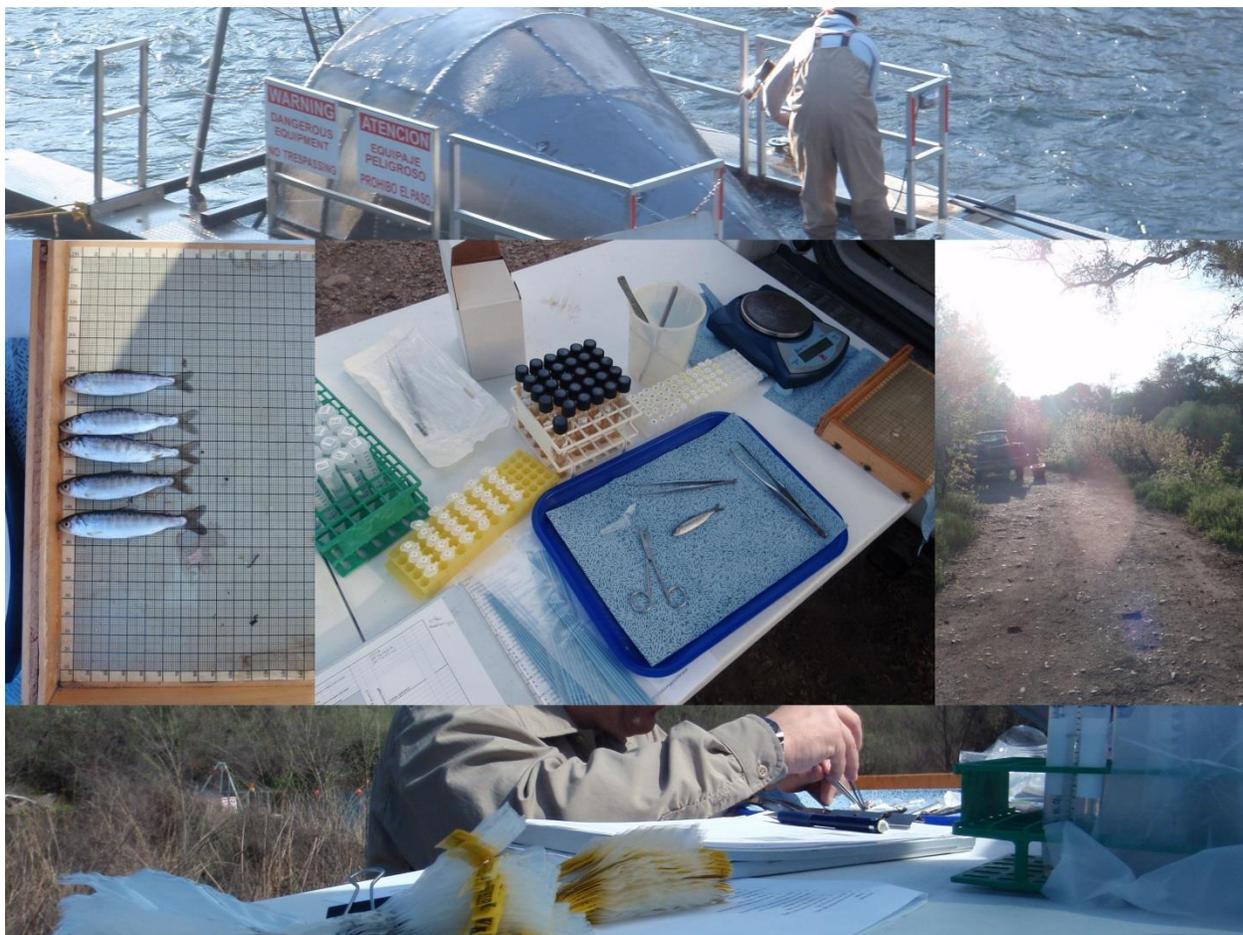


U.S. Fish & Wildlife Service

California Nevada Fish Health Center

FY2013 Technical Report: San Joaquin, Stanislaus, Tuolumne and Merced River Chinook Smolt Quality Assessment

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Summary

Juvenile Chinook smolts were sampled in March and April, 2013 in the Stanislaus, Tuolumne, Merced and San Joaquin Rivers. Sampling was intended to monitor smolt health and physiology changes that may affect survival during outmigration. Smolt health sampling and laboratory assays were conducted under protocols established for the USFWS National Wild Fish Health Survey (NWFHS, <http://www.fws.gov/wildfishsurvey/>). No bacterial or viral pathogens were detected. During April, *Tetracapsuloides bryosalmonae*, the causative agent of proliferative kidney disease (PKD), was detected in 80% of Merced, 7% of Stanislaus and 25% of mainstem San Joaquin River smolts. The majority of *T. bryosalmonae* infections were in the early stages with little or no associated kidney pathology. Smolts captured in April were larger, had higher Fulton condition factors and had higher gill Na⁺/K⁺-ATPase activity levels compared to fish sampled in March. No brain acetylcholinesterase inhibition due to pesticide exposure was detected in the fish sampled. The overall condition of smolts appeared good at the time of sampling; however, PKD could have eventually compromised survival and performance of the out-migrants, particularly in fish from the Merced River.

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Background

Health and performance of juvenile salmonid out-migrants (smolts) are major determinants of their survival. Infectious disease will reduce survival both in direct mortality and reduced physical performance (predator avoidance, saltwater adaptation). Contaminants and elevated water temperature are identified as stressors for salmonids in the San Joaquin River and Estuary (USFWS 2001). Both of these stressors have potential for immunosuppressive effects (Rice and Arkoosh 2002). Infection with the myxozoan parasite that causes Proliferative Kidney Disease (PKD), *Tetracapsuloides bryosalmonae*, was observed in 90-100% of naturally produced fish in a 2001 survey of Merced out-migrant salmonid health (Nichols and Foott 2002). Methyl mercury, elevated water temperature, nitrogenous input, agricultural runoff, spring irrigation return and their relationship to river flow are other potential issues for juvenile salmonids the San Joaquin River basin. This study examined smolt health during the critical spring outmigration using a suite of assays to detect pathogens (bacteria, virus, and parasites) and biomarkers of contaminate exposure.

Methods

The California-Nevada Fish Health Center performed health and physiological condition screening of Chinook salmon smolts in the San Joaquin River basin. Smolt health sampling and laboratory assays were conducted under protocols established for the USFWS National Wild Fish Health Survey (NWFHS, <http://www.fws.gov/wildfishsurvey/>). Sample sites included rotary screw traps near Oakdale on the Stanislaus River, Waterford on the Tuolumne River, Hopeton on the Merced River and a trawl site near Mossdale on the mainstem San Joaquin River. The three traps were operated by FISHBIO (Oakdale, CA) and the California Department of Fish and Wildlife conducted the Mossdale trawl. The Stanislaus, Tuolumne and Merced River traps were sampled on 27-28 March and again 16-17 April. The Mossdale trawl on the San Joaquin River was sampled on 30 April, 2013. Up to thirty juvenile Chinook salmon were targeted at each site per month, and fish greater than 70mm fork length (smolt size) fish were selected for sampling.

Lab assays included: bacteriology, virology and histopathology for pathogen prevalence and severity (USFWS and AFS-FHS 2010); gill Na⁺/K⁺-ATPase activity as an indicator of smolt development (McCormick 1993); brain acetylcholinesterase activity as biomarker of organophosphate pesticide exposure (Wheelock et al. 2005).

Results

Table 1 summarizes fork length (FL), weight (Wt) and Fulton condition factor (KFL). Increases in KFL were observed between March to April in fish from the Stanislaus (P<0.001) and Tuolumne (P=0.043), but not difference between months was observed in fish from the Merced (P=0.388).

Table 1. Fork length, weight and Fulton condition factor (KFL) for Chinook smolts captured on the Stanislaus, Tuolumne, Merced and San Joaquin Rivers during March and April, 2013.

Month	River	N	FL (mm)	Wt (g)	KFL (Wt*FL ⁻³ *10 ⁵)
March	Stanislaus	23	73.6 ±0.8	3.8 ±0.1	0.95 ±0.01
	Tuolumne	13	75.2 ±1.1	4.2 ±0.2	0.97 ±0.02
	Merced	30	75.8 ±0.9	4.2 ±0.2	1.00 ±0.01
April	San Joaquin	4	86.5 ±7.7	7.6 ±2.2	1.07 ±0.04
	Stanislaus	30	76.8 ±0.9	4.9 ±0.2	1.06 ±0.01
	Tuolumne	30	80.9 ±1.0	5.5 ±0.2	1.03 ±0.02
	Merced	30	82.8 ±1.1	5.9 ±0.3	1.02 ±0.01

No obligate bacterial or viral fish pathogens were detected in any of the fish sampled. No problems were observed in the gross external examination (gills, skin, fins, eyes). The only gross internal abnormality observed was minor kidney inflammation in one fish in the March Stanislaus River sample and one fish from the April Merced River sample. No associated *Renibacterium salmoninarum* (by DFAT) or *Tetracapsuloides bryosalmonae* (by histopathology) infections were detected in either of these individual fish; however, *Tb* infections were observed in other individuals from these locations (see histopathology results below).

Kidney tissue was examined by histopathology to determine the prevalence (Table 2) and severity (Table 3) of *T. bryosalmonae* infections. A significant increase in infection prevalence was observed in fish from the Merced River between March and April (P<0.001, Wilcoxon rank sum test), and no difference was observed between months on the Stanislaus (P=0.328, Wilcoxon rank sum test) and Tuolumne Rivers (no test needed). In April, Merced River smolts had a significantly greater prevalence of *T. bryosalmonae* infection compared to fish from the San Joaquin, Stanislaus or Tuolumne Rivers which were all similar (P<0.001, Kruskal-Wallis test). The severity of kidney lesions followed the same pattern as parasite prevalence, with Merced River smolts demonstrating a significant increase between March and April (P=0.0454, Wilcoxon rank sum test) and more severe lesions compared to fish from other locations (P=0.0487, Kruskal-Wallis test).

Table 2. Prevalence of *Tetracapsuloides bryosalmonae* parasite infections in juvenile Chinook from Stanislaus, Tuolumne, Merced or San Joaquin Rivers. Infections were rated as none (no parasites), light, moderate, or heavy based on number of parasites observed in the kidney by histopathology. Groups with different letter codes had significantly different mean infection ratings between Rivers (Kruskal-Wallis test, P<0.05), and asterisks indicate significant difference within a site between March and April (Wilcoxon rank sum test, P<0.05).

Month	River	N	None	Light	Moderate	Heavy	Group
March	Stanislaus	15	15 (100%)				*
	Tuolumne	13	13 (100%)				
	Merced	29	29 (100%)				
April	San Joaquin	4	3 (75%)	1 (25%)			b
	Stanislaus	30	28 (93%)	2 (7%)			b
	Tuolumne	23	23 (100%)				b
	Merced	30	6 (20%)	18 (60%)	4 (13%)	2 (7%)	a*

Table 3. Severity of kidney lesions associated with *Tetracapsuloides bryosalmonae* parasite infections in juvenile Chinook salmon smolts from the Stanislaus, Tuolumne, Merced and San Joaquin Rivers. Lesions were rated as normal (no lesion), Focal, Multifocal or Diffuse by histopathology. Groups with different letter codes had significantly different mean infection ratings between Rivers (Kruskal-Wallis test, $P < 0.05$), and asterisks indicate significant difference within a site between March and April (Wilcoxon rank sum test, $P < 0.05$).

Month	River	N	Normal	Focal	Multifocal	Diffuse	Group
March	Stanislaus	15	15 (100%)				*
	Tuolumne	13	13 (100%)				
	Merced	29	29 (100%)				
April	San Joaquin	4	4 (100%)				ab
	Stanislaus	30	30 (100%)				b
	Tuolumne	23	23 (100%)				b
	Merced	30	26 (87%)	3 (10%)	0	1 (3%)	a*

Median gill Na⁺/K⁺-ATPase (ATPase) activity levels ($\mu\text{mol ADP} \cdot \text{mg protein}^{-1} \cdot \text{hr}^{-1}$) ranged from 6.0 to 13.6, with significant differences observed between rivers (Figure 1) in both March and April ($P < 0.001$, Kruskal-Wallis test). ATPase activity levels increased significantly between March and April in smolts from the Stanislaus, Tuolumne and Merced Rivers ($P = 0.025$, $P < 0.001$ and $P < 0.001$ respectively, Wilcoxon rank sum test).

Acetylcholinesterase (AChE) – Median brain AChE activity in Chinook smolts ranged from 213 to 380 nmol AChE hydrolyzed $\cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ (Figure 2). In March, AChE activity levels in Merced smolts were significantly lower than fish from the Stanislaus or Tuolumne Rivers ($P < 0.001$, Kruskal-Wallis test); however, in April, AChE levels in Merced smolts were among the highest observed with fish from the mainstem San Joaquin River with the lowest ($P < 0.001$, Kruskal-Wallis test). AChE activity levels increased between March and April in fish from the Stanislaus, Tuolumne and Merced Rivers ($P < 0.001$ in all rivers, Wilcoxon rank sum test).

Discussion

Smolts sampled during April generally had greater KFL and gill ATPase levels compared to fish sampled in March. It was not known if these differences were an indication of better overall fitness in April fish or a temporary environmental effect with no biological significance. The lower KFL and gill ATPase observed in March smolts were not biologically significant or likely to impact outmigration performance.

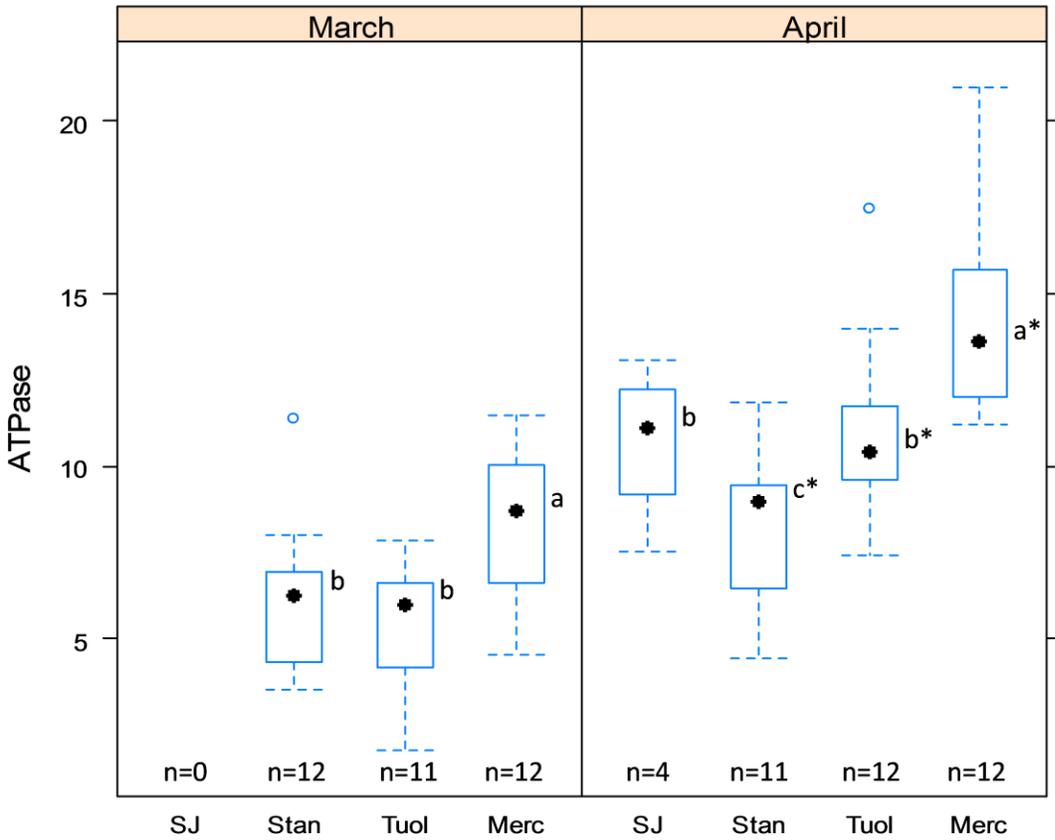


Figure 1. Box and whisker plot of median gill Na⁺/K⁺-ATPase activity (ATPase) values for juvenile Chinook salmon smolts from the San Joaquin (SJ), Stanislaus (Stan), Tuolumne (Tuol) and Merced (Merc) Rivers during March and April 2013. Different letter codes indicate a significant difference in mean ATPase activity between rivers within a month (March P=0.011, April P<0.001, Kruskal-Wallis test), and asterisks indicate significant difference within a site between March and April (Stan P=0.025, Tuol P<0.001, Merc P<0.001, Wilcoxon rank sum test).

The only significant pathogen detected was *Tetracapsuloides bryosalmonae*, the causative agent of proliferative kidney disease. This pathogen was detected during April in smolts from the Stanislaus, San Joaquin and Merced Rivers. Most of the infections were early stage with associated pathology (kidney lesions) observed on in a few of the fish from the Merced River. The *T. bryosalmonae* parasite is a known problem in the Merced River. In a 2012 health survey, moderate to heavy infections with significant associated pathology was not observed until mid-May (Nichols, Bolick and Foott 2012). Infection with this parasite does not necessarily lead to the death of the animal. Hedrick and Aronstien (1987) found that over 90% of *T. bryosalmonae* infected juvenile Chinook salmon transferred to full strength seawater were able to recover under laboratory conditions. Survival in the river may be much lower due to the anemia, kidney dysfunction and immune suppression linked to PKD (Angelidis et al. 1987, Hedrick and Aronstien 1987). This disease compromises the fish's performance in many areas (swimming, salt water entry, disease resistance) and decreases its potential for survival during out-migration.

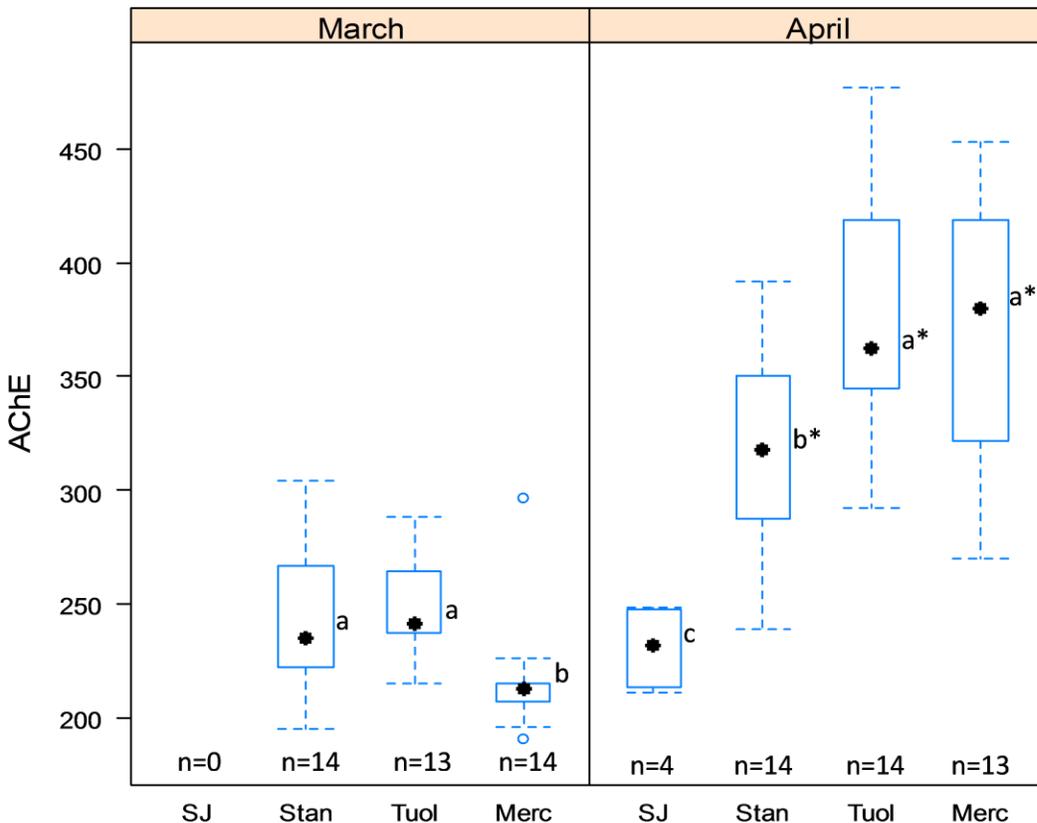


Figure 2. Box and whisker plot of median brain Acetylcholinesterase (AChE) activity ($\text{nmol AChE hydrolyzed} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$) for juvenile Chinook salmon smolts from the San Joaquin (SJ), Stanislaus (Stan), Tuolumne (Tuol) and Merced (Merc) Rivers during March and April 2013. Different letter codes indicate a significant difference in mean ATPase activity between rivers within the month (March $P < 0.001$, April $P < 0.001$, Kruskal-Wallis test), and asterisks indicate significant difference within a site between March and April (Stan $P < 0.001$, Tuol $P < 0.001$, Merc $P < 0.001$, Wilcoxon rank sum test).

It is not clear why AChE activity levels in fish captured in March were lower than fish sampled in April. The increase in AChE activity in fish sampled in April relative to March in this study is the opposite of what was observed in a 2012 study on juvenile Merced River Chinook (Nichols et al. 2012). Published literature also suggests AChE activity levels typically decline in older and larger fish (Phillips, Summerfelt and Atchinson 2002; Durieux et al. 2011). Fish size and age was likely not a significant factor in this study since only smolt size ($>70\text{mm FL}$) fish were targeted. There was also no relationship between FL and AChE activity within March or April (data not presented). The role of environmental temperature on AChE activity is unclear. Some studies suggest an increase in activity with higher water temperature, and no effect found by others (Durieux et al 2011; Phillips et al 2002). Whether the small increase in water temperature between March and April ($<2^\circ\text{C MDT}$, data not presented) was responsible for the differences in AChE activity, or activities in March were suppressed for some other reason deserves further study.

Acknowledgements

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