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Notes from the Editor

This summer issue of Volume 105 contains a number of interesting articles for fish and wildlife in California. The first is an important status update for Townsend's big-eared bats in our state, a timely article given that the fungus that causes white-nose syndrome was found for the first time in California. This disease has decimated bat populations in the eastern United States, and in July 2019, CDFW scientists confirmed that the fungus is now present in California. The second article is also timely as it discusses how largemouth bass respond to long-term reductions in water levels, a relevant topic given the increased likelihoods of prolonged droughts in California as the climate continues to warm. The reductions in water levels in this study were partly a result of the California drought that lasted from 2011-2016. The next article discusses the effects that managed flows which result in changes in water temperature and flow volume have on Chinook salmon. And lastly, researchers present a relatively new technique of using eDNA to determine predation on native rainbow trout by an invasive species.

As has been mentioned in recent issues of *California Fish and Game*, we continue to make changes to update and enhance the Journal. With this issue, we are announcing revised submission guidelines for authors. While, in the past, the guidelines have been published as an article in CFG, we have decided to use a different format and simply post them on our webpage; this will allow us to more easily update and make changes as necessary. The new guidelines can be found on our submission page: <https://www.wildlife.ca.gov/Publications/Journal/Submissions>. All manuscripts submitted after July 2019 should conform to these new guidelines.

Our Associate Editor team continues to grow. We have two new members of our AE team this issue. Karen Converse is an Environmental Scientist in our Wildlife Branch's Deer Program. She received her B.S. from California State University- Sacramento in 1999 and her M.S. in Biological Sciences with a concentration in Ecology, Evolution, and Conservation from the same school in 2013. Karen has been working for CDFW since she completed her bachelor's in 1999. Her background includes research in a variety of subjects including carnivores, big game, genetics, habitat management, and vegetation. Lauren Damon is currently a Senior Environmental Scientist (Specialist) in our Bay Delta Region. Lauren received her B.S. in Ecology and Evolution from the University of California, Santa Cruz in 2008 after which she began her career with CDFW. Her expertise includes San Francisco Estuary ecology and reproductive behavior of fish, for which she has a significant number of publications and professional presentations. We are excited for Karen and Lauren to join our expanding team of AEs!

Remember that we are working on three special issues this year: cannabis, fire, and human recreation and their impacts on fish and wildlife resources in the state. Please pass the word along to those you know who do research on these topics. If you would like to find out more about our Special Issues, please see our webpage: <https://www.wildlife.ca.gov/Publications/Journal/Special-Issues>.

Ange Darnell Baker, PhD

Editor-in-Chief

California Fish and Game

Assessment of the status of the Townsend's big-eared bat in California

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The California Department of Fish and Wildlife lists the Townsend's big-eared bat (*Corynorhinus townsendii*, COTO) as a Species of Special Concern and a Species of Greatest Conservation Need. The only California statewide field assessment of the species' status, however, was conducted in the 1980s and 1990s. Our goal was to quantify the current distribution of COTO in California by conducting a comprehensive roost assessment at sites visited during the previous statewide survey and a geographic expansion of that effort. We sampled during two complete winters (2014–15 and 2015–16) and three spring/summer/fall periods (2015 through 2017). We searched published and unpublished records and databases for records of COTO and communicated with biologists and other individuals to gather previously unreported and new records of occurrence and potential locations. We used the basic sampling units (sampling frame consisting of 10 x 10 km [100 km²] grid cells) of the North American Bat Monitoring Program (NABat) as the foundation of our survey and drew potential sampling cells by first dividing the state into Level III Ecoregions, and then randomly listing all cells in the state. Historical locations not known to be closed or otherwise uninhabitable were re-visited in the appropriate season. We surveyed 304 grid cells during this study, with 206 in summer and 98 in winter, and within

those cells surveyed approximately 620 potential roost sites. Statewide, we located the species in 209 active season roost sites without evidence of a maternity colony, 84 maternity sites, and 80 hibernacula. About one-half of the maternity colonies were in abandoned mines, 29% in natural caves, and the remaining colonies in various structures (e.g., buildings, bridges, culverts). For all sites visited, 58% had signs of human disturbance. We confirmed the species at 53 of 80 (66%) historical sites in summer and 37 of 63 (58%) historical sites in winter. We were able to determine the status of about two-thirds of the sites surveyed by Pierson and Rainey (1998) during the 1980-1990s period. Of those sites, we determined that about one-half remained active while the other half were inactive for a variety of reasons. Pierson and Rainey (1998) reported a total of 39-43 maternity colonies, while we documented at least 84 maternity colonies. Although our surveys identified substantially more maternity colonies than were known to Pierson and Rainey, we cannot conclude this indicates a substantial increase in site use because Pierson and Rainey were not able to cover the state in as intensive a manner as we could. Historical data for the "new" sites is not available and as such, we cannot state whether these colonies represent restriction or expansion of past occupancy. Additionally, our study did not focus on numbers of bats at a given site. As bats are long lived, the persistence of a maternity colony or bat presence at a site cannot be equated with viability of that particular population. Our surveys, along with other data known to exist on the species, indicated Townsend's big-eared bat remains distributed across much of California, in part because of their use of anthropogenic sites. Unless actively managed, however, available abandoned mines, and to a similar degree, buildings, will continue to decrease in number because of collapse or repurposing. Adequate foraging locations must also be available. We recommend allocating resources to implement long-term monitoring of the species, and so that individual owners-managers can be contacted and encouraged to work with agency personnel in protecting the bat resource through cooperative approaches.

Key words: California, *Corynorhinus townsendii*, distribution, impacts, status, trend, Townsend's big-eared bat

The Townsend's big-eared bat (*Corynorhinus townsendii*, COTO) is generally regarded as a bat species at high risk of endangerment throughout its range in western North America. The California Department of Fish and Wildlife (CDFW) has designated it as a Species of Special Concern (SSC) and also a Species of Greatest Conservation Need (SGCN) and it was recently (2012-16) the subject of a petition for listing as threatened or endangered under the California Endangered Species Act (CESA). The only statewide field assessment of the species' status was conducted in the 1980s and 1990s. Based on their statewide survey effort, which ended in 1991, Pierson and Rainey (1998) concluded that COTO had undergone a substantial population decline over the previous 40 years (i.e., since about 1950), with a 55% decline in maternity colonies, a 44% decline in the number of roosts, a 55% decline

in total abundance, and a 32% decline in average maternity colony size. As summarized by Pierson and Rainey (1998), the species is highly sensitive to human disturbance, particularly of maternity colonies. In addition to disturbance, the number of alternate roosts has been declining due to mine closures, renewed mining, timber harvest, cave commercialization, and general recreational exploration (Pierson and Rainey 1998, CDFW 2016).

Although the California Fish and Game Commission determined that COTO did not warrant a CESA listing in 2016, effective conservation and management of this species would benefit from a comprehensive management plan based upon a thorough survey of the current distribution and abundance of the species. A multi-state assessment and conservation strategy developed for COTO in the late 1990s (Pierson et al. 1999) recommended annual or biannual monitoring of selected sites across the species' range and monitoring of COTO numbers and roost conditions at all sites at 10-year intervals. Although some COTO roosts in California have been monitored at a variety of intervals in the past two decades, a comprehensive and extensive monitoring initiative at all known COTO sites in California has never been conducted.

This project aimed to quantify the current distribution of COTO in California by conducting a comprehensive roost assessment through revisiting the previous statewide survey sites and geographically expanding that effort. This project constituted a comprehensive assessment of this species using similar methods as used for the first statewide survey project more than two decades earlier and thus generated a comparable data set, save for colony count data, which were not collected. The project also expanded coverage to newly documented and potential habitat locations. This assessment provided information to CDFW's CESA Status Review (CDFW 2016) and will provide baseline data and recommendations to support CDFW to implement effective management actions that lead to conservation of the species.

Herein we provide a summary on the distribution, abundance, condition of historical and currently occupied sites, recommendations for maintaining or enhancing existing populations, and a discussion of likely future threats. Ancillary benefits of this study included increased jurisdictional interest in bats and associated habitat surveys, and additional public education on the status and value of bats through our contacts during surveys. Our specific objectives were: (1) assess the occupancy, activity, and condition of all known (historical and current) COTO roost locations; (2) design and conduct a stratified random sampling plan to determine occupancy of potential roosts based on known habitat features; (3) determine the current status and trend of the species relative to historical assessments; and (4) develop recommendations for research and management designed to enhance persistence of the species.

METHODS

The sampling scheme consisted of two primary components. First, to the degree feasible, all historical and roost sites not known to be destroyed (e.g., mine closure) were surveyed for current activity and condition. Second, we used a modification of a national bat survey protocol to generate a stratified random scheme for sampling to determine bat occupancy and abundance across its range in California. We initiated preliminary sampling in fall 2014 and field work continued through summer 2017, thus encompassing two complete winters (2014–15 and 2015–16) and three spring/summer/fall periods (2015 through 2017).

Historical data.—We searched the published and unpublished records and databases summarized below for records of COTO, and communicated with biologists (private,

government agency), cavers, and other individuals to gather previously unreported records of occurrence and potential locations. We gathered all existing site locations for COTO from the California Natural Diversity Database (CNDDDB) and cross-checked those with the literature to ensure no known records were missed. Pierson and Rainey (1998) summarized existing records through about 1998; here again we cross-checked their records with other known records (e.g., CNDDDB, literature prior to 1998). We attempted to gather all known literature records for COTO, which we then reviewed and cross-checked with existing data (e.g., CNDDDB, Pierson and Rainey 1998). We contacted State (e.g., CDFW, California Department of Parks and Recreation [CDPR]) and Federal (e.g., Bureau of Land Management [BLM], United States Forest Service [USFS], United States Fish and Wildlife Service [USFWS]) agency personnel to gather unpublished location records of COTO observations and locations of potential roosts known to them. Additionally, we used existing bat information networks (e.g., Western Bat Working Group) to request location data. Lastly, we have been conducting intensive winter and summer surveys throughout the Inyo and White mountain ranges (Inyo and Mono counties) and the adjacent eastern slopes of the Sierra Nevada Mountains since 2010, as a follow-up to surveys initiated in the 1990s; this work is ongoing and is continuing as of summer 2019. These data were incorporated into the overall analyses for this study (described below).

All location records (COTO observations) were entered into a FileMaker (FileMaker, Inc.) database and coded such that records can be summarized and also displayed (GIS mapping) by various characteristics including: record type (maternity, hibernacula, unknown), protected (e.g., gated or administrative protection) or unprotected, and timeframes (i.e., categorize data by 5-year blocks). As noted below, all new locations (not known to be previously visited) were entered into the database.

Selection of study cells for current occupancy.—We used the basic sampling units developed first by the Pacific Northwest Bat Grid (PNWBG) (Ormsbee et al. 2006) and subsequently incorporated into the North American Bat Monitoring Program (NABat) (Loeb et al. 2015) as the foundation of our survey. We focused on COTO hibernacula and maternity colonies; we did not attempt to sample general multi-species bat occurrence as is a goal of NABat. We used the NABat sampling frame that consists of 10 x 10 km (100 km²) grid cells that are the focal analytical unit for regional and range-wide assessments. Although we did not implement the specific NABat field survey protocol, our use of the same geographic grid boundaries will allow geographic or sampling comparisons between our data set and other studies using the NABat grid system.

We drew potential sampling cells (from 4,365 total in California) by first dividing the state into Level III Ecoregions, and then randomly listing all cells in the state, prioritizing visits to the lower numbered grid cells within each region where suitable habitat could be identified. Because little is known regarding COTO use of different areas for inactive and active periods throughout much of California, we did not make separate draws for each period.

After selecting the initial set of potential sampling cells, we screened each cell for the presence of potential roosts using GIS, visual examination of topographic maps, and Google Earth. Mine structures, including adits, shafts, and buildings, and other features such as publicly advertised caves are usually included on topographic maps. In our experience, these maps do not reveal about 5% of all mine structures. It is unlikely, however, if multiple mine structures occur in a concentrated location mapmakers would have completely missed them. Tools such as Google Earth were useful in some areas where lack of vegetation cover

allowed locating abandoned buildings and determining potential extent of a mine working (e.g., size of waste piles), although it was seldom possible to determine if the structure was open (i.e., portal not collapsed or otherwise accessible for occupancy by bats). As part of this desktop review process, where possible, we contacted jurisdictional biologists, land managers, recreational groups, and private landowners for additional information about the presence of bats and habitat.

Each cell was characterized as: (1) not environmentally suitable (i.e., too warm in winter [$>5^{\circ}\text{C}$] or too cold in summer [$<20^{\circ}\text{C}$], which was usually based on elevation or region), (2) environmentally suitable but no suitable roost sites, or (3) environmentally suitable with potential roost sites; these latter cells were included in the sampling schedule. Because of logistics, we usually could not visit each cell in the order it was selected. Accessibility issues, study timeframe and logistical constraints combined with the unexpected number of "new historical" sites (potential Townsend's occurrence records known to various jurisdictions, land managers and the caving community but not included in the CNDDb database of original historical records) that emerged through our data compilation and records requests, meant that not all sites or target cells were visited. Few jurisdictions refused access outright, with Joshua Tree National Park the only jurisdiction to refuse a submitted research use request. We did not target cells or historical sites that fell within jurisdictions where monitoring for bats is formalized and ongoing, as is the case with several National and State Park jurisdictions, because we wanted to focus our field efforts where surveying was not being conducted. Thus, our results may represent a conservative estimate of the distribution and status of COTO.

To the extent possible we used the presence of COTO as the basis for implementation of an adaptive surveying strategy. We implemented this additional surveying strategy because we were focusing on a single bat species, and to mimic to some degree the search within 15 km conducted by Pierson and Rainey around maternity roost sites. In such an adaptive strategy, all cells that meet basic selection criteria (i.e., habitat) adjacent to the occupied cell (see occupancy definition, below) would be surveyed; any additional occupied cells would then serve as the focal point for surveying the adjacent cells; and so forth until no occupied cells are located.

Indications of occupancy (e.g., guano; see below), acoustic identification, and visual identification can all indicate recent bat activity at a site. However, only certain methods provide reliable estimates of site bat abundance (e.g., internal counts of individuals; night emergence counts). For our sampling and implementation of the adaptive sampling scheme, we used the presence of even one bat to indicate occupancy of a sampling cell. Calculation of occupancy of sampling cells can later be determined using several different criteria for "presence" (see below under "Analyses"). Because we gathered at least two, and sometimes three, measures of presence (i.e., visual, guano, and rarely, acoustic) at each site, and never based occupancy assessment on acoustics alone, we minimized the probability of making false-positive acoustic detections in our occupancy estimation (Clement et al. 2014).

Selection of historical sites.—We re-visited historical locations not known to be closed in the appropriate season, where access and feasibility allowed; we included all historical sites in our analyses. Pierson and Rainey (1998) surveyed a 15-km radius around the original site if it was unoccupied; they deemed this an appropriate area because of the high site fidelity of colonies. Rather than a priori set a sampling limit, we applied our adaptive survey system (described above) to a former roost site. Variables used as the primary sampling

strata to exclude potential sampling areas included lakes, urban areas (the outskirts of cities were retained), and regions where COTO have not been shown to occur because of extreme seasonal conditions (e.g., high elevations in the Sierra Nevada).

We broke the initial broad-scale filter by “active” (spring/summer/fall) and “inactive” (winter/hibernating) periods. Elevation was the primary factor separating potentially suitable priority locations between summer and winter; elevation is, of course, correlated with seasonal changes in temperature in many regions. There were locations where potential winter and summer locations overlapped.

Field surveys.—Surveys were conducted from fall 2014 through summer 2017, thus encompassing three active (2015 through 2017) and three inactive (2014–15, 2015–16, and 2016–17) periods; most work occurred from fall 2014 through winter 2016–17. As logistically feasible, we attempted to sample an equal representation of geographic locations across the state each year (i.e., not concentrate in one geographic location each year).

Selected roost locations within a cell were visited in the active or inactive season, but in most cases not both, unless the roost had characteristics that might serve for both seasons. A cell with substantial elevation gain, or potential roosts with favorable characteristics (e.g., cold air flow for winter), could be visited in both seasons. This is because, while male COTO will often spend the inactive season at lower elevations, the ones that do so comprise a very small proportion of individuals and we could not expend the time and logistical effort to revisit locations that would potentially harbor only a few individuals (personal observation). This factor mostly came into play in areas with colder winter temperatures where we would expect overwintering bats to employ hibernation. For example, in the Inyo-White mountains and adjacent Sierra Nevada (Inyo Co.), most (>95%) individuals hibernate >2500 m, whereas maternity roosts are <2000 m elevation. Here again, our focused efforts on a broad spatial extent while recognizing we were missing some more local occupancy patterns. Because cells are only used for our general randomization as a basis for locating potential roosts, this strategy did not bias our survey in a substantial manner.

The Level III Ecoregions of California along with their USGS numerical designation (parentheses) and number of potential sampling cells were: Coast Range (1)—172; Cascades (4)—139; Sierra Nevada (5)—529; Southern/Central California Chaparral/Oak Woodlands (6)—805; Central California Valley (7)—467; Southern California Mountains (8)—158; Eastern Cascade Slopes and Foothills (9)—192; Central Basin and Range (13)—147; Mojave Basin and Range (14)—777; Klamath Mountains (78)—332; Northern Basin and Range (80)—50; Sonoran Basin and Range (81)—310; Southern California/Northern Baja Coast (85)—275.

Sampling occurred primarily throughout daylight hours by conducting internal surveys for the presence of bats or their sign. In few cases, we also conducted nighttime acoustic or visual exit surveys when safe entry of a site was not possible (see below). In addition to making an internal inspection for COTO, we recorded the occurrence of guano pellets or piles consistent with COTO sign. When a cluster of bats was encountered, we immediately exited the roost to minimize disturbance. During winter we attempted to count bats we could safely observe; our data thus represent a minimum estimate. We considered guano recognizable as COTO if it had unambiguous characteristic light golden patina and twisted shape, and more confidently when also occurring as a Gaussian-patterned pile below a domed section of a passage or other typical roost location. Such sign was considered indicative of maternity colony presence and was included in our final count of maternity sites.

In few situations, when entry could not be safely made and the field schedule allowed remaining in the area for evening emergence work, we used Pettersson Elektronik ultrasonic detectors (various models) to determine if COTO were present during spring/summer/fall. We based the specific placement and number of detectors on site characteristics, such as number of portal or cave openings, or exits from a building. Although such recordings are not appropriate for determining absolute abundance or absence, they can be used to establish presence. For example, a large number (e.g., >20) of separate recorded files at a portal near sunset during the appropriate time of year could suggest a maternity colony. Likewise, acoustic analysis during the late fall could indicate the potential location of large hibernacula; follow up internal surveys during winter could be conducted. COTO do not always echolocate, and when they do, their calls can be such low amplitude as to be nearly undetectable. Thus, a lack of acoustic detection, without corresponding visual confirmation of absence, was not used as confirmation of absence, nor were passively collected calls alone used to confirm the presence of a maternity colony, as acoustic data cannot confirm number of bats present.

We analyzed acoustic recordings using SonoBat software to recognize bat call sequences and identify them to species using a hierarchical decision engine trained on multiple time-frequency and time-amplitude parameters extracted from a library of >10,000 species-known recordings (Szewczak 2010). We used automated identifications with manual confirmation of species identifications using known call characteristics (Szewczak et al. 2011). Manual vetting is of particular importance for COTO because it vocalizes with lower amplitude compared to other bats, imparting lower automated acoustic detectability (Parsons and Szewczak 2009).

We gathered data on the estimated level of human disturbance at each roost. Because we were not monitoring human visitation at roosts, we made a visual estimate of disturbance based on proximity to easily travelled roads, footprints inside and outside roosts, trash, graffiti, and other signs. We then categorized our observations into none, slight (little or no fresh footprints or trash), frequent (numerous footprints, substantial trash), and continual (roosts readily accessible by road and open to visitation).

Other ongoing studies.—We gathered reports of the status of COTO surveys that were being conducted on a regular basis by resource agencies. In some cases, we did not need to conduct our own surveys because that work was being accomplished. We did not include those data directly in our databases because the data were not obtained within our sampling strategy. We do, however, report those data separately herein.

Analyses.—Pierson and Rainey (1998) focused their survey on what they termed “significant maternity colonies,” which they defined as >30 individuals. No definition was provided by them for hibernacula; rather, they sampled “a selection of known hibernating sites.” Thus, we could not know how many smaller (i.e., ≤30 individuals) maternity colonies they did not survey. Based on our results that found few maternity colonies of <30 bats, we doubt they excluded many sites. However, because they did not systematically look for previously unknown roost sites, but rather focused on known sites, our comparison with their findings cannot be taken as an overall assessment of the status (i.e., declining, stable, increasing) of COTO in California. Rather, our comparison with Pierson and Rainey is an assessment of change in status of the specific locations they surveyed and potential relocations of roost sites.

Basic occupancy was defined as the presence of ≥ 1 individual bat (visual detection, COTO guano, or acoustic identification) at a site within a cell. We present results on occupancy by several spatial extents (scales), including statewide (overall) and Level III Ecoregion. We also divided most data by season (active versus inactive). We summarized these data on the scale of the Ecoregion (i.e., ≥ 1 cell met the above criteria), and on the proportion of occupied versus unoccupied cells for each category for an Ecoregion.

We did not attempt to count (e.g., exit or emergence surveys) maternity colony size because our goal was to survey for presence; counting would have focused our attention in fewer survey cells. Although we were able to more thoroughly survey roosts in the winter, time, safety, and general logistical constraints often prevented us from conducting a complete internal survey. As such we chose to categorize hibernacula into several classifications of bat abundance (i.e., solitary, >1 to 5, and >5 bats). Pierson and Rainey conducted counts (emergence or internal) of number of individuals present at most of the maternity colonies they surveyed. Their counts were estimates, however, because they applied a correction factor rather than standardizing when the counts occurred; that is, some colonies were counted prior to young emerging, whereas others were counted after young started emerging. In addition to the logistical limitations that attempting to count individuals' places on a study (see above), because of the number of locations we wanted to visit, we decided to forego counts at maternity roosts. Thus, our presence-absence data provides location data on which future, more intensive studies of changes in abundance can be based.

RESULTS

Survey effort.—Although it varied by Ecoregion, our initial screening of the potential suitability of grid cells for survey indicated that usually 50% to 70% of the cells were within adequate environmental parameters for the species to occur during summer or winter. In the Sierra Nevada Ecoregion, for example, we deemed $\sim 70\%$ of the cells to be within acceptable environmental parameters for summer occupancy, but upon detailed examination (e.g., using Google Earth, topographic maps), concluded that only $\sim 25\%$ contained potential roost sites that were identifiable through desktop review and outreach means. Similarly, for winter, only about 50% were acceptable environmentally, with about 15% containing identifiable potential hibernacula.

Based on our initial screening of cells, we surveyed 304 grid cells during this study, with 206 in summer and 98 in winter (Table 1). The geographically small Ecoregion in the northeast, Northern Basin and Range, received no direct survey effort because we determined the sites of the few historical records were no longer viable (e.g., hotel torn down), and lack of readily identifiable potential roost sites. Similarly, the Central California Valley region received little effort because of the lack of potential roost sites (i.e., region primarily commercial-residential-urban and agriculture) and the extent of private land representing identification and accessibility obstacles to such habitat as might exist, given the scope of the study. Across all Ecoregions this study visited and surveyed approximately 620 potential roost sites (Figure 1).

Occupancy.—We located Townsend's big-eared bats in all Ecoregions of California; recent anecdotal sightings indicate their presence in the Northern Basin and Range. Statewide (all Ecoregions combined), we located the species in 209 active season roost sites without evidence of a maternity colony, 84 maternity sites, and 80 hibernacula (Figure 1).

TABLE 1.—Number of grid cells sampled during Townsend’s big-eared bat survey by Level III Ecoregion and season (different portions of cells with substantial elevation gain or other characteristics [see text] could be visited in both winter and summer).

Ecoregion (USGS no.)	No. cells in summer	No. cells in winter	No. cells total
Statewide	206	98	304
Coast Range (1)	11	5	16
Cascades (4)	9	5	14
Sierra Nevada (5)	36	20	56
Southern/Central California Chaparral/Oak Woodlands (6)	32	6	38
Central California Valley (7)	2	0	2
Southern California Mountains (8)	17	10	27
Eastern Cascade Slopes and Foothills (9)	10	1	11
Central Basin and Range (13)	8	27	35
Mojave Basin and Range (14)	42	12	54
Klamath Mountains (78)	28	7	35
Northern Basin and Range (80)	- ^a	-	-
Sonoran Basin and Range (81)	5	1	6
Southern California/Northern Baja Coast (85)	6	4	10

^aPotential cells were excluded from survey based on pre-screening.

The Mojave Basin and Range contained the most roost sites and maternity colonies, while the Central Basin and Range contained the most hibernacula. These data do not include the roost sites (of all purposes) known for some federal properties, including especially National Parks and Monuments in the northern portion of the state (see below).

Maternity structures.—About one-half of the maternity colonies were in abandoned mines (Table 2). The bulk of the remaining colonies were in natural caves (29%), which included limestone and other rock caves and lava tubes. Buildings, bridges, culverts, water flumes, tree basal hollows, and other structures accounted for the remaining locations. Types of roost structures were not surveyed in equal proportion, thus the proportion of colonies in each type of feature are not necessarily indicative of habitat preference.

Site condition and disturbance.—About 10% of all sites we visited had no potential roost habitat because of site removal (e.g., mine reclamation), portal collapse, structure removal or modification, regular human disturbance (e.g., recreational site), or other causes.

For all sites visited (with or without COTO or other bat species), few (2%) had continual human disturbance, but 22% had what we considered identifiable signs of frequent disturbance (Table 3). The remainder had signs of no (42%) or slight (34%) disturbance. Excluding the Central California Valley and Northern Basin and Range ecoregions because of small sample size due to few visited COTO cells, sites with frequent disturbance ranged from between ~10% and 38% (Table 3). For active maternity sites, overall 24% showed evidence of frequent disturbance while 41% showed only slight disturbance; the remaining 35% showed no evidence of disturbance.

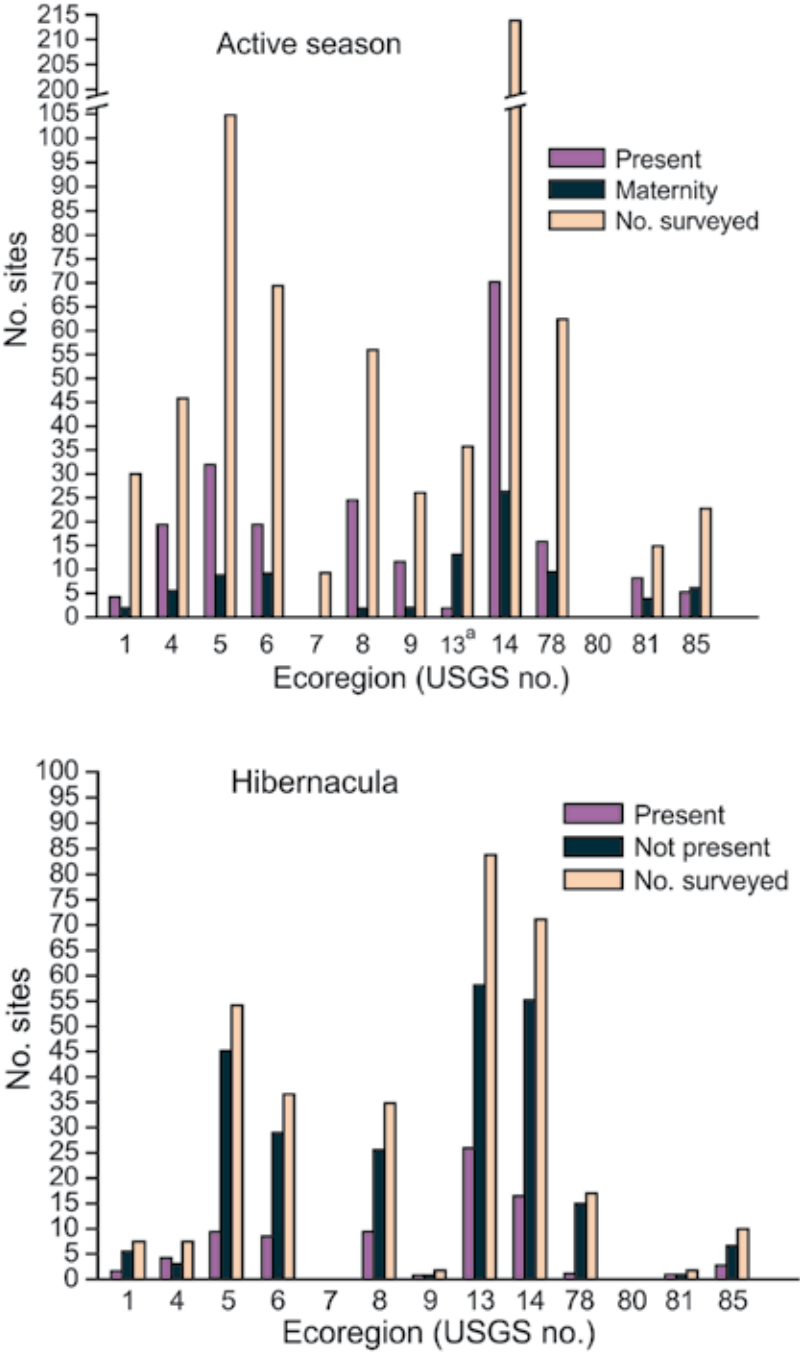


FIGURE 1.—Occupancy (no.) of sites surveyed for Townsend’s big-eared bats (COTO) by Ecoregion during active and inactive seasons. Active Season Central Basin and Range (13a) includes 10 maternity roosts not obtained through random cell selection.

TABLE 2.—Type of structure used by Townsend's big-eared bats for maternity roosts by Ecoregions.

Ecoregion (USGS no.)	Mine	Cave	Building	Other ^a
Statewide	44	25	10	7
Coast Range (1)		1	1	
Cascades (4)		6		
Sierra Nevada (5)	2	4	3	
Southern/Central California	3	3	3	
Chaparral/Oak Woodlands (6)				
Central California Valley (7)				
Southern California Mountains (8)	2			
Eastern Cascade Slopes and Foothills (9)		3		
Central Basin and Range (13)	11 ^b	2 ^c		
Mojave Basin and Range (14)	21	2	1	
Klamath Mountains (78)	2	4	2	
Northern Basin and Range (80)				
Sonoran Basin and Range (81)	3			1
Southern California/Northern Baja Coast (85)				6

^aBridges, culverts, water flumes, and other structures.

^bIncludes 8 maternity roosts not obtained through random cell selection.

^cNot obtained through random cell selection.

Overall historical.—Based on all data sources available to us (e.g., CNDDDB, unpublished reports, literature), we located the species at 53 of 80 (66%) historical sites in summer and 37 of 63 (58%) historical sites in winter (Figure 2); our totals include sites known to be closed or otherwise uninhabitable (i.e., unoccupied). Note these records include all roost purposes, including maternity, day and night roosts, and hibernacula. Ecoregions with the most historical occurrences indicated that about one-half to three-quarters of all historical roosts were still active, although the use of the roost could have changed; e.g., no longer maternity but some bats present. These data can best be viewed as a crude indication of continued availability of the roost site (e.g., mine still open).

Pierson and Rainey (1998).—We were able to determine the status of about two-thirds of the sites surveyed by Pierson and Rainey (1998) during the 1980-1990s period. Of those sites, we determined that about one-half remained active maternities while the other half were inactive for a variety of reasons, including portal collapse or exclusion (i.e., permanently collapsed by management activity), commercialization of the site, or high human visitation. We were not able to determine the status of the remaining one-third of the sites for various reasons (e.g., insufficient resources, could not obtain access permission, could not locate site).

Abundance in hibernacula.—Most (94%) hibernacula contained >1 individual, with the majority (63%) containing 1-5 bats (Table 4). Most relatively large (>5 bats) hibernacula were located in the Central Basin and Range (35%) and the Mojave Basin and Range (23%).

TABLE 3.—Level of disturbance at sites visited by Ecoregion for Townsend's big-eared bat, winter and summer combined. Approximate sample sizes are provided in Table 2.

Ecoregion (USGS no.)	Disturbance level (% of Sites)			
	None	Slight	Frequent	Continual
Statewide	42	34	22	2
Coast Range (1)	26	33	38	3
Cascades (4)	53	29	16	2
Sierra Nevada (5)	47	30	21	2
Southern/Central California Chaparral/Oak Woodlands (6)	46	32	22	0
Central California Valley (7)	22	0	56	22
Southern California Mountains (8)		49	30	20
Eastern Cascade Slopes and Foothills (9)	46	15	35	4
Central Basin and Range (13)	38	32	29	1
Mojave Basin and Range (14)	38	38	20	
Klamath Mountains (78)	46	38	16	0
Northern Basin and Range (80)	-	-	-	-
Sonoran Basin and Range (81)	25	50	25	0
Southern California/ Northern Baja Coast (85)	33	53	10	3

DISCUSSION

Pierson and Rainey (1998) summarized the known records of the species and reported that 46 maternity colonies were known prior to 1980, with most of the records made from the late 1940s to the 1960s. They could not locate 24 of the known colonies either at the original previous roost site or within the 15 km radius they searched. They also identified an additional 18–21 colonies during their surveys and through other means, bringing the total colonies known to them to 39–43. We documented at least 84 maternity colonies and determined that another 8 colonies occurred across multiple government land holdings.

Of the Pierson and Rainey maternity colonies that we could survey or otherwise determine status, about one-half were active, while the other one-half were inactive because the known colony roost site was no longer suitable (i.e., collapsed, destroyed, high human use). Overall, we did not determine the status of about one-third of their sites. Although our surveys identified substantially more maternity colonies than were known to Pierson and Rainey, we cannot conclude this indicates a substantial increase in site use because Pierson and Rainey were not able to cover the state in as intensive a manner as we could. As we did not count the number of bats in each of the extant colonies, we also cannot state whether the maternity colonies still present in historical locations have experienced any change in size. Given that Pierson and Rainey found COTO in only about half of historically occupied roosts, and our survey found about half of those still occupied, the data suggest a net decline of roost occupancy of about 75% since the 1950s. However, the fact there are twice or more colonies now known to exist establishes a new baseline for understanding and monitoring the species through time and provides a broad distribution of sites available as candidates

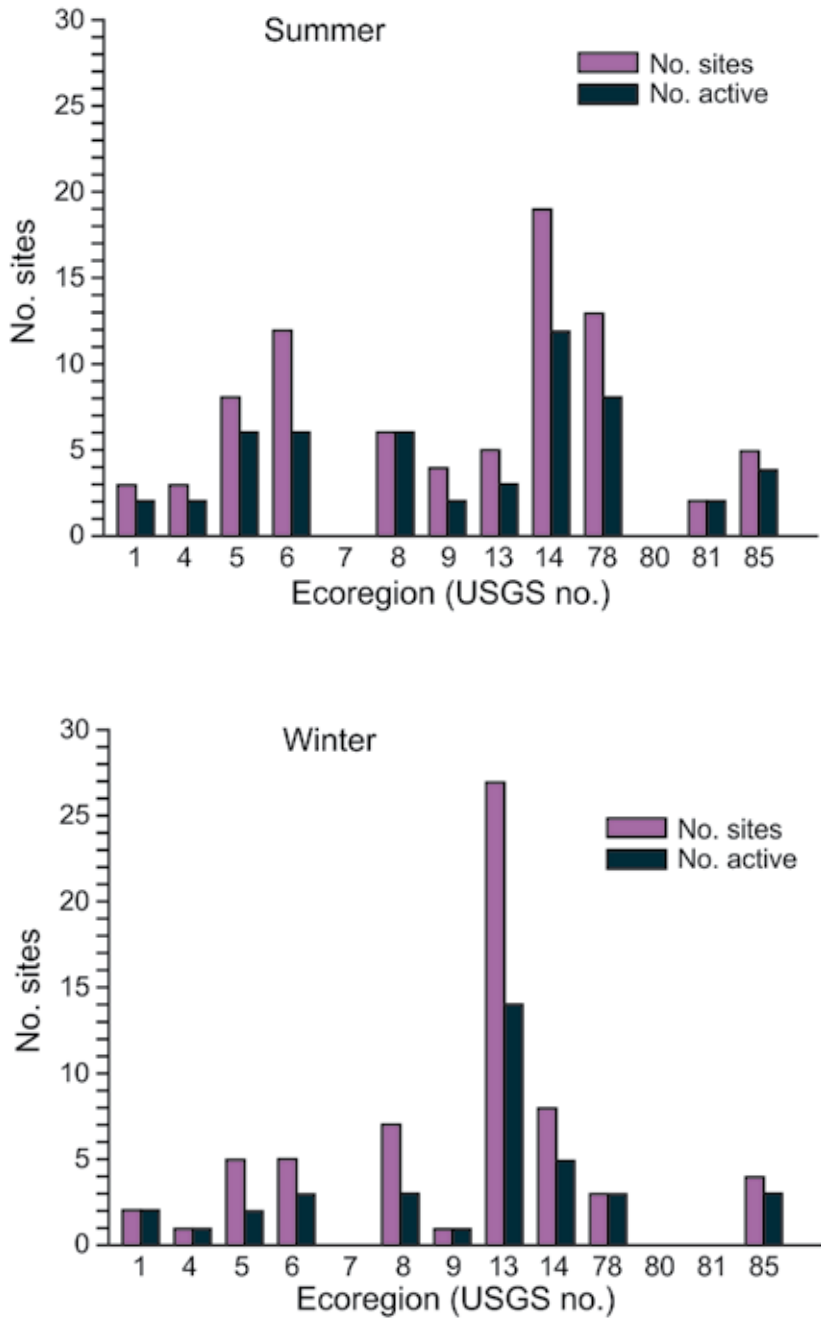


FIGURE 2.—Number of historic sites visited and determined to be active for summer and winter roosting Townsend’s big-eared bats.

TABLE 4.—Summary of hibernacula surveyed by minimum roost size category (1, 1-5, >5 bats) by Ecoregions for Townsend’s big-eared bat.

Ecoregion (USGS no.)	Number of sites		
	1	1-5	>5
Statewide	5	52	26
Coast Range (1)			1
Cascades (4)		2	4
Sierra Nevada (5)		9	
Southern/Central California			
Chaparral/Oak Woodlands (6)	2	3	3
Central California Valley (7)			
Southern California Mountains (8)		9	1
Eastern Cascade Slopes and Foothills (9)			1
Central Basin and Range (13)		18	9
Mojave Basin and Range (14)	3	6	6
Klamath Mountains (78)			2
Northern Basin and Range (80)			
Sonoran Basin and Range (81)		1	

for long-term monitoring efforts. We also speculate that colony relocation is occurring. The possibility of extirpation of a colony followed by recolonization requires research.

Based on the focus of Pierson and Rainey on known historical colonies, and lack of any randomization across potential roost locations, they could not draw a valid inference regarding the status of the species statewide. Thus, the Pierson and Rainey study is best viewed as an analysis of the status of previously known roosts, rather than a statewide assessment of status. Extension of their results statewide would require assuming that their sample represented conditions across a very broad spatial scale. Additionally, historical locations for most species are largely based on convenience sampling; that is, locations that are easy to access logistically. A sampling design based on an appropriate randomization method forces observers to traverse rugged terrain and often visit remote locations. Because prior to our work no broad-scale survey that incorporated randomization had been conducted, the Pierson and Rainey survey is by design biased towards readily accessible locations. As such, their design could also be biased towards human disturbance (selecting sites known to humans) as a cause for roost abandonment. Logistical and access issues may have inevitably introduced some level of the same bias to our efforts, though to a lesser degree given survey design.

Determining the overall trend of hibernacula or overwintering sites is problematic because Pierson and Rainey (1998) did not focus on the winter period. Additionally, although large hibernacula (i.e., >30 individuals in a single site) are known, most sites harbor far fewer individuals. Ongoing long-term research in the Inyo-White mountains is showing, for example, that individuals from a single maternity colony scatter across the landscape and occupy multiple hibernacula in numbers ranging from solitary individuals to several groups of up to 35 (M. Morrison, Texas A&M, unpublished data). Likewise, at Lava Beds National

Monument, there were 91 known hibernacula in 2017 with only nine sites having a mean abundance of >30 bats (K. Smith, pers. comm.; see also Weller et al. 2014). Especially on the west side of the Sierra Nevada, the species is known to frequently emerge from hibernation for short periods during winter when weather conditions permit (i.e., warm temperature periods). The use of a large number of sites, as well as potential movements between sites, makes it difficult to make conclusions on the status of wintering sites.

Pierson and Rainey did not use Ecoregions but rather divided the state into what they designated as nine study areas known to harbor populations of the species. They found the majority of bat colonies occurred in what they termed the lava flow area of the northeast (their Area 3, which roughly corresponds to Ecoregions 4, 9, and 80); the limestone caves and old mines of the Mother Lode and western Sierra (Area 4; Ecoregion 5); the abandoned mine workings in the eastern Sierra and western White Mountain foothills (Area 5; Ecoregion 13); and at various sites in the northern coastal areas and inner coast range (Area 1; Ecoregion 1 and 78). We also found a substantial number of maternity colonies in the region corresponding to their Area 5, and to a lesser extent, Areas 4 and 1. We did find substantially more colonies in their Area 6, which corresponded in part to our Ecoregion 14, the Mojave Basin and Range, likely because they did not emphasize the deserts for survey effort. We found relatively fewer colonies in the regions corresponding to their Area 3, which is likely because we did not include colonies known to exist on public lands (especially National Parks and Monuments) that are under regular monitoring by agency personnel (and would have biased our sampling strategy and comparison with Pierson and Rainey).

The type of structure used for maternity colonies that we found was somewhat different than that reported by Pierson and Rainey (1998:Fig. 2). Whereas we found about half of our colonies in abandoned mines and ~29% in natural caves, they found ~39% in mines and 43% in caves. We think this difference was due primarily to the lesser survey effort they expended in the Central Basin and Range and especially the Mojave Basin and Range Ecosystems relative to our efforts, rather than any shift in structure use by the species. Additionally, we are including maternity colonies from the Central Basin and Range that are part of an ongoing research effort by Morrison. Although we did not include known maternity colonies under study by others in north-central portions of the State, we learned that another 8 maternity colonies were active (Szewczak et al. 2018:Appendix C). Thus, although we cannot quantitatively assess, there does not appear to have been any shift in overall structure use. Moreover, we did not survey all structures in equal proportions; mine features dominated the survey effort. These percentage detections by structure type are not adjusted for distribution of effort.

Overall for all sites surveyed including maternities, ~70% showed no or slight disturbance due to human activities. Thus, ~30% of sites experience what we considered frequent disturbance. It is commonly assumed that timing of the disturbance is the primary factor determining the influence of human activities on bat occupancy. Because we conducted a one-time survey, it is not possible for us to evaluate the impact disturbance is having on the species, although almost three-fourths of the sites—including maternities—receive little disturbance. Because COTO occupy a large number of abandoned mines, survey access is becoming increasingly difficult and time consuming because mining roads and trails are seldom maintained. For example, there were many historical sites that could be accessed by vehicle during the Pierson and Rainey survey that now require long (>10 km one-way hikes); many locations cannot even be accessed by modern off-road vehicles (e.g., ATVs).

Conclusions and management implications.—CDFW (2016) published a status review of COTO, which included a comprehensive list of recommendations for research and management of the species. They summarized recommendations into seven broad categories: Research and monitoring needs, administrative actions, management of known roosts, landscape management actions, regulatory review of proposed development projects, public education and outreach, and health and disease issues.

Although we do not provide data on bat abundance, our surveys, along with other data known to exist on the species, indicated Townsend's big-eared bat remains distributed across much of California. Where naturally occurring structures are destroyed or highly disturbed, the species can exist in suitable anthropogenic sites. Suitable anthropogenic habitat, therefore, whether mines, buildings, or even bridges in some cases where the superstructure forms an appropriate cavern analog, appear to provide important refuge resources for the species. Numerous other variables play a role in the viability of a roost site, particularly for maternity roosts, such as distance to foraging habitat, or factors affecting vulnerability of a given colony to disturbance. Such evaluations reach beyond the scope of this study but must be considered in management approaches and warrant additional research.

Unless actively managed, abandoned mines, and to a similar degree, buildings, and other suitable roost structures, will continue to decline from deterioration and human use. Of greatest concern regarding the use of mines are Ecoregions 13 (Central Basin and Range) and 14 (Mojave Basin and Range), where most maternity sites occur in abandoned mines. Based on the variety of structures used by the species, including buildings and bridges, we anticipate that some colonies will be able to relocate locally when a mine becomes unusable. For natural roost structures, emphasis should be placed on managing and protecting natural roost structures. However, we also recommend that management entities consider use of artificial roost structures to replace or augment the availability of artificial roosts. Purpose-built artificial roosts should be designed for the particular climate and habitat onsite and proven to be suitable alternative structures for COTO or species with closely related roost requirements, such as vertical concrete towers (e.g., Mering and Chambers 2014) or wooden buildings with long-term maintenance plans. For artificial roost habitat to succeed as a replacement, long-term maintenance, and monitoring programs (including adequate funding) and adaptive management options are needed to ensure the artificial roost provides appropriate microclimates and volume and fulfills the same life history functions as the original roost. Alternative structures also provide an opportunity for efficient monitoring and colony study. In the Mojave Desert and Central Basin and Range, areas exist where natural caves are available but are frequently visited by humans for recreational use. Such natural roost sites should be closed from human visitation during either maternity season or winter season depending on use, while remaining available for human exploration during the inactive season.

Land management agencies, particularly the BLM, NPS, and USFS, have active programs for identifying, stabilizing, and protecting bat roost sites with an emphasis on the Townsend's big-eared bat. As emphasized by CDFW (2016), continuation and expansion of these programs provides a practical method of ensuring access to suitable roost sites by the species. Sites that are initially protected are, however, frequently vandalized by humans seeking entry into caves, mines, and abandoned buildings for recreational purposes, and thus require regular inspection and repair. During our statewide survey we frequently encountered vandalized sites that had been "protected" by gates or locks, but likely had been open to the

public for multiple years because agencies often lacked sufficient personnel and funding to conduct regular inspections. In addition to increasing the potential for bats to abandon such sites, many of the sites posed substantial risk of bodily harm to the public (e.g., falling into internal shafts). Thus, increased attention to previously protected sites would enhance conservation of COTO as well as other animals using the protected sites.

We were able to work with various private recreation groups, especially the caving community, in locating and accessing potential bat roosts. Because some individuals fear a loss of access to caving opportunities because of potential government actions, we encourage the relevant government agencies to work with these recreational groups to determine ways to allow continued site access while also protecting the bat resource (e.g., seasonal rather than permanent closures). Additionally, many roost sites exist on private lands, where identification of and communication with property owners-managers can require extensive time or prove futile. CDFW (2016) provided recommendations for enhanced public outreach and education, which we echo here. We recommend allocating resources so that individual owners-managers can be contacted and encouraged to work with agency personnel in protecting the bat resource in ways that are cooperative and do not create perceptions of infringing on personal property uses.

Disease is another area of research and monitoring that should be emphasized in the future (CDFW 2016). Although we did not identify any apparent disease issues (e.g., abandoned colonies were usually due to human disturbance), and COTO is not known to develop white-nose syndrome, the causative fungus, *Pseudogymnoascus destructans*, has been detected on the species in other states, and we found potentially susceptible *Myotis* species sharing hibernacula with COTO. Several locations we visited in the state in the course of this study had a high level of visitation yet no interpretive or cautionary signs to raise awareness and help protect the sites from human-caused pathogen spread. Land managers also showed varying degrees of knowledge regarding the white-nose syndrome threat, with some locations indicating the lack of interpretive signs was due to white-nose syndrome not yet occurring in California, and thus not needing to inform visitors. Like CDFW (2016), we recommend a systematic educational outreach effort to land managers and support for interpretive signs, decontamination stations, and gear loan options for visitors with gear from contaminated states. Various entities have conducted research showing the effectiveness of properly designed interpretive signs in altering visitor behavior (e.g., Duncan and Martin 2002).

We (see also CDFW 2016) encourage additional efforts to more fully understand the current and likely future status of the Townsend's big-eared bat in California including: (1) continued efforts to survey additional locations to identify roost sites, including other known historical sites; (2) periodic monitoring (including roost counts) of all maternity and selected hibernacula located during our survey (e.g., all sites could be visited over a moving 5-year period); (3) expanded coordination by CDFW with all land management agencies to promote protection and subsequent monitoring of the status of roost sites; (4) expanded communication with recreational groups that regularly access known or potential roost sites; (5) expanded communication and outreach to private land owners and managers who have roost sites on their properties; (6) development of a centralized, regularly updated database to track all of the monitoring efforts and roost locations from the groups above (i.e., we found in many cases, these data were not shared with CDFW); and (7) continued support for basic research on conservation-relevant aspects of the species' life history, such as disturbance vulnerability/resilience, seasonal movements, and foraging and roost ecology.

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Response of the catchable Largemouth Bass population to long-term water level reductions in Lake Perris, Riverside County, California

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Most of the Largemouth Bass (LMB) fisheries in California are within reservoirs that fluctuate annually. These reservoirs can experience prolonged reductions to the water level if cultural demands exceed the supply of water that normal climatic conditions can replace. These extended periods of reduced capacity may impact the fisheries by eliminating critical littoral habitat and limiting carrying capacity within the affected reservoirs. Lake Perris, California experienced a prolonged mandatory drawdown due to a dam remediation project exacerbated by drought conditions that eliminated nearly half of the water volume in the reservoir for over a 14-year period. Annual mark-recapture population estimates of catchable LMB (>305 mm TL) were conducted to monitor the response of the bass population and compared with the water levels over the duration of the 14-year dam remediation project. The estimated number of catchable Largemouth Bass responded in nearly synchronous decline along with the water level over the duration of the altered hydrology.

Key words: California, drawdown, drought, Lake Perris, Largemouth Bass, *Micropterus salmoides*, population estimate

Largemouth Bass (*Micropterus salmoides*; LMB) are one of the most popular and economically important freshwater sportfish in California. Of the 1.35 million freshwater anglers in California, 33% spent 6.69 million days pursuing LMB and other black bass species (U.S Fish and Wildlife Service 2013). Like many of the reservoirs in North America, the majority of the LMB fisheries in California reside within reservoirs which are utilized for water storage, flood control and generation of hydroelectric power in addition to recreational uses (Sammons and Bettolli 2000). Utilized mostly for non-fishery related purposes, many of these reservoirs fluctuate annually to accommodate the primary objectives of the reservoir. These fluctuations are typically annual and seasonally repetitive. However climatic or anthropogenic causes can alter the regular hydrological regime. Changes to the

hydrology have been found to affect year-class strength of various sport fish species: high water levels in spring have been found to improve year-class strength and low water levels have been found to negatively affect year-class strength. These studies have documented year-class strength based on age-0 fish (Heman et al. 1969, Aggus and Elliot 1975, Martin et al. 1981, Miranda et al. 1984, Willis 1986, Fisher and Zale 1991, Sammons et al. 1999, Jackson and Noble 2000, Sammons and Bettolli 2000); however, the age-0 year class may not correlate with the recruitment to catchable size after several years of influence from abiotic and biotic factors (Durocher et al. 1984, Maceina and Bettolli 1998, Allen et al. 1999, Tate et al. 2003). While modeling has been developed to predict LMB population trends (Orth 1979), models for predicting biological consequences of drawdowns have been considered inadequate. Primarily due to the lack of expensive long-term data documenting effects of water level drawdown on LMB populations (Ploskey 1986).

In July 2005, the California Department of Water Resources (DWR) identified potential seismic safety problems with the foundation of the Lake Perris Dam. DWR determined a portion of the dam's foundation would liquefy in the event of a magnitude 7.5 earthquake (Richter scale), which has the potential of a catastrophic dam failure. Based on these findings, DWR lowered the water level in Lake Perris from its maximum operating elevation of 484 m (1,588 ft) above mean sea level by 7.62 m (25 ft) until repairs to the dam's foundation are completed. The project was to be completed in 2012 (DWR 2007). However, delays extended the project until the end of 2017. This prolonged drawdown, resulting in a 41% reduction of the water volume and loss of much of the existing littoral habitat, was expected to severely reduce the LMB population. Mitigation measures, including changing the angling regulations and installing fishery habitat in the new drawn down littoral zone, were instituted to attempt to minimize anticipated reductions to the LMB population.

To monitor the effects of the drawdown to the LMB fishery, annual population estimates were initiated prior to the drawdown in 2005 and conducted annually thereafter through 2018 upon completion of the dam remediation project. These population estimates were intended to provide insight into the status of the catchable LMB population while the lake water level was lowered for the project. Various methods have been used to estimate fish populations throughout the United States, including angler catch, (Gablehouse and Willis 1986) underwater surveys, (Davis et al. 1997) or catch depletion techniques (Maceina et al. 1995). The primary methods for LMB population estimates have relied on mark-recapture and catch per unit of effort (CPUE) studies (Cooper 1981, Hightower and Gilbert 1984, Miranda et al. 1996, Isley and Tomasso 1998, Kershner and Marschall 1998, McInerny and Cross 1999, Granfors and Giusti 2011). For this study, mark-recapture methods were utilized to monitor the response of the catchable LMB within Lake Perris to the reduced water levels over the 14-year period.

MATERIALS AND METHODS

Study area.— Lake Perris (33° 52' N, 117° 09' W) is the termination of the eastern branch of the State Water Project operated by DWR and was first filled in 1974. The lake is located approximately 16 km (10 miles) southeast of the city of Riverside and 105 km (65 miles) east of Los Angeles at an elevation of approximately 484 m (1,588 ft) in the Perris Valley. At maximum pool, the lake occupies 2,292 surface acres and has 127,000 acre-feet of water storage. The lake drawdown of 7.62 vertical meters (25 ft) resulted in around 1,882

surface acres and 74,500 acre-feet of storage (59% capacity) at maximum capacity while repairs occurred (DWR 2007). A statewide drought further impacted the lake 2014-2017, further reducing the water volume to 53,500 acre-feet (42% capacity). The lake began to slowly refill in February 2018 after repairs were completed

Methods.— Mark-recapture using electrofishing was conducted in spring using a Smith-Root SR-18 electrofishing boat. The primary objective was to determine the population of catchable LMB of 305 mm (12 inches) total length (TL) and larger. Pulsed DC current at 60 pulses per second (6-8 amps) was used to put the fish into electro-narcosis. The boat crew consisted of two forward netters and a boat operator. LMB were netted and placed in the boat holding tank for processing and released back into the lake.

LMB larger than 305 mm were marked using a combination of pelvic fin clips (removal) and hole punches in the anal or second dorsal fin, each combination distinct to a given year of sampling (Granfors and Giusti 2011, Pine et al. 2012). The dorsal and anal fins were divided into thirds: front, middle and rear, for placement of the hole punch mark if used. Each yearly estimate was independent of any other estimates. Fish were marked with the fin clip combination unique to a given year and subsequently recorded as recaptures for that given year. Any fish marked in prior years were treated as unmarked fish and the new unique mark applied. The population was considered to be “closed” to estimate the population (Krebs 1999). Monthly lake storage data (acre-feet) at Lake Perris were collected from the DWR California Data Exchange and converted to elevation from lake capacity curve information provided by DWR.

The LMB population was estimated 2005-2018 using either Schumacher-Eschmeyer (SEM) or Petersen (P) statistical methods (Seber 1982, Krebs 1999). The 2005 pre-drawdown estimate sampled locations that were selected by dividing the lake shoreline into forty-one 0.40 km (0.25 mile) transects and randomly selecting 15 transects (37% of the available shoreline) for each of the mark-recapture efforts that year. The resulting 2005 pre-drawdown estimate was extrapolated to account for the remaining shoreline area that was not sampled. The 2006-2018 post-drawdown estimates sampled the entire shoreline primarily by electrofishing. The 2006-2008 estimates used SEM multiple mark-recapture sampling utilizing only electrofishing. However, the estimates 2009 and 2010 also incorporated catch and release bass fishing tournaments as an additional mark-recapture method to electrofishing to replicate a similar sampling methodology undertaken at nearby Diamond Valley Lake at the time (Granfors and Giusti 2011). Tournament data was collected to potentially increase the sampling area beyond the shallow areas of the lake through angling practices in deeper areas of the lake. The loss of staff and boat mechanical issues in 2011, disabled the ability to conduct a SEM multiple mark-recapture effort. Therefore, a Petersen mark-recapture estimate using only electrofishing was conducted to arrive at a population estimate that year. The estimates 2012-2018 resumed using SEM multiple mark-recapture, sampling the entire shoreline with electrofishing only.

LMB angling regulations were changed in 2006 to a two fish / 380 mm (15 inch) limit from five fish / 305 mm (12 inch) limit as a mitigation measure to limit harvest during the drawdown. Installation of 1,484 fishery habitat structures made of primarily citrus limbs were also placed in the lake from 2008 to 2016 as a mitigation measure for lost littoral habitat. These structures were placed into the drawn-down littoral zone to provide habitat for LMB and other sport-fish.

RESULTS

The 2005 pre-drawdown population estimate resulted in a SEM estimate of 21,726 LMB larger than 305 mm after extrapolation to shoreline area not sampled. The 2006 SEM analysis estimated a total of 13,472 LMB larger than 305 mm (62% of the 2005 reference population) and 5,802 LMB larger than 305 mm in 2008 (27% of the reference population) (Table 1). Catch and release tournament fishing was integrated into the sampling as a mark-recapture method in 2009. This additional sampling method accounted for 40% of the total fish collected that year and increased the estimate that year to 9,966 LMB. Tournament angling was again utilized as part of the 2010 mark-recapture efforts culminating in a population estimate of 5,642 LMB with tournaments accounting for only 20% of the total collected. The 2011 estimate relied upon a single mark-recapture Petersen method, resulting in an estimated 5,827 LMB larger than 305 mm. Estimates increased to 9,971 in 2012 and 11,169 in 2013, then decreased to 3,412 in 2015 and 3,631 in 2018 during drought years (Table 1).

TABLE 1.— Yearly catchable Largemouth Bass (*Micropterus salmoides*) population estimates, mark-recapture method, sampling methods and number of LMB collected from Lake Perris, CA 2005 -2018.

Year	Estimate	Method	Sampling Method	LMB collected
2005	21,726	SEM	Shoreline Transects	1,378
2006	13,472	SEM	Entire Shoreline	4,562
2007	9,596	SEM	Entire Shoreline	1,095
2008	5,802	SEM	Entire Shoreline	1,471
2009	9,966	SEM	Shoreline w/ tournaments	1,626
2010	5,642	SEM	Shoreline w/ tournaments	1,442
2011	5,827	PETERSON	Entire Shoreline	779
2012	9,791	SEM	Entire Shoreline	3,052
2013	11,169	SEM	Entire Shoreline	3,135
2014	7,518	SEM	Entire Shoreline	2,392
2015	3,412	SEM	Entire Shoreline	1,442
2016	4,678	SEM	Entire Shoreline	1,332
2017	5,447	SEM	Entire Shoreline	1,637
2018	3,631	SEM	Entire Shoreline	692

Lake Perris surface elevation was near full pool in 2005 averaging 483.4 m (1,586 ft; 96% capacity) until September when the mandated drawdown to 476.4 m (1,563 ft; 59% capacity) was initiated and was reached by November 2005 (Figure 1). Lake Perris elevation averaged 475.5 m (1,560 ft; 54% capacity) from 2006 to 2013. The 2014 statewide drought decreased the lake elevation to an average of 472.7 m (1,551ft; 42% capacity) from 2014-2017. Drought conditions ended in 2017, and water was available to refill the lake once the dam remediation project was completed in January 2018 (Figure 1).

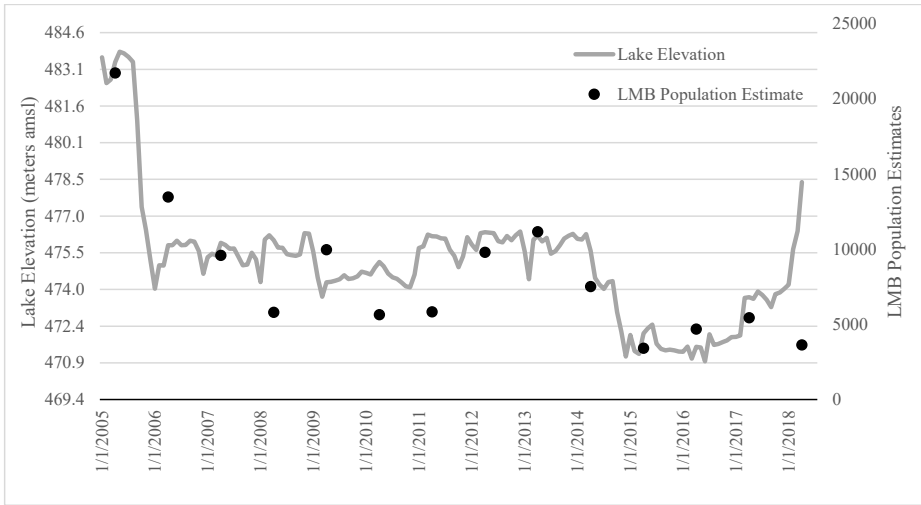


FIGURE 1.— Lake Perris Largemouth Bass (LMB; *Micropterus salmoides*) population estimates and lake elevation during a prolonged drawdown and drought 2005-2018.

DISCUSSION

Nationally there is a scarcity of quantitative data collected over many consecutive years on one waterbody which hinders testing of fisheries management hypothesis (Ploskey 1986). Because of this, effects of reservoir water level manipulation, as they relate to LMB recruited into the sport-fishery, are difficult to predict with any degree of confidence (Miranda et al. 1984, Ploskey 1986). Primarily due to the many biotic and abiotic variables that exist in reservoirs. Biotic factors such as spawning population size (Ricker 1954), suitable habitat availability (Durocher et al. 1984, Maceina and Bettoli 1998, Tate et al. 2003), body size (Aggus and Elliott 1975, Gutreuter and Anderson 1985, Miranda and Pugh 1997) and interspecific/intraspecific competition (Olson et al. 1995, Garvey et al. 2000) are known to affect recruitment. Abiotic factors, such as water level, are also important in understanding recruitment mechanisms of LMB, but the nature of its influence is not always obvious (Parkos and Wahl 2002). Reservoir hydrology plays a pivotal role in fish recruitment, as the amount of water in a reservoir regulates the potential for many underlying biotic variables to be exerted (Reinart et al. 1997, Sammons et al. 1999). This study documents 14 consecutive years of catchable LMB population estimates as they relate with drawn down water levels at Lake Perris, because the numbers and sizes of fish in a population determine the potential to provide benefits for recreational fisheries (Neumann et al. 2012).

Estimates of catchable LMB were chosen over monitoring age-0 or juvenile LMB, because of the recruitment variability that can occur with sport-fish to catchable size, even under normal hydrological conditions (Ploskey et al. 1996, Parkos and Wahl 2010). Ultimately, fisheries managers monitor species within a sport fishery by population size, angler catch rate, harvest rate, relative weights, and proportional size distribution (PSD) / relative size distribution (RSD) values of a fishery population. All these quantifiable attributes are taken from “adult” or “catchable” fish, not age-0, juveniles or sub-adults. Juvenile and sub-

adult LMB may not recruit into a sport fishery and reach a size of maturity to sustain the population or provide recreational benefits sought by anglers. Inability to recruit is due to the vast number of biological, limnological and environmental variables; which are regulated largely by hydrology, that can affect recruitment success of juvenile year classes (Gutreuter and Anderson 1985; Ploskey et al. 1996, Allen et al. 1999, Jackson and Noble 2000, Parkos and Wahl 2002, Parkos and Wahl 2010). The vast amount of uncertainty to whether juveniles or sub-adults will reach adulthood in a sport fishery prompted use of catchable population estimates to monitor the effect of the drawdown in Lake Perris.

The term “catchable” is often interchanged with the term “harvestable” because of Statewide or lake specific regulations used to manage sport-fish populations. Lake Perris regulations included a daily limit of five LMB larger than 305 mm (12 inches) per angler prior to the drawdown in 2005. Lake Perris regulations were temporarily changed to a daily limit of two LMB larger than 380 mm (15 inches) per angler for the duration of the drawdown. This management action was implemented to mitigate against a potential fishery collapse through unsustainable harvest. Though harvest rates of LMB at Lake Perris for the duration of the drawdown are unknown, angler survey data collected from nearby Diamond Valley Lake 2009-2017 and Lake Skinner 2014-2017 showed LMB harvest rates ranged from <1%-4% at both lakes (Q. Granfors unpublished data). All three lakes are frequented by the same pool of anglers targeting LMB, indicating predominately catch and release practices in the area. Another mitigation was the addition of brush pile structures to create habitat that was lost following the water reduction. These brush pile additions placed 2008-2014 consisted of 1,154 structures and were placed in the shallow littoral zone adjacent to areas where habitat was lost. However, drought in years 2014-2017 caused most of these shallow brush piles to be exposed to the air and thus unusable to fish. Therefore, 330 additional brush piles were placed 2015-2016 (22% of the total 1,484) in the drought induced littoral zone making them available to fish between 2015-2017. Despite the additional habitat, fishery habitat was very limited during these drought years. Similar to other studies that observed congregations of bass near brush habitat (Paxton and Stevenson 1979, Wege and Anderson 1979, Allen et al. 2014), many of the LMB collected by electrofishing 2015-2017 were concentrated near these brush piles. Electrofishing near many of the few remaining habitats in the water may have affected the population estimated in those years. Electrofishing around the limited areas where LMB congregated could have increased recapture rates of previously marked LMB relative to prior surveys where habitat areas were more numerous and spread out. This would result in more conservative estimates. However, electrofishing near areas where LMB congregate, also potentially increases sample size, which could result in a more thorough population estimate.

The initial 2005 population estimate ($N=21,726$) resulted from an extrapolated estimate derived from randomly sampling of 37% of the lake's shoreline over five sampling efforts. At that time, Lake Perris was near full pool, making sampling the entire shoreline impossible given budgetary and personnel constraints at the time. Transects sampled were chosen at random for each sampling effort, maximizing the assumptions for a closed population (Krebs 1999) given the entire lake was not sampled. Grinstead and Wright (1973) conducted a population estimate at Lake Eufaula Reservoir, Oklahoma, using the same transect extrapolation sampling methodology, and they found the estimates were biased toward underestimation. They based their conclusion on sampling bias that assumed all bass would be located near the shallow littoral zone where electrofishing occurs, and all sample

transects would be representative of the entire reservoir. Neither of these assumptions is accurate because bass populations are seldom evenly distributed due to environmental heterogeneity and behaviors regulated by competitive, predatory or reproductive actions (Miranda and Dibble 2002). Our 2005 estimate may also have underestimated the population size. However, underestimation is likely due to the same reasons identified in the Lake Eufaula study and not the sample size in general. The 2005 estimate collected, marked and examined 1,378 LMB over 305 mm (Table 1), indicating a sufficient number of LMB were collected if the population was near the assumed 20,000, based upon estimates conducted prior to 2005 (Robson and Reiger 1964, Krebs 1999). The 2006 estimate ($N=13,472$; Table 1) collected, marked and examined 4,562 LMB by sampling the entire shoreline. The 2006 sample size is exceedingly sufficient. Since the LMB population from 2007-2018 was likely never going to exceed the baseline 2005 population estimate of 21,726 fish at full pool or the 2006 estimate of 13,472 fish following initial drawdown, sample sizes of the remaining SEM multiple mark-recapture estimates should be considered adequate (Table 1). The much smaller sample size ($n=779$) of the 2011 single mark-recapture Petersen estimate ($N=5,827$) is well below all other sample sizes, however the estimate is similar to the preceding 2010 estimate ($N=5,642$).

All estimates from 2006-2013 during the drawdown ranged from 5,642-13,472 (26-62% of the reference estimate) and averaged 8,908 LMB which is 41% of the reference estimate. During the drought there was a reduced capacity to only 42% of the water volume, and this reduction was reflected in estimates that ranged from 3,412-7,518, which was 15-35% and averaged 4,937 LMB, 23% of the reference estimate. The 2018 estimate had the smallest sample size ($n=692$) of the study and second smallest estimate ($N=3,631$). I believe this may be due to the rapid refilling of the lake following several years of drought (Figure 2). The expansion of the lake would have resulted in the LMB population being more widely distributed and more difficult to sample.

The monitoring at Lake Perris between 2005-2018 has documented the response of the catchable LMB population during a drastic and prolonged drawdown at Lake Perris. Anticipated losses in the fishery resulting from the change in the lake were realized. Unanticipated drought further amplified reductions observed to the catchable LMB population. The severity of the drought eliminated nearly all existing fishery habitat throughout the lake, likely contributing to a reduction of the carrying capacity within the lake (Figure 2). The addition of mitigated fishery habitat appears to have concentrated LMB during drought years in areas where habitat was placed and sampled where water still covered this habitat during the years of drought. Many of the LMB collected during drought years were around the limited fishery habitat, indicating LMB preference for those areas. Carrying capacity is influenced by the most limiting resource, typically habitat or food. Had habitat mitigation efforts not been instituted, it is possible the reduction to the catchable LMB population brought on by the drawdown would have been greater. Estimated population size of catchable LMB appears to decline consistently with the declining water level and lake capacity (Figure 2). Typically, negative feedback on population density often involves time lags that lead to oscillations around carrying capacity (Miranda and Dibble 2002). However, these population estimates show little time lag effect upon the abundance of catchable LMB, as they relate to the water levels. Studies have shown LMB populations exist in dynamic equilibrium where density of bass populations change temporally but fluctuate near a recurring level in stable water level and habitat conditions (Inskip and Magnuson 1983, Maceina and

Bettoli 1998). Temporal changes include irregular fluctuations, increases and decreases over long periods and cyclic oscillations regulated by complex density-dependent and density-independent factors (Miranda and Dibble 2002). Reductions to the population in Lake Perris most likely manifested from dynamic equilibrium selective pressures affecting recruitment and carrying capacity over the study period through density-independent regulations (e.g. habitat & water volume loss) and density dependent regulations (e.g. competition for food) as the water receded.

Although sampling was conducted in a consistent manner, annual variability among population estimates as they relate to changing lake levels, may be partially due to sampling bias, habitat availability, fluctuations inherent in stable LMB populations, the inherent under-estimation in the statistical analysis used in estimating the population, or all of them in combination (Robson and Reiger 1964, Swingle et al. 1966, Grinstead and Wright 1973, Edwards et al. 1997, Miranda and Dibble 2002, Parkos and Wahl 2010, Michaletz and Siepker 2013). Therefore, even relatively minor lake level fluctuations of up to seven feet that occurred within the drawdown period likely influenced estimates obtained and LMB populations year to year. However, since the methods used, and sample sizes collected to monitor the LMB population in Lake Perris were consistent throughout, estimates likely represent the trend of abundance that occurred. Studies that have evaluated water level effect on the abundance of LMB populations have done so by examining abundance of age-0 and/or juveniles with uncertain assumptions of translation to the catchable population. This study has shown the estimated abundance of LMB as they relate to water level reductions where the fish collected have direct recreational value in the sport-fishery as catchable fish. Our results show an extreme and prolonged drawdown negatively affected the abundance of catchable LMB within Lake Perris, confirming the anticipated losses to the recreational fishery.

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Effects of managed flows on Chinook Salmon (*Oncorhynchus tshawytscha*) in relation to run-timing, fertility, and fluctuations in water temperature and flow volume

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We evaluated annual and seasonal patterns of run-timing in two genetically differentiated races of adult Chinook Salmon (*Oncorhynchus tshawytscha*) inhabiting the upper Trinity River, California. Our analysis provides evidence that highly managed flow regimes implemented since 2003 have 1) altered the pattern of run-timing in sympatric anadromous populations of spring- and fall-run Chinook Salmon, 2) resulted in significant differences among managed flow-types in relation to the taxon-specific “historical” post-dam baseline flow-type, 3) altered environmental measures of water temperature and flow volume, and 4) potentially affected hatchery-parental broodstock female average annual percent fertility associated with egg production. Additionally, counts of coded wire tagged spring- and fall-run adult Chinook Salmon were significantly correlated with total hatchery returns of all age classes of marked and unmarked fish, and all three groups of salmon exhibited a significant and negative decline in relative abundance since peaking in 2004. Trends in declining stocks of all Chinook Salmon coincided with establishment of the Trinity River Restoration Program in 2002 and subsequent highly managed flow releases including periodic pulsed augmentation flows beginning in 2003. Deviation away from the baseline flow pattern in run-timing occurred in spring-run fish by compression and movement of peak counts to earlier in the season; whereas in fall-run fish peak counts occurred earlier and later in the season relative to the baseline condition. Further, we show significant differences between the baseline flow pattern and managed flow hydrographs in both annual and seasonal measures of average daily water temperature, extremes in average daily water temperature, and average daily flow volume. Analyses of annual trends in hatchery records using generalized additive modeling also revealed a significant negative relationship between year and

average annual percent fertility of hatchery raised parental broodstock associated with hatchery egg-take in both spring- and fall-run Chinook Salmon. Declining concordant trends in fertility suggest that these seasonally disjunct and genetically differentiated races of Chinook Salmon are tracking conditions in the upper Trinity River in parallel, which may in part be a function of the potentially negative consequences of altered flow on riverine ecosystem processes and the fisheries resources they support.

Key words: coded wire tag, fall-run Chinook Salmon, fertility, hatchery broodstock, managed flows, pulse flows, spring-run Chinook Salmon, Trinity River

The Klamath River basin in Northern California has distinct populations of anadromous semelparous Chinook Salmon (*Oncorhynchus tshawytscha*). Trinity River is located in northwestern California and is the largest tributary in the Klamath River basin (Figure 1). Klamath River fall-run and Trinity River fall-run Chinook Salmon are allopatric populations, as both runs occur as adults in the Lower Klamath River and estuary during late summer and early fall. These populations separate geographically during migration upstream from the confluence of the Trinity River at the township of Weitchpec. Trinity River also has a sympatric population of spring-run Chinook Salmon that begins annual upstream migration as early as May. Separation of spring-run from fall-run Chinook Salmon in the Trinity River occurs through migration behavior and run-timing to the extent that these two runs appear to constitute separate genetic “races” (Kinziger et al. 2013). Historically, the size of the fall-run is typically much larger than the spring-run of Chinook Salmon. Artificial propagation programs in the upper Trinity River began in 1960 at the Lewiston Fish Trapping Facility, prior to completion of Lewiston Dam and Trinity River Hatchery (TRH) in 1963 (Murray 1962). Trinity River Hatchery has an operational egg-take allotment for spring-run Chinook of 3,000,000 eggs with a production goal of releasing 1.4 million (1,000,000 fingerlings and 400,000 yearlings); for fall-run Chinook the operational egg-take allotment is 6,000,000 (2,000,000 fingerlings and 800,000 yearlings; L. Glenn, personal communication 2016).

The Trinity River Restoration Program (TRRP), created by the Record of Decision, henceforth called “ROD” (USBR 2000), outlined a plan for restoration of 63.1 km (mainstem) of the upper Trinity River and its fish and wildlife populations (TRFES 1999). The Trinity River Mainstem Fishery Restoration Environmental Impact Statement was the basis for the ROD. The TRRP strategy for restoration included 1) flow management through manipulation of the annual hydrograph, 2) mechanical channel rehabilitation, 3) sediment management, 4) watershed restoration, 5) infrastructure improvements, 6) adaptive environmental assessment and monitoring, and 7) environmental compliance and mitigation. Timing, extent, volume of restoration flows, and annual water-year designations from 1995 through 2016 appear in Table 1. Information on the intended benefit of each restoration and pulsed flow augmentation hydrograph varies on an annual basis depending upon water availability and the particular restoration objective at the time of implementation (TRRP 2019). The primary objective of flow management on the upper Trinity River was to clean spawning gravels, build gravel bars, scour sand out of pools, provide adequate temperature and habitat conditions for fish and wildlife at different life stages, control riparian vegetation, and perform other ecological functions (TRRP 2019).

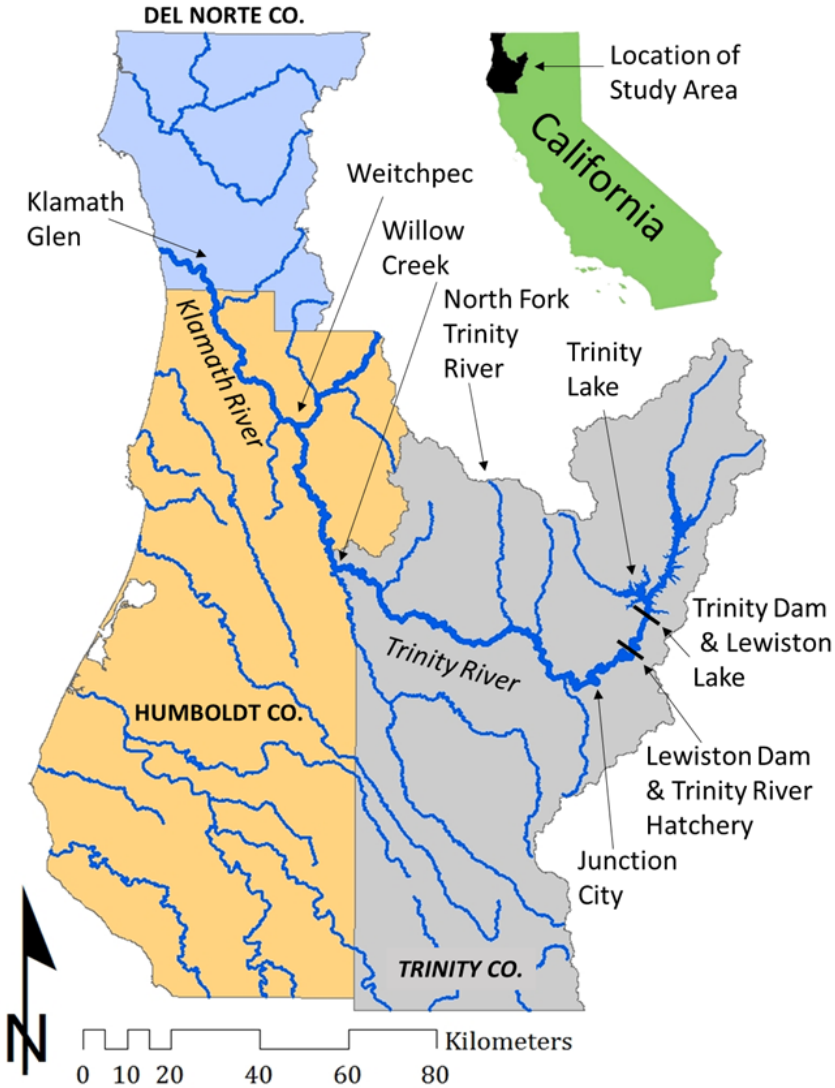


FIGURE 1.—Map showing counties, named tributaries (≥ 34 km in length), major towns, and facilities mentioned in the text along corridors of both the Klamath River and Trinity River.

In 2003 the TRRP began implementing restoration flows, henceforth called “ROD flows” to accomplish their stated restoration strategy (Magneson 2013). In conjunction with annual ROD flows, the TRRP and United States Bureau of Reclamation (USBR) implemented additional late-summer pulsed augmentation flows, henceforth called “pulse flows” in 2003, 2004, and 2012 through 2016. In regulated rivers pulse flows are often referred to as artificial freshets (Hasler et al. 2014) used to encourage fish holding in estuaries to move into the mainstem segment of the river or aid upstream movements through fish-ways (Peterson et al. 2017). However, pulse flows in the Trinity River have historically been implemented

TABLE 1.—Attributes of ascending and descending limbs of hydrographs that characterized baseline PreROD, ROD, and Pulse flows for years 1995 to 2017. Rate of flow measured in cubic meters per second (m³/s) and flow release in hectare meters. For each hydrograph, a bench indicated a temporary holding steady of flow release volume and flattening of the hydrograph for at least one day. Rapidness indicated a steep and immediate increase or decrease in rate of flow, relative to a more prolonged or gradual increase or decrease in rate of flow. Abbreviations: NA = no data, shape of the ascending and descending limbs of the hydrographs: R = rapid, G = gradual, B = number of benches, and 2P = double peak. Digital data to verify online printed hydrographs for 1994 and 2001 were not available through the California Department of Water Resources, California Data Exchange Center website for the time-period encompassing by all 3-flow-types.

Year	Water year- type	Low release magnitude (m ³ /s)	Shape as- cending limb	Peak release magnitude (m ³ /s)	Restoration release (hect- are m)	Shape descending limb	Low release magnitude (m ³ /s)	Date and duration to base-flow	Total days
PreROD Flow (1994 - 2002)									
1995	NA	14	R	131	NA	G	27	25 Apr-22 May	28
1996	NA	9	R	144	NA	G, 1B	14	10 May-9 Jun	31
1997	NA	10	R	62	NA	G, 3B	13	2 May-2 Jul	62
1998	NA	47	R	192	NA	G	13	24 May-27 Jul	65
1999	NA	15	G	71	NA	G	13	8 May-18 Jul	72
2000	NA	9	R	66	NA	G	13	8 May-27 Jul	81
2002	normal	9	R	171	59,540	G	13	27 Apr-25 Jun	28
ROD Flow (2005 - 2011, 2017)									
2005	wet	8	R, 2B	197	79,880	G, 1B	13	27 Apr-22 Jul	87
2006	extra wet	8	G, 5B	286	99,900	G, 2B	13	16 Apr-22 Jul	98
2007	dry	8	R	135	55,963	G	13	25 Apr-25 Jun	62
2008	dry	9	R, 1B	183	80,016	G, 3B	20	22 Apr-15 Jul	85
2009	dry	8	R	125	54,952	G, 4B	12	24 Apr-6 Jul	74
2010	wet	9	R	194	81,003	G, 3B	12	22 Apr-2 Aug	102
2011	wet	7	R, 2B	329	89,033	G, 2B	13	26 Apr-1 Aug	98
2017	extra wet	9	R, 2P	326	101,536	G	13	22 Apr-11 Aug	112

TABLE 1.—Continued.

Year	Water year- type	Low release magnitude (m ³ /s)	Shape as- cending limb	Peak release magnitude (m ³ /s)	Restoration release (hect- are m)	Shape descending limb	Low release magnitude (m ³ /s)	Date and duration to base-flow	Total days
Pulse Flow (ROD-segment + pulsed augmentation flow; 2003, 2004, 2012 - 2016)									
2003	wet	9 + 13	R + R	73.9 + 51	55,272 + 4,194	G, 2B + G	12 + 13	29 Apr-22 Jul + 23 Aug-18 Sep	85 + 27
2004	wet	9 + 16	R + R	175.6 + 485	80,300 + 4,465	G, 4B + G	12 + 14	4 May-22 Jul + 21 Aug-14 Sep	80 + 25
2012	normal	9 + 13	R, 2B + R, 1B	172.2 + 39	79,817 + 4,811	G, 4B + R, 1B	13 + 13	4 Apr-26 Jul + 12 Aug- 20 Sep	114 + 40
2013	dry	8 + 13	R, 1B + R	125.2 + 74	55,741 + 2,294	G, 2B + R, 1B	13 + 13	13 Apr-25 Jun + 24 Aug-20 Sep	74 + 28
2014	critically dry	9 + 12	R, 1B + R, 1B	96.6 + 97	45,701 + 7,993	G, 1B + R, 1B	13 + 13	21 Apr-26 Jun + 15-25 Sep	67 + 11
2015	dry	9 + 19	R + R, 1B	240.8 + 83	55,593 + 5,908	G, 3B + R 1B	13 + 13	21 Apr-1 Jul + 20 Aug- 21 Sep	72 + 31
2016	wet	9 + 14	R, 2P + R, 1B	283.3 + 35	87,429 + 4,835	G + R, 2B	13 + 13	20 Apr-2 Aug + 24 Aug-28 Sep	105 + 36

based on the expectation of 1) large annual runs of fall-run Chinook Salmon into the lower and upper Klamath River basins, or 2) drier than normal hydrological conditions determined by water-year designations (Table 1). A third justification for this action was to specifically reduce the potential threat of a reoccurrence of the large pathogen-related fish kill that occurred in 2002 (Magneson and Chamberlain 2015, USBR 2015), although there have been no quantitative studies post-2003 to evaluate whether pulse flows have actually prevented another fish die-off in the lower Klamath River. Further, no study has demonstrated a clear relationship between pulse flows and fish movement (Thorstad and Heggberget 1998, Thorstad et al. 2003, Hasler et al. 2014, Peterson et al. 2017).

Recent information suggests that velocities and higher turnover rates of water associated with the magnitude and duration of additional water provided by pulse flows are more important than quality of additional cold water from the Trinity River intended to stimulate fish to move for prevention of disease in the lower Klamath River (Strange 2010, Peterson et al. 2017). Additionally, within the context of the National Environmental Protection Act, numerous assessments have determined no significant impact to populations of salmonids in the Trinity River from implementation of either ROD or Pulse flows, or a combination of both (USBR 2015, USBR 2016, and references therein). Importantly, however, there has been no assessment of the potential effects of intensely managed flow regimes on the pattern of seasonal run-timing or impacts to female reproductive performance, relative to the post-dam baseline flow pattern (henceforth called “PreROD flows”), for any population of adult anadromous species of salmonid in the Trinity River.

Thus, our specific objectives were fourfold. First, we compared annual counts of known-race coded wire tagged and adipose fin clipped (“marked”) hatchery recoveries of adult Chinook Salmon (henceforth called “CWT”), to expanded estimates of CWT adult fish, and all Chinook Salmon that return to the hatchery. This category includes all CWT adult marked and all age classes of marked and un-marked fish (henceforth called “total TRH returns”). Second, we assessed the annual distribution and relative abundance of both races of CWT adult Chinook Salmon and evaluated seasonal patterns in run-timing to identify any concordant patterns common to both races. Third, we tested three research hypotheses of significant differences among designated flow-types in relation to 1) annual and seasonal counts of Chinook Salmon, 2) environmental variables reflecting fluctuations in water temperature and flow volume, and 3) average annual percent fertility determined at the hatchery ($AAPF = ([\text{total eggs taken} + \text{total eggs culled}] / \text{total eggs after pick})$):

H_1 : Annually managed flow regimes (hydrographs) implemented by the ROD have altered the run-timing of spring- and fall-run Chinook Salmon in the upper Trinity River.

H_2 : Annually managed flow regimes implemented by the ROD differ significantly among flow-types and have altered 1) average daily water temperature, 2) extremes in average daily water temperature, and 3) average daily flow volume in the upper Trinity River.

H_3 : Annually managed flow regimes implemented by the ROD have altered hatchery-parental broodstock female AAPF associated with egg production, which includes both hatchery-origin and potentially an unknown number of “wild” natural-origin fish mixed in with the hatchery egg collection.

Our fourth objective was to assess the potential impact of these extrinsic water temperature and flow volume variables on AAPF of hatchery parental broodstock. Importantly, we maintain that AAPF constitutes a “baseline” for performance-based comparisons, developed under controlled hatchery conditions, with in-river spawning of hatchery- and natural-origin stocks of both spring- and fall-run Chinook Salmon inhabiting the upper Trinity River.

MATERIALS AND METHODS

Study area.—Trinity River is located in northwestern California and is the largest tributary of the Klamath River (Figure 1). Construction of Trinity and Lewiston dams occurred in the early 1960s. Trinity Dam creates Trinity Lake (NAD 83, Zone 10N, UTM 519,964.7 m east and 4,516,719.7 m north), storing up to 3,022 m³ of water (USFWS and HVT 1999). Lewiston Lake, formed by Lewiston Dam, is located 11.8 km downstream of Trinity Dam (river kilometer [rkm] 180; UTM 517,489.4 m east and 4,508,408.4 m north), which serves as a re-regulating reservoir for flow into the Trinity River and diversion into the Sacramento River Basin, comprising the Trinity River Division of the Central Valley Project. Lewiston Dam is the uppermost limit of anadromous fisheries on the Trinity River. From Lewiston Dam, the Trinity River flows approximately 180 kilometers before joining the Klamath River at the township of Weitchpec (UTM 440,575.2 m east and 4,559,590.2 m north). The Klamath River flows for an additional 70 rkm before entering the Pacific Ocean west of Klamath Glen. The upper Trinity River is the stretch from the confluence of the North Fork Trinity River to 63.1 km up stream to Lewiston Dam. Trinity River Hatchery is located immediately below Lewiston Dam; it releases approximately 4.3 million Chinook Salmon, 300,000 Coho Salmon, (*Oncorhynchus kisutch*), and 448,000 juvenile anadromous Rainbow Trout (*Oncorhynchus mykiss*) (henceforth called “steelhead”) into the Trinity River annually. Data presented herein, derive from counts of Chinook Salmon obtained at the hatchery as part of annual single mark recapture estimates conducted for both races of Chinook Salmon.

Flow year-types.—We used our designated flow-types to test our research hypotheses 1) baseline post-dam PreROD flows (1982 - 2002), 2) ROD flows (2005 - 2011, 2017), and 3) Pulse flows (2003, 2004, 2012 - 2016). Late summer pulsed flows were intended to cue up-river seasonal migration of Chinook Salmon out of the lower Klamath River to reduce risk of the epizootic of the ciliate parasite *Ichthyophthirius multifiliis*. Prior to 2003, there were no annually managed ROD or Pulse flows. Importantly, we note that each Pulse flow event was accompanied by a single ROD flow hydrograph (ROD flow plus Pulse flow), beginning in 2003. Thus, for each Pulse flow, effects of each pulsed augmentation are not completely separable or independent from effects of its companion ROD flow.

Since 2001, total restoration releases have included flows for 1) restoration, 2) Tribal Ceremonial Boat Dances, and 3) late summer pulse flows (Table 1). Ceremonial Tribal Boat Dance flows occur in odd years in ROD flows and just prior to any pulsed flow augmentation in Pulse flow years (Figure 2A). They are evident in each hydrograph, amount to $\leq 0.6\%$ of the total release into the Trinity River (TRRP 2019) and are included herein as Pulse flows tier off the trailing ends of Ceremonial Boat Dance flow hydrographs when they occur. Shapes of the ascending limbs of ROD flow hydrographs were mostly rapid (19/15) with few years in which there were benches (7/15), all of which were associated with managed flows (Table 1). In contrast, shapes of the descending limbs of ROD flow hydrographs were generally gradual with numerous “benches” associated with virtually all managed flows (14/15). We designated benches in these hydrographs as indicating stabilization of water release for one or more consecutive days. There were two double peaked ROD flows (2016 and 2017; Table 1). All Pulse flows had rapid ascending hydrographs and at least one bench. Similarly, all descending limbs of Pulse flows were rapid with at least one bench. Spring and summer base flow releases historically equate to 13 m³/second.

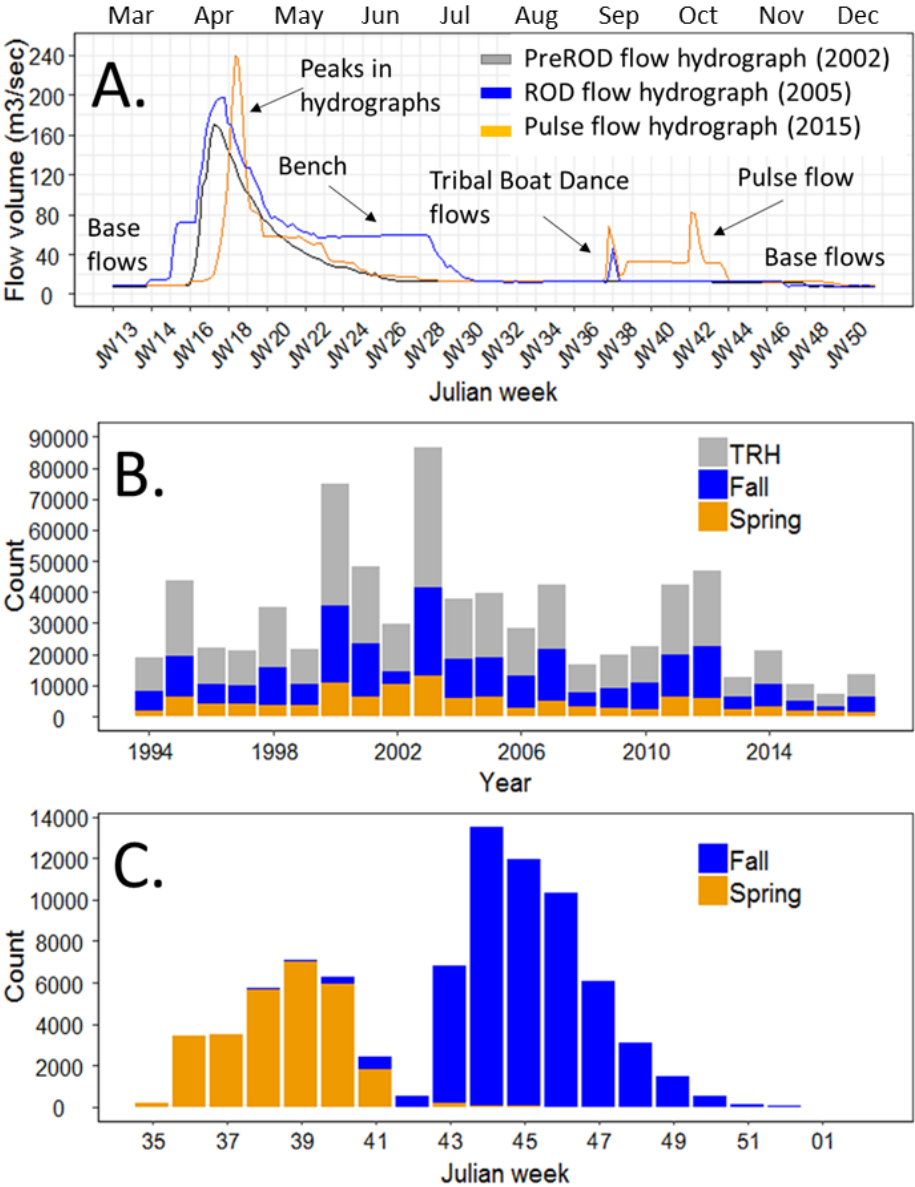


FIGURE 2.—Line and bar graphs of A) examples of PreROD, ROD, and Pulse flow-type hydrographs showing characteristics described in Table 1. Also shown is the approximate timing of Julian weeks (JW) in relation to months. B) Annual fluctuations in total Trinity River Hatchery returns of all age classes of marked (CWT) and un-marked Chinook Salmon ($n = 401,667$), and expanded estimates of CWT adult spring-run ($n = 114,720$) and fall-run Chinook Salmon ($n = 246,813$). C) Seasonal fluctuations in Julian week hard-counts of both races of CWT adult Chinook Salmon.

ROD flows generally occurred annually from late April to August, whereas conjoining Pulse flows mostly occurred from August to September. Actual timing, magnitude, and duration of ROD and Pulse flows varied in hydrologic characteristics, cubic meters per second (m^3/s), and shape and duration of the hydrograph annually depending upon the specific intent of the management action (Table 1). Average duration of ROD flows approximated 89.8 days (range 62.0 - 112.0 days) from mid-April to early August and averaged approximately 221.9 m^3/s (range 124.9 - 328.6 m^3/s) of flow at the top end of the hydrograph. Average duration of Pulse flows approximated 28.3 days (range 11.0 - 40.0 days) from mid-August to late September, and averaged approximately 61.1 m^3/s (range 35.3 - 97.0 m^3/s) of flow at the top end of the hydrograph. For the same general monthly period, average duration of baseline PreROD flows approximated 52.4 days (range 28.0 - 81.0 days) from late April to late July, and averaged approximately 119.6 m^3/s (range 62.3 - 192.3 m^3/s) of flow at the top of the hydrograph. Water summary data and typical flow release diagrams (hydrographs) teared to water-year type are available at the TRRP website (TRRP 2019, <http://www.trrp.net>).

Study design.—We compared counts of total TRH returns to known age and race CWT spring- and fall-run adult Chinook Salmon recovered from the hatchery. We “expanded” the counts of these CWT adult Chinook Salmon by standardizing, using specific CWT release group multipliers (expansion coefficients). This process generated expansion estimates for total TRH returns based on ratios of the total number of individual fish released for each release group for both spring- and fall-run adult CWT adult Chinook Salmon, divided by the total number of CWT adult fish released for each CWT value. Telemetered digital flow volume (m^3/s) and water temperature data (degrees centigrade [$^{\circ}\text{C}$]) were derived from the US Geological Survey (USGS) and the USBR Lewiston Water Quality Gauge (LWS), upper Trinity River at river-km 178.2 (UTM 516,634 m E and 4,507,678 m N, elevation 558 m), 1.7 rkm downriver from the Lewiston Dam and the hatchery. We downloaded digital data on average daily water temperature (ADWT) and average daily flow volume (ADFV) from the California Department of Water Resources, California Data Exchange Center (DWR 2018).

We used the LWS gauge data for several specific reasons. First, water temperature in the hatchery main intake is monitored daily and water temperature throughout the facility to the river effluent has historically been within 1 degree (D. Muir, personal communication). Thus, we assumed that thermal regimes in the hatchery closely mimic immediate in-river water temperatures, as both derive from water sources immediately out of Lewiston Lake. Second, this gauge is the “standard” used in all National Environmental Protection Act assessments and flow augmentations analyses of in-river average water temperature, specific to the upper Trinity River out of Lewiston Dam since 1997 (Zedonis 2009 and included references). Second, this gauge provides the best, most consistent, and detailed long-term digital information reflecting water temperature conditions used during each hatchery phase of egg production, juvenile grow-out, and release of all hatchery-produced Chinook Salmon assessed herein. Third, virtually all hatchery raised Chinook Salmon spawn within the first 3.2 km below the Lewiston Dam and hatchery (Rupert et al. 2016). Fourth, this section of the upper Trinity River consistently contains the largest densities of hatchery-origin Chinook Salmon spawning redds (Rupert et al. 2017a and 2017b). Thus, this gauge provides the best location for measuring water temperature conditions nearest to the hatchery for in-river spawning of hatchery-origin Chinook Salmon, and it is highly likely that other natural-origin co-occurring anadromous species of salmonids also spawn in this segment of the reach. Although punctuated up-and-down measurements using average daily water temperature may

approximate a bi-monthly framework for viewing average water temperature in the upper Trinity River, this metric does not identify or illustrate extremes in water temperature. Instead, we used the range of extreme variation in average daily water temperatures (minimum to maximum) to address the need for an index of water temperature variability (ADWTVI).

Statistical analyses.—A Shapiro-Wilk test of the hypothesis that annual counts of total TRH returns, and spring- and fall-run CWT adult Chinook Salmon derive from a normally distributed population was rejected for each group of fish (spring-run: $W = 0.85$, $P < 0.01$, $n = 24$; fall-run: $W = 0.85$, $P < 0.01$, $n = 24$; total TRH returns: $W = 0.88$, $P = 0.01$, $n = 24$). In all three groups of fish, counts were skewed significantly to the right, consistent with a Poisson distribution. Thus, all subsequent non-regression statistical analyses of count data used non-parametric methods (McDonald 2014). Spearman's rank correlation (r_s) was used to calculate strength and direction of the relationship between two variables, expressed as a monotonic relationship, whether linear or not (Corder and Foreman 2014). Although we provide and analyze expanded estimates of the relative abundance of fish based on CWT adult Chinook, we used non-expanded CWT adult fish for all follow-on statistical analyses and comparisons because these data are not estimates but hard-counts (Kilduff et al. 2015).

We analyzed trends in seasonal count data by use of Julian weeks (JW), defined as one of seven consecutive-day-sets of 52 weekly periods in a calendar year, beginning 01 January of each year. This procedure allowed inter-annual comparisons of identical weekly periods. Extra day in leap years was included in the ninth week. Wilcoxon signed-rank test computed from two-sided probabilities using approximate normal variates (Z) evaluated the hypothesis that the median difference between pairs of Julian weeks was zero among different flow-types for each race of Chinook Salmon (Hasler et al. 2014, McDonald 2014). To determine if timing of seasonal migration in ROD and Pulse flows deviated from the baseline PreROD flow condition, we calculated a Percent Deviation Index (PDI) from total hard-counts:

$$\begin{aligned} \text{PDI for ROD flows} &= \% \text{ROD flow count} - \% \text{PreROD flow count} \\ \text{PDI for Pulse flows} &= \% \text{Pulse flow count} - \% \text{PreROD flow count} \end{aligned}$$

To evaluate the specific pattern in timing of migration, we tested the hypothesis (H_1) that counts of spring- and fall-run Chinook Salmon identified during individual Julian weeks were significantly different between flow-types. We attempted to standardize sampling effort by including in our analysis only those pairwise comparisons that had a sample size ≥ 5 for each flow-type. Pairwise comparisons of non-zero counts using Julian weeks as attributes were then evaluated by use of the Dwass-Steel-Chritchlow-Fligner (*DSCF*) 2-tailed test (Critchlow and Fligner 1991) combined with the Holm adjustment for unplanned multiple comparisons (Holm 1979). We assessed annual trends in continuously distributed linear measures of water temperature and flow volume from 1995 through 2018, and seasonally by use of Julian weeks specifically from the flow schedule implemented from previous managed hydrographs (Table 1, JW13 - JW40).

We generated regression models for total TRH returns and counts of CWT adult Chinook Salmon by use of Generalized Additive Models (GAMs, R Core Team 2019, Wood 2017). GAMs are a semi-parametric extension of Generalized Linear Models (GLM) that are less restrictive in assumptions about the underlying distribution of data; thus providing an effective technique for assessing non-linear relationships between response and explana-

tory variables (Madsen and Thyregod 2011). Instead of estimating single parameters as in a GLM, a general unspecific function relates predicted transformed y -values to predictor values (x -values). GAM models assume the dependent variable is dependent on the univariate smooth term (function) of the independent variable, rather than the independent variable itself (Hastie and Tibshirani 1990). We used default settings degrees of freedom in our GAM-based analyses of counts and a thin plate-regression spline base-function as our smoothing technique with 10 degrees of freedom (k). This method generally gives 1) the best mean square error performance and optimal smoother of any given basis dimension; and 2) it is advantageous because it is flexible and avoids the need to make prior assumptions about the shape of the function (Schluter 1988, Wood 2003, Wood 2017).

Response curves generated from each GAM show the relationship between the fitted function to the response scaled to zero. Y -axes based on partial residuals indicate the relative influence of each predictor on the prediction on the base of partial residuals. Smooths were “centered” to ensure model identity and summed to zero over covariate values. Statistics reported from each GAM were 1) χ^2 - or F -statistic (approximate significance of smooth terms) including P -value and 95% confidence band for the spline line (Nychka 1988); 2) adjusted regression coefficient for the model (R^2 adj.), 3) estimated residual degrees of freedom (*Ref. d.f.*), and 4) proportion of null deviance explained (*Dev.Exp.*). We used Spearman’s rank correlation coefficient as a follow-on procedure to assess strength and significance of trends in counts delineated by smooth terms, because GAMs lack a statistical inference procedure and formal parameter of goodness of the fit, which makes interpretation of output potentially complicated (Diankha and Thiaw 2016). Because our count data were over-dispersed, we used the negative binomial error-structure (family = “nb” [link = “log”]) in construction of GAM models to establish the relationship between response variables and smoothed functions of predictor variables (Peterson et al. 2017, Wood 2017). In contrast, we used the gamma error-structure (family = “Gamma” [link = “log”]) to assess annual and seasonal (JW) fluctuations in ADWT, ADWTVI, and ADFV (Appendix I) and their mean values when compared to AAPF (Appendix II), as visual inspection of Q-Q plots of standardized residuals showed that all variables were only near normal in their distributions. Thus, we used non-parametric statistics to assess relationships among environmental variables, flow-types, and AAPF for each genetic race of Chinook Salmon. We evaluated the species-specific relationships between AAPF (response variable) and explanatory extrinsic water temperature and flow volume attributes individually and in paired combinations. We used the Akaike information criterion modified for overdispersed count data adjusted for small sample uncertainty ($QAIC_c$) to select the most parsimonious GAM models for comparisons between AAPF and various individual and combined water temperature and flow volume effects (Akaike 1973, Burnham and Anderson 1998).

We assumed that the highest-ranking model was the most parsimonious given the limitations of our data, and subsequent models were ranked in relation to the most parsimonious model. Fourteen water temperature and flow volume models resulted from our investigation of the relationship between AAPF and environmental attributes (Table 2). This analysis allowed identification of potentially useful models, variables, and variable combinations potentially affecting AAPF in hatchery- and natural-origin stocks of both races of Chinook Salmon. We used autocorrelation analysis of residuals derived from GAM analyses of annual and seasonal Julian week counts to investigate the relationship of each time point to each previous time point in the distribution of annually and seasonally consecutive counts

TABLE 2.—Summary of approximate significance of smooth terms and statistics derived from generalized additive model (GAM) regressions of total Trinity River Hatchery returns and coded wire tagged (CWT) annual and Julian week (JW) counts of adult Chinook Salmon in relation to 1) average annual percent fertility (AAPF), 2) average daily water temperature (ADWT), 3) average daily water temperature variability index (ADWTVI), and 4) average daily flow volume (ADFV). For the GAM regression (χ^2) “nb” = a negative binomial error structure and GAM regression (F) “Gamma” = a gamma error structure. The Akaike information criterion modified for overdispersed count data adjusted for small sample uncertainty ($QAIC_c$) was used to select the most parsimonious GAM models.

GAM (family = “nb”)	GAM χ^2	Ref.d.f.	P-value	n	R^2 (adj.)	Dev.Exp.	$QAIC_c$
<u>Counts vs years (1994 - 2017)</u>							
Total TRH returns ~ year	18.9	3.3	< 0.01	24	0.27	46.1%	487.2
Spring-run ~ year	16.6	3.3	< 0.01	24	0.26	44.1%	367.3
Fall-run ~ year	16.3	3.6	< 0.01	24	0.25	44.3%	404.3
<u>Counts vs years (2003 - 2017)</u>							
Total TRH returns ~ year (2003 - 2017)	16.1	1.0	< 0.01	15	0.50	53.4%	302.9
Spring-run ~ year (2003 - 2017)	32.1	4.3	< 0.01	15	0.71	77.3%	234.7
Fall-run ~ year (2003 - 2017)	12.6	1.0	< 0.01	15	0.47	46.6%	253.8
<u>Counts vs Julian weeks (JW35 - JW02)</u>							
Spring-run ~ JW	67.9	4.2	< 0.01	12	0.85	82.3%	214.4
Fall-run ~ JW	664.2	5.7	< 0.01	19	0.93	98.0%	264.4
GAM (family = “Gamma”)	GAM F	Ref.d.f.	P-value	n	R^2 (adj.)	Dev.Exp.	$QAIC_c$
<u>AAPF vs year (1994 - 2014)</u>							
Spring-run AAPF ~ year	6.7	3.8	< 0.01	20	0.51	60.9%	130.1
Fall-run AAPF ~ year	5.2	3.0	< 0.01	19	0.46	50.6%	129.6
<u>Environmental variables vs year</u>							
ADWT ~ year	162.2	4.0	< 0.01	4,347	0.13	12.7%	12,040.7
ADWTVI ~ year	226.8	4.0	< 0.01	4,347	0.16	15.0%	8100.4
ADFV ~ year	10.8	4.0	< 0.01	4,458	0.01	1.6%	38838.5
<u>Environmental variables vs Julian week</u>							
ADWT ~ JW	413.2	4.0	< 0.01	4,347	0.28	28.1%	11228.7
ADWTVI ~ JW	274.3	4.0	< 0.01	4,347	0.16	18.1%	7935.7
ADFV ~ JW	442.0	4.0	< 0.01	4,458	0.38	47.7%	35846.1
<u>Between environmental variables</u>							
ADWT ~ ADWTVI	444.6	4.0	< 0.01	4,347	0.29	29.3%	11158.0
ADWT ~ ADFV	536.7	4.0	< 0.01	4,315	0.30	32.6%	10846.1
ADWTVI ~ ADFV	842.7	4.0	< 0.01	4,315	0.31	40.2%	6529.3

TABLE 2.—Continued.

	<u>Run-specific AAPF vs each environmental variable</u>						
Spring-run AAPF ~ ADWT	11.8	3.9	< 0.01	20	0.64	74.6%	122.9
Spring-run AAPF ~ ADWTVI	8.1	2.4	< 0.01	20	0.50	53.9%	127.1
Spring-run AAPF ~ ADFV	0.5	1.0	= 0.48	20	0.02	2.6%	137.9
Fall-run AAPF ~ ADWT	13.7	3.9	< 0.01	19	0.68	79.0%	119.7
Fall-run AAPF ~ ADWTVI	7.6	1.6	< 0.01	19	0.35	37.3%	129.3
Fall-run AAPF ~ ADFV	0.2	1.0	= 0.63	19	0.78	1.2%	136.4
	<u>Run-specific AAPF vs combinations of environmental variables</u>						
	<u>Spring-run AAPF ~ ADWT + ADWTVI</u>						
ADWT	3.6	7.1	< 0.01	20	0.74	84.5%	124.6
ADWTVI	2.0	3.4	= 0.05	20			
	<u>Spring-run AAPF ~ ADWT + ADFV</u>						
ADWT	11.4	3.9	< 0.01	20	0.62	75.1%	127.9
ADFV	0.1	1.0	= 0.73	20			
	<u>Spring-run AAPF ~ ADWTVI + ADFV</u>						
ADWTVI	7.2	2.4	< 0.01	20	0.47	54.2%	130.9
ADFV	0.0	1.0	= 0.98	20			
	<u>Spring-run AAPF ~ WT + ADWTVI + ADFV</u>						
ADWT	6.8	3.8	< 0.00	20	0.72	83.9%	130
ADWTVI	3.7	2.2	=0.05	20			
ADFV	0.4	1.0	=0.56	20			
	<u>Fall-run AAPF ~ ADWT + ADWTVI</u>						
ADWT	9.8	3.8	< 0.01	19	0.74	83.8%	118.9
ADWTVI	6.3	1.0	= 0.03	19			
	<u>Fall-run AAPF ~ ADWT + ADFV</u>						
ADWT	13.7	3.9	< 0.01	19	0.66	79.2%	125.2
ADFV	0.5	1.0	= 0.83	19			
	<u>Fall-run AAPF ~ ADWTVI + ADFV</u>						
ADWTVI	10.8	1.0	< 0.01	19	0.36	38.9%	131.4
ADFV	1.3	1.0	= 0.27	19			
	<u>Fall-run AAPF ~ ADWT + ADWTVI + ADFV</u>						
	10	3.8	< 0.00	19	0.76	85.70%	123.6
	8.0	1.0	< 0.01	19			
	1.7	1.0	= 0.22	19			

of total TRH returns and each race of Chinook Salmon (R Core Team 2019). A follow-on Box-Pierce (χ^2) test evaluated the extent of stationarity of the time series model (Box and Pierce 1970, Fuller 1976, Ljung and Box 1978). We accepted overall statistical significance at $P \leq 0.10$ because sample sizes were generally small, despite the risk of higher false signals (Rubin and Cole 2009, Noymer 2008, Bartley et al. 2012, McDonald 2014). Moreover, we wanted a more “relaxed” threshold to act as an early “warning signal” given the importance of assessing annual and seasonal trends that have immediate management and economic implications in reference to oceanic conditions, regional climate change, local drought, and flow manipulation (Good et al. 2005, Gallagher et al. 2012).

RESULTS

Fluctuations in annual counts and expanded estimates.—From 1994 to 2017, the hatchery recovered 27,821 (6.9%) spring-run and 55,196 (13.7%) fall-run CWT adult Chinook Salmon, which equates to 20.7% ($n = 401,667$) of all Chinook Salmon recovered from the hatchery (total TRH returns). Annual expanded estimates for CWT adult spring-run fish totaled 114,720 (28.56%) and 246,813 (61.5%) for fall-run fish. For both races of CWT adult fish, the combined expanded estimate represented 90.0% of all Chinook Salmon that returned to the hatchery between 1994 and 2017, which equates to an average multiplier of 4.35493. Prior to brood year 2000, the percent of marked pre-release juvenile Chinook Salmon averaged 13.5% (range 11.3% [1998] - 15.1% [1999]) of total TRH returns annually. However, beginning with brood year 2000 production (2001 release groups), the hatchery implemented an annual 25.0% “constant fractional marking rate” of pre-release juveniles. Nevertheless, the full effect of 25.0% constant fractional marking at the hatchery did not occur until 2005, with the parental brood year 2000 age 5 cohort.

Analysis of counts of Chinook Salmon returning to the hatchery and counts of spring-run and fall-run CWT adult fish fluctuated considerably on an annual basis (Figure 2B). Peak counts in total TRH returns occurred in 2003, 2000, 2001, and 2012. Whereas the smallest number of Chinook Salmon occurred in 2016, 2015, 2013, and 2017. From 1994 to 2017, 50.0% ($n = 24$) of the years had counts smaller than recorded during the massive fish die-off that occurred in the lower Klamath River in 2002 (CDFG 2004). Follow-on rank correlations between total TRH returns and expanded estimates of CWT adult fish were significant and positive for both races of Chinook Salmon (spring-run: $r_s = 0.90$, $P < 0.01$, $n = 24$; fall-run: $r_s = 0.92$, $P < 0.01$, $n = 24$), as well as the combined expanded estimate using both races of CWT adult Chinook Salmon simultaneously ($r_s = 0.99$, $P \leq 0.01$, $n = 24$). Similarly, the relationship between total TRH returns and counts of CWT adult spring- and fall-run fish also were significant and positive ($r_s = 0.88$, $P < 0.01$, $n = 24$ and $r_s = 0.79$, $P < 0.01$, $n = 24$, respectively) even though combined counts of CWT adult fish represent only 20.7% of total TRH returns.

Autocorrelation analyses of residuals for annual counts showed that all time points were contained within approximate 95% confidence levels of significance for each correlation in the autocorrelation function correlograms. These results were corroborated by the lack of significance in Box-Pierce tests for total TRH returns ($\chi^2 = 1.11$, $P = 0.29$, $d.f. = 1$), and CWT adult spring-run ($\chi^2 = 0.48$, $P = 0.49$, $d.f. = 1$), and fall-run fish ($\chi^2 = 2.0$, $P = 0.16$, $d.f. = 1$). Thus, we found no evidence against time dependency for counts; as all three groups of Chinook Salmon appeared to represent stationary series of relatively constant autocorrelation

structure over time for the consecutive sequence of dates analyzed herein. Importantly, a stationary series for hatchery production likely will always occur for hatchery raised Chinook Salmon as TRH releases fingerlings and yearlings based on egg-take allotments established in the 1980s to meet fixed mitigation goals of 7,000 returning adult fall-run Chinook to the hatchery irrespective of annual hatchery escapement. Plots of partial residuals showed that annual counts of Chinook Salmon were nonlinear, and well defined by response curves for total TRH returns, and both races of CWT adult fish (Figure 3A, 3B, and 3C). All smooth terms were significant and deviance explained was $\geq 44.1\%$ for each group of fish (Table 2). Additionally, there was a negative relationship between year and total TRH returns ($r_s = -0.44$, $P < 0.05$, $n = 24$), and counts of spring-run CWT adult Chinook Salmon ($r_s = -0.43$, $P < 0.05$, $n = 24$), but not between year and counts of fall-run CWT adult fish ($r_s = -0.04$, $P > 0.10$, $n = 24$). For the annual sampling period shared by both races of CWT adult Chinook Salmon, the correlation between counts was significant and positive ($r_s = 0.64$, $P < 0.01$, $n = 24$), indicating that both races exhibited concordant patterns in timing of annual migration.

However, for the annual sampling period from 2003 to 2017, a stronger negative trend was evident between year and counts in all groups of Chinook Salmon (total TRH returns: $r_s = -0.67$, $P = 0.01$, $n = 15$; spring-run: $r_s = -0.72$, $P < 0.10$, $n = 15$; fall-run: $r_s = -0.65$, $P = 0.01$, $n = 15$). Similarly, all smooth terms were significant and deviance explained was $\geq 46.6\%$ (Table 2). As expected, the strength of the correlation between counts of each group of fish from 2003 to 2017 was strong, significant, and positive (spring-run vs. total TRH returns: $r_s = 0.91$, $P < 0.01$, $n = 15$; fall-run vs. total TRH returns: $r_s = 0.99$, $P < 0.01$, $n = 15$; spring-run vs. fall-run: $r_s = 0.85$, $P < 0.01$, $n = 15$). Thus, all age classes of marked (CWT) and un-marked fish have declined dramatically in relative abundance since 2003. Importantly, this concordant trend in declining stocks of Chinook Salmon began before the two disjunct periods of three consecutive years of regional drought as indicated by water year-type for 2007-2009 and 2013-2015 (3A, 3B, 3C; Table 1, TRRP 2019).

Seasonal fluctuations in run-timing.—Seasonal trends in counts of spring- and fall-run CWT adult Chinook Salmon also fluctuated on a weekly basis. In spring-run fish, fluctuations in seasonal run-timing ranged from early September through late November (JW36 - JW48, Figure 2C). Migrating fish occurred most frequently in the upper Trinity River and counted at the hatchery from early to mid-September through early October. There was a primary peak in late September, followed by an abrupt decline by mid-October, with some fish lingering in the upper reach into mid-November. In contrast, seasonal run-timing in fall-run CWT Chinook Salmon ranged from late September through early January (JW36 - JW01). Migrating fall-run fish occurred most frequently from mid-October through mid-November (Figure 2C), with the primary peak occurring in late October, followed by an abrupt decline through late November and early December.

Autocorrelation analyses of residuals for Julian week counts showed that all time points were contained within approximate 95% confidence levels of significance for each correlation in the autocorrelation function correlograms and by the Box-Pierce tests for both CWT adult spring-run ($\chi^2 = 0.16$, $P = 0.69$, $d.f. = 1$) and fall-run ($\chi^2 = 0.25$, $P = 0.62$, $d.f. = 1$) fish. Plots of partial residuals showed that seasonal trends in Julian week counts of spring- and fall-run CWT adult Chinook Salmon were significant, nonlinear, and well defined by response curves, with deviance explained $\geq 82.3\%$ (Figure 3D and 3E, Table 2). Follow-on ranked correlation found a significant negative relationship between Julian weeks and counts of CWT adult spring-run fish ($r_s = -0.62$, $P < 0.01$, $n = 12$) but not fall-run fish

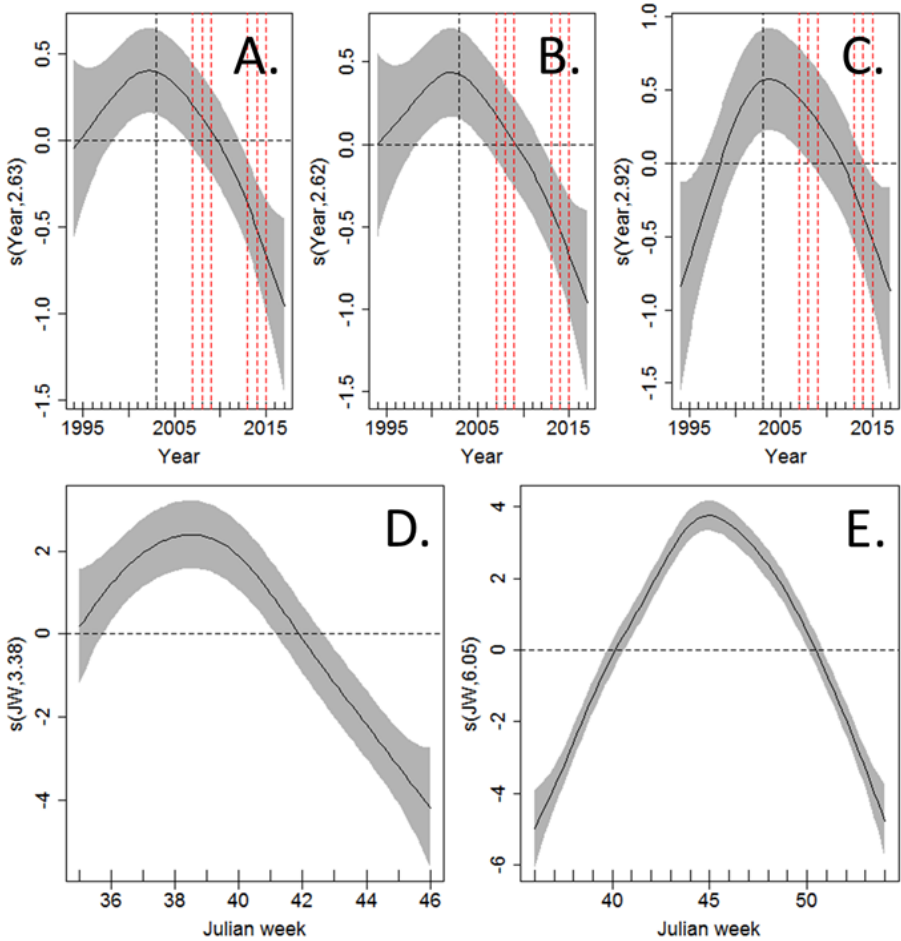


FIGURE 3.—Response curves using GAM regression of annual fluctuations in the distribution of A) total Trinity River Hatchery returns, B) CWT adult spring-run, C) CWT adult fall-run fish, and seasonal fluctuations in Julian week (JW) counts of D) CWT adult spring-run and E) CWT adult fall-run Chinook Salmon. X-axes are labeled with the covariate name (cov) and y-axes by the covariate name and estimated degrees of freedom (edf) of each smooth ($s[cov,edf]$). Plots show the relationship of the fitted GAM function to the response scaled to zero. Shaded areas indicate 2-times the point-wise standard error for each curve surrounding each fitted GAM function (black lines). Horizontal black dashed lines measure extremes above and below the mean. Vertical dashed black lines reference year 2003 and vertical dashed redlines reference 2-periods of three consecutive dry water-years reflective of regional drought (Table 1)

($r_s = 0.06$, $P > 0.10$, $n = 19$). As expected, the relationship between counts of CWT adult spring- and fall-run fish was significant but negatively correlated for the weekly sampling period shared by these two seasonally semi-disjunct races of Chinook Salmon ($r_s = -0.68$, $P < 0.05$, $n = 11$).

Deviation in run-timing from the baseline flow-type.—For CWT adult spring-run Chinook Salmon, counts of each flow-type were 1) baseline PreROD flow ($n = 12,296$); 2) ROD flow ($n = 7,248$) and 3) Pulse flow ($n = 8,277$; Figure 4A). Deviation away from the baseline PreROD flow pattern in run-timing relative to other managed flow-types was

predominantly negative and occurred through 1) reduction in number of fish at the ascending limb of the baseline hydrograph, and 2) addition of fish along the declining central segment and trailing end of the baseline hydrograph (Figure 4C). Analysis of planned median differences in Wilcoxon sign-ranked tests of pairs of Julian week counts showed a significant overall difference in run-timing between baseline PreROD and ROD flows ($Z = 2.6$, $P < 0.01$, $n = 12$) and between PreROD and Pulse flows ($Z = 1.8$, $P = 0.08$, $n = 12$), but not between ROD and Pulse flows ($Z = 1.0$, $P = 0.36$, $n = 12$). However, unplanned pairwise comparisons of individual Julian weeks showed several significant differences between flow-types. For example, of the 12 total Julian weeks encompassing the entire CWT adult spring-run (years 1994 - 2017), 66.7% (8/12) had sample sizes ≥ 5 for each flow-type (Table 3); of which four pair-wise comparisons showed significant differences between PreROD and ROD flows (JW43 - JW44) and PreROD and Pulse flows (JW40 - JW41, Figure 4C).

Counts of CWT spring-run adult Chinook Salmon, encompassing all deviations, both positive and negative, away from baseline PreROD flow pattern in run-timing, ranged from 5,156 fish (ROD flows) to 5,683 fish (Pulse flows, Table 4). Thus, the combined influence of both ROD and Pulse flow hydrographs affected 10,839 spring-run CWT adult Chinook Salmon relative to the baseline PreROD flow condition. This number equates to an estimated 47,203 total adult fish using the expansion coefficient or 11.8% of total TRH returns from 1994 to 2017. Strength of the correlation between spring-run fish affected by ROD flows and those affected by Pulse flows was significant and positive ($r_s = 0.77$, $P < 0.01$, $n = 12$), indicating that both ROD and Pulse flows altered run-timing in the baseline PreROD flow pattern in parallel (Figure 4C).

For fall-run CWT adult Chinook Salmon, counts of each flow-type were 1) baseline PreROD flow ($n = 18,086$), 2) ROD flow ($n = 18,902$), and 3) Pulse flow ($n = 18,208$; Figure 4B). Timing of migration for both ROD and Pulse flows also deviated from the historical baseline PreROD flow pattern of migration. Here, deviations were mostly positive and occurred through 1) addition of fish at both the ascending (early) and descending (late) limbs of the baseline PreROD flow hydrograph, and 2) reduction of fish within the center of the baseline PreROD flow hydrograph (Figure 4D). Median differences in planned pairs-wise comparisons of Julian week counts showed no significant overall difference in run-timing between baseline PreROD and ROD flows, PreROD and Pulse flows, or ROD and Pulse flows ($Z = 1.2$, $P = 0.24$, $n = 19$; $Z = 0.46$, $P = 0.67$, $n = 19$; $Z = 0.66$, $P = 0.52$, $n = 19$, respectively). However, of the 19 Julian weeks encompassing the entire fall-run for CWT adult Chinook Salmon, 11 (57.9%) had sample sizes ≥ 5 for each flow-type (Table 3). Of these unplanned pairwise comparisons 7 (63.6%) showed significant differences between PreROD and the ROD flows (JW39, JW43, JW45, JW47), PreROD and Pulse flows (JW41), and ROD and Pulse flows (JW46, JW47, JW48; Figure 4D).

Counts of fall-run CWT adult Chinook Salmon encompassing all deviations, both positive and negative, away from the baseline PreROD flow condition, ranged from 5,008 fish (ROD flows) to 3,412 fish (Pulse flows, Table 4). Hence, the combined influence of both ROD and Pulse flow hydrographs affected 8,420 fall-run CWT adult Chinook Salmon, relative to the baseline PreROD flow pattern of migration, which equates to an estimated 36,669 total fish using the expansion coefficient or 9.1% of total TRH returns from 1994 to 2017. Importantly, however, the relationship between fall-run fish affected by ROD flows and those fish affected by Pulse flows was significant and negatively correlated it was not strong ($r_s = -0.41$, $P < 0.08$, $n = 19$; Figure 4D). These results indicate that ROD and Pulse

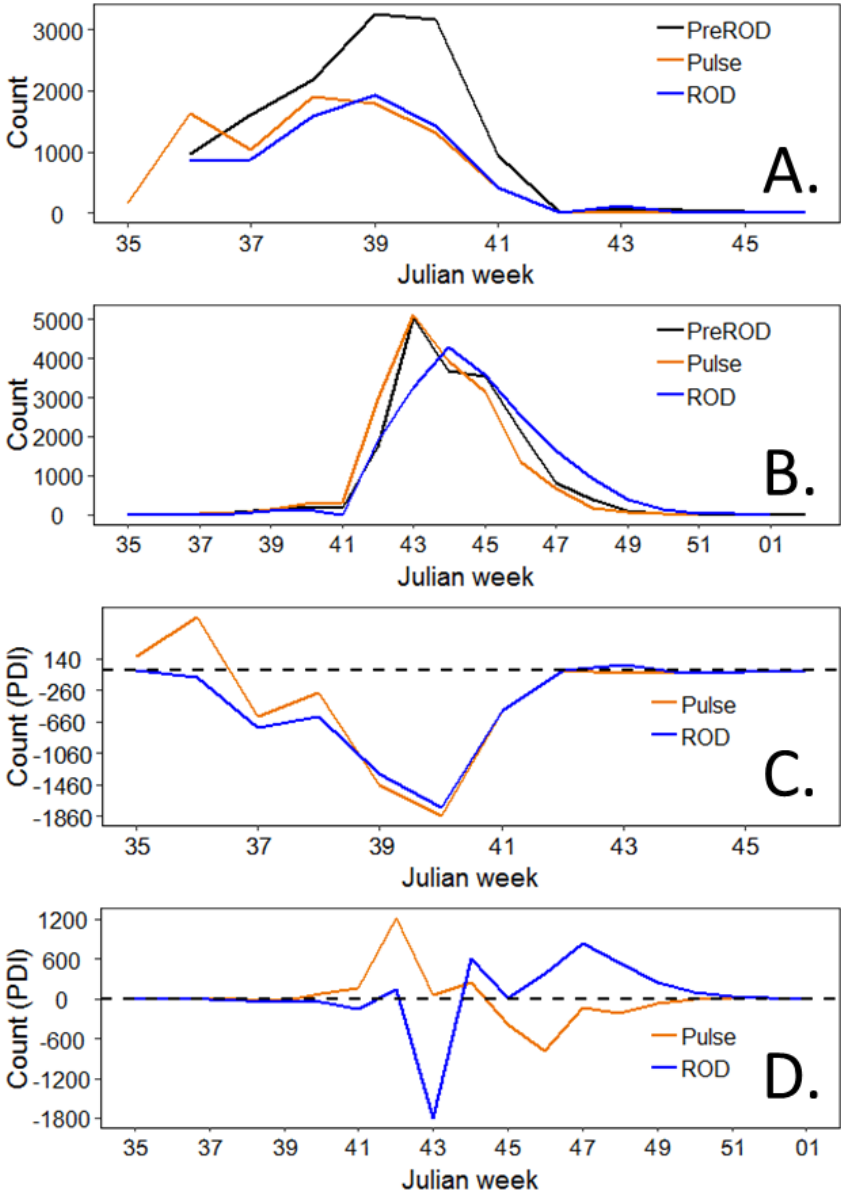


FIGURE 4.—Line graphs of seasonal fluctuations in counts of CWT adult A) spring-run and B) fall run Chinook Salmon; and counts of CWT C) adult spring-run and D) adult fall-run fish affected by both ROD and Pulse flows relative to the baseline PreROD flow pattern of run-timing, expressed as a function of the Percent Deviation Index (PDI). ROD and Pulse flow lines that plot above or below dashed horizontal black lines on the y-axes indicate addition or subtraction of fish from the taxon-specific baseline pattern of run-timing associated with the specific managed flow-type.

TABLE 3.—Results of the Dwass-Steel-Chritchlow-Fligner (*DSCF*) pairwise comparisons of nonzero coded wire tag marked (CWT) spring- and fall-run adult Chinook Salmon using Julian week (JW) counts as attributes to assess significance between baseline flow-types from 1994 to 2017. Only pairwise comparisons with Julian week count sample sizes ≥ 5 were included in our analyses. We adjusted raw *P*-values using the Holms adjustment method separately for each race and JW. Only those comparisons that were significant ($P \leq 0.10$) appear in the table.

Julian week	Flow group (<i>i</i>)	<i>n</i>	Flow group (<i>j</i>)	<i>n</i>	DSCF statistic	<i>P</i> -value
Spring-run Chinook salmon						
JW37	Pre-ROD	9	Pulse	7	3.1	0.08
JW38	Pre-ROD	8	Pulse	7	3.1	0.07
JW39	Pre-ROD	9	Pulse	7	3.3	0.05
	ROD	8	Pulse	7	2.9	0.09
JW40	Pre-ROD	9	Pulse	7	4.0	0.01
JW41	Pre-ROD	9	Pulse	6	7.2	0.00
JW43	Pre-ROD	6	ROD	6	3.8	0.02
JW44	Pre-ROD	8	ROD	6	4.2	0.01
Fall-run Chinook salmon						
JW41	Pre-ROD	8	ROD	6	3.3	0.06
	Pre-ROD	8	Pulse	6	3.6	0.03
JW43	Pre-ROD	6	ROD	7	4.1	0.01
	ROD	7	Pulse	7	2.9	0.10
JW45	Pre-ROD	8	ROD	7	4.0	0.01
	ROD	8	Pulse	7	3.1	0.07
JW46	ROD	8	Pulse	7	3.3	0.05
JW47	Pre-ROD	8	ROD	7	5.0	0.00
	ROD	8	Pulse	7	4.1	0.01
JW48	Pre-ROD	8	ROD	7	3.1	0.07
	ROD	8	Pulse	7	3.6	0.03

flows altered run-timing in the baseline PreROD flow pattern of fall-run CWT adult fish at different times during the season (JW41 - JW44). Thus, annually managed hydrographs have affected approximately 20.9% (83,872/401,667) of total TRH returns of spring- and fall-run adult Chinook Salmon, relative to the baseline PreROD flow pattern since 2003.

Fluctuations in annual reproductive attributes of hatchery parental brood-stock.—An average of 1,125.2 ($n = 27,028$) spring-run and 1,972.9 ($n = 43,403$) fall-run marked (CWT) and un-marked adult female Chinook Salmon were spawned annually at the hatchery from 1994 to 2017. Spring- and fall-run fish differed significantly in total number of adult females spawned (AFSPW: $Z = 4.1$, $P < 0.01$, $n = 22$), a function of generally larger run-size in fall-run fish (Figure 2B and 2C) and AAPF ($Z = 1.7$, $P = 0.08$, $n = 22$), but not in average eggs per ounce (AEPOZ; $Z = 0.65$, $P = 0.52$, $n = 22$), or average eggs per female (AEPF: $Z = 0.05$, $P = 0.96$, $n = 22$). All reproductive attributes except AEPOZ were significantly correlated between spring- and fall-run adult Chinook Salmon (AEPF: $r_s = 0.80$, $P < 0.01$; AFSPW: $r_s = 0.52$, $P = 0.02$; AAPF: $r_s = 0.50$, $P = 0.02$; AEPOZ: $r_s = 0.33$, $P = 0.13$).

TABLE 4.—Total counts (positive and negative) and total cumulative counts (positive + negative) by Julian week (JW) of spring- and fall-run CWT adult Chinook Salmon affected by ROD and Pulse flows, relative to the pattern in run-timing typical of taxon-specific baseline PreROD flows.

Julian week	Spring-run affected			Fall-run affected		
	ROD flows	Pulse flows	Total affected	ROD flows	Pulse flows	Total affected
JW35	0	165	165	0	0	0
JW36	-100	667	767	1	2	3
JW37	-733	-589	1,322	-3	-1	4
JW38	-594	-286	880	-4	3	7
JW39	-1,326	-1,469	2,795	-38	-7	45
JW40	-1,742	-1,858	3,600	-44	-21	65
JW41	-530	-530	1,060	-53	83	136
JW42	-8	-1	9	-160	168	328
JW43	54	-45	99	140	1,208	1,348
JW44	-45	-50	95	-1,794	53	1,847
JW45	-19	-19	38	597	250	847
JW46	-5	-4	9	24	-397	421
JW47	0	0	0	370	-795	1,165
JW48	0	0	0	837	-124	961
JW49	0	0	0	537	-215	752
JW50	0	0	0	256	-68	324
JW51	0	0	0	96	-16	112
JW52	0	0	0	40	-1	41
JW01	0	0	0	12	0	12
JW02	0	0	0	2	0	2

Average annual percent fertility began to decrease in 2005 in spring-run and in 2006 in fall-run Chinook Salmon (Figure 5A), and in both taxa it continued to decline through 2014. An increase in AAPF occurred in 2015 coincidental with changes in the methodology used in fertilization and incubation of fertilized eggs in response to declining fertility rates in Chinook Salmon eggs brood years 2012 to 2014 (personal communications L. Glenn, 2014; S. McCarn and S. Ballard, 2015 and 2017, respectively). The 2015 increase in AAPF continued through 2016, followed by a slight downturn in 2017. Prior to the change in measurement protocol, a well-defined downward trend in AAPF was evident in both races of adult Chinook Salmon (Figure 5B and 5C). In each group of fish, plots of partial residuals of AAPF versus year were nonlinear, well defined by response curves, significant, with deviance explained $\geq 50.6\%$ (Table 2), indicating a significant negative annual trend in AAPF in both races of Chinook Salmon. This suggests that the pattern of AAPF was similar for both races, when AAPF drops for one race it also drops for the other race and vice versa.

Additionally, autocorrelation analyses of AAPF for hatchery collected and reared Chinook Salmon eggs showed that all time points were contained within approximate 95% confidence levels of significance for each correlation in the autocorrelation function correlograms. This result was corroborated by follow-on Box-Pierce tests for both CWT adult spring-run ($\chi^2 = 1.7$, $P = 0.19$, $d.f. = 1$) and fall-run Chinook Salmon ($\chi^2 = 0.97$, $P = 0.37$, $d.f. = 0.32$). Thus, for the sequence of consecutive dates assessed herein, annual estimates of

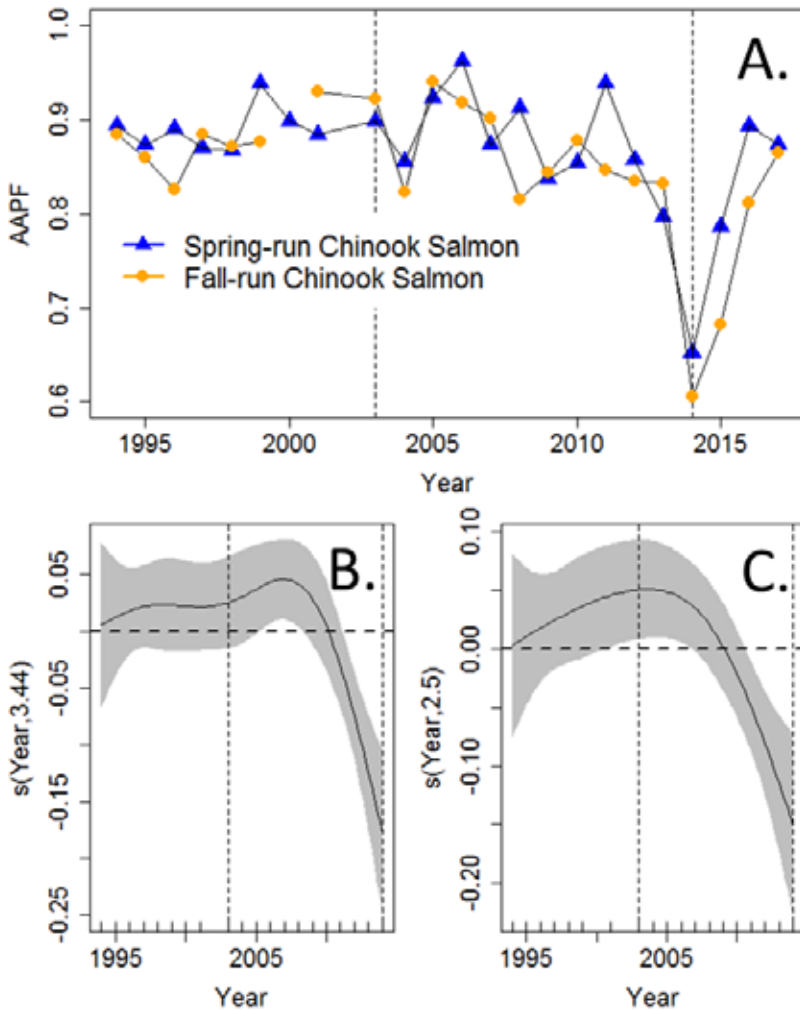


FIGURE 5.—Line graphs of seasonal fluctuations in A) average annual percent fertility (AAPF) of adult female spring-run and fall-run Chinook Salmon measured at the Trinity River Hatchery, and response curves generated from regression using GAM for adult B) spring-run and C) fall-run fish. Plots show the relationship of the fitted function to the response scaled to zero. Shaded areas indicate 2-times the point-wise standard error for each curve surrounding each fitted GAM function (black lines). Vertical dashed black lines identify year 2003 and 2014.

AAPF for spring- and fall-run Chinook Salmon represented a stationary series of relatively constant autocorrelation structure over time.

Fluctuations in water temperature and flow volume associated with flow-types.—Regression using GAM showed a pattern of nonlinear variation in each variable used to assess annual trends in water temperature and flow volume (Figure 6). All smoothed year terms were significant for each annual response curve (ADWT, ADWTVI, ADFV), but deviance explained was not particularly robust among environmental variables examined (Table 2). Correlation analysis also indicated that annual trends in environmental variables were

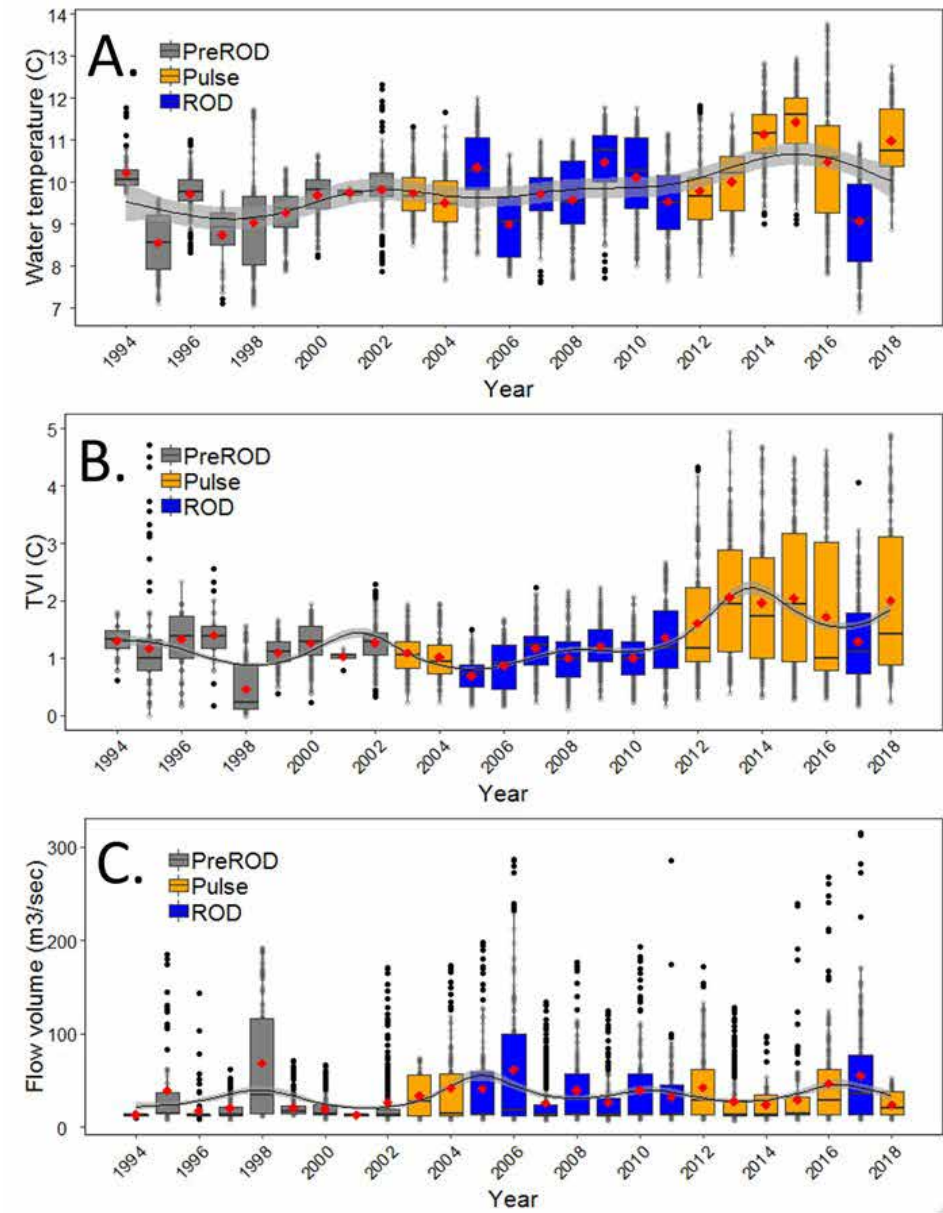


FIGURE 6.—Regression of annual fluctuations in average daily A) water temperature (ADWT), B) temperature variability index (ADWTVI), and C) flow volume (ADFV) for each flow-type (PreROD, ROD, and Pulse). Boxplots display annual distributions of continuously distributed variables (gray colored points), including minimum, first quartile, median (horizontal line), third quartile, and maximum. Shaded areas indicate 2-times the point-wise standard error for each curve surrounding each fitted GAM function (black lines). Black “dots” at the end of the boxplot represent outliers and red diamonds represent the mean value of the distribution by year.

significant and positive for ADWT ($r_s = 0.34$, $P < 0.01$, $n = 4,347$), ADWTVI ($r_s = 0.18$, $P < 0.01$, $n = 4,347$), and ADFV ($r_s = 0.07$, $P < 0.01$, $n = 4,458$), but the strength of the correlations were not particularly strong. These results show that all three environmental variables have increased over time for the above sequence of Julian weeks, with increases in flow volume exhibiting the weakest annual trend. As expected, whereas the relationship between ADWT and ADWTVI was significant and positive ($r_s = 0.46$, $P < 0.01$, $n = 4,347$), both variables were inversely correlated with ADFV ($r_s = -0.38$, $P < 0.01$, $n = 4,315$ and $r_s = -0.67$, $P < 0.01$, $n = 4,315$, respectively). Thus, as flow volume increased average water temperature and the range of variability in extreme water temperature decreased moving toward the end of seasonal run-timing for both races of Chinook Salmon.

Kruskal-Wallis (H) non-parametric one-way analysis of variance (ANOVA) rank sum test identified significant overall differences among flow-types in all environmental variables, also reflected in both the mean and variance for each environmental variable (Table 5). For example, mean values of ADWT, ADWTVI, and ADFV were 1) PreROD flows (9.5° C, 1.1° C, 27.2 m³/s, $n = 1,334$), 2) ROD flows (9.8° C, 1.0° C, 42.2 m³/s, $n = 1,447$), and 3) Pulse flows (10.3° C, 1.5° C, 36.9 m³/s, $n = 1,280$). Additionally, planned post-hoc multiple pairwise comparisons found significant differences between all flow-types in each environmental variable except between ROD and Pulse flows in ADWTVI and ADFV (Table 5). Mean values of each measure of water temperature were higher in Pulse flows than in baseline PreROD or ROD flows. Similarly, variance in ADWT and ADWTVI was higher in Pulse flows (1.3° C, 0.91° C) than in PreROD flows (0.61° C, 0.28° C) or ROD flows (1.00° C, 0.26° C). We note that in 2017, ROD flows resulted in lower values for both water temperatures variables and higher flow volume than in Pulse flow-years, consistent with the seven consecutive ROD flows from 2005 to 2011 (Figure 6). Whereas in the 2018 Pulse flow this pattern was reversed among environmental variables.

Nonlinear patterns of seasonal Julian week counts were also evident in each measure of water temperature and flow volume, as all smooth terms were significant for each response curve and deviance explained ranged from 18.1% (ADWTVI) to 47.7% (ADTV; Table 2). Here, volume of flow was driving the response in extreme variability in water temperature; and although significant, none of the relationships were strong between Julian week counts and ADWT ($r_s = 0.41$, $P \leq 0.01$, $n = 4,347$), ADTVI ($r_s = 0.11$, $P \leq 0.01$, $n = 4,347$), or ADFV ($r_s = -0.11$, $P \leq 0.01$, $n = 4,458$). Shapes of the response curves showed elevated levels of ADWT and ADWTVI associated with the ROD-segments of Pulse flows across virtually the full spectrum of Julian week counts (Figures 7A and 7B). Elevated levels of ADWT and particularly ADWTVI were evident at the onset and during the decline in the ROD-segments of pulsed augmentations (JW13 - JW17, JW26 - JW38), which encompassed the entire spring-run and early onset of run-timing in fall-run Chinook Salmon (Figure 2C). However, ADWTVI decreased on the trailing end of the ROD-segments of Pulse flows (JW34 - JW37), then increased during the actual pulsed augmentations (JW38 - JW40). These extremes in water temperature variability are a function of an increase in ADFV during the same period (Figure 7C).

As expected, ROD flows exhibited the largest mean flow volume (42.2 m³/s) compared to PreROD (27.2 m³/s) or Pulse flows (36.9 m³/s), a pattern particularly evident for Julian week 17 to 24 (Figure 7C). ROD flows also exhibited greater variance in ADFV (2,225.7 m³/s) than either PreROD (1,132.9 m³/s), or Pulse (1,288.3 m³/s) flows. Apparent also was the large and concentrated distribution of outliers in ADFV clearly associated with year

TABLE 5.—Non-parametric Kruskal-Wallis (*H*) rank sum one-way analysis of variance (ANOVA) tests followed by the Chritchlow-Fligner (*DSCF*) post-hoc multiple pair-wise comparisons tests of means between flow-types using 1) average daily water temperature (ADWT), 2) average daily water temperature variability index (ADWTVI), 3) and average daily flow volume (ADFV) for Julian weeks 13 to 40 (1995 - 2017; Table 1). Sample sizes for PreROD, ROD, and Pulse flows were *n* = 1,403, *n* = 1,510, and *n* = 1,545, respectively.

ADWT (<i>H</i> = 321.8, <i>P</i> < 0.01, <i>d.f.</i> = 2)			
Group(<i>i</i>)	Group(<i>j</i>)	<i>DSCF</i>	<i>P</i> -Value
Pre-ROD	Pulse	26.294	0.00
Pre-ROD	ROD	43.035	0.00
Pulse	ROD	16.052	0.00
ADWTVI (<i>H</i> = 132.1, <i>P</i> < 0.01, <i>d.f.</i> = 2)			
Group(<i>i</i>)	Group(<i>j</i>)	<i>DSCF</i>	<i>P</i> -Value
Pre-ROD	Pulse	-2.337	0.03
Pre-ROD	ROD	10.481	0.00
Pulse	ROD	14.498	0.00
ADFV (<i>H</i> = 63.5, <i>P</i> < 0.01, <i>d.f.</i> = 2)			
Group(<i>i</i>)	Group(<i>j</i>)	<i>DSCF</i>	<i>P</i> -Value
Pre-ROD	Pulse	6.651	0.00
Pre-ROD	ROD	15.577	0.00
Pulse	ROD	12.784	0.00

2002 through 2016 (Figure 6C), which corresponded to 2002 the year of the massive fish kill in the lower Klamath River and all ROD and Pulse flows post-2002 to present. Further, the influence of Pulse flows and Ceremonial Tribal Boat Dance flows was clearly evident in ADFV, which spiked just prior to the “historical” peak in run-timing of spring-run adult Chinook Salmon (JW39, Figure 2A and 2C), and early arriving CWT fall-run adult fish (JW38 - JW40), which overlapped the trailing end of the spring-run (Figure 7C).

Average annual percent fertility as a function of water temperature and flow volume.— We assessed fluctuations in annual ADWT, ADTVI, and ADFV (1994 - 2017) separately and in combination, against annual fluctuations in AAPF using GAM (Table 2). Plots of partial residuals suggested concordant declining trends in AAPF for each environmental variable to each fitted GAM function for each race of Chinook Salmon (Figure 8A - 8B, and 8D - 8E, respectively). Shapes of response curves reflected a negative effect of ADWT and ADWTVI on AAPF, as smooth terms were significant for each water temperature gradient in each regression model, particularly ADWT (Table 2). Thus, as ADWT and ADWTVI increase AAPF decreases in relation to both environmental variables; whereas AAPF showed no significant trend in relation to variation in annual volume of flow. For each race of Chinook Salmon measures of model fit (*QAIC_c*) were most efficient (parsimonious) using ADWT followed by ADWTVI, individually, in pairs, or in combination with ADFV, in the fitted function as nonlinear descriptors of the behavior in AAPF (Table 2).

For both races of Chinook Salmon, univariate histograms (rugs) at the base of each plot, showed that data points for ADWT were concentrated in their distribution between 9.0° C to 10.5° C (Figure 8A and 8C); whereas data points for ADWTVI ranged primarily

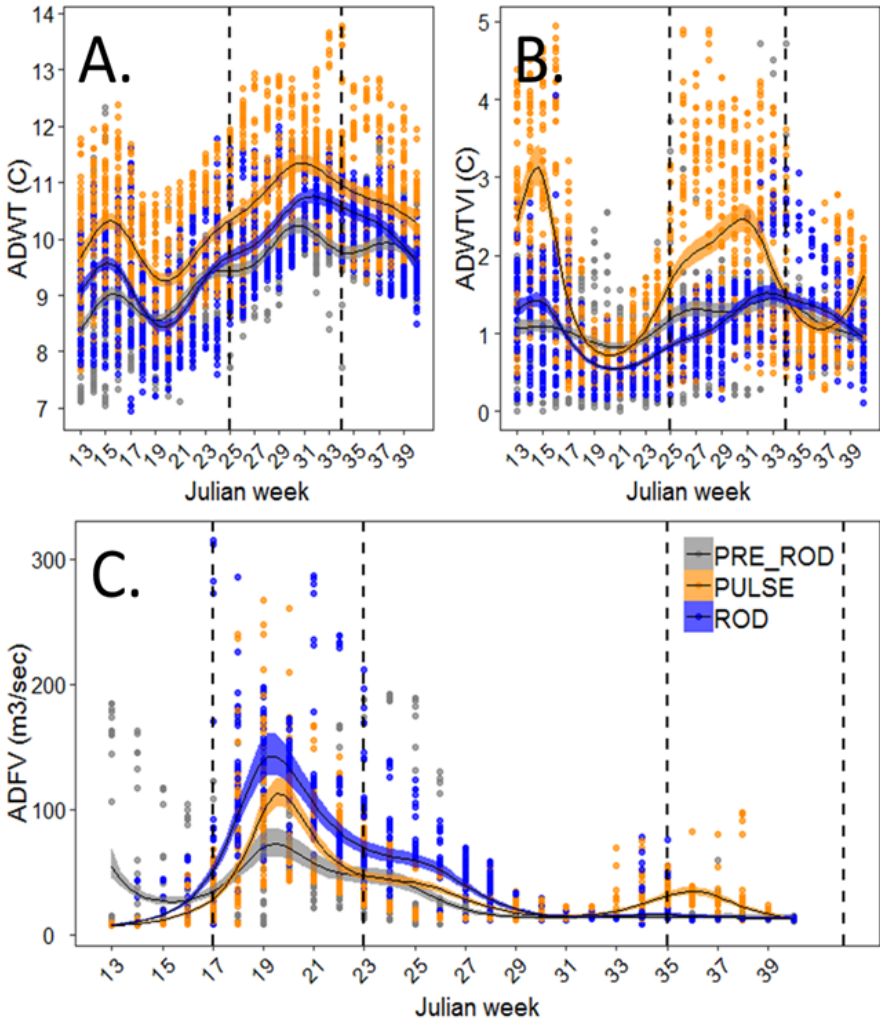


FIGURE 7.—Regression of seasonal Julian week (JW) counts among flow-types in response to average daily A) water temperature (ADWT), B) temperature variability index (ADWTVI), and flow volume (ADFV). Plots show the relationship of the fitted GAM function to each response variable. Shaded areas indicate 2-times the point-wise standard error for each curve surrounding each fitted function (black lines). Vertical (x-axis) black dashed lines correspond to Julian weeks referenced in the text.

between 1.0° C to 1.4° C (Figure 8B and 8D). Plots of partial residuals using GAM found no significant trends in AAPF in response to ADFV for either race of Chinook Salmon (Figure 8C and 8F, respectively), and most flow volume ranged between 19 m³/sec and 43 m³/second. Further, a significant positive relationship between spring- and fall-run fish suggests that AAPF may be a function of average and extreme measures of variability in water temperature, as AAPF in both races of fish tracked fluctuations in water temperature in a similar way, irrespective of changes in measurement initiated in 2015. For example, regression analysis using of AAPF between these two taxa resulted in GAM deviance explained ranging from 74.6% from 1994 to 2017 ($F = 18.2$, $Ref.df = 3.1$, $P < 0.01$, $n = 22$) to 73.2% from 1994 to

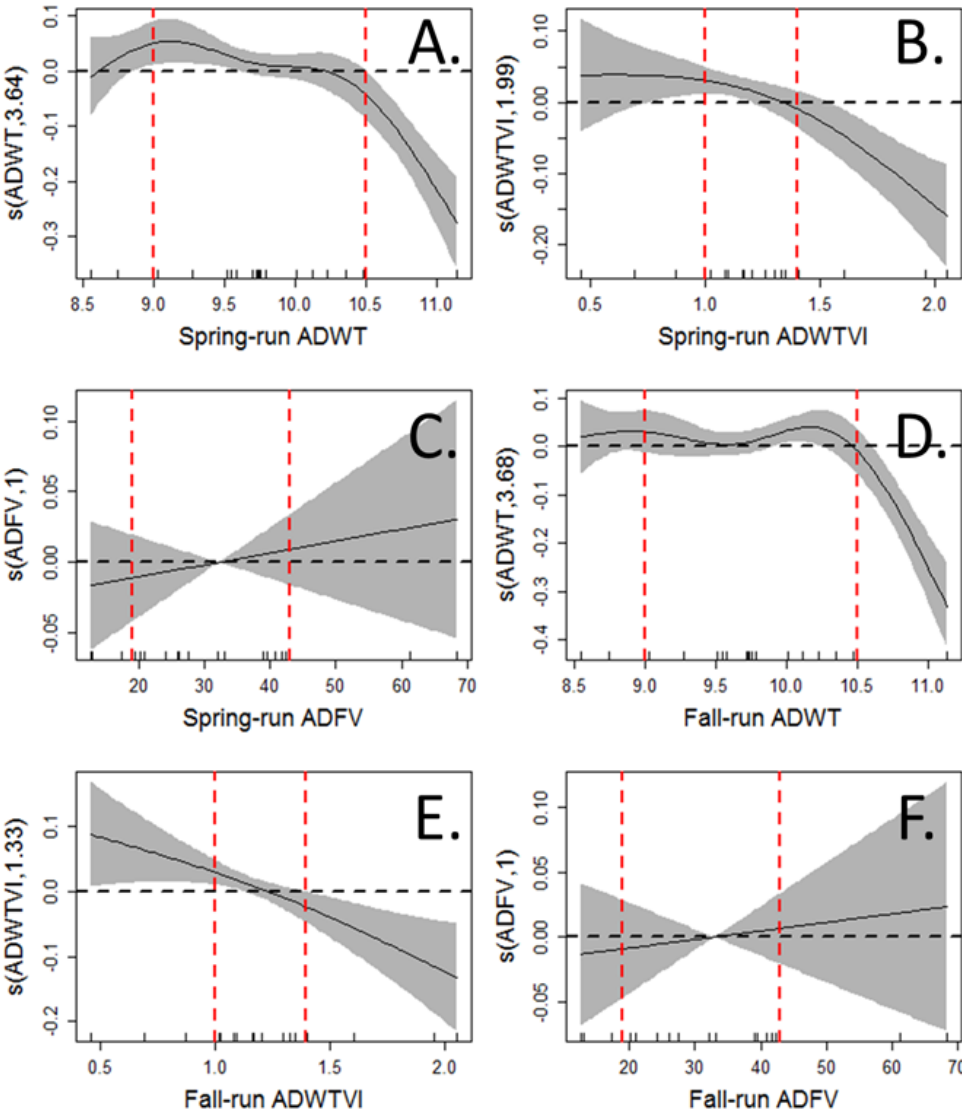


FIGURE 8.—Regression of seasonal Julian week (JW) fluctuations in average annual percent fertility (AAPF, 1994 - 2014) in response to 1) average daily water temperature (ADWT), 2) temperature variability index (ADWTVI), and flow volume (ADTV) for spring-run (8A, 8B, and 8C, respectively) and fall-run Chinook Salmon (8D, 8E, and 8F, respectively). GAM labels *x*-axes with the covariate name (cov) and *y*-axes by the covariate name and estimated degrees of freedom (edf) of each smooth ($s[\text{cov}, \text{edf}]$). Plots show the relationship of the fitted function to the response scaled to zero. Shaded areas indicate 2-times the point-wise standard error for each curve surrounding each fitted GAM function (black lines). Vertical red dashed lines on *x*-axis correspond to various Julian weeks referenced in the text.

2014 ($F = 21.8$, $Ref.df = 2.1$, $P < 0.01$, $n = 19$). Similarly, follow-on correlation analysis of AAPF between taxa also showed and significant and positive relationship ($r_s = 0.48$, $P \leq 0.01$, $n = 22$) for the period 1994 to 2017, irrespective of changes in the methodology used by the hatchery to measure this parameter.

Effects of flow-types on returning progeny.—We found no significant difference in the proportion of fish assigned to each age class (ages 2 - 5) between total TRH returns, and expanded estimates of CWT adult spring- or fall-run Chinook Salmon from 1994 to 2017 (Table 6, Figure 9). Age class 3 followed by age class 4 composed the greatest proportion of fish in each group of Chinook Salmon. Similarly, except for age class five in spring-run Chinook Salmon, there was no significant correlation between year and age class for any group of fish or a significant difference among flow-types in the proportion of age classes for either race of Chinook Salmon (Table 6). However, unlike the direct effects of ROD and Pulsed flows on annual and seasonal fluctuations in run-timing, potential direct effects of managed hydrographs and their resulting temperature regimes on same-season reproduction are not immediately evident in annual counts of spring- or and fall-run Chinook Salmon, age 2, 3, 4, or 5-year old fish. For example, in 2012, a total of 24,374 Chinook Salmon returned to the hatchery (Table 7). These fish represented the four age cohorts (age 2, 3, 4 and 5-year-old fish) for hatchery-raised parental broodstock of brood-years 2007 through 2010. However, returning progeny of the 2012 Chinook Salmon hatchery-raised broodstocks comprise brood-years 2014 (age 2), 2015 (age 3), 2016 (age 4), and 2017 (age 5). Similarly, Chinook Salmon hatchery-raised parental broodstock in 2017 were derived from brood-years 2012 through 2015 parental brood-stock origin and will have returning progeny in 2019 through 2022.

DISCUSSION

Implications of annual and seasonal fluctuations in counts.—Results of our analyses showed a highly significant correlation between counts of known race CWT adult Chinook Salmon and total TRH returns of marked (CWT) and unmarked hatchery-origin fish of all age classes. Combined expanded estimates of CWT Chinook Salmon represented 90.0%

TABLE 6.—Results of the 1) Kruskal-Wallis (H) rank sum tests ($n = 24$, $df = 2$) for differences in age classes and flow-types for Trinity River Hatchery total returns, and spring- and fall-run Chinook Salmon; and 2) Spearman's rank correlations (r_s) between percentages of various age classes and year for both runs of Chinook Salmon.

2012 ($n = 24,374$)				2013 ($n = 6,430$)				2014 ($n = 10,813$)			
Parental stock		Returning progeny		Parental stock		Returning progeny		Parental stock		Returning progeny	
Age	Year	Age	Year	Age	Year	Age	Year	Age	Year	Age	Year
5	2007	2	2014	5	2008	2	2015	5	2009	2	2016
4	2008	3	2015	4	2009	3	2016	4	2010	3	2017
3	2009	4	2016	3	2010	4	2017	3	2011	4	2018
2	2010	5	2017	2	2011	5	2018	2	2012	5	2019
2015 ($n = 5,341$)				2016 ($n = 3,650$)				2017 ($n = 7,013$)			
Parental stock		Returning progeny		Parental stock		Returning progeny		Parental stock		Returning progeny	
Age	Year	Age	Year	Age	Year	Age	Year	Age	Year	Age	Year
5	2010	2	2017	5	2011	2	2018	5	2012	2	2019
4	2011	3	2018	4	2012	3	2019	4	2013	3	2020
3	2012	4	2019	3	2013	4	2020	3	2014	4	2021
2	2013	5	2020	2	2014	5	2021	2	2015	5	2022

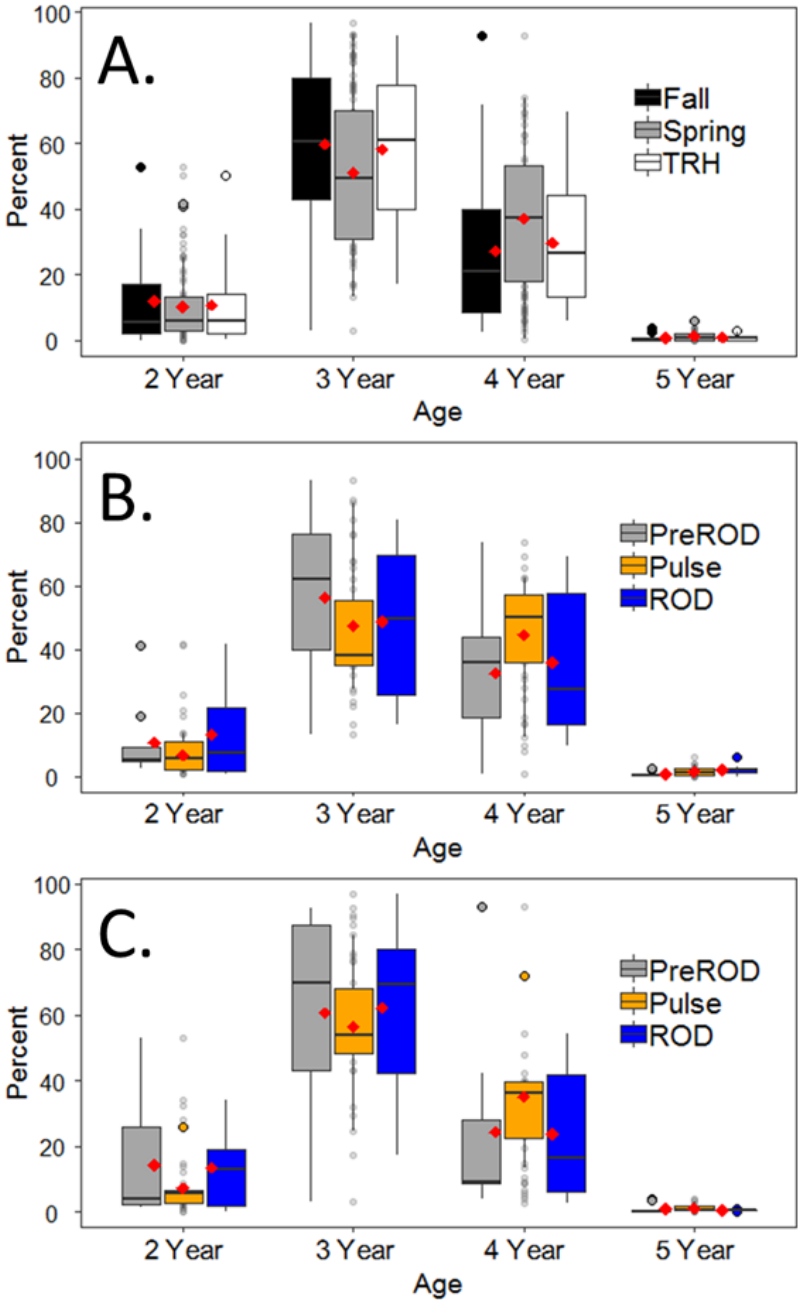


FIGURE 9.—Distribution of age classes, expressed as a percentage of counts for A) total TRH returns ($n = 401,667$), spring-run ($n = 114,768$), and fall-run ($n = 246,809$) Chinook Salmon; and in relation to different flow-types for B) spring-run (baseline PreROD = 50,835; ROD = 29,881; Pulse = 34,052) and C) fall-run (baseline PreROD = 95,952; ROD = 77,560; Pulse = 73,297) Chinook Salmon (1994 - 2017). Boxplots display annual distributions of continuously distributed variables (gray colored points), including minimum, first quartile, median (horizontal line), third quartile, and maximum. Colored “dots” at the ends of boxplots represent outliers as per flow-type and red diamonds represent the mean value of the distribution by year.

TABLE 7.—Annual counts of total returning Trinity River Hatchery spawned Chinook Salmon, age class, year of returning progeny, and parental source of broodstock for fish spawning from 2012 through 2017.

2012 (<i>n</i> = 24,374)				2013 (<i>n</i> = 6,430)				2014 (<i>n</i> = 10,813)			
<u>Parental stock</u>		<u>Returning progeny</u>		<u>Parental stock</u>		<u>Returning progeny</u>		<u>Parental stock</u>		<u>Returning progeny</u>	
Age	Year	Age	Year	Age	Year	Age	Year	Age	Year	Age	Year
5	2007	2	2014	5	2008	2	2015	5	2009	2	2016
4	2008	3	2015	4	2009	3	2016	4	2010	3	2017
3	2009	4	2016	3	2010	4	2017	3	2011	4	2018
2	2010	5	2017	2	2011	5	2018	2	2012	5	2019
2015 (<i>n</i> = 5,341)				2016 (<i>n</i> = 3,650)				2017 (<i>n</i> = 7,013)			
<u>Parental stock</u>		<u>Returning progeny</u>		<u>Parental stock</u>		<u>Returning progeny</u>		<u>Parental stock</u>		<u>Returning progeny</u>	
Age	Year	Age	Year	Age	Year	Age	Year	Age	Year	Age	Year
5	2010	2	2017	5	2011	2	2018	5	2012	2	2019
4	2011	3	2018	4	2012	3	2019	4	2013	3	2020
3	2012	4	2019	3	2013	4	2020	3	2014	4	2021
2	2013	5	2020	2	2014	5	2021	2	2015	5	2022

of total TRH returns from 1994 to 2017. We show that the pattern of annual fluctuations in counts of known race CWT adult fish was an accurate reflection of the annual hard-count of Chinook Salmon recovered from the hatchery, even though CWT adult fish only represent an average of 20.6% of total all TRH returns over the period we sampled. As such, we view the management practice of coded-wire tag marking of hatchery-raised fish as a reliable surrogate for assessing relative trends in populations of both races of Chinook Salmon, relative to un-marked hatchery-origin natural area spawning fish, particularly during years of low run size. Normally, fall-run Chinook Salmon represent the largest component of fish returning to the hatchery. However, in 2002, 2016, and 2017 returns of CWT adult spring-run Chinook Salmon at the hatchery exceeded counts of CWT adult fall-run Chinook Salmon. Potential explanations for these counts may be a function of the large die-off in 2002 of Chinook Salmon in the lower Klamath River or over harvesting of fish. In 2016 and 2017 there was no commercial ocean harvest or in-river sport harvest in 2017, except in the Hoopa Valley where Tribal take (*n* = 1,660), exceeded the allowed quota (*n* = 163, Thom 2018). Further, trends in relative abundance by use of GAM showed a concordant and significant downward monotonic trend in counts from 2003 to 2017 in all CWT adult spring- and fall-run, and total TRH returns of Chinook Salmon, coincidental with establishment of the TRRP in 2002 and subsequent ROD and periodic Pulse flows beginning in 2003.

Implications of seasonal fluctuations in counts in relation to flow-type.—Results of our analyses indicate that evidence exists to support the hypothesis (*H₁*) that annually managed flow regimes implemented by the ROD have altered the pattern and timing of migration of spring- and fall-run Chinook Salmon in the Trinity River in a significant and concordant way beginning in 2003. Seasonal fluctuations in migration of each race of CWT adult Chinook

Salmon not only showed significant differences between baseline PreROD flows and ROD and Pulse flows, but we also observed significant differences between ROD and Pulse flows in both overall and individual Julian week comparisons, and in the number of counts affected by managed flows relative to the baseline condition. Thus, Pulse flows in combination with their associated companion ROD-segment appears to represent an important and independent factor affecting the temporal distribution of Chinook Salmon relative to a “purely” ROD flow hydrograph. As such, we maintain that annual ROD flows in combination with Pulse flow hydrographs have had significant direct and cumulative effects on the pattern of migration and run-timing of spring- and fall-run CWT adult Chinook Salmon in the upper Trinity River, relative to the pattern reflected in baseline PreROD flows. We also see these same annual and seasonal trends and flow effects in the sympatric potamodromous migration of the non-anadromous population of Brown Trout (*Salmo trutta*) in the upper Trinity River (Sullivan and Hileman 2018).

We hypothesize that effects of annually managed hydrographs have likely also alter run-timing of un-marked hatchery-origin and natural-origin spawning Chinook Salmon, which spawn in-river predominantly within the first 15 km of the upper Trinity River below Lewiston Dam and the hatchery, and increasingly down-river and outside mainstem TRRP restoration sites (Rupert et al. 2017a and Rupert et al. 2017b). Peterson et al. (2017) used a variety of environmental attributes to assess the relative influence of managed pulse flows to explain the magnitude of daily counts and proportions of fall-run Chinook Salmon in the Stanislaus River, California. They concluded that although managed pulse flows resulted in immediate increases in daily passages, the measured response was brief, representing only a small portion of the total run, relative to a stronger response between migratory activity and discharge levels. We view this phenomenon to be more reflective of annual ROD flows acting in concert with companion ROD-segments that are part of each Pulse flow hydrograph, as opposed to short-term pulsed augmentations. Viewed collectively, the combined influence of ROD and Pulse flow-types affected, both positively and negatively, the pattern of run-timing in both spring- and fall-run Chinook Salmon in a significant and concordant way, similar to synchrony in survival rates of Chinook Salmon and Coho Salmon in response to ocean conditions linked to coastal food webs (Kilduff et al. 2015, NOAA 2017).

Other potential covariates.—Although we focused specifically on the potential effects of a riverine system subjected to highly managed flow regimes, other covariates not studied also likely affect annual and seasonal patterns of relative abundance and timing of migration in salmonids in the upper Trinity River. For example, factors responsible for decreasing stocks of anadromous salmonids in both the Trinity and Klamath rivers, reference recent ocean conditions and regional drought (Dettinger and Cayan 2014, Diffenbaugh et al. 2015, Mann and Gleick 2015, Adams et al. 2017, NOAA 2017). However, yet to be fully documented or realized, is the degree to which these conditions pose threats to inland fisheries, as a function of changing climate. Importantly, we have shown that since 2001, 38.9% of regional water-years had “dry” or “critically dry” designations, including two periods of three consecutive dry water-years (Table 1). However, although major tributaries of the Trinity River Basin below the North Fork Trinity River may have suffered from the effects of drought, the mainstem Trinity River, which ends administratively at the confluence of the North Fork Trinity River, did not. This was because management of annual hydrographs resulted in release of flows down the Trinity River throughout all drought-years and tributary accretion of water for this segment of the mainstem is less relative to inflow from major

tributaries below the mainstem.

Impact of altered run-timing on hatchery management.—Run-size in Chinook Salmon has been a topic in numerous discussions focused on late summer hydrological augmentations (Turek et al. 2004, Strange 2010, NOAA and USFWS 2013). Yet, these considerations have not occurred in coordination with hatchery management practices. As such, what are the potential ramifications of altering the baseline PreROD flow run-time pattern on the distribution of 1) Chinook Salmon, 2) in-river spawning by hatchery-origin stocks, and 3) natural-origin spawning of “wild” Chinook Salmon? Historically, during baseline PreROD flows, timing of female egg ripening at the hatchery resembled a bell-shaped curve (unripe-ripe-overly ripe), with overly ripe females removed from the spawning matrix once egg-take allotments were approached (3 million spring- and 6 million fall-run Chinook Salmon). However, late summer pulse flows (2012 - 2016) have narrowed ripening curves such that they become negatively skewed, in which a high percentage of early arriving adult females have delayed gametic maturation (“green” females; L Glenn and J. Hileman, personal observations 2015). This situation narrows the spawning window and logistically limits flexibility in managing ripeness in females, particularly when most early arriving and unripe spring-run female Chinook Salmon suddenly become overly ripe (L. Glenn, personal communication, 2015). Additionally, during pulse flows, once spring-run females ripen, fall-run “green” females started arriving in abundance. A shift in ripening curves becomes even more problematic logistically because the hatchery is under a mandate to kill 100% of the Chinook Salmon that come through the hatchery fish trap, as there are no options for separating anesthetized fish, which are either killed or placed in holding tanks for gametic ripening.

Further, holding fish over in cement ripening ponds as a buffer in mitigating potentially low hatchery returns and increasingly earlier arriving spring- and fall-run Chinook Salmon directly exacerbates this practice, because holding ponds can only accommodate approximately 800 fish each. If there are too many unripe females early in a season, the hatchery is limited to holding females for a short duration for ripening before incurring highly density dependent mortality. Thus, if unripe female fish “trickle” into the hatchery for prolonged periods of time, held-over fish are processed multiple times for ripeness with ripe females removed for processing and unripe individuals put back into holding ponds. Consequently, if fall-run Chinook begin arriving prior to the annual temporal spawning break between races (October 15 and October 25), they are dispatched due to limited viability of adult fish in holdover ponds over the duration of the spawning break. Thus, Chinook Salmon are low-graded, such that at the end of egg-take for spring-run Chinook Salmon, all fish that look fresh “chrome” are euthanized. Thus, actions of managed flow regimes that alter the pattern and timing of migration in hatchery-raised Chinook Salmon matter tremendously, when these actions affect hatchery management logistical constraints and government mandates.

At present, there has been no comprehensive effort to collect data to measure in-river individual Chinook female ripeness. However, although, our study was not intended to examine relative adult female productivity between hatchery fish, or in-river spawning by hatchery or natural-origin “wild” fish (Hughes and Murdoch 2017), our analysis is an important step in identifying annual trends in measures of reproductive performance in Chinook Salmon, which has direct application for in-river spawning of both hatchery and natural-origin fish. By proxy, it is highly likely that similar ripening curves have shifted in both in-river spawning of hatchery-origin stocks and natural-origin spawning of Chinook Salmon.

This effort is important due to redd superimposition (Fukushima et al. 1998). For

example, in the upper Trinity River, redd superimposition mostly occurs below and within 3.2 km of Lewiston Dam, which consistently contains the largest densities of hatchery-origin Chinook and Coho Salmon (Rupert et al. 2016, Rupert et al. 2017a, Rupert et al. 2017b). This is also the area most affected by hydrological changes (Pulse flows) and extreme fluctuations in water temperature. Thus, if pulsed augmentation flows affect spawning Chinook Salmon at the hatchery, we predict that these actions will likely have an even greater effect on natural-area spawning redds. We agree with Rupert et al. (2016), that redd superimposition likely limits reproductive success of spring-run Chinook Salmon more than any other race or species of anadromous salmonid in the upper Trinity River (Hendry et al. 2003). Estimates of superimposition from 2015 to 2017 revealed significant and highly density dependent relationships (Rupert et al. 2016, Rupert et al. 2017a and 2017b) with severe superimposition occurring in relative proximity to Lewiston Dam where most in-river spawning of spring-run Chinook Salmon likely occurs.

Implications of fluctuations in water temperature.—Natural environmental influences determine river flow and water temperature in unregulated rivers, whereas in regulated rivers, flow and water temperature regimes can be highly altered by dam operations and water redistribution (Ward and Stanford 1979). Discharge of water from Lewiston Dam involves complex issues affected by political, economic, environmental, and biological factors. These processes are important role in regulating water temperatures downstream in the upper Trinity River. In-river water temperature is one of the most important environmental variables affecting salmonid biology (Carter 2005, Magnuson 2014, Magnuson and Chamberlain 2015). It influences feeding and growth rates (Hicks 2002, USEPA 2003), metabolism (Fry 1971, Beiting and Fitzpatrick 1979), development, run-timing in anadromous and potamodromous migration in nonanadromous taxa (Hicks 2002, Beeman et al. 2012), spawning and rearing (USEPA 2001a, USEPA 2003), and availability of food (Ligon et al. 1999). Fluctuations in water temperature can block migration (CDFG 2004), and cause stress and lethality in fish (Elliot 1981, Li et al. 1994, USEPA 2001b, Myrick and Cech 2004, Barthelow 2005) leading to potential for disease in juvenile and adult salmonids (Guillen 2003, Lynch and Risley 2003, CDFG 2004, True et al. 2010). Moreover, depending on the flow release configuration of a dam (e.g., surface-spill, selective gates, or hypolimnetic), thermal characteristics of pulse flows can vary widely and must be considered relative to effects on all aquatic biota (Reiser et al. 2008).

Numerous reports have addressed fluctuations in water temperature in the upper Trinity River, but only in the context of average daily water temperature (Zedonis and Turner 2006, Zedonis and Turner 2008, Zedonis 2009, Scheiff and Zedonis 2010, Scheiff and Zedonis 2012, Magnuson 2013, USBR 2015). This was the metric used to determine potential direct, indirect, and cumulative effects to the affected environment associated with supplemental flow releases from Lewiston Dam in 2015 and 2016 (USBR 2016). Yet there has been no attempt to equate fluctuations in average daily water temperature or extremes in average daily water temperature in the upper Trinity River, to quantify, document, or evaluate potential effects to run-timing or reproductive output in anadromous salmonids associated with very divergent flow regimes initiated in 2003. As such, we do not agree that effects of ROD or Pulse flow augmentations on river stage, in-river water temperature, or the biology of this riverine system are fairly well known (Zedonis 2001), particularly as relates to Chinook Salmon.

Herein, we provide evidence in support our hypothesis (H_2) that annually managed

flow regimes implemented by the ROD differ significantly among flow-types, which has altered average daily water temperature, and extremes in water temperature and volume of flow in the upper Trinity River. Further, we maintain that a major issue associated with declining stocks of Chinook Salmon is in part a function of extreme fluctuations in seasonal and daily maximum and minimum water temperatures associated with managed hydrographs initiated in 2003, in conjunction with diversions of water through Lewiston Dam into the Trinity River Division of the Central Valley Project (CVP). For example, from 2001 to 2017, 21,690,290 acre-feet of water was released from Lewiston Lake, of which 49.5% was diverted into the upper Trinity River below Lewiston Dam, and 50.5% was diverted to the CVP (Sacramento River, TRRP 2019), a river system never connected to the Klamath River Basin, but is part of the inter-basin water transfer program. Yet we are unaware of any analyses that address the overall effects to variable water temperature on migration timing, reproductive performance, or spawning of hatchery-origin or in-river natural-origin salmonids from this diversion policy relative to water released into the upper Trinity River.

To assist in this process we provide evidence in support of our hypothesis (H_3) that annually managed flow regimes implemented by the ROD may effect hatchery-parental broodstock fertility associated with egg production at the hatchery, which includes both hatchery-origin and potentially an unknown number of “wild” natural-origin fish mixed in with the hatchery egg collection. For example, GAM analyses revealed 1) the potential importance of highly variable water temperatures in relation to a declining trend in AAPF in both races of hatchery-origin Chinook Salmon eggs, 2) significant differences in fluctuations in average daily water temperatures between flows, and 3) extreme fluctuations in maximum and minimum water temperatures on a daily or prolonged punctuated weekly basis. That eggs of both races of hatchery-reared Chinook Salmon exhibited concordant declining trends in AAPF in response to post-PreROD hydrological regimes suggests that these seasonally and genetically differentiated races of Chinook Salmon are tracking conditions in the upper Trinity River in parallel (Kinziger et al. 2013, USBR 2015). Viewed collectively, these results lead us to hypothesize that reduction in fertility in eggs of hatchery-reared Chinook Salmon is likely a function of extreme fluctuation in daily water temperature within the upper Trinity River, exacerbated by hydrological events attributable to annually managed flows. We believe that these issues need further investigation, particularly as relates to the potential effects on in-river 1) female reproductive viability in all salmonid populations in the upper Trinity River, including delayed maturation of females and reduced average annual fertility, and 2) spawning by hatchery-origin and natural-origin fish.

Additionally, evidence suggests that in-river hatchery-origin and natural-origin fish appear particularly susceptible to extreme fluctuations in water temperature. For example, from 2015 to 2017, in-river natural-origin spawning Chinook Salmon produced the lowest redd count and fewest Salmon carcasses recovered since inception of carcass surveys in 2002 (Gough and Rupert 2016, Rupert et al. 2017a, Rupert et al. 2017b). Although natural-origin Chinook Salmon spawn throughout the mainstem Trinity River, redds constructed by hatchery-origin fish were highly skewed toward the hatchery, as the number of hatchery-origin redds has decreased since 2002 throughout the mainstem and within TRRP restoration reaches (Rupert et al. 2017a and Rupert et al. 2017b). “Natural-origin” fish are those individuals that emerge as juveniles from each redd, as opposed to hatchery-reared individuals (Rupert et al. 2017a and 2017b). However, for the Trinity River, there is no accounting for 1) hatchery-origin fish not adipose fin clip marked in the 25% constant fractional pre-lease marking; or

2) Chinook Salmon that emerge from redds produced by ‘hatchery-origin’ parental stocks. Potential factor(s) responsible for reproductive trends described herein remain to be identified by more refined examination and experimentation, particularly as relates to in-river production of juvenile salmonids based on 1) quality of redds and 2) hatchery-origin versus natural-origin spawning (Hughes and Murdoch 2017). This need is timely, since from 2015 to 2017 total hatchery returns, CWT counts, and expanded estimates derived from CWT fish were the lowest recorded since 1994 for both races of Chinook Salmon (CDFW 2017a and CDFW 2017b). Similarly, natural-origin redd abundance, predicted to increase following restoration actions (TRRP and ESSA 2009, Rupert et al. 2017a), has not changed significantly from 2002 to 2016. Instead, natural-origin Chinook Salmon have reduced spawning activity in upper reaches of the mainstem and shifted spawning into more mid-river sections below restoration reaches, with no clear post-restoration response to TRRP rehabilitation sites with respect to abundance of natural-origin redds (Rupert et al. 2017a and Rupert et al. 2017b).

Implications of future returns in progeny in relation to flow-types.—Logistical constraints associated with management operations at hatcheries are integral to coordination and successful in-river management and restoration activities associated with overall viability of anadromous salmonid stocks in the upper Trinity River. Historically, such integration and coordination of hatchery operations has not been part of the Record of Decision (ROD) for the TRRP or a significant component of the long-term plan to protect salmonids in the Klamath River Basin (USBR 2015, USBR 2016). Unlike direct effects of ROD flows and Pulse flow augmentations on run-timing, effects of managed flows that cause altered and highly variable water temperature regimes that potentially impact reproductive output are not manifested in annual counts of Chinook Salmon until age 2-, 3-, 4-, and 5-year old fish. For example, 24,374 spawning Chinook Salmon returned to the in 2012. This cohort was comprised of individuals from brood-years 2007 through 2010; however, returning progeny of this cohort comprise brood-years 2014 (age 2), 2015 (age 3), 2016 (age 4), and brood-year 2017 (age 5). Similarly, counts documented in 2015 are in part a reflection of ROD flows and augmented Pulse flows implemented in 2012 and 2013, and progeny produced in 2016 are primarily a reflection of managed flows. In contrast spawning Chinook Salmon in 2017 will be entirely a reflection of managed flows with returning progeny in 2019 through 2022.

Thus, we hypothesize that flow management resulting in highly variable water temperatures that potentially affect AAPF in consecutive ROD and Pulse flows since 2012 will continue to influence annual counts of 2-, 3-, 4-, and 5-year old fish through 2022. Similarly, 3 to 4 brood-year cycle shifts and delays in response time also occurred in the distribution of redd counts in response to restoration activities in the upper Trinity River (Rupert et al. 2017a and Rupert et al. 2017b). We agree that the final judgment of success or failure of flow manipulations in managed river systems may ultimately be the actual numbers of returning naturally produced salmonids to the Trinity River and Klamath River Basin in general (Zedonis and Newcomb 1997). However, we also view hatchery-produced stocks as an integral baseline for performance-based comparisons with natural-origin stocks in evaluating the overall status of spring- and fall-run Chinook Salmon in the upper Trinity River. Development and enhancement of juvenile salmonid rearing habitat, as well as increasing numbers of juvenile salmonids produced within the upper Trinity River is a mandate of the TRRP. However, that mandate does not preclude the necessary requirement of assessing the long-term effects of managed ROD flows

and pulsed augmentation flows on run-timing or reproductive performance of hatchery spring- and fall-run Chinook as part of TRRP management actions.

Such issues have historically not been part of the overall effects analysis of the USBR Long-term Plan to Protect Salmon in the lower Klamath River EIS (USBR 2016), even though flows designed to facilitate such protection originate in the upper Trinity River. As of 25 July 2016, there are plans to address these issues for adult salmonids in the upper Trinity River or as part of any proposed environmental impact assessment (M. Paasch, personal communication, 2016). Further, in the most recent effects analysis, potential alteration of baseline PreROD pattern of run-timing in spring-run and fall-run Chinook Salmon, as well as sympatric populations of listed Coho Salmon and steelhead, were not examined in the most recent National Environmental Protection Act finding of no significant impact (USBR 2015, USBR 2016). Comparing the frequency distribution of migration parameters before, during, and after ROD and Pulse flow events would seem critical in discerning if a change in flow acts as a temporary stimulus or retardant to migration, or acts to alter the post-dam baseline seasonal run-time distribution pattern that we address herein. We contend that it is imperative that these issues be incorporated into future management and effects analyses of the overall impact of managed hydrographs associated with the upper Trinity River, particularly given the historically small run-size recorded for both races of Chinook Salmon since 2015.

Management recommendations.—Effective evaluation of annually managed flows and pulse flow augmentation on anadromous salmonids requires carefully designed field studies that include appropriate controls, which test specific hypotheses relevant to anadromous salmonids and their life history requirements. An integrated strategy includes comparative analyses and synthesis of potential impacts of managed flow regimes on timing of migration, population size, age structure, reproductive output, and survival of juvenile outmigrants (Peterson et al. 2017, Cyril 2018). Additionally, similar studies conducted concurrently on non-anadromous “control” species not affected by marine conditions may provide additional insight to flow management not observed in anadromous taxa (Sullivan and Hileman 2018). Close inspection of historical flow and project operational records in tandem with long-term biological data can provide insight into potential effects of planned management of pulse flow augmentation. A comparison of frequency distributions of migration parameters before, during, and after pulsed augmentation flows would help determine if a change in flow regimes acts either as a temporary stimulus or as a retardant to migration. Further, integration and coordination of experimental hatchery management and stocking practices with planned flow management, implemented of restoration actions, and other geomorphological events is crucial to this process. Comparative experimental studies on hatchery-raised fish conducted simultaneously with in-river natural-area spawning hatchery and “wild” fish would allow better understanding of relative annual population productivity in combination with managed flow releases used for conservation purposes in all relevant and connected segments of this highly regulated river system (Hughes and Murdoch 2017). Investigations of reproductive viability in female in-river Chinook Salmon from both hatchery parental brood-stocks and natural-area spawning fish will require refined methods of experimentation.

Long-term datasets that include a wide range of environmental conditions are particularly effective in allowing model linkages between environmental quality and biology of fish occurrence and production if rehabilitation of degraded riverine systems is a desired goal. However, altering measurement parameters used to evaluate reproductive performance (i.e.

AAPF) becomes problematic when it prevents monitoring of historical information critical in assessing trends in population viability, particularly when disconnected from in-river hydrological and geomorphological management of focal species. Herein, the change in measurement parameters used at the hatchery beginning in 2015 dramatically affected use of post-2014 data in adequately assessing the historical sequence we analyzed.

Research of this nature should continue for the longevity of any active large-scale geomorphological restoration program given the multitude of intrinsic and extrinsic co-variables impinging upon this riverine system. This need is particularly relevant given documented fluctuations in influential ocean conditions, climate change, regional drought, random drift, ongoing flow and water management policies, and increased environmental degradation and pollution of watersheds from illegal growing of marijuana throughout the upper Trinity River Basin (Welsh 2011, Kilduff et al. 2015, Rupert et al. 2017a, Rupert et al. 2017b, Mourad et al. 2018). Such actions are an integral part of any coordinated and monitored science-based adaptive management program, which is in large measure the original vision of the Record of Decision (USBR 2000), which outlined a plan for restoration of the Trinity River and its populations of fish and wildlife.

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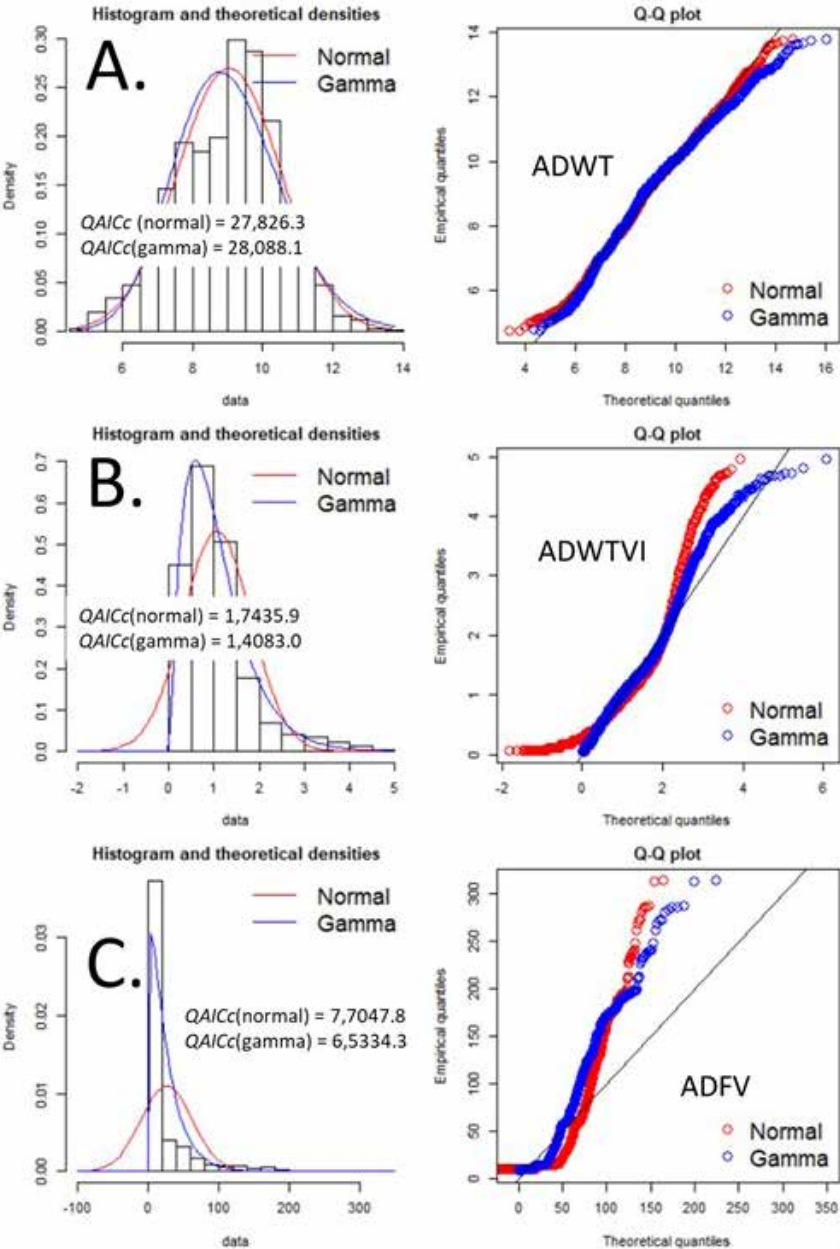
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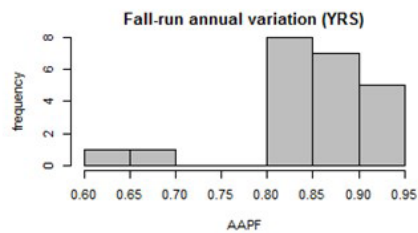
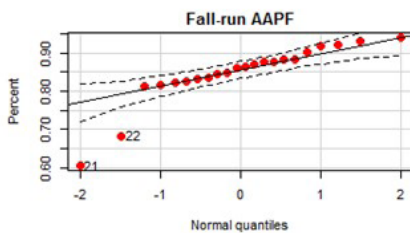
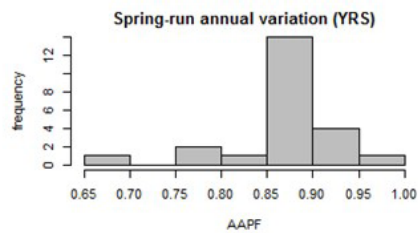
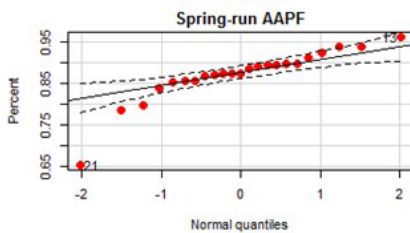
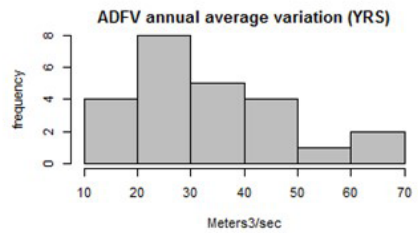
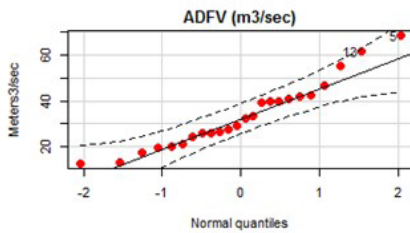
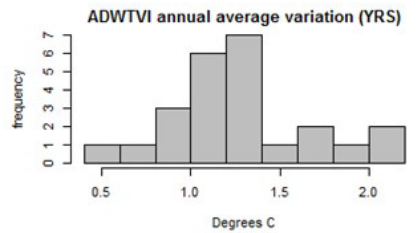
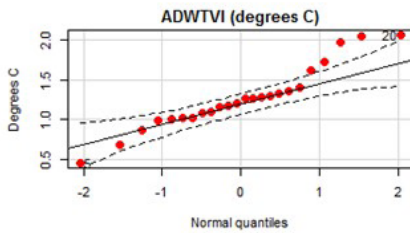
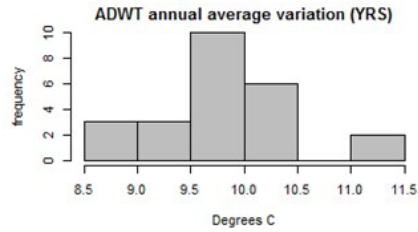
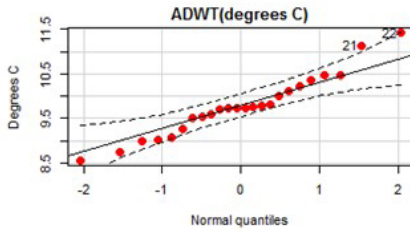
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APPENDIX I.—Normal quantiles plots and histograms of the frequency distribution both annually and seasonally (Julian weeks) of raw data for 1) averaged daily water temperature (ADWT, $n = 4,347$), 2) average daily water temperature variability index (ADWTVI $n = 4,347$), and 3) average daily flow volume (ADFV, $n = 4,458$). Dashed black lines represent 95% confidence limits for the fitted normal quantile plots for each variable. We assume normality if all red points fall approximately along the reference black line.



APPENDIX II.—Normal quantiles plots and histograms of the frequency distribution of average annual values for 1) averaged daily water temperature (ADWT, $n = 24$), 2) average daily water temperature variability index (ADWTVI, $n = 24$), 3) average daily flow volume (ADFV, $n = 24$), and average annual percent fertility (AAPF) for adult female spring-run ($n = 23$) and fall-run ($n = 22$) Chinook Salmon. Dashed black lines represent 95% confidence limits for the fitted normal quantile plots for each variable. We assume normality if all red points fall approximately along the reference black line. Outliers in AAPF represent the smallest annual measurements recorded for each race of Chinook Salmon.



Using eDNA to validate predation on native *Oncorhynchus mykiss* by invasive Sacramento pikeminnow (*Ptychocheilus grandis*)

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Key words: aquatic invasive species, diet, eDNA, non-native fish, *Oncorhynchus mykiss*, predation, qPCR, rainbow trout, steelhead

Traditional methods for assessing fish predation have the potential to underestimate the occurrence of important prey items due to rapid digestion and evacuation rates (Deagle et al. 2005, Ley et al. 2013). Visual examination of gut contents is a common method used to determine fish diet and predation rates of fish (Hyslop 1980, Hartleb and Moring 1995), but in many cases the results only represent a short window of feeding activity. For example, laboratory studies have reported larval and early life stage fish become unidentifiable after less than two hours post-ingestion at water temperatures of 16–20°C (Schooley et al. 2008, Legler et al. 2010).

Initial investigations have demonstrated that genetic analysis can lengthen the gut content detection window for prey items (Ley et al. 2013). Studies analyzing DNA collected from stomach samples have been able to detect and identify larval fish species up to 48 hours after ingestion even when the gut appeared empty (Hunter et al 2012), while juvenile fish DNA has been detected more than 100 hours after ingestion (Brandl et al. 2016). Prey DNA detection half-life (where prey DNA is detected in half of the predators after ingestion) occurred at 26 hours for larval Delta smelt (*Hypomesus transpacificus*) fed to Mississippi silverside (*Menidia audens*) and 66 hours for whole juvenile Chinook salmon (*Oncorhynchus tshawytscha*) fed to striped bass (*Morone saxatilis*), under controlled temperatures (18°C) using qPCR DNA analysis (Brandl et al. 2016).

In this study, we assessed evidence for predation by non-native Sacramento pikeminnow (*Ptychocheilus grandis*) (hereafter referred to as “pikeminnow”) on California red-legged frog (CRLF) (*Rana draytonii*) and steelhead/rainbow trout (*Oncorhynchus mykiss*) in Chorro Creek using traditional assessments and genetic analysis of samples collected from pikeminnow stomachs.

Chorro Creek is a tributary to Morro Bay on the California central coast (Figure 1). The watershed drains 111 km² and provides important habitat for two federally listed aquatic species, CRLF and *O. mykiss*. The Chorro Creek watershed has several factors that provide a higher potential for *O. mykiss* recovery and resiliency than in other nearby watersheds, including perennial and continuous flows in the mainstem downstream of a waste water treatment plant that provides year-round migratory connectivity to a productive estuary (Morro Bay), dense riparian canopy, moderate summer water temperatures, and a relatively small urban footprint. However, Chorro Creek also supports a self-sustaining population of non-native pikeminnow, which have been reported to prey on juvenile *O. mykiss* and frogs (Brown and Brasher 1995, Brown and Moyle 1996, Nakamoto and Harvey 2003). The presence of pikeminnow in the Chorro Creek watershed may inhibit *O. mykiss* recovery by reducing juvenile survival through predation and competition for food and habitat. Although other non-native fish species have been observed in Chorro Creek (e.g., largemouth bass [*Micropterus salmoides*]), they have not established stable populations and are only rarely observed (D. Michniuk, CDFW, pers. comm. 2017). There are no native predators to *O. mykiss* in Chorro Creek.

Pikeminnow larger than 200 mm (Standard Length, [SL]) feed almost exclusively on fish and crayfish (Brown and Brasher 1995). Pikeminnow typically reach this size by the end of their third year (Moyle 2002). Moreover, as juveniles, pikeminnow have a diet and habitat distribution similar to juvenile *O. mykiss*, leading to likely competition for food



FIGURE 1.—Chorro Creek watershed and Sacramento pikeminnow sample locations.

between juveniles of these two species. Reese and Harvey (2002) found a reduction in *O. mykiss* growth of more than 50% when pikeminnow were present compared to growth without pikeminnow.

The ability to identify fish prey items using visual examination of pikeminnow stomachs is expected to be limited by their high digestive rates. Pikeminnow have rapid digestion rates with gastric evacuation times reported to range from 36 hours at 10°C, 17 hours at 15°C, and 14 hours at 20°C after consuming juvenile Chinook salmon (*Oncorhynchus tshawytscha*) (Vondreck 1987). This suggests that in Chorro Creek, where water temperatures range from approximately 10°C during the winter to 20°C during the summer (Kitajima 2016), a juvenile *O. mykiss* consumed by a pikeminnow would have exited the stomach after about 17 hours and the ability to visually detect a fish prey item and identify it to species would be significantly shorter.

Pikeminnow were captured from pool habitats within four sections of Chorro Creek in fall 2017 and spring 2018 (Figure 1 and Table 1). Sampling efforts during fall were conducted to coincide with low flows in Chorro Creek, while spring efforts were conducted to coincide with *O. mykiss* fry emergence (based on visual observations from snorkel dives). Piscivorous-size (> 200 mm SL) pikeminnow were targeted by angling with lures that imitate juvenile *O. mykiss* or were captured using spearfishing. Only pikeminnow greater or equal to 165 mm SL were retained for analysis. After capture, the location, species, fish length (SL), and sample ID were recorded. All captured pikeminnow were euthanized (following AVMA [2013] guidelines), measured, and processed. To conduct stomach analysis and collect DNA samples, biologists used a pair of sterile gloves before processing fish, and gloves were disposed of after handling each fish. A sterile scalpel was used for the initial cut into the body cavity, after which the scalpel was disposed of. Once the cavity was open, a sterile scalpel was used to remove and then open the stomach. All recognizable prey items were recorded. Large items were removed, and the stomach and intestines were rinsed with 95% ethanol. Runoff from the stomach and intestine rinsing was captured in a 5 mL Eppendorf tube and stored on ice for DNA analysis (described below). Each fish was processed with new sterile items (i.e., gloves and scalpels). A single stomach sample was also obtained from a Sacramento sucker (*Catostomus occidentalis*) incidentally captured during fall 2017. This fish was used as a control sample during analysis because Sacramento sucker are not piscivorous, and therefore would not be expected to contain DNA from *O. mykiss* or CRLF.

Vials containing the stomach contents were wiped down with 10% bleach and 70% ethanol twice to remove DNA from the outside of the container. The stomach content samples were thoroughly mixed, and 2 mL of the sample was poured into a new 5 mL vial for DNA extraction. One stomach sample included a fish which could not be visually identified to species; therefore, two DNA extraction samples were collected from this stomach: a tissue sample of the unknown fish and the ethanol rinse runoff from the stomach. Samples were placed under a laminar flow hood to evaporate all ethanol from the samples. Three negative extract controls were created using fresh 100% ethanol and followed the same extraction procedure with the field samples to evaluate any potential cross-contamination between samples. Once the ethanol was evaporated, DNA was extracted from the samples using Qiagen's DNeasy Blood and Tissue Kit, modifying the manufacturer's protocol by using a larger volume of the initial buffer and lysing solutions to account for the larger volume of starting material. Each DNA extract was eluted into 200 µL of AE buffer solution and stored at -20°C until further analysis.

TABLE 1.—Visual and DNA detections of prey items found in stomach samples collected from Sacramento pikeminnow in Chorro Creek, California during fall 2017 and spring 2018.

Sample date	Capture location ^a	Species	Standard length (mm)	Visual assessment of gut contents ^b	<i>O. mykiss</i> DNA copies detected ^c
Fall 2017 sampling effort					
9/9/17	CCER	Pikeminnow	385	crayfish	No
9/9/17	CCER	Pikeminnow	335	empty	Yes (10.2)
9/9/17	CCER	Pikeminnow	345	crayfish	No
9/9/17	CCER	Pikeminnow	280	crayfish	No
9/9/17	CCER	Pikeminnow	238	crayfish	No
9/9/17	CCER	Pikeminnow	340	empty	No
9/9/17	CCER	Pikeminnow	370	empty	No
9/9/17	CCER	Pikeminnow	325	empty	Yes (27.1)
9/9/17	CCER	Pikeminnow	220	empty	No
9/13/17	CalPoly	Pikeminnow	390	empty	No
9/13/17	CalPoly	Pikeminnow	285	crayfish	Yes (3.8)
9/13/17	CalPoly	Pikeminnow	260	empty	No
9/13/17	CalPoly	Pikeminnow	287	crayfish	No
9/13/17	CalPoly	Pikeminnow	285	crayfish	No
9/14/17	CalPoly	Sacramento sucker	195	empty	No
Spring 2018 sampling effort					
4/20/18	CalPoly	Pikeminnow	220	empty	No
4/20/18	CalPoly	Pikeminnow	355	empty	Yes (9.0)
4/20/18	CalPoly	Pikeminnow	200	empty	No
5/4/18	CalPoly	Pikeminnow	290	empty	No
5/4/18	CalPoly	Pikeminnow	280	crayfish & beetle	No
5/4/18	CalPoly	Pikeminnow	270	crayfish	No
5/4/18	CalPoly	Pikeminnow	360	multiple crayfish	No
5/8/18	CalPoly	Pikeminnow	325	crayfish	No
5/9/18	Camp SLO	Pikeminnow	250	crayfish parts	Yes (6.3)
5/9/18	Camp SLO	Pikeminnow	280	whole crayfish	Yes (3.5)
5/9/18	Camp SLO	Pikeminnow	300	crayfish parts	No
5/9/18	Camp SLO	Pikeminnow	360	crayfish	No
5/9/18	Camp SLO	Pikeminnow	300	crayfish	No
5/9/18	Camp SLO	Pikeminnow	320	crayfish	No
5/9/18	Camp SLO	Pikeminnow	290	empty	No
5/9/18	Camp SLO	Pikeminnow	280	crayfish	No
5/9/18	Camp SLO	Pikeminnow	280	empty	No

TABLE 1 continued.

Sample date	Capture location ^a	Species	Standard length (mm)	Visual assessment of gut contents ^b	<i>O. mykiss</i> DNA copies detected ^c
5/9/18	Camp SLO	Pikeminnow	248	empty	No
5/10/18	Water Treatment	Pikeminnow	305	crayfish	No
5/10/18	Water Treatment	Pikeminnow	315	unidentified fish	Yes (85,350) ^d
5/10/18	Water Treatment	Pikeminnow	322	crayfish	No
5/10/18	Water Treatment	Pikeminnow	270	empty	No
5/10/18	Water Treatment	Pikeminnow	290	empty	No
5/10/18	Water Treatment	Pikeminnow	220	empty	No
5/10/18	Water Treatment	Pikeminnow	165	empty	No
5/10/18	Water Treatment	Test Blank	na	na	No

^a Locations shown on Figure 1.

^b Visual assessment of gut contents includes items identified visually from dissected fish stomachs.

^c Number of DNA copies detected is included in parenthesis for positive *O. mykiss* detections.

^d Value reported is from stomach sample, a tissue sample from the unidentified fish was also analyzed and was positive for *O. mykiss* DNA with 495,500 DNA copies detected.

Species-specific DNA markers were used to assess the presence of *O. mykiss* (Brandl et al. 2015) and CRLF (Halstead et al. 2018) in the stomach samples. Markers were developed from mitochondrial genes cytochrome oxidase 1 and cytochrome b for *O. mykiss* and cytochrome b for CRLF. Brandl et al. (2015) and Halstead et al. (2018) demonstrated that their marker sets were specific to the targeted species and do not amplify DNA from closely related species that may occur within the study area. All assays used TaqMan MGB probes (Life Technologies) in singleplex reactions on a StepOnePlus quantitative PCR machine (Applied Biosystems). To ensure maximum detection, we optimized primer and probe concentrations for our assay protocol using a synthetic double-stranded DNA (gBlock) (Integrated DNA Technology, San Diego), created from the targeted species' DNA sequences available from the NCBI database. The primer and probe concentrations for each species-specific marker was optimized for use in a 25 μ L reaction using the qPCR thermal cycles 50°C for 2 minutes, 95°C for 10 minutes, and 50 cycles of 95°C for 15 seconds and 60°C for 1 minute, as follows:

O. mykiss optimized reaction: 5 μ L of template DNA, 0.6 μ M forward primer, 0.3 μ M reverse primer, 0.2 μ M probe, 1x Taqman Environmental Master Mix 2.0.

CRLF optimized reaction: 5 μ L of template DNA, 0.3 μ M forward primer, 0.6 μ M reverse primer, 0.25 μ M probe, 1x Taqman Environmental Master Mix 2.0.

Each marker was tested on tissue from the targeted species to confirm positive detec-

tion. Trials were performed to test the sensitivity of each marker using the gBlock with the optimized primer and probe concentrations. Serial 1:5 dilutions were tested in duplicate using reactions with a high concentration, that ranged from over 20 million DNA copies ($5\text{E}-03$ ng/reaction) for both species down to 27 thousand DNA copies ($5\text{E}-06$ ng/reaction) for CRLF and 35 thousand DNA copies ($5\text{E}-06$ ng/reaction) for *O. mykiss*, and a low concentration, that ranged from approximately 30 copies ($5\text{E}-09$ ng/reaction) to less than one DNA copy per reaction ($5\text{E}-11$ ng/reaction), for both species.

Samples were tested for PCR inhibitors using an internal positive control assay (TaqMan Exogenous Internal Positive Control, Applied Biosystems) in their initial assay. All stomach content samples were tested in duplicate for *O. mykiss* and CRLF. Each assay plate included the set of field samples, three extract negative controls, three negative PCR template controls, and three 1:10 dilution standards run in triplicate from the synthetic DNA fragments. Dilution standards were based on previously described sensitivity trials and included the lowest concentrations that were consistently detected for each marker. Samples were prepared in a clean room and moved into the post-PCR area for loading of the standards. Separate laboratory equipment was dedicated to either the pre- or post-PCR stations, which were in separate rooms to avoid potential introduction of high copy number material (synthetic gBlock DNA and amplicon) into field or controls samples. A sample was considered positive if either replicate displayed amplification before 45 cycles. Fifty cycles were included in the PCR cycle protocol to visually confirm that any positives after 40 cycles developed a complete amplification curve.

Stomach samples were analyzed from 39 pikeminnow captured in Chorro Creek, including 14 fish captured in fall 2017, and 25 fish captured in spring 2018. Captured pikeminnow ranged in size from 165 to 390 mm (SL). Based on growth rates reported in Moyle 2002, the size range of these fish correlates to fish ranging from two-years to over five-years in age. Visual observations of pikeminnow stomach contents generally identified stomach contents as either empty or containing crayfish. Only one pikeminnow was observed to have a fish in its stomach but the fish could not be visually identified to species due to the level of digestion.

Trials to test sensitivity showed positive detections during each of the high concentration dilution series replicates for both CRLF and *O. mykiss*. The low DNA concentration dilutions detected CRLF with only one DNA copy present in the reaction, whereas *O. mykiss* presence was detected with only four DNA copies present (Table 2). The positive control tissue samples from *O. mykiss* and CRLF amplified using their respective marker. The standard curves for all runs had efficiencies between 80-102%, $R^2 \geq 0.98$, and intercepts between 38 and 41 cycles.

Oncorhynchus mykiss DNA was detected in seven of the thirty-nine pikeminnow stomach samples (18%) (Table 1), confirming pikeminnow predation of *O. mykiss* in Chorro Creek. Crayfish parts were visually observed in 20 of the pikeminnow stomach samples (51%). The stomach sample from the pikeminnow with the fish in its stomach that could not be visually identified had the highest number of *O. mykiss* DNA copies detected while the tissue sample from this fish amplified nearly 500,000 DNA copies (Table 1). The proportion of pikeminnow stomach samples with positive detections for *O. mykiss* was similar in spring (21%) and fall (16%) (Table 3). No samples were inhibited, and all positive samples amplified before 38 cycles. No extract controls or negative template PCR controls were positive for *O. mykiss* DNA. No stomach contents were positive for CRLF.

TABLE 2.—Summary of the detection trials for low concentrations of the targeted synthetic DNA sequences (gBlock) for CRLF and *O. mykiss*. Values represent the number of DNA copies or ng of DNA per qPCR reaction. Each DNA concentration was tested in duplicate and any detection is listed with its associated Ct value(s).

Detection Trial	DNA Concentration	Detection ^a
CRLF		
#1	27 DNA copies (5E-09 ng)	Yes (Ct: 34.0; 33.2)
#2	14 DNA copies (2.5E-09 ng)	Yes (Ct: 33.7; 32.8)
#3	3 DNA copies (5E-10 ng)	Yes (Ct: 35.5; 35.0)
#4	1 DNA copy (2.5E-10 ng)	Yes (Ct: 36.8; 34.8)
#5	<1 DNA copy (5E-11 ng)	Yes (Ct: 36.7)
<i>O. mykiss</i>		
#1	35 DNA copies (5E-09 ng)	Yes (Ct: 33.7; 35.1)
#2	18 DNA copies (2.5E-09 ng)	Yes (Ct: 34.7; 36.0)
#3	4 DNA copies (5E-10 ng)	Yes (Ct: 37.0)
#4	2 DNA copies (2.5E-10 ng)	No
#5	<1 DNA copy (5E-11 ng)	No

^a Positive detections are listed with the associated Ct value for each positive detection out of two replicates

In this study, visual gut observations revealed no *O. mykiss* in pikeminnow stomachs, but DNA detections found *O. mykiss* DNA in 7 of 39 pikeminnow stomach samples. Visual assessments found crayfish in twenty pikeminnow gut samples (51%), but crayfish exoskeletons and statoliths are more resistant to digestion than fish parts, requiring nearly twice as long to digest compared to fish (Schneider 1973). This suggests that studies using only a visual examination of the gut contents may bias the extent of predation on specific prey items based on the type of prey item being assessed and the time of sampling.

Results of this study suggest that genetic analysis of fish diet items is more reliable than the use of visual analysis. The methods of detection used in this study, allowed us to detect individual species from a slurry of mixed prey items found in stomach samples with eDNA while, historically, DNA analysis of stomach content required pieces of tissue from prey items and each piece had to be tested individually to identify the specific prey item. While visual observation may only provide reliable detection of prey items within a few hours of consumption (Schooley et al. 2008, Legler et al. 2010), qPCR can detect prey items consumed for up to a few days (Hunter et al 2012, Brandl et al. 2016). The ability to significantly extend the detection period of specific prey items makes qPCR a valuable technique for assessing fish diet and predation.

Researchers are still grappling with the potential for false positive and false negative eDNA results. Several recent papers have modeled the likelihood of false positives and negatives using eDNA (Lahoz-Monfort et al. 2015, Guillera-Aroita et al. 2017, Dorazio and Erickson 2017). Each of these papers explored a different approach and mathematical model, depending on the experimental design (e.g., whether they had known positive and

TABLE 3.—Proportion of Sacramento pikeminnow stomach samples with positive detections of *O. mykiss* DNA during fall 2017 and spring 2018 collection efforts in Chorro Creek, California.

Effort	Pikeminnow stomach samples	<i>O. mykiss</i> Detections	Proportion of samples with positive detections
Fall 2017	14	3	21%
Spring 2018	25	4	16%
Total	39	7	18%

negative field samples). A widely accepted model to accurately estimate the likelihood of false positives and false negatives has not yet been established.

In this study, the potential for false positives was controlled for by using sterile single use equipment at each step of eDNA sample collection and negative sample controls were used at each step of the eDNA process. None of the negative controls (i.e., stomach content DNA extraction or stomach content PCR template controls) were positive for any of the qPCR assays. This indicates that there was no contamination in the samples and provides confidence in the positive samples. The potential for false negatives were reduced by using highly sensitive markers and by processing samples in duplicate. Since DNA in stomach samples is expected to be much more concentrated than DNA in water samples, duplicate processing seemed sufficient to detect our target specie. However, since some of the samples had a positive detection for only one out of the two replicates, there is potential that additional samples may have had a positive detection if a third round of replication had been performed.

Pikeminnow predation rates appeared similar between seasons, although the overall sample size was low. Predation rates are likely to vary throughout the year in response to seasonal shifts in water temperature, metabolic rates, and variation in juvenile *O. mykiss* abundance associated with periods of outmigration and fry emergence. DNA detections do not indicate the size or number of prey items consumed. Therefore, positive DNA detections may have resulted from consumption of one *O. mykiss* or multiple *O. mykiss* per pikeminnow. Based on the high predation observed in this study, the *O. mykiss* population size in the watershed is likely being limited as a direct result of pikeminnow predation. Furthermore, individuals that remain are likely those that have found refuge in available cover or reside in habitat less optimal for pikeminnow, such as shallow runs and riffles.

There is a remote potential for *O. mykiss* DNA to end up in crayfish which may scavenge on dead *O. mykiss*. However, the likelihood of this occurring is expected to be extremely low because it requires a crayfish to find and consume a dead *O. mykiss*, for that crayfish to be consumed by a pikeminnow, and for the *O. mykiss* DNA to remain intact after being in two digestive tracts. The likelihood of this occurring once, let alone on seven separate occasions, is unlikely. Crayfish parts were observed in seventeen pikeminnow stomach samples where *O. mykiss* DNA was not detected, and crayfish were observed in three of the seven samples that had positive detections for *O. mykiss* DNA.

Our results did not detect any CRLF from the pikeminnow samples. Frogs are reported to be an important prey item for pikeminnow in the Eel River (Brown and Moyle 1996). During a prior study, a frog was found in the stomach of a pikeminnow within Chorro Creek, although, it could not be visually identified to species (Stillwater Sciences, unpublished data). CRLF tadpoles and egg-masses are most susceptible to predation and were

likely present at the locations sampled during the spring but not during the fall. Tadpoles and eggs are expected to digest at a higher rate than *O. mykiss* because of their soft bodies, which may make it more difficult to detect these prey items through DNA techniques and nearly impossible through traditional visual techniques. Additionally, CRLF may be less abundant than *O. mykiss* in this system based on fish monitoring efforts (Stillwater Sciences 2017 and 2018). It is possible that we may have detected CRLF in pikeminnow stomachs if we had a larger sample size.

Further research to determine DNA detection half-life specific to *O. mykiss* consumed by pikeminnow would help estimate predation rates for these predator-prey interactions. Our method was successful for use on non-listed species which can be sacrificed, but a modified approach may be required when sacrificing the predators is not feasible or desirable. For example, others have applied similar techniques to samples collected using nonlethal methods such as gastric lavage on species of concern (Barnett et al. 2010) to detect prey components.

The use of DNA samples can be used to assess predation of *O. mykiss* by pikeminnow and may be more reliable than visual examination of gut contents. In this study, pikeminnow predation levels on *O. mykiss* are likely substantial based on the proportion of positive DNA samples while the visual inspections suggested otherwise.

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