ONCORHYNCHUS MYKISS SCALE COLLECTION AND AGE DETERMINATION STUDY REPORT DON PEDRO PROJECT FERC NO. 2299











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Oncorhynchus mykiss Scale Collection and Age Determination Study Report

TABLE OF CONTENTS Section No. Description Page No. 1.0 INTRODUCTION......1-1 1.1 Background1-1 Relicensing Process1-3 1.2 1.3 2.0 STUDY GOALS AND OBJECTIVES......2-1 3.0 4.0 4.1 4.2 4.3 5.0 5.1 5.2 6.0 7.0 STUDY VARIANCES AND MODIFICATIONS......7-1 8.0

List of Figures

Figure No.	Description	Page No.
Figure 1.1-1.	Don Pedro Project location.	1-2
Figure 4.1-1.	Fish schematic showing area (oval) where scale samples were taken from fish (modified from Columbia Basin Fish and Wildlife Authority 1999).	m 4-2
Figure 5.1-1.	<i>O. mykiss</i> age-at-length relationship for the lower Tuolumne Rive between RM 52 and 30.	er 5-2
Figure 5.2-1.	Relationship between scale radius and fork length for <i>O. mykiss</i> collected in this study.	ed 5-3
Figure 5.2-2.	Incremental growth rate between annuli of <i>O. mykiss</i> collected in th study	iis 5-4

Figure 6.0-1.	Age-at-length data from Zimmerman et al.'s (2009) analysis of Tuolumne River <i>O. mykiss</i> otoliths. Note the four-year age class includes all fish four years old and older.	. 6-1
Figure 6.0-2.	Combined age-at-length relationship from <i>O. mykiss</i> otoliths and scales in Zimmerman et al. (2009) ages 1–3 fish and this study	. 6-3
Figure 6.0-3.	Mean and standard deviation growth exhibited by cohort-year (i.e., the year in which the fish was hatched) and by age for the three age groups of <i>O. mykiss</i> sampled from the lower Tuolumne River for this study in 2012	. 6-4
Figure 6.0-4.	Estimated growth by age for four age groups of <i>O. mykiss</i> collected for this study in the lower Tuolumne River.	. 6-5

List of Tables

Table No.	Description	Page No.
Table 4.1-1.	O. mykiss scale sampling dates and locations, Tuolumne River, 2012	
Table 5.1-1.	Age and size ranges of <i>O. mykiss</i> in the lower Tuolumne River betwee RM 52 and 30	een 5-1
Table 5.2-1.	Minimum, maximum, and average back-calculated fork length at anr and growth rates to annuli for <i>O. mykiss</i> in the lower Tuolumne River	nuli 5-3
Table 5.2-2.	Back-calculated incremental growth rates between annuli of <i>O. mykiss</i> the lower Tuolumne River.	s in 5-3
Table 6.0-1.	Size ranges of fish in this study (W&AR-20) compared to those report by Zimmerman et al. (2009).	ted 6-2
Table 6.0-2.	Combined Zimmerman et al. (2009) and W&AR-20 age and size ranges <i>O. mykiss</i> .	s of 6-2

List of Attachments

Attachment A Scale Sample Collection, Age, and Growth Data

CDPR	California Department of Parks and Recreation		
CDSOD	California Division of Safety of Dams		
CDWR	California Department of Water Resources		
CE	California Endangered Species		
CEII	Critical Energy Infrastructure Information		
CEQA	California Environmental Quality Act		
CESA	California Endangered Species Act		
CFR	Code of Federal Regulations		
cfs	cubic feet per second		
CGS	California Geological Survey		
CMAP	California Monitoring and Assessment Program		
CMC	Criterion Maximum Concentrations		
CNDDB	California Natural Diversity Database		
CNPS	California Native Plant Society		
CORP	California Outdoor Recreation Plan		
CPUE	Catch Per Unit Effort		
CRAM	California Rapid Assessment Method		
CRLF	California Red-Legged Frog		
CRRF	California Rivers Restoration Fund		
CSAS	Central Sierra Audubon Society		
CSBP	California Stream Bioassessment Procedure		
CT	California Threatened Species		
CTR	California Toxics Rule		
CTS	California Tiger Salamander		
CVRWQCB	Central Valley Regional Water Quality Control Board		
CWA	Clean Water Act		
CWHR	California Wildlife Habitat Relationship		
Districts	Turlock Irrigation District and Modesto Irrigation District		
DLA	Draft License Application		
DPRA	Don Pedro Recreation Agency		
DPS	Distinct Population Segment		
EA	Environmental Assessment		
EC	Electrical Conductivity		

EFH	Essential Fish Habitat
EIR	Environmental Impact Report
EIS	Environmental Impact Statement
EPA	U.S. Environmental Protection Agency
ESA	Federal Endangered Species Act
ESRCD	East Stanislaus Resource Conservation District
ESU	Evolutionary Significant Unit
EWUA	Effective Weighted Useable Area
FERC	Federal Energy Regulatory Commission
FFS	Foothills Fault System
FL	Fork length
FMU	Fire Management Unit
FOT	Friends of the Tuolumne
FPC	Federal Power Commission
ft/mi	feet per mile
FWCA	Fish and Wildlife Coordination Act
FYLF	Foothill Yellow-Legged Frog
g	grams
GIS	Geographic Information System
GLO	General Land Office
GPS	Global Positioning System
HCP	Habitat Conservation Plan
HHWP	.Hetch Hetchy Water and Power
HORB	.Head of Old River Barrier
HPMP	.Historic Properties Management Plan
ILP	Integrated Licensing Process
ISR	Initial Study Report
ITA	Indian Trust Assets
kV	kilovolt
m	meters
M&I	Municipal and Industrial
MCL	Maximum Contaminant Level
mg/kg	milligrams/kilogram

mg/L	milligrams per liter
mgd	million gallons per day
mi	miles
mi ²	square miles
MID	Modesto Irrigation District
MOU	Memorandum of Understanding
MSCS	Multi-Species Conservation Strategy
msl	mean sea level
MVA	Megavolt Ampere
MW	megawatt
MWh	megawatt hour
mya	million years ago
NAE	National Academy of Engineering
NAHC	Native American Heritage Commission
NAS	National Academy of Sciences
NAVD 88	North American Vertical Datum of 1988
NAWQA	National Water Quality Assessment
NCCP	Natural Community Conservation Plan
NEPA	National Environmental Policy Act
ng/g	nanograms per gram
NGOs	Non-Governmental Organizations
NHI	Natural Heritage Institute
NHPA	National Historic Preservation Act
NISC	National Invasive Species Council
NMFS	National Marine Fisheries Service
NOAA	National Oceanic and Atmospheric Administration
NOI	Notice of Intent
NPS	U.S. Department of the Interior, National Park Service
NRCS	National Resource Conservation Service
NRHP	National Register of Historic Places
NRI	Nationwide Rivers Inventory
NTU	Nephelometric Turbidity Unit
NWI	National Wetland Inventory

NWIS	National Water Information System
NWR	National Wildlife Refuge
NGVD 29	National Geodetic Vertical Datum of 1929
O&M	operation and maintenance
OEHHA	Office of Environmental Health Hazard Assessment
ORV	Outstanding Remarkable Value
PAD	Pre-Application Document
PDO	Pacific Decadal Oscillation
PEIR	Program Environmental Impact Report
PGA	Peak Ground Acceleration
PHG	Public Health Goal
PM&E	Protection, Mitigation and Enhancement
PMF	Probable Maximum Flood
POAOR	Public Opinions and Attitudes in Outdoor Recreation
ppb	parts per billion
ppm	parts per million
PSP	Proposed Study Plan
QA	Quality Assurance
QC	Quality Control
RA	Recreation Area
RBP	Rapid Bioassessment Protocol
Reclamation	U.S. Department of the Interior, Bureau of Reclamation
RM	River Mile
RMP	Resource Management Plan
RP	Relicensing Participant
RSP	Revised Study Plan
RST	Rotary Screw Trap
RWF	Resource-Specific Work Groups
RWG	Resource Work Group
RWQCB	Regional Water Quality Control Board
SC	State candidate for listing under CESA
SCD	State candidate for delisting under CESA
SCE	State candidate for listing as endangered under CESA

SCT	State candidate for listing as threatened under CESA
SD1	Scoping Document 1
SD2	Scoping Document 2
SE	State Endangered Species under the CESA
SFP	State Fully Protected Species under CESA
SFPUC	San Francisco Public Utilities Commission
SHPO	State Historic Preservation Office
SJRA	San Joaquin River Agreement
SJRGA	San Joaquin River Group Authority
SJTA	San Joaquin River Tributaries Authority
SPD	Study Plan Determination
SRA	State Recreation Area
SRMA	Special Recreation Management Area or Sierra Resource Management Area (as per use)
SRMP	Sierra Resource Management Plan
SRP	Special Run Pools
SSC	State species of special concern
ST	California Threatened Species under the CESA
STORET	Storage and Retrieval
SWAMP	Surface Water Ambient Monitoring Program
SWE	Snow-Water Equivalent
SWRCB	State Water Resources Control Board
TAC	Technical Advisory Committee
TAF	thousand acre-feet
ТСР	Traditional Cultural Properties
TDS	Total Dissolved Solids
TID	Turlock Irrigation District
TLSRA	Turlock Lake State Recreation Area
TMDL	Total Maximum Daily Load
ТОС	Total Organic Carbon
TRT	Tuolumne River Trust
TRTAC	Tuolumne River Technical Advisory Committee
UC	University of California

- USDOCU.S. Department of Commerce
- USDOIU.S. Department of the Interior
- USFSU.S. Department of Agriculture, Forest Service
- USFWSU.S. Department of the Interior, Fish and Wildlife Service
- USGSU.S. Department of the Interior, Geological Survey
- USR.....Updated Study Report
- UTM Universal Transverse Mercator
- VAMP.....Vernalis Adaptive Management Plan
- VELBValley Elderberry Longhorn Beetle
- VRMVisual Resource Management
- WPTWestern Pond Turtle
- WSA.....Wilderness Study Area
- WSIPWater System Improvement Program
- WWTPWastewater Treatment Plant
- WY.....water year
- $\mu S/cm \ldots microSeimens \ per \ centimeter$

1.0 INTRODUCTION

1.1 Background

Turlock Irrigation District (TID) and Modesto Irrigation District (MID) (collectively, the Districts) are the co-licensees of the 168-megawatt (MW) Don Pedro Project (Project) located on the Tuolumne River in western Tuolumne County in the Central Valley region of California. The Don Pedro Dam is located at river mile (RM) 54.8 and the Don Pedro Reservoir has a normal maximum water surface elevation of 830 ft above mean sea level (msl; NGVD 29). At elevation 830 ft, the reservoir stores over 2,000,000 acre-feet (AF) of water and has a surface area slightly less than 13,000 acres (ac). The watershed above Don Pedro Dam is approximately 1,533 square miles (mi²). The Project is designated by the Federal Energy Regulatory Commission (FERC) as project no. 2299.

Both TID and MID are local public agencies authorized under the laws of the State of California to provide water supply for irrigation and municipal and industrial (M&I) uses and to provide retail electric service. The Project serves many purposes including providing water storage for the beneficial use of irrigation of over 200,000 ac of prime Central Valley farmland and for the use of M&I customers in the City of Modesto (population 210,000). Consistent with the requirements of the Raker Act passed by Congress in 1913 and agreements between the Districts and City and County of San Francisco (CCSF), the Project reservoir also includes a "water bank" of up to 570,000 AF of storage. CCSF may use the water bank to more efficiently manage the water supply from its Hetch Hetchy water system while meeting the senior water rights of the Districts. The "water bank" within Don Pedro Reservoir provides significant benefits for CCSF's 2.6 million customers in the San Francisco Bay Area.

The Project also provides storage for flood management purposes in the Tuolumne and San Joaquin rivers in coordination with the U.S. Army Corps of Engineers (ACOE). Other important uses supported by the Project are recreation, protection of the anadromous fisheries in the lower Tuolumne River, and hydropower generation.

The Project Boundary extends from RM 53.2, which is one mile below the Don Pedro powerhouse, upstream to RM 80.8 at an elevation corresponding to the 845 ft contour (31 FPC 510 [1964]). The Project Boundary encompasses approximately 18,370 ac with 78 percent of the lands owned jointly by the Districts and the remaining 22 percent (approximately 4,000 ac) owned by the United States and managed as a part of the U.S. Bureau of Land Management (BLM) Sierra Resource Management Area.

The primary Project facilities include the 580-foot-high Don Pedro Dam and Reservoir completed in 1971; a four-unit powerhouse situated at the base of the dam; related facilities including the Project spillway, outlet works, and switchyard; four dikes (Gasburg Creek Dike and Dikes A, B, and C); and three developed recreational facilities (Fleming Meadows, Blue Oaks, and Moccasin Point Recreation Areas). The location of the Project and its primary facilities is shown in Figure 1.1-1.



Figure 1.1-1. Don Pedro Project location.

1.2 Relicensing Process

The current FERC license for the Project expires on April 30, 2016, and the Districts will apply for a new license no later than April 30, 2014. The Districts began the relicensing process by filing a Notice of Intent and Pre-Application Document (PAD) with FERC on February 10, 2011, following the regulations governing the Integrated Licensing Process (ILP). The Districts' PAD included descriptions of the Project facilities, operations, license requirements, and Project lands as well as a summary of the extensive existing information available on Project area resources. The PAD also included ten draft study plans describing a subset of the Districts' proposed relicensing studies. The Districts then convened a series of Resource Work Group meetings, engaging agencies and other relicensing participants in a collaborative study plan development process culminating in the Districts' Proposed Study Plan (PSP) and Revised Study Plan (RSP) filings to FERC on July 25, 2011 and November 22, 2011, respectively.

On December 22, 2011, FERC issued its Study Plan Determination (SPD) for the Project, approving, or approving with modifications, 34 studies proposed in the RSP that addressed Cultural and Historical Resources, Recreational Resources, Terrestrial Resources, and Water and Aquatic Resources. In addition, as required by the SPD, the Districts filed three new study plans (W&AR-18, W&AR-19, and W&AR-20) on February 28, 2012 and one modified study plan (W&AR-12) on April 6, 2012. Prior to filing these plans with FERC, the Districts consulted with relicensing participants on drafts of the plans. FERC approved or approved with modifications these four studies on July 25, 2012.

Following the SPD, a total of seven studies (and associated study elements) that were either not adopted in the SPD, or were adopted with modifications, formed the basis of Study Dispute proceedings. In accordance with the ILP, FERC convened a Dispute Resolution Panel on April 17, 2012 and the Panel issued its findings on May 4, 2012. On May 24, 2012, the Director of FERC issued his Formal Study Dispute Determination, with additional clarifications related to the Formal Study Dispute Determination issued on August 17, 2012.

This study report describes the objectives, methods, and results of the *O. mykiss* Scale Collection and Age Determination Study (W&AR-20) as implemented by the Districts in accordance with FERC's SPD and subsequent study modifications and clarifications. On January 17, 2013, the Districts filed the Initial Study Report for the Don Pedro Project. The U.S. Department of the Interior, Fish and Wildlife Service (USFWS) filed comments on the Initial Study Report on March 11, 2013; the Districts replied to study comments on April 9, 2013. The USFWS comment referred to use of the W&AR-20 data in the W&AR-10: O. mykiss Population Study Report; data used in the model are fully described in the W&AR-10 study report. In order to clarify data analyzed in this study, the Districts edited the W&AR-20 O. mykiss Scale Collection and Age Determination Study Report to correct an error regarding the Zimmerman et al. (2009) O. mykiss age classes. Documents relating to the Project relicensing are publicly available on the Districts' relicensing website at www.donpedro-relicensing.com.

1.3 Study Plan

The continued operation of the Don Pedro Project may contribute to cumulative effects to the salmonid fish habitat in the lower Tuolumne River, including the quantity and quality of physical habitat available for *O. mykiss*, potentially affecting populations in the lower Tuolumne River.

As part of the *Oncorhynchus mykiss Population Study* (W&AR-10), the Districts will incorporate fish age and growth analyses into the development of population models, relying primarily on length-frequency analysis (e.g., MacDonald and Pitcher 1979) of *O. mykiss* observed during snorkel surveys of the past several years (e.g., TID/MID 2011). At the request of relicensing participants, the Districts also agreed to collect scales from *O. mykiss* in the lower Tuolumne River downstream of La Grange Dam to refine the age composition and growth estimates as detailed in the W&AR-20 Study Plan. The results of this exercise (age-at-length relationship based on scale analysis) will provide more comprehensive *O. mykiss* length data to develop a representative population age structure as part of the interrelated *O. mykiss Population Study* (TID/MID 2011).

Consistent with the Districts agreement to undertake this study, FERC in its December 22, 2011 Study Plan Determination directed the Districts to file a study plan for FERC approval after consultation with relicensing participants, within 60 days of the SPD. On February 28, 2012, the Districts filed their study plan. FERC subsequently approved the study plan as proposed by the Districts on July 25, 2012. FERC recommended that the Districts collect *O. mykiss* data, including scales, to verify their age and growth, but only if the Districts were able to obtain authorization from NMFS to collect scales from *O. mykiss* in the lower Tuolumne River. The Districts were able to conduct this study by operating under FISHBIO's existing Endangered Species Act (ESA) section 10(a)(1)(a) permit that allowed take of up to 80 *O. mykiss*. The Districts carried out the Scale Collection and Age Determination Study consistent with the FERC-approved study plan.

2.0 STUDY GOALS AND OBJECTIVES

The goal of this study is to use scales to estimate the age-at-length relationship of *O. mykiss* in the lower Tuolumne River. Objectives in meeting this goal include:

- Collecting, preserving, and analyzing O. mykiss scales to estimate ages of individual fish, and
- Developing an age-at-length relationship for the Tuolumne River *O. mykiss* population.

3.0 STUDY AREA

The study area included the Tuolumne River from the La Grange Dam (RM 52) downstream to Robert's Ferry Bridge (RM 39.5). *O. mykiss* were collected by angling in the reach that extended from La Grange Dam to Turlock Lake State Recreation Area (TLSRA) at RM 42. In addition, a single sample was collected from the rotary screw trap (RST) survey near Waterford (RM 30).

4.0 METHODOLOGY

4.1 Sample Collection

The study plan proposed that "length data and scale samples will be obtained from up to 75 fish using 15 individuals per 100 mm size-group (i.e., 50-150 mm, 150-250 mm, 250-350 mm, 350-450 mm, and 450–550 mm) encountered during sampling." Six O. mykiss sampling efforts were conducted by angling from February 13 through April 9, 2012. One O. mykiss was also obtained from ongoing RST monitoring at Waterford during June 2012 (Table 4.1-1). O. mykiss were collected from pool and riffle-tail habitats by angling as required by FISHBIO's ESA Section 10(a)(1)(a) permit. Fish were collected from the 50-150 mm, 150-250 mm, 250-350 mm, 350-450 mm, and 450-550 mm size groups encountered during sampling. However, only two fish (one from the Waterford rotary screw trap) were collected from the 50-150 mm size class, likely due to this cohort being generally too small to take a hook and bait. No fish were captured from the 450–550 mm size group, probably due to the inherent difficulty in catching old fish that are few in number and have experience with hooks. In addition, continuing to try and collect fish to fill in the 50-150 and 450-550 mm size groups would have required capturing large numbers of O. mykiss in the already filled 150-250 mm, 250-350 mm, 350-450 mm categories. That could have potentially resulted in injury, and possibly mortality, to a significant number of fish, so the sampling was halted.

The survey crew recorded the date, location (GPS coordinates), and habitat type at each sampling location. Upon capture, each fish was photographed and transferred to a measurement cradle for positive identification. Data recorded for each fish included fork length (FL, mm), total length (TL, mm), sex (if possible), and any marks that would aid in determining hatchery versus wild origin (e.g., adipose fin clip).

Sample Event	Sample Period	Method	Location
1	February 13	Angling	La Grange Powerhouse to Basso Bridge
2	February 16	Angling	Basso Bridge to TLSRA ¹
3	March 12	Angling	Basso Bridge to TLSRA ¹
4	April 3	Angling	Basso Bridge to TLSRA ¹
5	April 4	Angling	La Grange Dam to Basso Bridge
6	April 9	Angling	Basso Bridge to TLSRA ¹
7	June 2	Trap	Waterford rotary screw trap

 Table 4.1-1.
 O. mykiss scale sampling dates and locations, Tuolumne River, 2012.

¹ Turlock Lake State Recreation Area

In accordance with the study plan, scale sampling was limited to *O. mykiss* greater than 50 mm FL. Removing scales from fish smaller than 50 mm may increase the risk of injury. Scales were removed from the region between the posterior end of the dorsal fin and the lateral line on the left side, roughly two scale rows above the lateral line (Figure 4.1-1) (RIC 1997, Stokesbury et al. 2001). Prior to scale removal, mucous and debris were cleaned from the sampling location for ease in scale processing (Schneider et al. 2000). Scales were removed by scraping a dull knife from the anterior to posterior of the sample area (RIC 1997). Approximately 10 scales were removed per fish, with the fish released immediately following sampling. Knives were cleaned with ethanol between each fish sampled to prevent cross-contamination.

Scales from each fish were placed in individual "Rite in the Rain" envelopes clearly labeled with species, site location, total and fork length, date, condition, and any other applicable information. Envelopes were pressed flat to reduce scale curling and increase analytical accuracy.



This Illustration is based on a fish specimen of 150 mm fork length.

Figure 4.1-1. Fish schematic showing area (oval) where scale samples were taken from fish (modified from Columbia Basin Fish and Wildlife Authority 1999).

4.2 *O. mykiss* Age Analysis

Scales were prepared for analysis by qualified staff according to standard procedures described by Drummond (1966). Scales were transferred from envelopes onto glass slides. The best scales were arranged towards the top of the slide, with all scales oriented the same direction. Care was taken to insure that all scales were laid flat, not curled. A second glass slide was then placed on top and both slides were taped together. Each slide was labeled with the sample identification number and date.

Slides containing scales were examined under a microscope at 25x magnification, and digital images were generated and enhanced for each scale examined using AmScope Corporation's ToupView®Version 3.2 software to improve contrast and make scale annuli more apparent. In general, age was estimated based on the number of annuli on the three best scales from each sample; however, some samples lacked three readable scales, such as in cases where scales had been regenerated (regenerated scales were excluded from the aging analysis). In those instances, fish age was based on the best available one or two scales. Annuli were identified at a 20 degree angle from the anterior-posterior scale axis. The age of fish was determined by counting the number of annuli between the scale focus and the outer margin, as described in DeVries and Frie (1996) and results were recorded in a Microsoft Excel® spreadsheet.

4.3 Growth Determination

Individual fish growth was estimated based on the distance between the scale focus and each annulus along the scales' longest posterior axis. Measurements were made to the nearest micrometer using a calibrated scale for 25x magnification power. Individual fish lengths at previous ages were back-calculated using the Fraser-Lee method, as described in DeVries and Frie (1996).

$$L_i = \left(\frac{L_c - a}{S_c}\right)S_i + a$$

Where:

 $L_{i =}$ back-calculated length of the fish when the *i*th increment was formed, $L_c =$ Fork length of the fish at capture, $S_c =$ scale radius at capture, $S_i =$ scale radius at the *i*th increment, and $\alpha =$ intercept parameter (fish size at time of scale focus development).

A relatively accurate intercept parameter (α) could not be obtained from this study's dataset due to the relatively small overall sample size (n = 47), low numbers of samples in the smallest and largest size classes, and capture method bias (primarily angling); it was therefore necessary to review available literature to obtain a representative intercept parameter. The intercept parameter (α = 36.65) used in this study was obtained from 1,956 rainbow trout (resident *O. mykiss*) collected during electrofishing efforts in the years 1994, 1996, and 1997 on the Sacramento River upstream of Lake Shasta (Glowacki 2003).

5.0 **RESULTS**

5.1 *O. mykiss* Age-at-length

The Districts were able to collect 53 *O. mykiss* for sampling (See Attachment A). Scale samples were obtained from 48 *O. mykiss* collected during the study of which 47 were suitable for analysis (the non-suitable sample contained only regenerated scales). No scales were taken from five fish because sufficient numbers of fish in their size class had already been collected.

Angling was the more successful of the two sampling methods permitted to collect *O. mykiss*, (angling and RST). However, angling is biased toward larger, older age classes. Susceptibility to angling decreases with smaller, typically younger fish. Only two samples were obtained from *O. mykiss* younger than age 2+: (1) an age-1+ fish collected by angling, and (2) an age-0+ fish captured in the Waterford RST; therefore, no size range could be determined for these age classes (Table 5.1-1). No fish from the 450–550 mm size group were captured. Overall, the size of captured fish ranged from 78 mm FL (age 0+) to 450 mm FL (age 4+) and included fish from five age classes (age 0 to age 4) (Table 5.1-1, Figure 5.1-1, Attachment A).

001		
Age	Number Sampled	Fork Length Range (mm)
0+	1	78
1+	1	150
2+	16	194–270
3+	17	267–370
4+	12	365–450

Table 5.1-1.Age and size ranges of O. mykiss in the lower Tuolumne River between RM 52 and
30.



Figure 5.1-1. O. mykiss age-at-length relationship for the lower Tuolumne River between RM 52 and 30.

5.2 Growth Rates

The results of the scale analysis show a strong positive relationship between fish length and scale size (Figure 5.2-1). This relationship allowed for back-calculating fish size from scale data.

Growth rates for *O. mykiss* captured in this study were calculated using the Fraser-Lee method, as described in DeVries and Frie (1996). The growth rates presented in Table 5.2-1 below are based on the back-calculated lengths of individual fish when their annuli were formed (See Attachment A for raw data). Frequency distributions of back-calculated lengths at annuli formation are typically less than the lengths at time of capture (i.e., when the scale was collected) due to the growth of fish between the time of most recent annulus formation and time of scale sampling.



Figure 5.2-1. Relationship between scale radius and fork length for *O. mykiss* collected in this study.

Table 5.2-1.	Minimum, maximum, and average back-calculated fork length at annuli and
	growth rates to annuli for O. mykiss in the lower Tuolumne River.

Age	Back-calculated Fo	ork Length (mm) at nuli	Annual Growth Rate (mm) to Annuli				
	Range	Average	Range	Average			
1	87–127	109	51-90	73			
2	147–212	182	51–92	72			
3	217-291	257	49–94	74			
4	298-382	331	61–98	78			

 Table 5.2-2.
 Back-calculated incremental growth rates between annuli of *O. mykiss* in the lower Tuolumne River.

Annual Growth	Number of Fish at Annuli Age										
Range (mm)	Age-1	Age-2	Age-3	Age-4							
49–60	6	9	6	0							
61–70	11	10	4	2							
71-80	19	14	9	6							
81–90	10	11	6	2							
91–100	0	1	4	2							



Figure 5.2-2. Incremental growth rate between annuli of *O. mykiss* collected in this study.

6.0 DISCUSSION AND FINDINGS

In general, age-at-length datasets often show substantial overlap between cohorts, which is typical in fish populations, while mean age-at-length increases each year. This is due to differences in individual growth rates which may be related to fish density, food resource abundance, water temperature, suspended sediment, disease, environmental stress, territorial competition, or other factors (Harvey et al. 2006, Bjornn and Reiser 1991, Newcombe and Jensen 1996).

A separate age-at-length data set for *O. mykiss* in the Tuolumne River was developed by Zimmerman et al. (2009). These authors analyzed otoliths from 151 fish collected between 1996 and 2008 in an attempt to determine the maternal origin and migratory history of *O. mykiss* found in Central Valley rivers. However, Zimmerman et al. (2009) combined all fish four years old and older into the single four year old age class (Figure 6.0-1). This combining of the oldest age classes limited the study's comparability with the W&AR-20 age and length data to only those fish three years old and younger.



Figure 6.0-1. Age-at-length data from Zimmerman et al.'s (2009) analysis of Tuolumne River *O. mykiss* otoliths. Note the four-year age class includes all fish four years old and older.

The one-to-three year old fish analyzed in this study (W&AR-20) were generally of a smaller size than those collected by Zimmerman et al. (2009) (Table 6.0-1 and Figure 6.0.2). This may be due to differences in the time of sample collection; the fish in this study were collected during the winter and early spring when annuli would be forming and only early season growth occurred, while Zimmerman et al. (2009) samples were collected between October and May when

substantial growth would have followed annulus formation. For example, a two-year old fish captured in March (just after annulus formation) would be smaller than if that same two-year old fish were captured in October to January, following a growing season that extended through the spring summer, and fall.

Dissimilarities in collection methods between this study and Zimmerman et al. (2009) resulted in differences in sample sizes and fish lengths. This study primarily used angling (one RST capture) as a collection method, resulting in a smaller sample size. This is because many fish in the 50-150 mm size class are generally too small to take a hook and bait. No fish were captured from the 450-550 mm size group, probably due to the inherent difficulty in catching old fish that are few in number and have experience with hooks. Zimmerman et al. (2009), on the other hand, was able to employ rotary screw traps, angling, electrofishing, beach seining, and carcass surveys that allowed a larger number and broader range of sizes to be collected.

Due to permitting restrictions, the W&AR-20 sample size was too small to represent the full range of fish lengths at given ages. Therefore, the Zimmerman et al. (2009) and this study's age and fork length data were combined to develop an age-at-length relationship that was based on a larger dataset (Table 6.0-2 and Figure 6.0-2).

Table 6.0-1.	Size ranges of fish in this study (W&AR-20) compared to those reported by
	Zimmerman et al. (2009).

	St	tudy W&AR-20		Zimmerman et al. (2009)				
Age	Minimum FL (mm)	Maximum FL (mm)	No. of Fish	Minimum FL (mm)	Maximum FL (mm)	No. of Fish		
0	78	78	1			0		
1	150	150	1	145	199	37		
2	194	270	16	200	315	37		
3	267	370	17	320	395	37		
4	365	450	12	-	-	-		

Note: Age four fish from Zimmerman et al. (2009) were not included in this table due to that study combining all age four and older fish into the single age four category.

Table 6.0-2.	Combined	Zimmerman	et	al.	(2009)	and	W&AR-20	age	and	size	ranges	of	<i>0</i> .
	mykiss.												

Age	Number Sampled	Fork Length Range (mm)
0	1	78
1	38	145–199
2	53	194–315
3	54	267–395
4	12*	365–450

*Includes only W&AR-20 age four fish.



Figure 6.0-2. Combined age-at-length relationship from *O. mykiss* otoliths and scales in Zimmerman et al. (2009) ages 1–3 fish and this study.

Annual growth appeared consistent and comparable for each of the four years and each of the three age groups of *O. mykiss* collected for this study. Growth exhibited during the first and second years was very similar for all three age groups that dominated the sample (i.e., age 2, age 3 and age 4) (Figure 6.0-3). The mean observed growth during the first year varied less than 3 mm, ranging from 70 mm for age 2 fish (in 2010) to 73 mm for age 3 fish (in 2009). Similarly, mean growth during the second year varied about 2 mm among the three age groups, ranging from 72 mm for age 3 fish in 2010 to 74 mm for age 2 fish in 2011. Annual growth observed for each age group present during 2009 through 2011 was also very similar (Figures 6.0-3 and 6.0-4). Mean annual growth ranged from 74 mm (age 2) to 78 mm (age 4) in 2011, 69 mm (age 4) to 72 mm (age 3) in 2010 and was the same for both the age 3 and age 4 groups in 2009. Growth varied very little among years as well. The combined mean growth for all age groups present ranged from 70 mm in 2010 to 76 mm in 2011.



Figure 6.0-3. Mean and standard deviation growth exhibited by cohort-year (i.e., the year in which the fish was hatched) and by age for the three age groups of *O. mykiss* sampled from the lower Tuolumne River for this study in 2012.



Figure 6.0-4. Estimated growth by age for four age groups of *O. mykiss* collected for this study in the lower Tuolumne River.

7.0 STUDY VARIANCES AND MODIFICATIONS

Consistent with permit requirements, the Districts proposed in their Study Plan that up to 75 fish would be collected. The Districts were able to collect 53 fish using approved sampling methods, of which 48 were sampled. No scales were taken from five fish because sufficient numbers of fish in their size class had already been collected. Permit requirements that limited the collection methods to angling and RST resulted in fewer samples per size group and limited the number of fish collected in the smallest and largest size classes.

The objectives for this study were met; scale data were used to estimate ages of individual fish, and an age-length relationship for the Tuolumne River *O. mykiss* population was developed. In addition, incremental annual growth rates for each age class were developed. The data from this study, and the information from from Zimmerman et al (2009), are sufficient as input for developing a representative population age structure as part of the interrelated *O. mykiss Population Study* (TID/MID 2011).

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STUDY REPORT W&AR-20 ONCORHYNCHUS MYKISS SCALE COLLECTION AND AGE DETERMINATION

ATTACHMENT A

SCALE SAMPLE COLLECTION, AGE, AND GROWTH DATA

W&AR-20	Scale Age	e and Gro	wth Data													
			Longth		from	from	from	from								
			Lengin	radius of	mom pusiess to	mom puclear to	mom	muclear to	length at	length at	length at	length at		Zodyc	Berlan	Artur
	Sex		capture	scale	1st annuli	2nd annuli	3rd annuli	4rth annuli	1st annuli	2nd annuli	3rd annuli	4th annuli	1st vr growth	erowth	erowth	erowth
Sample #	(M/F)	Age	(FL)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)
1	unk	2	250	1.2542316	0.4198873	0.899964			108	190			108	82		
2	unk	3	342	1.5463918	0.3632222	0.816351	1.2694797		108	198	287		108	89	89	
3	F	4	370	2.3705227	0.495193	0.9997602	1.5715656	2.0888396	106	177	258	330	106	71	80	73
4	м	4	365	1.9467634	0.44234	0.9901702	1.4157276	1.7765524	111	204	275	336	111	92	72	61
5	м	4	400	1.904819	0.4627188	0.830736	1.1567969	1.5511868	125	195	257	333	125	70	62	75
6	F	4	390	2.2332774	0.4051786	0.7839847	1.2467034	1.8652601	101	161	234	332	101	60	73	98
7	M	3	370	2.0895828	0.4878926	0.8296092	1.2194318	1.7421482	114	169	231	315	114	55	62	83
8	M	4	440	2.4310717	0.456725	0.9170463	1.2718772	1.7705586	112	189	248	330	112	76	59	83
9	unk	2	230	1.2379286	0.5089187	1.0270079			116	197			116	81		
10	unk	2	270	1.4466075	0.5492328	1.0483457			125	206			125	81		
11	unk	2	235	1.2114601	0.5244306	0.9826421			123	198			123	75		
12	F	3	335	2.0206665	0.4878926	1.0093503	1.3414049	1.7669624	109	186	235	298	109	77	49	63
13	unk	3	345	2.0079118	0.4937305	1.0628386	1.6561856		112	200	291		112	87	91	
14	unk	2	215	1.0964157	0.4680173	0.9431072			113	190			113	77		
15	F	3	340	2.1525054	0.4165308	0.7956126	1.461532		95	149	243		95	53	94	
16	M	3	360	1.7142172	0.4512467	0.8193119	1.3004196		122	191	282		122	69	91	
17	unk	2	194	0.9050587	0.3236634	0.6353392			93	147			93	54		
18	F	4	370	2.1637497	0.4155598	0.8031647	1.3545912	1.8700551	101	160	245	325	101	60	85	79
19	unk	2	220	0.8772596	0.4021218	0.7790578			121	199			121	79		
20	unk	2	240	1.2853632	0.3708583	0.8209902			95	167			95	71		
21	F	3	345	1.9575641	0.4639175	0.9721889	1.5715656		110	190	284		110	80	94	
22	M	4	365	1.841393	0.3996883	0.8400863	1.2965716		108	186	268		108	79	81	
23	F	3	370	1.885531	0.4591225	0.8151522	1.1364181		118	181	238		118	63	57	
24	unk	2	220	1.091321	0.4599856	0.7607049			114	164			114	51		
25	F	3	335	2.1457684	0.553824	0.991369	1.5248142		114	174	249		114	61	74	
26	F	3	355	1.8104891	0.4039799	0.7624071	1.1771757		108	171	244		108	63	73	
27	F	4	370	1.9488492	0.3883961	0.7722848	1.1850156	1.647087	103	169	239	318	103	66	71	79
28	F	4	375	1.58076	0.4017861	0.6792136	0.9546871	1.2979381	123	182	241	314	123	59	59	73
29	F	4	405	2.121194	0.44234	0.9158475	1.3665788	1.8269	113	196	274	354	113	82	78	80
30	unk	2	230	1.1304244	0.3104771	0.7120595			90	158			90	69		
31	м	na	445													
32	unk	2	230	1.1088468	0.2912971	0.6629106			87	152			87	65		
55	unk	1	150	0.8475186	0.4483337				97				97			
34	unk	4	450	2.2236874	0.4866938	0.9458164	1.3521937	1.8568689	127	212	288	382	127	85	76	94
35	unk	2	220	1.360585	0.47111	1.0668904	1 4024472		100	180	240		100	80	73	
30	r M	,	343	2.1/8154/	0.42/9549	0.9/45664	1.49244/9		37	1/5	248		9/	01	/3	
3/	M	2	330	2.000713	0.400/13	0.800370	1.017022		102	191	2/4		102	64	63	
20	unk	2	223	1 1062550	0.32903/2	0.04/5208			105	100			105	94		
29	unk	2	250	1.1903358	0.3826014	0.8840/99	1.0620222		105	194	217		105	57	50	
40	unk	2	20/	1.3041815	0.30/2000	0.9370395	1.0030392		101	193	21/		101	3/	36	
41	unk	2	247	1.074083	0.3943898	0.0379280			115	195			115	80		
43	unk	3	302	1 497243	0.425557	0.888278	1 364181		112	194	278		112	82	84	
44	unk	3	270	1 444409	0.522656	0.890874	1 238312		121	181	237		121	50	56	
45	unk	3	287	1 3989451	0 3883961	0.7420283	1 1268281		106	169	238		106	63	69	
46	unk	3	335	1 5616159	0.4099736	0.8343323	1 2757372		115	196	280		115	81	84	
47	unk	3	345	1.9669264	0.5248981	0.9973000	1,4367059		119	192	262		119	73	70	
48	unk	0.5	78	0.3776073			2.430.030								~~	
~																
									1+	2+	3+	4+				
								Min	87	147	217	298	87	51	49	61
								Max	127	212	291	382	127	92	94	98
								Average	109	182	257	331	109	72	74	78
L																