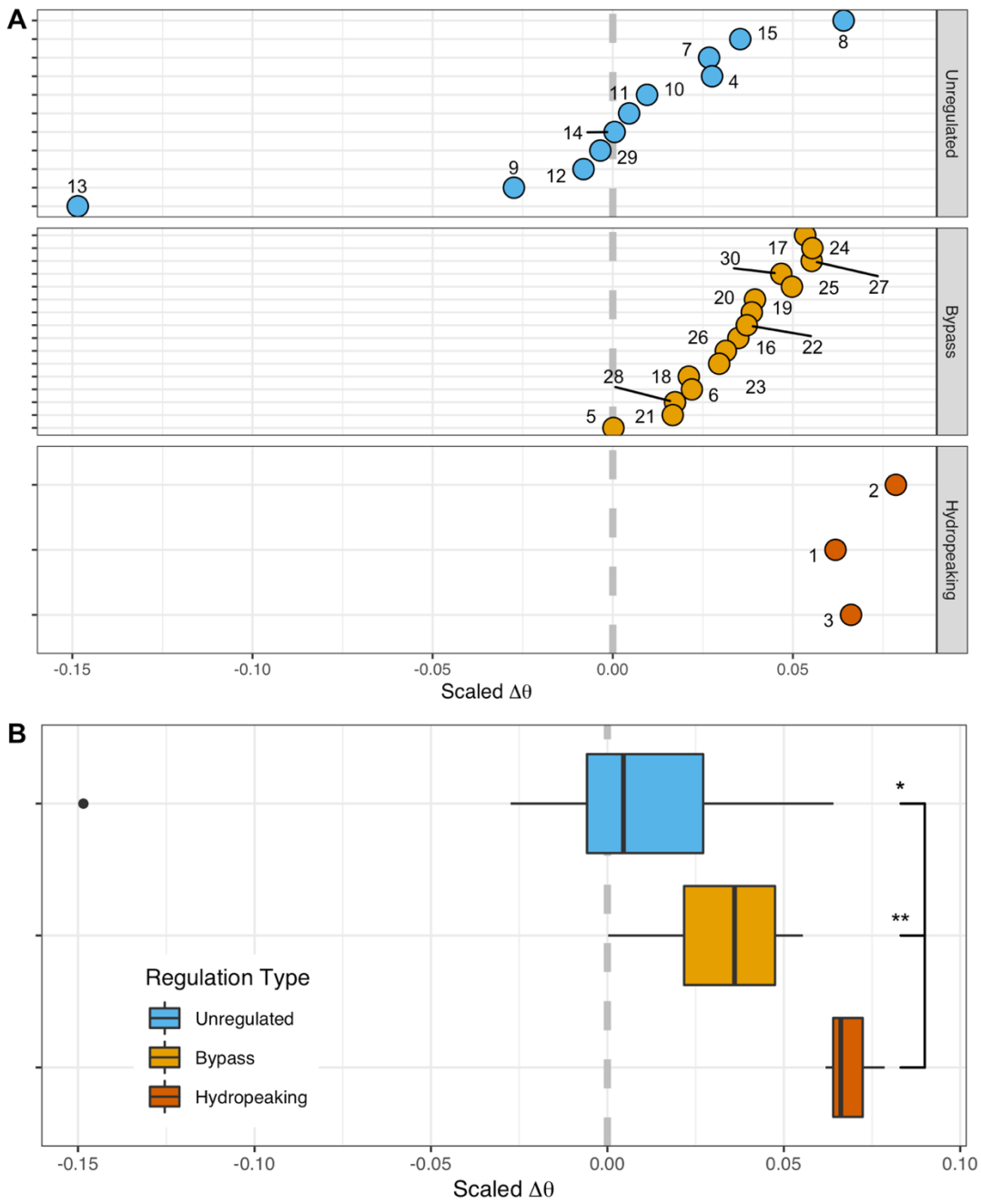



Figure 5. Relationship between river regulation and genetic diversity trajectory in *R. boylii*. A) Assessment of genetic diversity trajectories using scaled Δθ ($\theta_\pi - \theta_S$ / [$\theta_\pi + \theta_S$] / 2) for each sampling location; B) Boxplots of scaled Δθ and pairwise significance between regulation groups using a pairwise Wilcoxon rank sum test with Bonferroni

827 correction ($P < 0.05$). Negative values represent trends of increasing genetic diversity,

828 positive values represent trajectories of diversity loss, values near zero are stable.