

SAN JOAQUIN RIVER AGREEMENT

VERNALIS ADAPTIVE MANAGEMENT PLAN

ANNUAL TECHNICAL REPORT



2008

Figure 1-1



Figure 2-1





2008 ANNUAL TECHNICAL REPORT

On implementation and Monitoring of the San Joaquin River Agreement and the Vernalis Adaptive Management Plan

Prepared by
San Joaquin River Group Authority

Prepared for the
California Water Resources Control Board
in compliance with D-1641

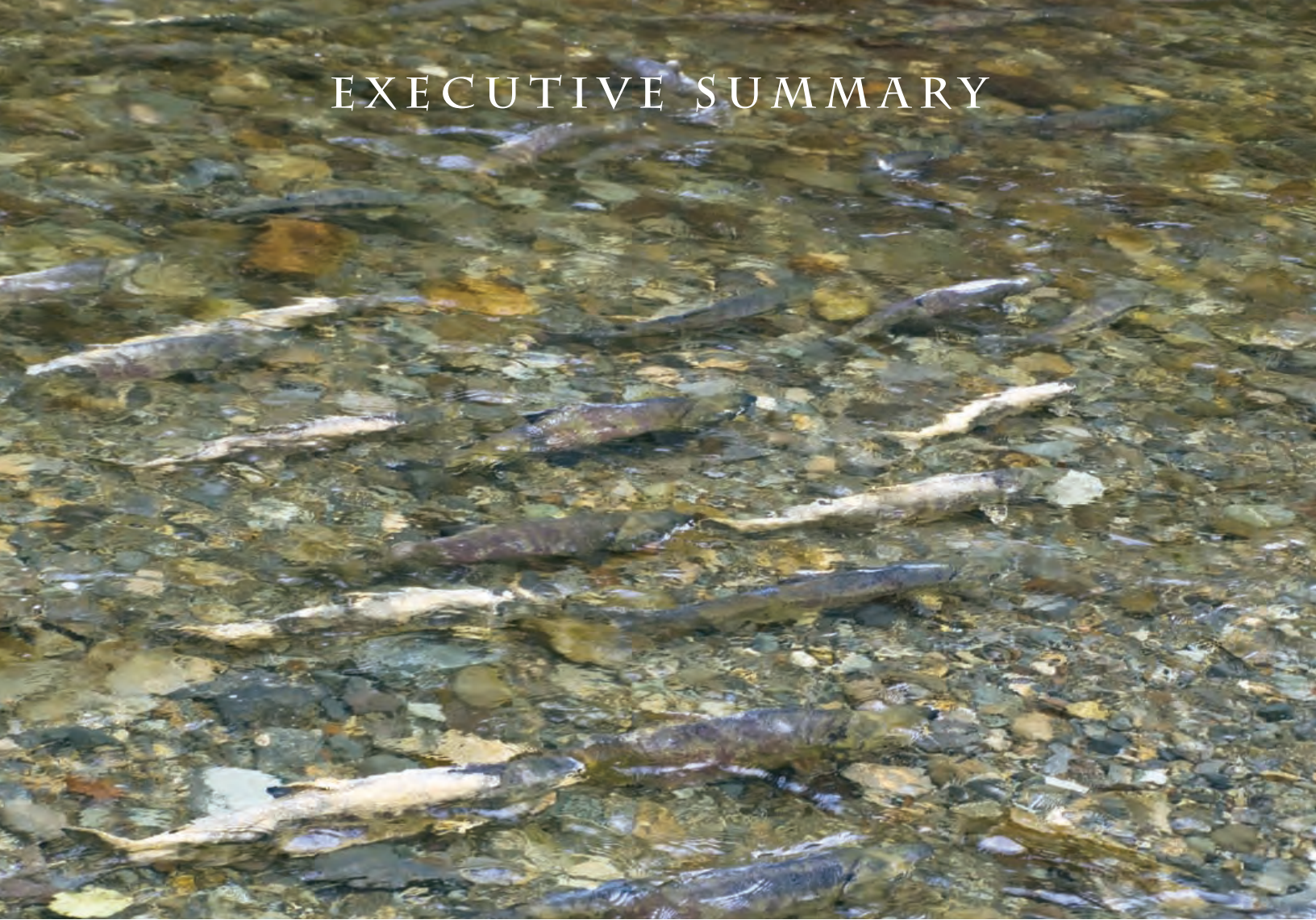
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EXECUTIVE SUMMARY



The San Joaquin River Agreement (SJRA)  is the cornerstone of a history-making commitment to implement the State Water Resources Control Board (SWRCB) 1995 Water Quality Control Plan (WQCP) for the lower San Joaquin River and the San Francisco Bay-Delta Estuary (Bay-Delta). Vernalis Adaptive Management Plan (VAMP), officially initiated in 2000 as part of SWRCB Decision 1641, is a large-scale, long-term (12-year), experimental-management program designed to protect juvenile Chinook salmon migrating from the San Joaquin River through the Sacramento-San Joaquin Delta.  The VAMP is also a scientific experiment to determine how salmon survival rates change in response to alterations in San Joaquin River flows and State Water Project (SWP)/Central Valley Project (CVP) exports with the installation of the Head of Old River Barrier (HORB).

The 2008 VAMP relied on the acoustic telemetry and tracking methodology to monitor the migration of salmon smolts through the Delta. The SJRA technical committee (SJRTC) developed an acoustic telemetry monitoring program, relying on 1,000 acoustic tagged

salmon smolts. The VAMP test period was conducted over the period of April 22-May 22 to allow the test fish to increase in size to better accommodate the acoustic tag to body weight ratio standard of less than 5 percent. The Water Year 2008 winter was dry in the San Joaquin River watershed, with seasonal precipitation in the San Joaquin Hydrologic Region (Cosumnes, Mokelumne, Stanislaus, Merced and San Joaquin Rivers) measuring only 85% of average on April 1, 2008. The forecasted April-July runoff as of April 1 in the four basins above Vernalis (Stanislaus, Tuolumne, Merced and San Joaquin) ranged from 78% to 84% of average. With the dry conditions for the current year and the very dry antecedent conditions, the forecasted mean flow without VAMP in the San Joaquin River near Vernalis for the VAMP target flow period of April 22 through May 22 was approximately 2,000 cubic feet per second (cfs), resulting in a VAMP target flow at the minimum value of 3,200 cfs. The water districts of Oakdale Irrigation District, South San Joaquin Irrigation District, Modesto Irrigation District, Turlock Irrigation District, Merced Irrigation District and San Joaquin Exchange

Contractors Water Authority provided 78,930 acre-feet of supplement water to support the VAMP target flow.

The 2008 Annual Technical Report consolidates the annual SJRA Operations and the Vernalis Adaptive Management Plan (VAMP) Monitoring Reports. 2008 represents the ninth year of formal compliance with SWRCB Decision 1641 (D-1641). D-1641 requires the preparation of an annual report documenting the implementation and results of the SJRA program. Specifically, this 2008 report includes the following information on the implementation of the SJRA: the hydrologic chronicle; management of any additional SJRA water; the acoustic telemetry experimental design; flow monitoring in the lower San Joaquin River, Old River, and Delta; results available to date of the juvenile salmon acoustic tag study; discussion of complementary investigations; and conclusions and recommendations.

Technical problems with the electronic transmitters substantially increased the data processing time and likely will bias estimates of survival and travel times. Results on the survival, distribution and behavior of acoustic tagged fish will be made available in a future stand-alone report.

The VAMP is intended to employ an adaptive management strategy using current knowledge to protect Chinook salmon as they migrate through the Delta, while gathering information to allow more efficient protection in the future. 2008 represented the second year of a monitoring program relying fully on the use of acoustic telemetry technology. With the assistance of the United States Geological Survey (USGS) the key monitoring stations at Jersey Point and Chipps Island were deployed in 2008. Resources from the USGS Columbia River Research Laboratory (CRRL) provided tagging training and helped with refinement to the experimental design. In addition, the technical committee adopted a water quality monitoring study to address questions of potential fish mortality near the Stockton Waste Water Treatment Plant. With concerns for the protection of endangered delta smelt the Head of Old River Barrier was not installed in 2008. Specific experimental objectives of VAMP 2008 included:

- Evaluation of migration path selection at the San Joaquin River – Old River flow split at the Head of Old River and at the San Joaquin River – Turner Cut split under the 2008 flow conditions.
 - Monitoring predator behavior in the Delta (near the CVP export facility).
 - Evaluation of fish mortality across Clifton Court Forebay between the Clifton Court Forebay inlet structure and the Skinner Fish Facility.
 - Study water quality conditions near the Stockton Waste Water Treatment Plant Chinook
 - Establish a new release site on the San Joaquin River near Stockton.
 - Evaluation of acoustic tag reliability and tag battery life.
 - Health and physiological testing of VAMP fish at the Merced River Hatchery (MRH) to evaluate the incidence of disease.
- Quantification of Chinook salmon smolt survival along individual river segments between Durham Ferry, Stockton Waste Water Treatment Plant (WWTP), Jersey Point and Chipps Island by detection of acoustic signals from transmitters implanted in the test fish.





Condition and short term mortality of “dummy tagged” salmon held for 48 hours in net pens at Durham Ferry and Stockton.

The VAMP design provides for a 31-day pulse flow (target flow) in the San Joaquin River at the Vernalis gage along with a corresponding reduction in SWP/CVP exports. The magnitude of the pulse flow is based on an estimated flow (existing flow) that would occur during the pulse period absent the VAMP. As part of the implementation planning, the VAMP hydrology and biology groups meet regularly throughout the year to review current and projected information on hydrologic conditions occurring within the San Joaquin River watershed. This facilitates communication and coordination for both the VAMP Chinook salmon smolt survival experiments and for scheduling streamflow releases on the Tuolumne, Merced, and Stanislaus rivers to facilitate the experimental investigations and protection for juvenile salmon within the tributaries.

Hydrologic conditions in 2008 were drier than any previous VAMP years. In the March 14 operation plan the Existing Flow was forecasted to be between 2,240 and 3,220 cfs calling for a VAMP target flow of either 3,200 or 4,450cfs. As the planning proceeded in subsequent weeks the forecasted Existing Flow declined to near 2,000 cfs calling for the VAMP target flow of 3,200 cfs. A ruling by the Federal Court to protect the endangered delta smelt prohibited the installation of the spring HORB. In planning for the VAMP the SJRA Technical Committee recommended delaying the start of the VAMP pulse period from the default date of April 15th to April 22nd in an effort to provide larger sized fish for the implantation of acoustic tags. Along with evaluating survival through the Delta the study was designed to measure survival along selected segments of the San Joaquin River between Durham Ferry and Chipps Island.

With assistance of the U.S. Geological Survey the acoustic receiver stations at Jersey Point and Chipps Island were installed for the 2008 VAMP. Thus estimates of survival to Jersey Point and Chipps Island were possible.

As in prior years computerized temperature recorders were employed at the MRH, in the transport trucks, at the release sites and throughout the lower San Joaquin River and Delta for a continuous record of temperatures encountered by the migrating test fish. Overall the average temperature at all sites remained below 20° C, which is considered suitable for salmon smolts.

At the time of preparing this report the survival results from the 2008 experiment are not yet available. A separate report on the survival will be prepared for submittal to the SWRCB.

With experience in the acoustic telemetry technology and with assistance from the USGS a more defined experimental model was developed for 2008. The statistical model was based on the release-recapture models of the past with a route-specific survival component. With the use of double-detection arrays at the receiver locations an estimate of the detection probability was made at each double-array site. The detection probability allowed for estimating test fish distribution at channel junctures and separated the survival probabilities for each channel reach.

A tag life study was conducted to determine any bias in the survival estimates caused by premature failure of the acoustic tags. A random sample of 50 tags was collected for testing. These were programmed and subsequently detected continuously over time in the same standard manner as used in the field. As a result of higher than expected failure among the original batch of tags the manufacturer provided replacement tags. Of the original tags and replacement tags 21 percent and 12 percent failed within the first 11 days. All tags expired within 20 days. This higher than expected tag failure during the operational phase will bias the 2008 VAMP fish survival because tag failure cannot be separated from fish mortality.

Similar to prior VAMP experiments a small group of test fish were collected and evaluated to assess the potential mortality due to PKD. In 2008 40 test fish were randomly “dummy tagged” (non-functioning tags) and transported to the U.S. Fish and Wildlife’s California/Nevada Fish Health Center. Holding tank conditions at the USFWS laboratory were matched to field conditions at the time for both water temperature and salinity levels. Cumulative mortality to Proliferative Kidney Disease (PKD) was 20 percent in 2008.

The decline in fish production at the MRH and the continued concern for the abundance of delta smelt will greatly influence future VAMP designs. A priority in designing the 2008 acoustic monitoring study was to generate similar estimates of survival so that results could be compared to those generated from the previous coded wire tag studies.

CHAPTER 6

COMPLEMENTARY STUDIES RELATED TO THE VAMP

Throughout 2008 several fishery studies were conducted to advance the understanding of juvenile salmon abundance and survival in the San Joaquin River basin. Following are summary reports of the information developed in each study.

Review of Juvenile Salmon Data from the San Joaquin River Tributaries to the South Delta during January through June, 2008

Contributed by Tim Ford, Turlock and Modesto Irrigation Districts, and Chrissy Sonke, FISHBIO Environmental

The VAMP includes protective measures for San Joaquin River (SJR) smolts during a 31-day period in April and May, and evaluations are conducted annually to determine how these measures (i.e., river flow and exports) relate to delta survival. However, juvenile salmon from the spawning areas of the Stanislaus, Tuolumne, and Merced Rivers (referred to here as tributaries) can migrate to the SJR and delta over a longer season that may range from January to June. Their migration and rearing patterns vary among tributaries and among years in response to flow releases, runoff events, turbidity, and other factors.

During 2008, rotary screw trapping was conducted near the confluences of the Stanislaus, Tuolumne, and Merced Rivers with the SJR. Seining was also conducted in the SJR from below the head of Old River (HOR) to upstream of the Tuolumne River confluence. This review presents data from those rotary screw traps (RST) and seining to identify the presence and movement of juvenile salmon from the tributaries into the mainstem San Joaquin River relative to observations at the Mossdale Trawl and in CVP and SWP salvage facilities. Salmon were assigned to lifestage category based on a forklength scale, where <50 mm= fry, 50-69 mm= parr, and ≥ 70 mm= smolt.

Stanislaus River RST monitoring was conducted at River Mile (RM) 9 (Caswell site) between January 21st and June 26th; Tuolumne River RST monitoring was

conducted at RM 5 (Grayson site) between January 29th and June 4th; and Merced River RST monitoring was conducted at RM 2 (Hatfield site) between March 3rd and June 5th. Weekly seining during Jan-Jun was done at up to 8 sites from River Mile 51 (Dos Reis) to River Mile 83 (North of Tuolumne River) and two other sites were seined every two weeks from mid-January to late May at River Mile 78 and 90. Trawling was conducted in the San Joaquin River at Mossdale near RM 54 (downstream of the tributaries, and just upstream of the HOR) with a schedule of three days/week, January 2nd through March 30th; five days per week, March 31st through June 6th; and three to five days per week during the remainder of June. Although salvage data of unmarked salmon does not distinguish which salmon originate from the San Joaquin tributaries, they can be compared to timing, abundance, and size of salmon collected in the San Joaquin basin monitoring. Flow and rainfall patterns in the basin are shown in Figure 6-1.

Overall, Chinook outmigrant abundance in 2008 was extremely low in the San Joaquin Basin (i.e., 483 juvenile Chinook captured in the three tributaries), consistent with the low 2007 adult returns (i.e., total basin estimated escapement was about 1,195). Estimated escapement to the San Joaquin Basin during fall 2007 was only 21% of the previous year, and was the lowest observed since the 1987-1992 drought.

At the Stanislaus River RST, there were no obvious peaks in fry movement (Figure 6-2) and fry catch never exceeded eight fish per day. RST sampling was not conducted during the fry outmigration period on the Merced River (Figure 6-3). A seasonal peak catch of fry (n=69) at the Tuolumne River RST (Figure 6-4) occurred on February 29th following increasing runoff from rain

Figure 6-1
San Joaquin Basin Flows and Rainfall, 2008

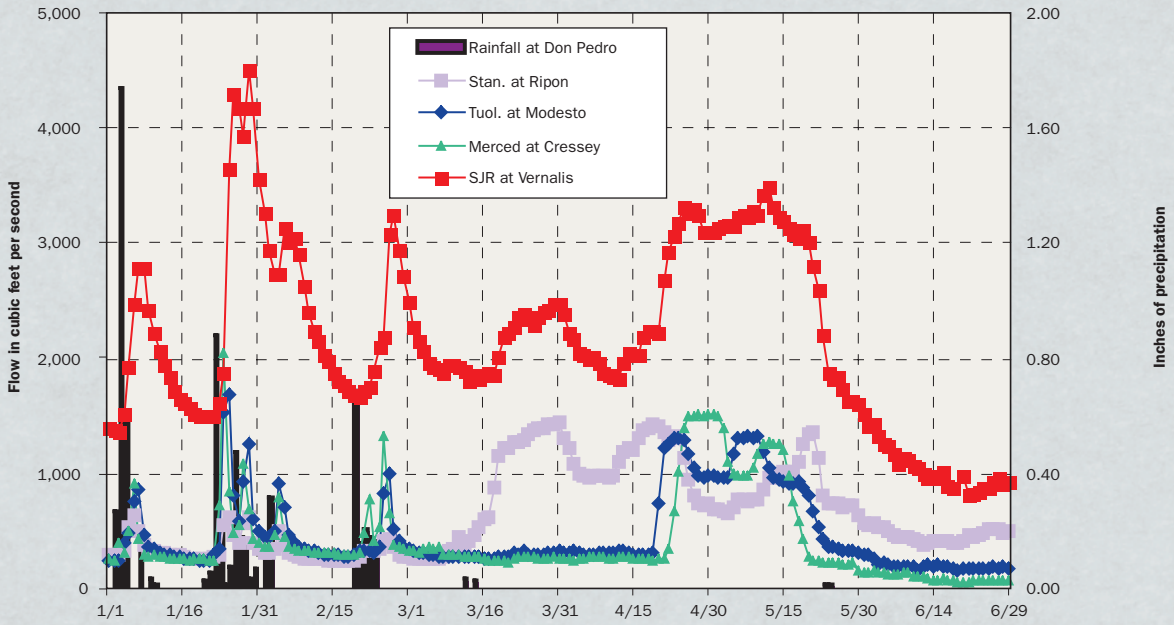


Figure 6-2
Stanislaus screw trap catch of unmarked juvenile Chinook salmon



Figure 6-3
Merced screw trap catch of unmarked juvenile Chinook salmon

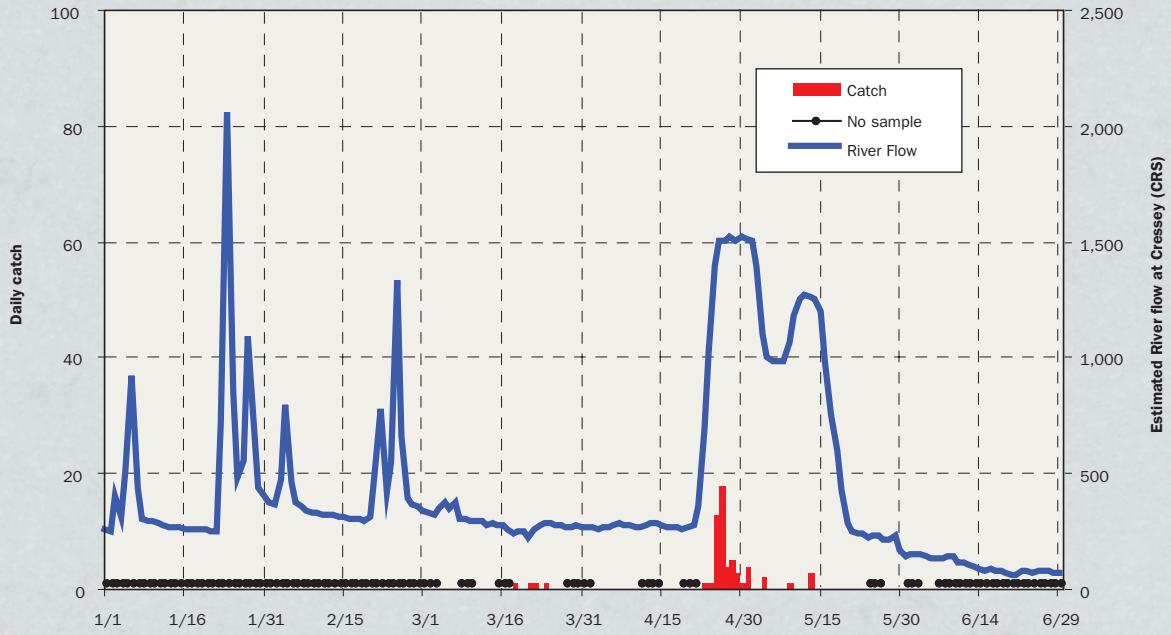
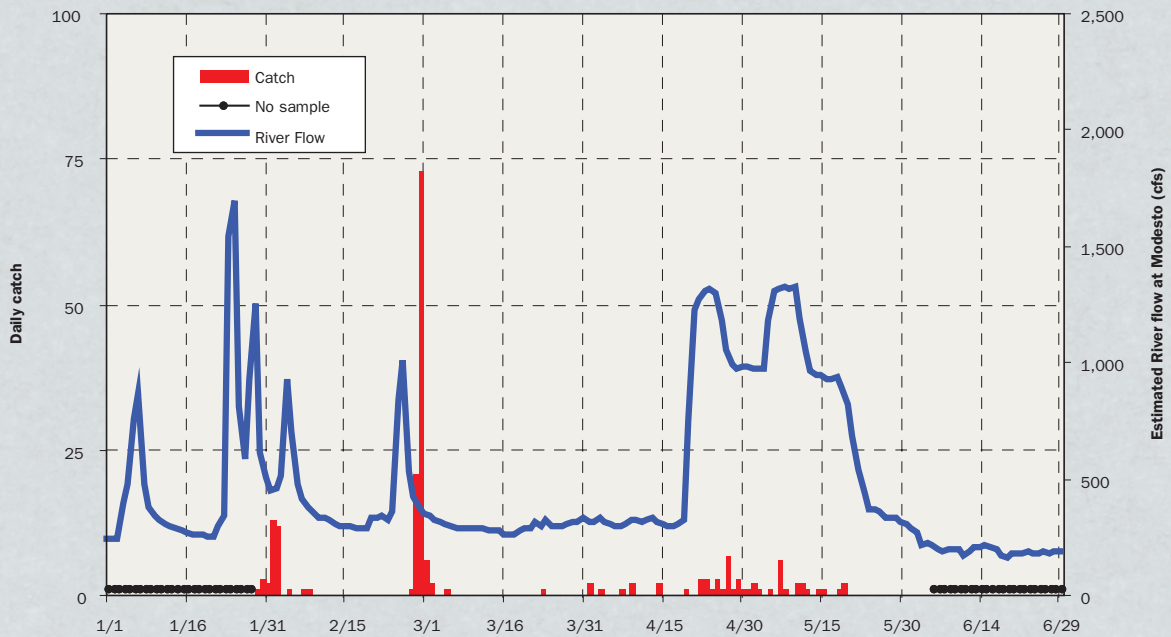


Figure 6-4
Tuolumne screw trap catch of unmarked juvenile Chinook salmon



events during February 20th - 24th; this peak represents 54% of all fry and 36% of all juveniles captured in the three tributary RSTs combined. A smaller elevated catch was observed under similar conditions on the Tuolumne River in early February. Only two salmon fry were captured in the Mossdale trawl (Figure 6-5) and numbers salvaged at the CVP and SWP facilities (Figures 5-12 and 5-13) were also low which is consistent with the low numbers of fry migrating out of the Stanislaus and Tuolumne Rivers.

Small, seasonal peak catches during the parr/smolt outmigration period were observed on the Merced River on April 26th (Figure 6-3) and 30 Apr on the Stanislaus River (Figure 6-2), and were subsequently detected at Mossdale during April 28th through May 2nd. At the Tuolumne River RST there were no obvious peaks in parr/smolt catch. Seasonal peak parr/smolt catch occurred at Mossdale on May 16th (Figure 6-5), coincident with peak recovery of marked salmon from several releases in the lower Merced River at Hatfield Park. This “Pied Piper effect”, where natural migrants are stimulated to migrate with released hatchery salmon, is a trend commonly observed in the RSTs. Seining in the SJR captured only three salmon: one (82 mm) at Route 132 (RM 77) on January 10th; one (74 mm) at Dos Reis (RM 51) on April 18th; and one (84 mm) at Mossdale (RM 56) on April 22nd. Very low catches of juvenile salmon were observed by mid-May in the Tuolumne and Merced Rivers, and by the end of May in the Stanislaus River and the San Joaquin River at Mossdale.

Average size in RST and trawl catches (Figure 6-6) shows that most fish observed prior to mid-March averaged <50 mm fork length (FL). In contrast, average size in the salvage prior to late March shows that most fish were substantially larger than those emigrating from the San Joaquin Basin. Although salvage operations are relatively less effective at capture of fry, the absence of fry in the salvage combined with low abundance of fry observed at upstream monitoring locations suggests that few fry of San Joaquin Basin origin were entrained by the pumps during 2008. Instead it appears that salvage during January through March was dominated by larger fish of other runs from the Sacramento Basin (Figure 5-16). Average size at all locations typically increased by early April to >80 mm FL (Figure 6-6).

To obtain more useful information on salmon movement into the Delta, daily monitoring at the lower end of each of the three San Joaquin tributaries and at Mossdale for the entire season (roughly January through June) is a high priority. Further evaluation of the trawl and salvage efficiency on smaller juvenile salmon is necessary. These data would help to refine existing protective measures for fry to smolts, if warranted, and to identify alternative

strategies that may protect a larger proportion of the juvenile salmon population migrating from the San Joaquin tributaries.

2008 Mossdale Trawl Summary

*Contributed by H. Steve Tsao
California Department of Fish and Game*

Introduction

Since 1988 DFG has conducted monitoring of the fall-run Chinook salmon smolt out-migrant population in the San Joaquin River from the HOR to about two miles downstream of Mossdale Landing County Park (RM 56) (Figure 6-7). This essential measurement of timing and production for out-migrating fall-run Chinook salmon smolts has been performed at this location to:

- 1) Determine annual salmon smolt production in the San Joaquin Basin,
- 2) Develop smolt production trend information,
- 3) Determine timing and magnitude of smolt out-migration into the Delta from the San Joaquin tributaries.
- 4) Document the occurrences of other species including listed species such as steelhead and delta smelt.

Methods

Sampling is performed with a 6 x 25 foot (1.87m x 7.6m) Kodiak trawl net. The Kodiak trawl uses two boats to pull a net equipped with spreader bars, wings, and a “belly” in the throat of the net (to improve capture vulnerability). The cod end of the trawl net is secured using a rope. The sampling intensity was 5 days a week from April 1st to June 6th, and 3 days a week from June 9th to June 30th. The entire sampling period was from April 1st to June 30th, 2008 with a total of 58 sample days out of the study period of 91 days. All trawling occurred during daylight hours, starting between 0700 to 0800 hours. A sampling day usually consisted of 10 tows at 20 minutes per tow. A sampling day may have been extended if a trawl efficiency test was conducted. Sampling was also conducted 3 days per week from July to March by the USFWS Stockton Office.

All fish were identified to species and enumerated. The first 30 per tow of all species, except Chinook salmon, were also measured. Chinook salmon were checked for dye mark. All non-marked Chinook salmon were considered “natural” for the purpose of this study. All Chinook salmon were measured (fork length, mm).

Water temperature, turbidity, weather, and beginning tow time were recorded for each tow. Velocity was

Figure 6-5
 Mossdale kodiak trawl catch of unmarked juvenile Chinook salmon

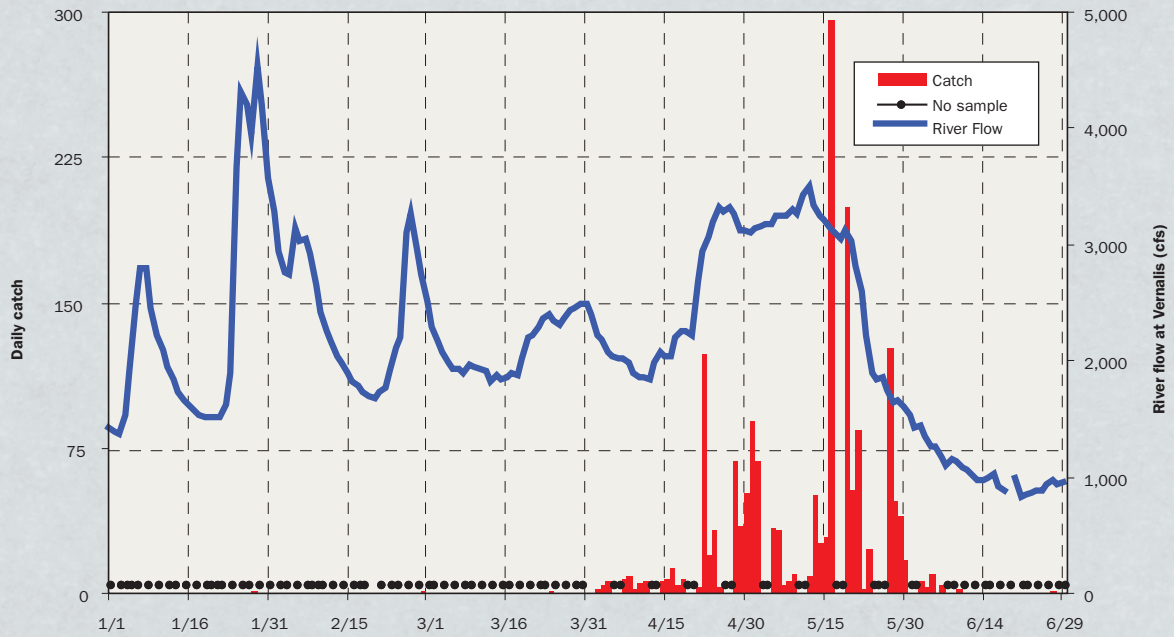
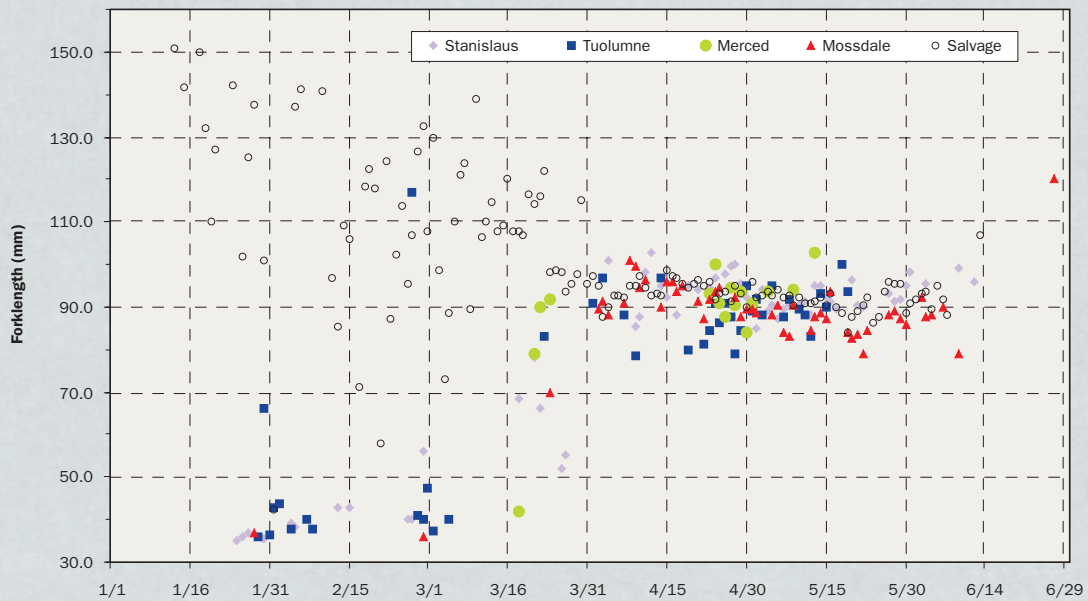


Figure 6-6
 Daily average forklength of unmarked juvenile Chinook salmon



recorded by using a General Oceanics Inc model 2030R digital flow meter. A Garmin GPS Map 172c was used to map the location of all sampling tows. This mapping was done to evaluate differences in catch rate verses tidal influence throughout the sampling area (Figure 6-8). The tidal information at Grant Line Canal was provided by NOAA. The mean daily river flow data that is used in this report were taken from the U.S. Geological Survey mean daily stream flow gauge at Vernalis.

Analysis

Smolt Production Index Calculation (Smolt/ac-ft Method)

The 2008 natural smolt production from the San Joaquin River drainage was estimated by two different methods. The first method, smolt production index calculation (smolt/ac-ft method) involves taking the actual number of non-marked Chinook salmon and dividing by the actual volume sampled to get Chinook/ac-ft. This number is then expanded by the daily mean flow recorded at Vernalis for a 5-hour index and expanded again for a 24-hour daily estimate. These daily average smolt densities are then expanded by multiplying by the daily mean flow recorded at Vernalis. Production for days not sampled within the study period was estimated by averaging smolt/ac-ft for the two days before and two days after the non-sampled period.

The natural smolt index estimates (E_i) are calculated as follow:

$$E_i = \sum_{i=1}^{n=91} \left[\left(\frac{C_i}{V_{Ti}} \right) (V_{Pi}) \left(\frac{24}{5} \right) \right]$$

Where:

- EI = Smolt Production Index Estimation
- n = days in the index period
- C = daily non-marked Chinook catch
- VT = daily volume of trawl sampled
- VP = daily 5-hour volume of water passing Mossdale
- i = ith Day

The 95% confidence interval around this index was calculated as +1.96 x the Standard Deviation of the mean smolt density (smolt/ac-ft) in the trawl catch over the 91 days.

Vulnerability Expansion Estimation (Single Year Population Ratio Method)

The second method, vulnerability expansion calculation (single year population ratio method), which DFG believes to be a more accurate estimate due to the uneven distribution of smolts in the channel, is determined based on the recapture rates of dye marked vulnerability release groups. There were 5 vulnerability

test groups in 2008 (Table 6-1). A population ratio was calculated based on these 5 test groups. The population ratio was used to calculate a 5-hour index, and extrapolated to a 24-hour seasonal estimate (Figure 6-9). Production for days not sampled within the study period were estimated by averaging smolt catch and minutes towed for the two days before and two days after the non-sampled period.

The population ratio (r) was calculated as follow:

$$r = \frac{\sum_{i=1}^n y}{\sum_{i=1}^n x} = \frac{\bar{y}}{\bar{x}}$$

Where:

- r = population ratio
- n = number of vulnerability test groups
- y = number of marked fish captured
- x = number of marked fish released (effective release)
- i = ith day

$$E_v = \sum_{i=1}^{N=91} \left\{ \left[\frac{(C_i/r)}{(T_i/300)} \right] \left(\frac{24}{5} \right) \right\}$$

Where:

- EV = Vulnerability Expansion Estimation
- r = population ratio
- C = daily non-marked Chinook catch
- T = tow duration
- i = ith day
- N = number of days sampled

For the purpose of the analysis, vulnerability to the trawl was assumed to be from the beginning of the first tow that fish were detected to the end of the last tow the fish were detected on the day of release. Detection of the test group of fish subsequent to the day of release was not used in the analysis (this was less than 5 fish total for all releases). Travel time from release point to the trawl, time vulnerable to the trawl, and the percent vulnerability as related to flow were determined for each test group (Table 6-1).

Results

Between April 1 and June 30, 2008, 1,696 non-marked Chinook salmon smolts were captured in the Mossdale trawl. Daily capture of non-marked salmon ranged from 0 to 296 individuals with an average of 35. Figure 6-9 shows the expanded daily catch of non-marked Chinook. Average forklength of non-marked Chinook was 88.3 mm and ranged from 58 - 129 mm.

The smolt production estimate for the San Joaquin basin was 188,652 using the smolt production index

Figure 6-7
Location Map of Mossdale Trawl Area in Lower San Joaquin River, 2008

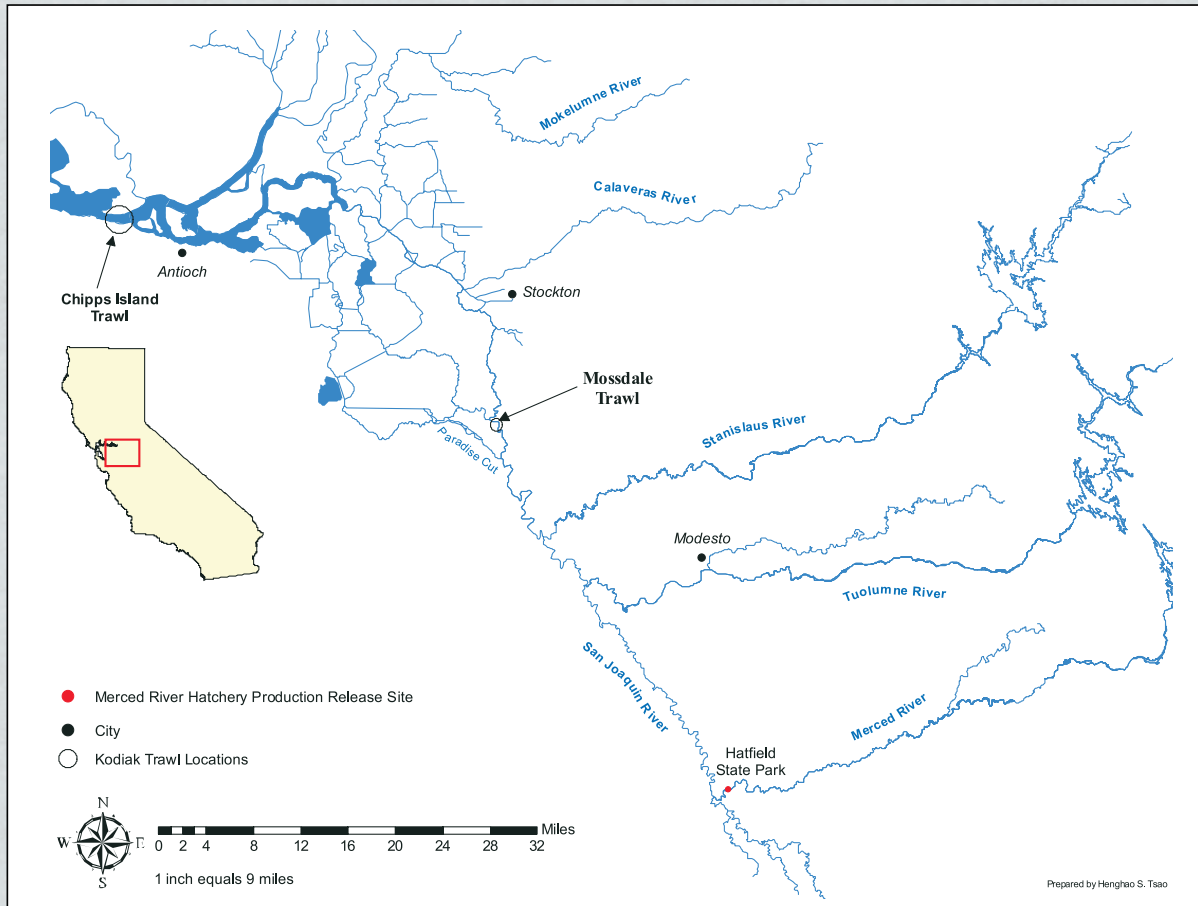


Figure 6-8
GPS Trace of Sampling Tows During Mossdale Trawl, 2008



Table 6-1
Dye marked smolt release from Merced River Hatchery for vulnerability studies in the San Joaquin River at Mossdale Landing, April through May, 2008

Release Date /Time	Water Temp. (°C) Truck/River	Effective # Released	Number Recovered	Streamflow (cfs) at Vernalis	Beginning and Ending Recovery Time
15-Apr-08 9:00	10/16	2009	80	2010	10:54 14:35
22-Apr-08 8:22	9.5/13	5017	149	2822	10:14 15:21
6-May-08 7:01	11/17	2830	110	3126	9:24 14:28
20-May-08 7:45	13/20	4992	371	3046	9:11 14:15
27-May-08 7:00	12.5/16.5	2999	324	1805	8:48 14:59

estimation, and 285,887 using the vulnerability expansion estimation (Table 6-2). The vulnerability expansion estimation is thought to be more accurate than the smolt/ac-ft index method because it should account for an uneven distribution of migrating smolts in the river channel.

Four steelhead/ rainbow trout (RBT) were captured during the 2008 sampling period. All RBTs were measured and returned to the river. Forklength ranged from 214- 251 mm (240 mm average), and all samples were in the stage of smolting.

2008 Water Quality Survey in Support of the Vernalis Adaptive Management Plan (VAMP)

Contributed by Sharon Borglin, Jeremy Hanlon, Justin Graham, Chelsea Spier, Kennedy Nyugen, Remie Burks and William Stringfellow

*Ecological Engineering Research Program,
University of the Pacific*

Introduction

During the 2008 Vernalis Adaptive Management Plan (VAMP) fish release the Ecological Engineering Research Program (EERP) at the University of the Pacific (UOP) conducted a water quality sampling program (WQ) to determine ambient conditions in the San Joaquin River (SJR) near the City of Stockton Waste Water Treatment Plant (WWTP). This study was commissioned following detection of numerous immobile fish tags in the SJR near the WWTP during the 2007 VAMP fish release. In 2007 during mobile monitoring in the SJR from Mossdale to the Stockton Deep Water Ship Channel, a high number of acoustic transmitters were detected at a very small, localized site adjacent to the Stockton

WWTP outfall (SJRGA, 2007; Vogel, 2007). The area of concern was 0.75 miles downstream of the Highway 4 Bridge, 1.7 miles upstream of the Stockton Deep Water Ship Channel, and adjacent to a railroad bridge and the Stockton WWTP outfall. A total of 116 tags were found at this site which included some fish from all of the upstream releases made on the SJR during the proceeding two weeks. This may be a minimum number lost at that location as the mobile monitoring was done on May 17 and 18, 2007 after the battery life of some of the tags from the first week fish release may have ended. These tags were motionless, indicating the tags were either in dead fish or had been defecated by a predator. An investigation by the Regional Water Quality Control Board and an independent investigation commissioned by the City of Stockton (RBI Inc, 2007) found that the WWTP was in compliance with discharge permit requirements during the 2007 VAMP period. The cause of the high fish mortality observed in this area in 2007 could not be determined, but the local concentration of immobile tags strongly suggests that this area was a hostile environment for juvenile salmon in May 2007.

To determine if water quality could have been a factor in the mortality observed, EERP conducted a monitoring program in the SJR to determine physical and chemical water quality in the area of high fish mortality seen in 2007. This study was conducted to establish a more complete picture of water quality conditions during the period of the 2008 VAMP fish releases from May 1st to May 15th, 2008. In this report we present flow and water quality data collected in a six-mile reach of the San Joaquin River between Brandt Bridge (River Mile 46.7) and Burns Cutoff near Channel Point (River mile 40.5) (Figure 6-10).

Figure 6-9
Expanded daily catch of non-marked Chinook based on vulnerability estimates and flow at Vernalis, 2008

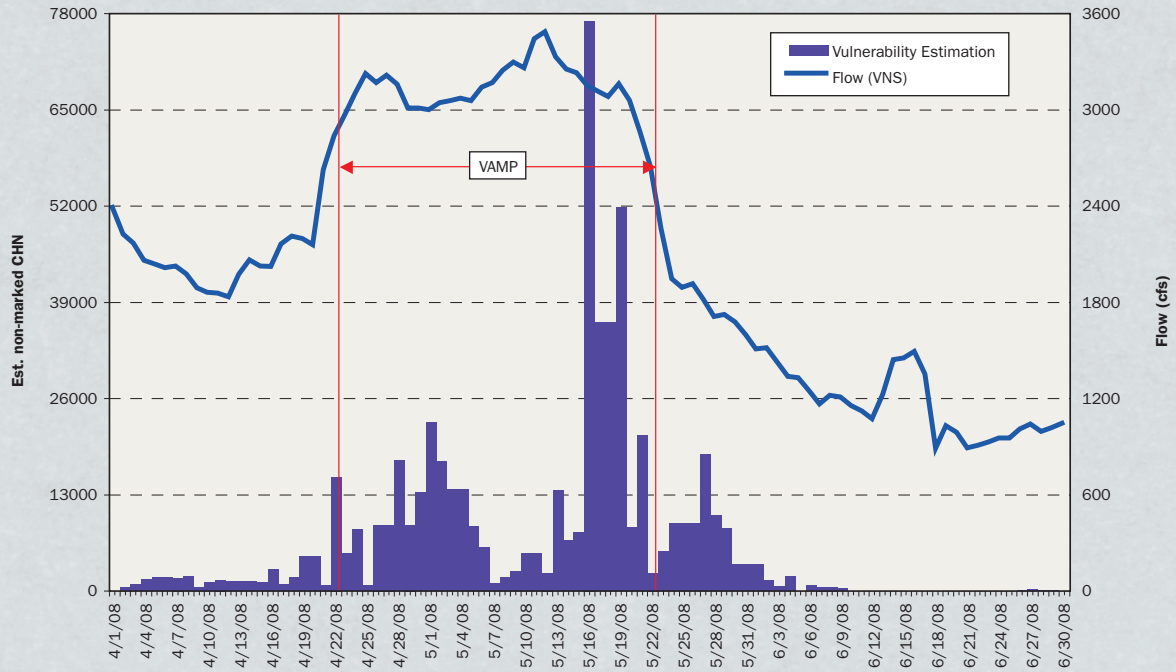


Figure 6-10
Map of study area showing sampling sites

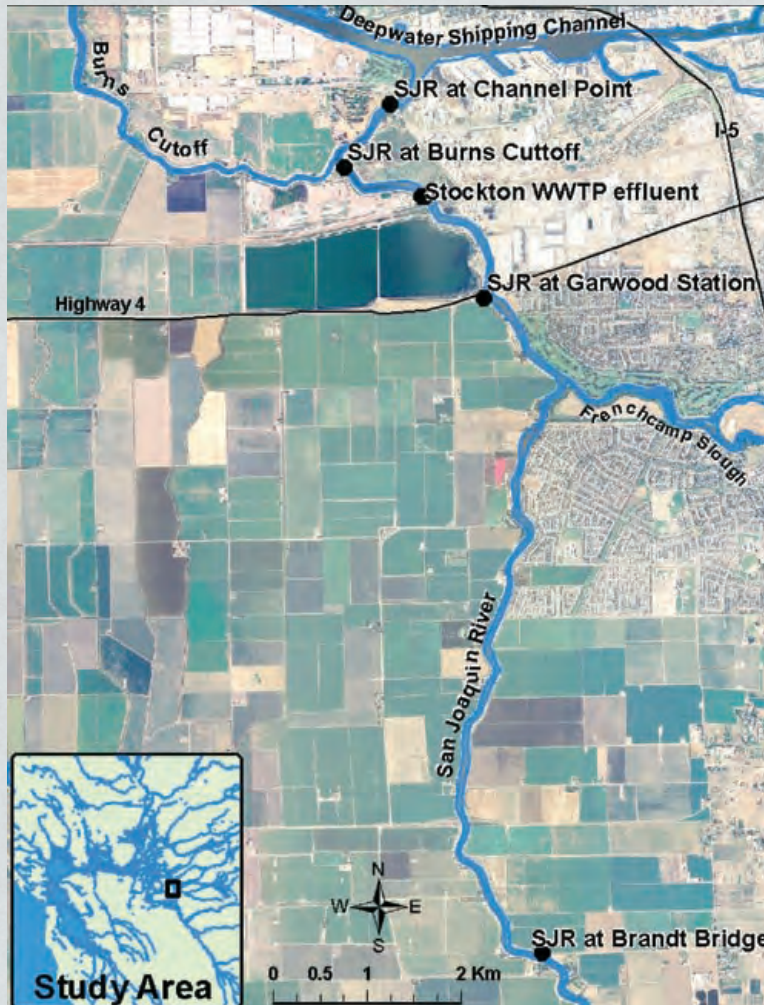


Table 6-2
Smolt Production seasonal estimates and sampling period for the duration of the study.

Year	Sampling Period (Days)	Percentage of Day Sampled (%)	Smolt/ac-ft Estimate	"Vulnerability Smolt Production Annually Population Ratio Method (95% confidence range)"
2008	91	63.7	188,652 + 8,010	285,886 : (29,043 - 67,432)
2007	75	76	273,798 + 7,490	920,006***
2006	75	85.3	848,394 + 12,888	1,808,143 : (1,749,531- 1,866,755)
2005	89	80.9	363,800 + 14,700	621,403 : (388,884- 1,119,550)
2004	61	88.5	92,500 + 66,500	297,348 : (191,222- 665,160)
2003	88	80.7	107,500 + 60,300	368,424 : (277,626- 545,121)
2002	74	87.8	229,100 + 557,100	2,254,647 : (1,455,066- 5,179,591)
2001	103	78.6	279,800 + 286,000	928,996 : (586,790- 2,228,789)
2000	88	81.8	211,100 + 181,900	484,703**
1999	119	71.4	146,900 + 63,500	438,979**
1998	99	67.7	1,075,000 + 562,800	2,844,637**
1997	92	69.6	168,600 + 89,400	635,517**
1996	89	85.4	381,900 + 626,900	1,155,319**
1995	60	78.3	1,108,900 + 2,640,000	3,361,384**
1994	63	73	67,500 + 62,200	453,245**
1993	83	61.4	54,200 + 21,800	269,035**
1992	72	44.4	23,600 + 6,300	280,395**
1991	59	66.1	*	538,005**
1990	82	69.5	*	263,932**
1989	54	100	*	4,241,862**

* Data is currently being reevaluated.

** 1989-2000 estimates based on the natural log of all vulnerability tests (1989-2005).

*** 2007 estimates based on the natural log of all vulnerability tests (1989-2006)

Materials and Methods

To document ambient river conditions during the 2008 VAMP fish releases, water quality grab samples were collected daily from May 1st through 15th, 2008 on the outgoing tide at four sites along the SJR; one upstream background site (Brandt Bridge), a site directly above the WWTP (Garwood), a site located at the WWTP outfall (Outfall), and a site downstream of the WWTP (Burns Cutoff). Samples of the WWTP effluent were also collected (Figure 6-10).

In conjunction with the grab sample data, continuous water quality sondes were deployed at four SJR sites; DO-01 Channel Point (for DO-194 Burns Cutoff), DO-84 SJR at Garwood Bridge, DO-127 SJR at Brandt Bridge, and DO-195 SJR at Stockton WWTP Outfall (Figure 6-10). All sampling was coordinated with fish-cage and fish tagging studies conducted as part of the 2008 VAMP research program. Fish cages were located at Brandt Bridge, Stockton WWTP outfall, and Burns Cutoff (Nichols and Foott, 2008).

River and effluent flows

River flow data was collected at two continuous water quality sampling locations, DO-84 SJR at Garwood Station and DO-127 SJR at Brandt Bridge. The flow measurement station SJR at Brandt Bridge (station ID: BDT) is operated by the Department of Water Resources (DWR). The flow measurement station SJR at Garwood Bridge (station ID: SJG) is operated by USGS. Data from both sites was taken directly from the CDEC website. Flow data was measured and recorded by DWR or USGS every fifteen minutes at the two stations. Effluent flow data for the WWTP was recorded from the flow meter at the water quality sampling site DO-193 WWTP during grab sampling events.

Daily Water Quality Monitoring

Samples were collected in bottles attached to a pole and were depth integrated by moving the bottle between the top and bottom of the water column during filling. All grab samples were collected by boat on an outgoing tide. Samples of WWTP discharge were collected at the same time as the other grab samples.

In addition to the grab samples, water quality field data was collected at each location using an YSI 6600 multi-parameter sonde connected to an YSI 650 MDS handset (YSI Inc., Yellow Springs, CO). While the sonde logged water quality data, water samples were collected according to established protocols (Graham and Hanlon, 2008; Puckett, 2002). A description and photo documentation of sampling activities were also made.

Samples were returned to the EERP laboratory and analyzed for the constituents listed in Table 6-3. All data in the project was collected in accordance with rigorous, Surface Water Ambient Monitoring Program (SWAMP) compatible, QA/QC procedures (Puckett, 2002; Stringfellow, 2005; Borglin et al., 2006; California Department of Fish and Game, 2007). Briefly, samples were received by the laboratory the same day they were sampled, logged in and inspected for damage, and stored at 4°C until filtering and analysis. All filtration and preservation of samples were completed within 24 hours. Samples were collected, preserved, stored, and analyzed by methods outlined in Standard Methods for the Analysis of Water and Wastewater (Clesceri et al., 1998; Clesceri et al., 2005).

Continuous Water Quality Monitoring

Daily WQ grab sampling was supplemented with deployment of continuous monitoring devices (YSI 6600 sondes) monitoring chlorophyll a, pH, dissolved oxygen, and other key WQ parameters at 15-minute intervals (Table 6-3, field measurements). The sonde depth averaged around 3 ft but varied with the tidal cycle (Graham and Hanlon, 2008). Sondes were not deployed in the Stockton WWTP effluent or at the confluence of the SJR and Burns Cutoff. Continuous data for the Burns Cutoff location was collected at Channel Point, approximately 0.5 miles downstream. Data for pH was obtained from permanently installed monitors at the WWTP site and recorded at the time of field sampling.

Quality Assurance Summary

Quality assurance samples were run in parallel with all the sample analysis. Quality assurance samples included some or all of the following: a lab duplicate, field duplicate, matrix spike, matrix spike duplicate, calibration check standards, laboratory control standard, trip and lab blanks. From May 1st through May 15th, 2008, 98.9% of all laboratory quality assurance and 100% of all field quality assurance checks were within passing range. Proficiency check samples, standards with unknown concentration to the laboratory analyst, were run before the start and at the end of the project. All results were within established acceptable ranges.

Table 6-3
List of field and laboratory measurements made as part of the Water Quality Survey in Support of the Vernalis Adaptive Management Plan (VAMP) May 2008.

Field	Laboratory
Spec Cond mS/cm	Chlorophyll-a (Chl-a), ug/L
TDS mg/L	Pheophytin-a ug/L
DO mg/L	Alkalinity, mg CaCO3/L
pH	Total Organic Carbon (TOC), mg/L
ORP mV	Dissolved Organic Carbon (DOC), mg/L
Turbidity	Total Suspended Solids (TSS), mg/L
Sonde Chl-a ug/L	Volatile Suspended Solids (VSS), mg/L
	Total Nitrogen (TN) mg/L
	Total Ammonia Nitrogen (TAN), mg/L
	Nitrate Nitrogen (NO3-N), mg/L
	Nitrite Nitrogen (NO2-N), mg/L
	Total Phosphate (TP) mg/L
	Soluble Reactive Phosphate (PO4-P) mg/L

Findings: Water Quality

Field and laboratory results collected during the daily, grab sampling events are displayed in Tables 1 and 2 in Appendix D. All data was collected successfully with the exception of the loss of a set of chlorophyll samples on May 6th due to a refrigeration problem. Chlorophyll was quantified on this date by in-field sonde measurements at the time of sampling and continuous deployment sonde measurements, so no information was lost.

Concentrations of Total Ammonia Nitrogen (TAN) are shown in Figure 6-11. Concentrations of nitrite (NO₂-N) and nitrate (NO₃-N) over time showed similar trends. In the box and whisker plot in Figure 6-11, the central horizontal line in the box represents the data median, the box represents the upper and lower quartiles (representing ± 25% of the data) and the whiskers are the data maximum and minimum. The plots are aligned with the sites from upstream to downstream on the x-axis and include only the river sites. The top plot is the data from days when the WWTP was not discharging into the SJR, and the bottom plot is from days where there was WWTP discharge. The average WWTP value is reported above the lower plot.

Figures 6-12 and 6-13 show a time integrated 3-D plot of the TAN and NO₃-N data, respectively. In the foreground, the x-axis represents the 15 days of sampling time increasing from left to right. The z-axis

Figure 6-11

Total Ammonia Nitrogen (TAN) concentration in the SJR upstream and downstream of the Stockton WWTP measured by the EERP laboratory in support of the 2008 VAMP project

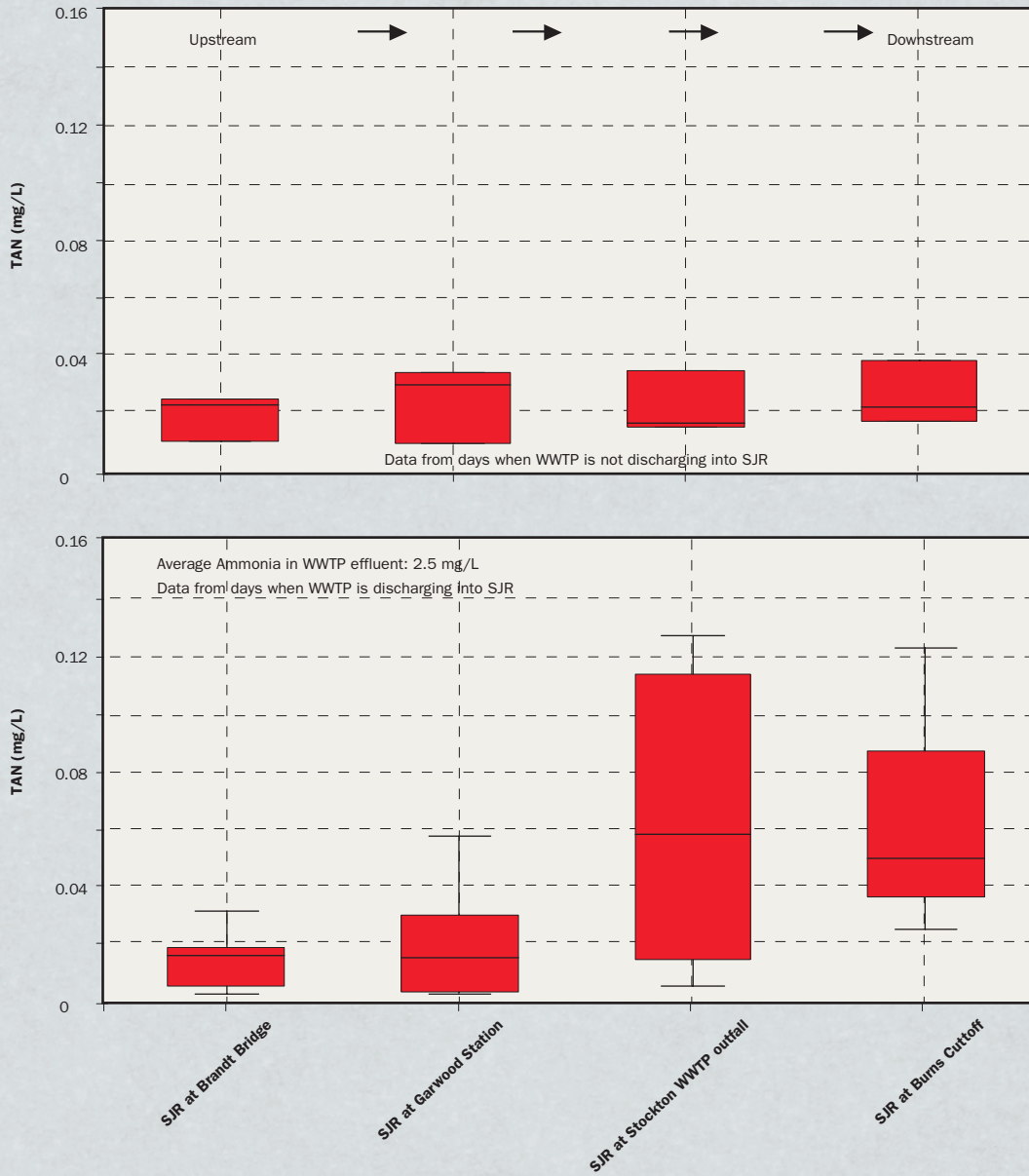


Figure 6-12

3-D plot of the Total Ammonia Nitrogen (TAN) concentration in the SJR upstream and downstream of the Stockton WWTP measured by the EERP laboratory in support of the 2008 VAMP project. The yellow ribbon represents treatment plant effluent concentration.

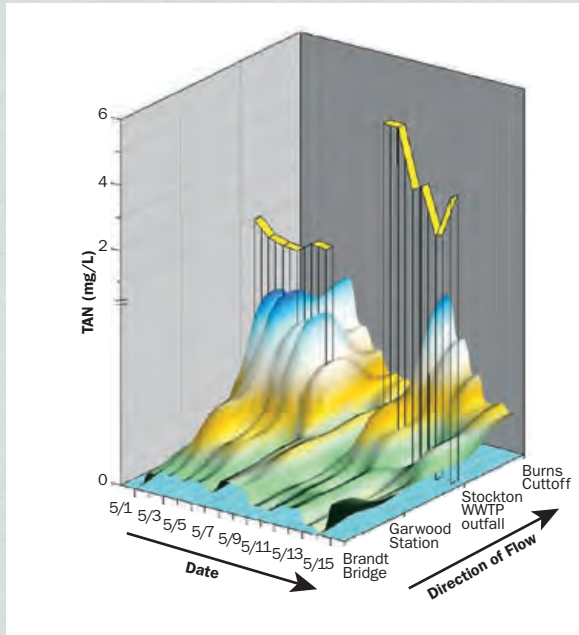


Figure 6-13

3-D plots of Nitrate-N concentration in the SJR upstream and downstream of the Stockton WWTP measured by the EERP laboratory in support of the 2008 VAMP project. The yellow ribbon represents the treatment plant effluent concentration.

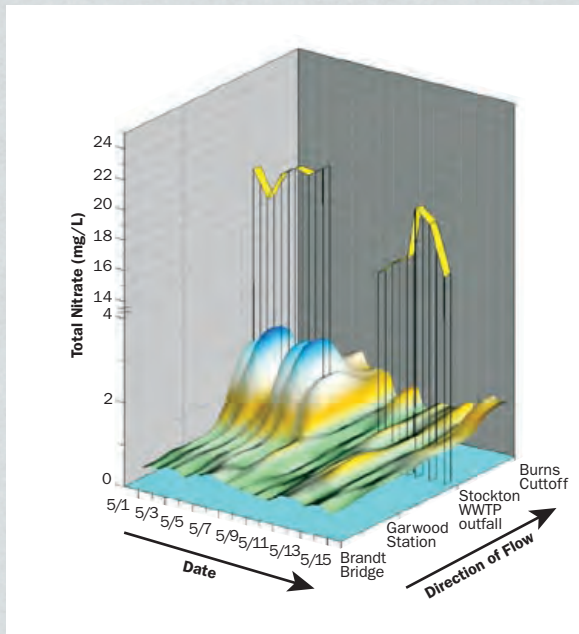


Table 6-4
Observed ammonia concentrations (TAN and un-ionized NH³) and chronic criteria concentration (CCC) in the SJR during VAMP (May 1 May 15, 2008)

Sample Site	Average TAN (mg N/L)	Maximum TAN (mg N/L)	Average NH ³ (mg N/L)	Maximum NH ₃ (mg N/L)	Average CCC (mg TAN/L)	Minimum CCC (mg TAN/L)
Brandt Bridge (DO-127)	0.0159	0.0312	0.0005	0.001	2.949	1.847
Garwood (DO-84)	0.0202	0.0575	0.0007	0.002	2.634	1.496
SJR @ Stockton WWTP effluent (DO-195)	0.055	0.126	0.0011	0.002	3.105	1.734
Burns Cut (DO-194)	0.0541	0.1216	0.0014	0.005	3.088	1.67
Stockton WWTP effluent (DO-193)	2.5052	5.3232	0.0055	0.014	6.367	4.892

represents the relative position of each sample point in the river from upstream in the foreground towards downstream in the background. The y-axis represents the measured value of the indicated parameter. It can be seen from this data that after the discharge from the WWTP shut down both the TAN and NO₃-N values diminished at the outfall river site DO-195. It was observed during field sampling that when the WWTP resumed discharging that the plume had a different character, with less aeration and it is possible that there was less mixing at the sample location, so the sample collected was not as influenced by the WWTP effluent. The Burn's cut site, DO-194, shows similar patterns before and after the WWTP shutoff.

Figures 6-12 and 6-13 indicate that concentrations of N compounds in the SJR are higher downstream during periods of the Stockton WWTP discharge. A two-tailed statistical t-test with unequal variance was performed to compare water quality at the upstream reference station (DO-127 Brant Bridge) and the downstream station (DO-194 SJR at Burns Cutoff) to see if differences in water quality upstream and downstream of the WWTP were significant. The t-test shows that all measured nitrogen compounds (TAN, nitrate, and nitrite) show a significant increase in concentration downstream of the WWTP discharges into the SJR.

Statistical comparisons were made between the upstream and downstream stations separately for the period of time when the WWTP was discharging and when it was not discharging. The results indicate that for many water quality variables, concentrations at upstream and downstream locations were statistically different only when the WWTP was discharging. These variables included specific conductance, alkalinity, total and dissolved organic carbon, ammonia-N, nitrate-N, nitrite-N, and total-N. This result further supports the conclusion that WWTP discharge can influence ambient water quality conditions in the SJR.

Other parameters with significant increases after the WWTP began discharging (95% confidence interval) include volatile suspended solids, phosphate-P and turbidity. Those parameters with no significant change include total suspended solids, mineral solids, chlorophyll, total P, dissolved oxygen, alkalinity, chlorine, and pH.

Findings: Ammonia and Fish Toxicity

The regulatory criteria for ammonia in water were established by USEPA (1999) and consist not of a single value but an equation that is dependent on several factors: (1) the presence of salmonids (2) presence of early life stages (3) pH and (4) temperature. The complex nature of ammonia toxicity is due to the fact that the ammonium ion (NH₄⁺), which is predominant at low pH, is much less toxic than ammonia (NH₃) which is predominant at higher pH. The chronic criteria concentration (CCC) was calculated for all the sites during the VAMP period assuming early life stages and salmonids present using the following equation given by USEPA (1999):

$$CCC = 0.854 \cdot \left(\frac{0.0676}{1 + 10^{7.688 - \text{pH}}} + \frac{2.912}{1 + 10^{\text{pH} - 7.688}} \right) \cdot \text{MIN} (2.85, 1.45 \cdot 10^{0.028 \cdot (25 - T)})$$

Where T = temperature. For example, on May 3rd site DO-195, the SJR at the WWTP outfall, had a pH of 7.58 and temperature of 17.04°C, resulting in a CCC for this site of 4.05 mg/L TAN. The measured value of TAN on this date was 0.126 mg/L. The overall average CCC value for the river was found to be 2.9 +/- 0.8 mg TAN/L. The observed concentrations of TAN, NH₃-N, and the CCC for each location are given in Table 6-4. As can be seen from this table, TAN levels were well below the CCC during the VAMP period. Also note that the CCC is higher in the WWTP effluent samples due to the lower pH in those samples. The acute criteria are calculated in a similar manner and are generally one order of magnitude larger than the CCC.

The 1999 USEPA guidelines for CCC use fish death as a measurable endpoint after 30 days of exposure in a controlled system. However, it has been shown that values of ammonia below the CCC can cause changes in fish behavior that can influence fish survivability in real systems. Several studies have found that levels of TAN from 0.001 – 0.25 mg/L are detrimental to the swimming speed of salmonids, therefore reducing survivability (Buhl, 2002; Randall and Tsui, 2002; McKenzie et al., 2003; Passell et al., 2007). In addition, some studies have shown that when fish are swimming, feeding, or stressed by additional contaminants such as Cu, salt, chlorine or other environmental conditions they will have increased sensitivity to ammonia (Randall and Tsui, 2002; Passell et al., 2007). Cu at levels as low as 0.08 µM (5 µg/L) has been shown to increase the level of ammonia in blood because it interferes with the ion regulatory systems and impairs ammonia excretion and increases internal ammonia production from food digestion (McKenzie et al., 2003). While Cu was not measured in this study, historical data shows an average value of 1.7 µg/L Cu in the SJR, with measured values ranging from 1 to 70 µg/L (DWR, 2004; Buck et al., 2007).

Conclusions

Water quality monitoring was performed in the SJR adjacent to the Stockton WWTP during the 2008 VAMP fish release. Results demonstrate that the WWTP discharge has an effect on water quality especially in respect to N compounds, including TAN. However, concentrations of ammonia are below the CCC established by the USEPA for protection of fish health. Further study is needed to determine if a combination of water quality constituents, including salts, Cu, and chlorine, could combine to create conditions that would reduce the survivability of salmonids in the SJR.

Survival and Physiological Evaluation of Chinook Salmon held in the San Joaquin River near the Stockton Wastewater Treatment Plant, May 2008.

Contributed by Ken Nichols and J. Scott Foott, USFWS, CA-NV Fish Health Center

Introduction

As a component of the 2008 Vernalis Adaptive Management Plan (VAMP) study on reach-specific survival and distribution of migrating Chinook salmon in the San Joaquin River and delta, the CA-NV FHC conducted a bioassay to assess acute water quality effects on salmon. In 2007, acoustic tags from juvenile Chinook salmon were detected “not moving” near the Stockton Waste Water Treatment Plant (WWTP). Due to the mortality observed in 2007, aquatic bioassays were conducted in the critical reach near the WWTP during the initial 24 hours of the 2008 VAMP study releases.

Methods

Fish

Juvenile Chinook salmon used in this study were reared at the California Department of Fish and Game Merced River Hatchery (MRH) and were cohorts of the acoustic tagged Chinook used in the VAMP survival and distribution studies. Exposures began on the same days as the acoustic tagged fish were released at Durham Ferry. The first exposure began on May 1st and the second exposure on May 8th. Prior to transport, weight and fork length were measured from 20 fish of the tank population used for the study. This data was not collected at the exposure sites to speed necropsy. Another 60 salmon were transported in an aerated 80 gal tank to the bioassay sites in 6 live cages (10 fish / cage). Total transport time averaged 2 hours.

Sites

The three exposure sites were at the Stockton WWTP outfall (WWTP), Burn's Cut about 0.5 miles downstream of the water treatment plant (Downstream), and a control site at Bryant Bridge approximately 8 miles upstream of the WWTP (Control). Two live cages were placed at each site. Temperature and dissolved oxygen were measured at each site using a Hach HQ10 portable LDO meter at the end of each exposure.

Figure 6-14
Photomicrograph of Microvesicular hepatocyte vacuoles in the liver of juvenile fall Chinook salmon. H&E stain.

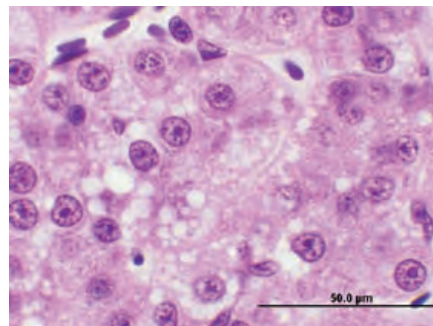


Figure 6-15
Photomicrograph of epithelial edema in the gill of juvenile fall Chinook salmon. H&E stain.



Table 6-5
Histological evaluation of gill, liver, and kidney from MRH salmon used as sentinels for VAMP release 1 (April 29th - May 1st). Data recorded as number of fish showing abnormality over total fish sampled at 4 or 24h at the control, WWTP outfall, or downstream of outfall. Also listed is the incidence of *T. bryosalmonae* (Tb) infection and number of infected fish showing severe interstitial hyperplasia (inflammation).

	Control		WWTP		Downstream	
	4h	24h	4h	24h	4h	24h
Epithelial edema in >10% of gill	3/10	1/10	1/10	1/9	0/5	1/5
Vacuolated hepatocytes	3/9	3/8	2/9	2/9	2/4	1/5
Incidence of Tb infection	80%	100%	50%	66%	80%	80%
Interstitial inflammation	1/8	3/10	0/10	0/9	0/5	0/5

Table 6-6
Histological evaluation of gill, liver, and kidney from MRH salmon used as sentinels for VAMP release 2 (May 6 th- May 8 th). Data recorded as number of fish showing abnormality over total fish sampled at 4 or 24h at the control, WWTP outfall, or downstream of outfall. Also listed is the incidence of *T. bryosalmonae* (Tb) infection and number of infected fish showing severe interstitial hyperplasia (inflammation).

	Control		WWTP		Downstream	
	4 hr	24 hr	4 hr	24 hr	4 hr	24 hr
Epithelial edema in >10% of gill	0/10	0/10	1/10	0/10	2/10	0/10
Vacuolated hepatocytes	1/10	1/10	3/10	0/10	5/10	3/10
Incidence of Tb infection	80%	90%	60%	90%	70%	100%
Interstitial inflammation	1/10	0/10	0/10	0/10	0/10	2/10

Table 6-7
Blood chemistry data for 2008 VAMP fish used in live cage bioassays at the Stockton Waste Water Treatment Plant outfall (WWTP) 0.5 miles downstream of the WWTP (Downstream) and 8 miles upstream of the WWTP (Control). Exposures corresponded with the 2008 VAMP release groups on May 1st and May 8 and fish were held in live cages at the sites for 4 and 24 hours before sampling. Data presented as mean ± SE (n).

Exposure	Time	Site	HCT (%)	TP (mg/dl)	Cl- (mEq/l)
May 1st	4 hrs	Control	39.0±1.9 (10)	29.3±2.1 (10)	124±3 (10)
		WWTP	40.5±1.3 (10)	37.2±3.4 (10)	134±6 (10)
		Downstream	45.6±4.5 (5)	29.1±1.7 (5)	138±10 (5)
	24 hrs	Control	33.5±1.5 (10)	29.0±2.1 (9)	148±11 (9)
		WWTP	36.1±1.9 (10)	25.6±1.1 (10)	128±3 (10)
		Downstream	35.8±0.5 (4)	26.5±1.2 (4)	117±1 (5)
May 8th	4 hrs	Control	42.0±0.9 (10)	32.5±1.1 (10)	120±7 (10)
		WWTP	42.9±1.7 (9)	35.6±1.2 (10)	117±5 (10)
		Downstream	40.3±1.2 (10)	33.6±1.1 (10)	117±6 (10)
	24 hrs	Control	38.3±1.4 (9)	25.0±0.8 (9)	119±1 (9)
		WWTP	42.0±0.8 (10)	28.4±1.6 (10)	110±6 (10)
		Downstream	34.3±1.2 (10)	31.0±4.0 (10)	123±3 (10)

Sampling

One live cage at each of the 3 sites was sampled after 4 and the other at 24 hours post exposure. Fish were euthanized in an overdose of MS222 and immediately bled into heparinized microhematocrit tubes from the severed caudal peduncle. Blood was used to prepare a blood smear, assay for methemoglobin, centrifuged to obtain the hematocrit value and collect plasma. Plasma was held on dry ice for frozen transportation back to -80°C storage. Tissues were collected for histology, and samples of gill, liver and kidney collected and frozen in liquid nitrogen for further analysis, if needed, by Dr. Inge Warner (UC Davis).

Assays

Histopathology – The gills, viscera (intestinal tract, pyloric caeca, heart, liver and spleen) and posterior kidney were rapidly removed from the fish and immediately fixed in Davidson's fixative, processed for 5 mm paraffin sections and stained with hematoxylin and eosin (Humason 1979). All tissues for a given fish were placed on one slide and identified by a unique code number. Each slide was examined at low (40X) and high magnification (400X).

Methemoglobin (methHb) Assay – Elevated nitrite levels can induce methemoglobinemia in fish (Wedemeyer 1996). A blood sample was tested for percent methemoglobin using a method modified from Fairbanks and Klee (1994). In short, 20 ml of blood was diluted in 1980 ml phosphate buffer (0.067M, pH 6.7). The samples was mixed and split into 2 cuvetts (A and B). A solution of 20% $K_3Fe(CN)_6$ was added to cuvet B and allowed to react for 2 min. The absorbance (630 nm) of both cuvetts was then read in a spectrophotometer (A_1 and B_1) using 50% phosphate buffer in water as a blank. A drop of neutralized cyanide (6% acetic acid and 5% sodium cyanide in water) was added to all cuvetts. The sample was mixed and absorbance read again (A_2 and B_2). The percent methHb was then calculated as $100 * (A_1 - A_2) / (B_1 - B_2)$.

White blood cell count – Blood smears were stained with a Diff-Quick stain kit (Dade-Behring, Newark DE) and read at 1000X magnification. A total of 100 white blood cells were counted and identified to lymphocyte, thrombocyte, neutrophil, or monocyte. The ratio of leukocytes to granulocytes (neutrophil) was calculated.

Plasma total protein and chloride – Plasma was stored at -80°C until analyzed. Total protein was measured using colorimetric analysis reagents from Point Scientific (Canton, Michigan, kit T7528) and bovine serum

albumin as a standard. Plasma chloride was measured using colorimetric analysis reagents from Point Scientific (kit C7501).

Results

Fish

Average (SE) fork length and weight was 96.8mm (0.3mm) and 9.8g (0.1g) for the May 1st exposure, and 106.0mm (0.6mm) and 12.3g (0.2g) for the May 8th exposure. All fish survived the exposures. Due to a failure of the anchor system, one of the two live cages was lost at the Burn's Cut site in the May 1st exposure. The 10 fish from the remaining live cage were split for the 4 and 24 hr samples.

Sites

The Stockton Waste Water Treatment Plant was discharging effluent during the May 1st (first) exposure period, but the plant was not discharging during the May 8th (second) exposure. The plant was down for maintenance and did not discharge for the entire 24 hour exposure period. Dissolved oxygen measurements at the exposure sites ranged from 7.8-9.8 mg/L. Water temperature measurements ranged from 17.1 to 19.6°C on the May 1 exposure and 19.4 to 21.5°C on the May 8th exposure.

Assays

Histopathology

Most of the fish in the experiment were infected with *Tetracapsuloides bryosalmonae* (Tb) with associated kidney inflammation apparent in only a few samples (Tables 6-5 and 6-6). There was no difference in Tb infection between exposure groups or sites. The incidence of microvesicular hepatocyte vacuoles (Fig. 6-14) in the liver was higher in fish exposed May 1st compared to fish exposed on May 8th. Similarly, edema of the gill epithelial layer (Figure 6-15) was noted in a few fish from all groups. These changes were observed in fish from all exposure sites with no evidence of a difference between sites.

MetHb assay

This assay failed to perform under field conditions. Results were highly variable ranging from negative values to well over 100% MetHb.

Hematocrit

Values were all within normal range (25-55 %, Table 6-7). The only significant difference detected was between the May 8th Downstream and WWTP 24 hour exposure groups ($P < 0.001$, ANOVA). None of the fish had HCT values suggesting anemia.

White blood cell count

No obvious differences between groups were observed in the WBC counts, but a high number of lysed cells made counts difficult and possibly biased. This assay was not used in any analysis.

Plasma total protein

No differences were detected between fish at any of the sites in the May 1st exposure groups or May 8th exposure groups ($P > 0.05$, ANOVA) (Table 1). A potential hyperproteinemia (≥ 40 mg/dl) was observed in several (7 of 107) fish. These fish were noted at all sites and no pattern in exposure time or location was evident (data not shown)

Plasma chloride

A difference was observed in the May 1st (24 h) exposure groups between the WWTP and the control site ($P = 0.028$, ANOVA). No differences were detected in any of the other exposure groups. Three of the 10 fish in the May 1st WWTP 24 h exposure group were hyperchloremic (> 140 mEq/L) which caused this group to stand out. In total, 7 of 108 fish were potentially hyperchloremic and were detected in samples from all 3 sites. One fish from the May 8th Downstream 24 h exposure group appeared hypochloremic.

Discussion

The purpose of this VAMP study component was to determine if there was localized acute mortality or morbidity associated with the WWTP effluent as hypothesized in 2007. None of the fish in this study died and no significant site specific sub-lethal effects were identified. In past monitoring of VAMP study fish, the most significant health finding for study fish was infection with *T. bryosalmonae* (Foott et al. 2007). While the incidence of *T. bryosalmonae* (causative agent of Proliferative Kidney Disease or PKD) was high in the all the exposure groups, it appeared that the infections were in early stages and would not influence the fish's performance. The MRH Fall Chinook become infected

with *T. bryosalmonae* at the hatchery, and mortality due to PKD does not occur until June after the VAMP studies are completed (Foott et al. 2007). Elevated plasma protein and chloride values (hyperproteinemia and hyperchloremia) were observed in fish from all sites and both exposure periods. These elevated plasma chemistry values were likely not a result of the exposure site, but rather changes due to our handling of the fish or samples. Possible explanations of these elevated plasma chemistry values include: plasma reduction due to shock, contamination of the plasma in the field, or desiccation of the plasma.

A difference in HCT values was detected between the WWTP and downstream sites during the May 8th exposure, but the difference was not large enough to have any biological significance as none of the fish were anemic. Elevated HCT can result from the stress response of splenic contraction and erythrocyte swelling (Wells and Weber 1991). The histopathological changes observed in the gills did not appear to be related to exposure site, and were more likely artifacts of delayed fixation. Hepatocyte vacuoles appeared to be a mix of fat and glycogen. This condition is not atypical for hatchery salmon fed high energy diets.

Two of the assays attempted in this study did not perform well enough to be included in the analysis. The metHb assay relied on the ability to quickly and consistently process the blood sample in the field. Several factors which may have interfered with the metHb assay including: multiple fish were processed at a time delaying some steps; warm weather likely caused blood to clot and reagents react and degrade faster than expected; bright sunlight and dust may have interfered with the spectrophotometer. This assay may perform much better in the lab where a single fish could be processed at a time and environmental conditions could be better controlled. The white blood cell count assay was impaired by high numbers of smudge and ghost cells which may have been affected by field conditions including temperature and delayed processing.