

# STANISLAUS RIVER JUVENILE CHINOOK SALMON SURVIVAL STUDY

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*Prepared for:*

United States Fish and Wildlife Service

Anadromous Fish Restoration Program

*Prepared by:*

Steven Zeug, Kirsten Sellheim, Jenny Melgo, and Joseph Merz

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## EXECUTIVE SUMMARY

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This report describes the results of a three year mark-recapture experiment with juvenile (age 0) fall run Chinook salmon in the Lower Stanislaus River (LSR). Radio telemetry of hatchery juveniles was used to (1) estimate the survival of tagged juvenile Chinook salmon (FL size range 72-114 mm) in 50, 16, and 8 km increments in the LSR, (2) associate biotic and abiotic characteristics of the study reach with variation in survival of tagged juveniles including; flow, temperature, fish size and release timing, (3) identify areas of disproportionate mortality that could be targeted by management actions, and (4) associate mortality locations with specific habitat features (e.g. diversions, agricultural drains, irrigation pumps, assumed predator pools etc.). The study area extended from the Oakdale Recreational Area ( $\approx$  rkm 64.4) to Caswell Memorial State Park ( $\approx$  rkm 14.8). The  $\approx$  50 km reach was further divided into three  $\approx$  16.5 km segments and six  $\approx$  8 km sub-reaches. A total of 1,228 radio tagged salmon were released over all years. Each spring, two tagged salmon releases were performed where one release occurred early (typically in late March or early April) and one release occurred later in the migration period (usually late April or early May). Releases were performed at the upstream end of each of the six 8 km sub-reaches. Stationary receivers were deployed at the downstream end of each 16 km segment to detect fish as they moved through the study reach. Additionally, mobile surveys were performed at regular intervals after each release to gain additional information on specific locations of tagged salmon and movement over time within each sub-reach.

Survival was estimated both from detections at the stationary receivers and mobile detections in separate statistical models. This was done to take advantage of the strengths and limit the biases associated with each method. Survival from stationary detections was based on the length of stream traveled between release and receiver station (i.e. a function of distance traveled) whereas survival from mobile detections was based on the time between surveys (i.e. survival was a function of time since release). Significant spatial variation in survival was detected in all years. However, the scale at which the differences occurred varied by the method of detection used. Survival in the 16 km reaches ranged from a low of 21% in Reach 2 to a high of 72% in Reach 1. This suggests that although survival was low in some reaches, there is potential for it to be increased significantly through rehabilitation activities. Survival estimated from mobile detections indicated significant variation in survival among 8 km sub-reaches in all years whereas survival from stationary detections yielded significant differences among sub-reaches only in the two most downstream sub-reaches. Mobile detections suggested that over the first three days after release, survival per day ranged from a low of 0% to a high of 100%; again suggesting significant potential for improvement. Reach 1 never had a daily survival value less than  $70\% \cdot \text{day}^{-1}$  supporting the finding that survival was greater in the most upstream reach in all years.

Estimated movement rates of juvenile salmon through the study reach varied considerably depending on the method of detection used. Stationary receivers yielded movement rates that ranged from a low of  $18 \text{ km} \cdot \text{day}^{-1}$  in Reach 2 to a high of  $51 \text{ km} \cdot \text{day}^{-1}$  in Reach 1. These estimates are similar to estimates for actively migrating Chinook salmon in other Central Valley rivers (Michel et al. 2013). Movement rates calculated from mobile data were considerably lower ranging from a low of  $0.88 \text{ km} \cdot \text{day}^{-1}$  in Reach 3 to a high of  $9.2 \text{ km} \cdot \text{day}^{-1}$  in Reach 1. The variation is likely the result of stationary receivers failing to pick up fish that were exhibiting

rearing or holding behavior and mobile surveys failing to detect fish that moved quickly out of the study reach between mobile surveys.

Analysis of survival as a function of biotic and abiotic characteristics followed an information theoretic approach where candidate models were constructed to represent different hypotheses related to influences on juvenile salmon survival in the LSR. Model selection of data from stationary receivers indicated that there was more variation among release years than there was in release timing (early vs. late), sub-reaches or along gradients of environmental variation (e.g., flow, temperature). Analysis of mobile detections indicated that many juvenile salmon exhibited holding/rearing behavior during some releases that likely biased stationary survival estimates because they remained alive but did not pass stationary receivers and thus appeared as mortalities in the stationary models. Survival analysis from the mobile detections indicated that the greatest mortality occurred in the first few days after release. Additionally, there was significant variation in survival among the 8 km sub-reaches ( $0-100\% \cdot \text{day}^{-1}$ ). Spatial analysis of last known detections relative to random point placement yielded significant clustering of last known detections close to the four release sites in the two most downstream experimental reaches. This confirmed indications from the mobile data that mortality often happens rapidly after release. This pattern was not found at the two release locations in the most upstream 16 km reach suggesting this was not purely a release effect but was also influenced by the quality of habitat into which the fish were released. Finally, a “hot spot” analysis of last known detections indicated that there were a disproportionate number of last known detections in the 8 km Sub-reach “2b”, located between rkm 33-40. This reach generally had the lowest survival during most release events. Analysis of bed elevation data suggested that the location of last known detections were in significantly deeper areas than expected in the most downstream reach and the lower half of the middle reach. Deep habitats are known predator holding locations and the lower half of the study area appears to have a large proportion of these habitats. Combined, these data indicate that survival is lower in downstream reaches that have greater proportions of deep habitats. Sub-reach 2b in particular demonstrated low survival for juvenile Chinook salmon and should be targeted for future rehabilitation.

## INTRODUCTION

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The San Joaquin River system, located in California's Central Valley, once supported escapements of adult Chinook salmon (*Oncorhynchus tshawytscha*) numbering in the hundreds of thousands (Yoshiyama *et al.* 2001). These historic runs exhibited a rich ecological diversity, and Chinook salmon were present throughout the river system year-round due to broad variation in life history strategies and high diversity and abundance of available habitat. Chinook salmon are among several native Central Valley anadromous fish populations undergoing widespread decline (Moyle *et al.* 2008) with spring- and winter-run Chinook salmon listed under the Federal Endangered Species Act and fall- and late fall-run Chinook salmon currently considered as species of concern (NMFS 2004). Declines of Chinook salmon populations have been linked to a variety of anthropogenic impacts including overharvest and reduction of in river habitat quality and quantity caused by dams, gravel mining, water quality degradation due to urban development and agricultural runoff, and, introduction of non-native piscivorous fish (Nehlsen *et al.* 1991; Yoshiyama *et al.* 2001; Williams 2006).

Commercial harvest of salmon by European settlers became widespread starting in the 1850's, and marked declines were observed in some tributaries as early as the 1870's (Yoshiyama *et al.* 1998). In recognition of this decline, laws to protect salmon populations and prevent over-fishing were passed during the late nineteenth and early twentieth century; however, these laws were not effectively enforced and illegal overharvesting was widespread for many decades (Yoshiyama *et al.* 1998). Hatchery supplementation has been widely implemented as a surrogate for declining wild salmon populations, but this only compounds the problem by compressing run timing and stock complexity and potentially reducing the survival of natural populations through density-dependent mechanisms (Augerot *et al.* 2005, Unwin 1997, Unwin and Glova 1997). Although spring-run Chinook salmon was historically the dominant race in the San Joaquin River and its tributaries, dam construction has prevented passage to critically important staging areas and spawning grounds. As a result, the spring-run life history is now largely considered extirpated from this region (Yoshiyama *et al.* 2001; Williams 2006), although small numbers have been observed in the lower Stanislaus River (LSR), a tributary to the San Joaquin River, in recent years (Anderson *et al.* 2007).

Dams have caused widespread degradation of river habitat worldwide by interrupting natural hydrological and sediment transport regimes and disrupting complex ecological processes that depend on flow fluctuations (Poff *et al.* 1997, Brown and Bauer 2010). Native fish populations are particularly impacted by flow regulation because they have life cycles adapted to seasonal flow fluctuations (Moyle *et al.* 2011). In California's Mediterranean climate, flow regulation generally results in a reduction in magnitude and duration of peak winter and spring runoff (Kondolf and Batalla 2005, Nilsson *et al.* 2005). This in turn decreases inundation frequency and duration of off-channel habitats that are important for juvenile salmonid rearing (Sommer *et al.* 2001, Jeffres *et al.* 2008) and low spring flows can increase juvenile residence time in the river and eliminate flow-related cues that trigger salmonid outmigration (Jager and Rose 2003). Several studies have observed positive association between flow and juvenile salmon migration through a given river reach (Brandes and McClain 2001, Perry *et al.* 2010, Zeug *et al.* 2014). Managing flows to more closely mimic natural conditions may provide a way to reduce the negative effects of flow regulation; however, insufficient data exists relating to juvenile salmonid survival, growth, migration timing, and the relative contribution of different life stages to provide



a basis for determining optimum flow timing and magnitude needed for out-migrating juvenile salmonids (Downs *et al.* 2011). Further, the relative contribution of specific sources of juvenile mortality and the distribution of mortality sources within the river system is not well understood (Kondolf *et al.* 2001).

In many Central Valley rivers, including the Stanislaus, historic gold and gravel mining greatly altered geomorphic and hydraulic conditions. As gold was retrieved from river sediments, discarded tailings were piled on floodplains (Clark 1970). These actions inverted in-channel gravel composition and disconnected side channels and floodplains, heavily impacting salmon spawning and rearing habitat (Kondolf 1997). Dredged channels and pits reduce flow turbulence and velocity; it has been hypothesized that this negatively affects juvenile salmonids by increasing travel times, providing favorable habitat for invasive predators, and reducing dissolved oxygen concentrations to harmful levels in the late spring and early fall when temperatures are high. Increased travel times for juvenile salmonids passing through areas with poor water quality and high predator concentrations in slow-flowing channels lacking cover may foster high rates of juvenile mortality.

Water temperature and dissolved oxygen conditions can become stressful for juvenile salmonids during outmigration in the late spring to early summer when air temperatures increase (Myrick and Cech 2004). Laboratory experiments have demonstrated that juvenile Chinook salmon exhibit decreased growth, impaired development, and increased vulnerability to predators at temperatures above 17°C (Marine and Cech 2004). Water quality has also been degraded in many Central Valley streams due to agricultural and urban runoff (Weston *et al.* 2004, Zhang *et al.* 2008, Ensminger *et al.* 2013). Weston *et al.* (2004) conducted a study of toxicity in river sediments throughout California's Central Valley and reported that pyrethroid pesticides were detected in 75% of the sites, with 14% of sites sampled showing extreme sediment toxicity in laboratory experiments. Zhang *et al.* (2008) identified the Stanislaus River as being particularly high-risk for water pollution from pesticide runoff compared with other San Joaquin River tributaries due to land use practices, precipitation, geomorphology, and soil type. However, it is unknown to what extent poor water quality caused by suboptimal temperature, dissolved oxygen, or agricultural and urban runoff impacts outmigrating salmonids.

Non-native piscivorous fish are highly abundant throughout California's Central Valley, and predation is believed to be a major contributor to juvenile salmonid mortality in this system (Lindley and Mohr 2003, Nobriga and Feyrer 2007, NMFS 2014). Striped bass *Morone saxatilis* were introduced in 1879 and are currently managed as a sport fishery (Mason 1882, Parks 1978). Other common piscivorous fish such as non-native black bass *Micropterus* spp. and native Sacramento pikeminnow *Ptychocheilus grandis* may also impact juvenile salmonid survival, especially in degraded channels. A positive relationship between piscivorous fish abundance and water depth is well documented in the scientific literature, and deep pools are known to facilitate predation (Power *et al.* 1985; Brown and Moyle 1991; Gelwick *et al.* 1997). Although shallow habitats may increase predation risk from avian and mammalian piscivores, it has been hypothesized that non-native fish are the dominant predators of juvenile salmonids in the lower Stanislaus River (Kondolf 1997). However, few studies to date have explicitly measured the density and distribution of piscivorous fish or the impact of predation relative to other factors on juvenile salmonid survival (but see Cavallo *et al.* 2012 and Sabal *et al.* 2016).

Historically, the lower Stanislaus River was a dynamic river system, characterized by depositional and scour features and a diversity of off-channel salmonid rearing habitat. Following extensive dam construction, the active channel became relatively static and entrenched due to the factors described above (Kondolf *et al.* 2001). Rotary screw trap data from the upstream and downstream sections of the outmigration corridor of the Stanislaus River shows a significant reduction in juvenile abundance in the downstream trap, suggesting that mortality is generally high as juvenile salmonids migrate downstream through the river system (Zeug *et al.* 2014). This study also identified several positive correlates with survival, including greater cumulative flow and higher variance in flow during the migration period.

The present study was designed to provide a scientifically robust assessment of juvenile Chinook salmon survival within the LSR migratory corridor over several seasonal (e.g., late winter versus spring), flow (e.g., high versus low), and population (e.g., migration timing, size) conditions. Four broad goals were addressed by this study including: (1) use radio telemetry technology to estimate reach-specific survival for two distinct size-classes of juvenile Chinook salmon in the LSR in 8, 16 and 50 km increments and determine whether there is spatial variation in survival along the 50 km study reach, (2) associate biotic and abiotic characteristics of reaches with greater suspected mortality to explore whether reach-specific differences in these characteristics contribute to differences in survival along the LSR migratory corridor, (3) use mobile telemetry surveys to identify the location of salmonid mortality during outmigration at the finest resolution possible (habitat unit scale) to identify problem areas that could be targeted for future management actions and (4) track experimental fish using mobile radio telemetry surveys and monitor their movement and behavior during their outmigration in relation to potential sources of mortality (e.g., deep water habitats, agricultural return drains, irrigation pumps, etc.). To address these four broad goals we directly tested the following null hypotheses:

H<sub>0</sub>1: The survival of Chinook salmon juveniles does not differ among sub-reaches of the LSR.

H<sub>0</sub>2: Survival is constant throughout the migration period.

H<sub>0</sub>3 The spatial distribution of last known detections is random throughout the study reach.

H<sub>0</sub>4: Survival estimates are not related to migration speed of juvenile Chinook salmon.

H<sub>0</sub>5: Survival is not associated with identified biotic or abiotic characteristics.

Although estimation of survival probabilities and identification of areas exhibiting high juvenile mortality are the primary goals of this multi-year study, we also seek to describe other aspects of juvenile migration, including travel times and migration speeds, which will inform fisheries and flow management and habitat restoration efforts that aim to rehabilitate LSR salmonid populations.

## METHODS

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### Study area

The Stanislaus River flows along the western slopes of the Sierra Nevada Mountains southwest into the San Joaquin River. It drains approximately 284,899 hectares and 40% of its basin is above snowline (Kondolf *et al.* 2001). The confluence of the Stanislaus and San Joaquin rivers is near the southern end of the Sacramento-San Joaquin Delta in California's Central Valley (Figure 1). The lower Stanislaus River, heretofore referred to as the LSR, is a 95 km stretch of river available to anadromous salmonids between the first upstream barrier at Goodwin Dam (~91.4 meters above mean sea level) and the San Joaquin River confluence. For this study, the two rotary screwtraps (i.e., Oakdale, located at rkm 64.4; and Caswell, located at rkm 14.8) delineate the approximate upstream and downstream ends of the Stanislaus River outmigration corridor (Figure 2).

Using available literature specific to the Stanislaus River (e.g., Aceituno 1990; Demko *et al.* 1998; Kondolf *et al.* 2001) and information obtained from local experts, we separated the outmigration corridor into three reaches based on physical features that are meaningful to migrating juvenile salmonids and represent key landmarks that provide access and landmarks as reference points (Figure 2). Each reach is between 16.0-19.4 rkm in length. Reach 1 begins just downstream of the Hwy 120 bridge, and includes gravel ponds at the Oakdale Recreation Area and agricultural drains near Riverbank. Reach 2 begins at McHenry Bridge and includes several areas with deep water habitat and at least one agricultural drain. Reach 3 begins several km above the Hwy 99 Bridge and ends just above the Caswell rotary screw trap (Figure 2).

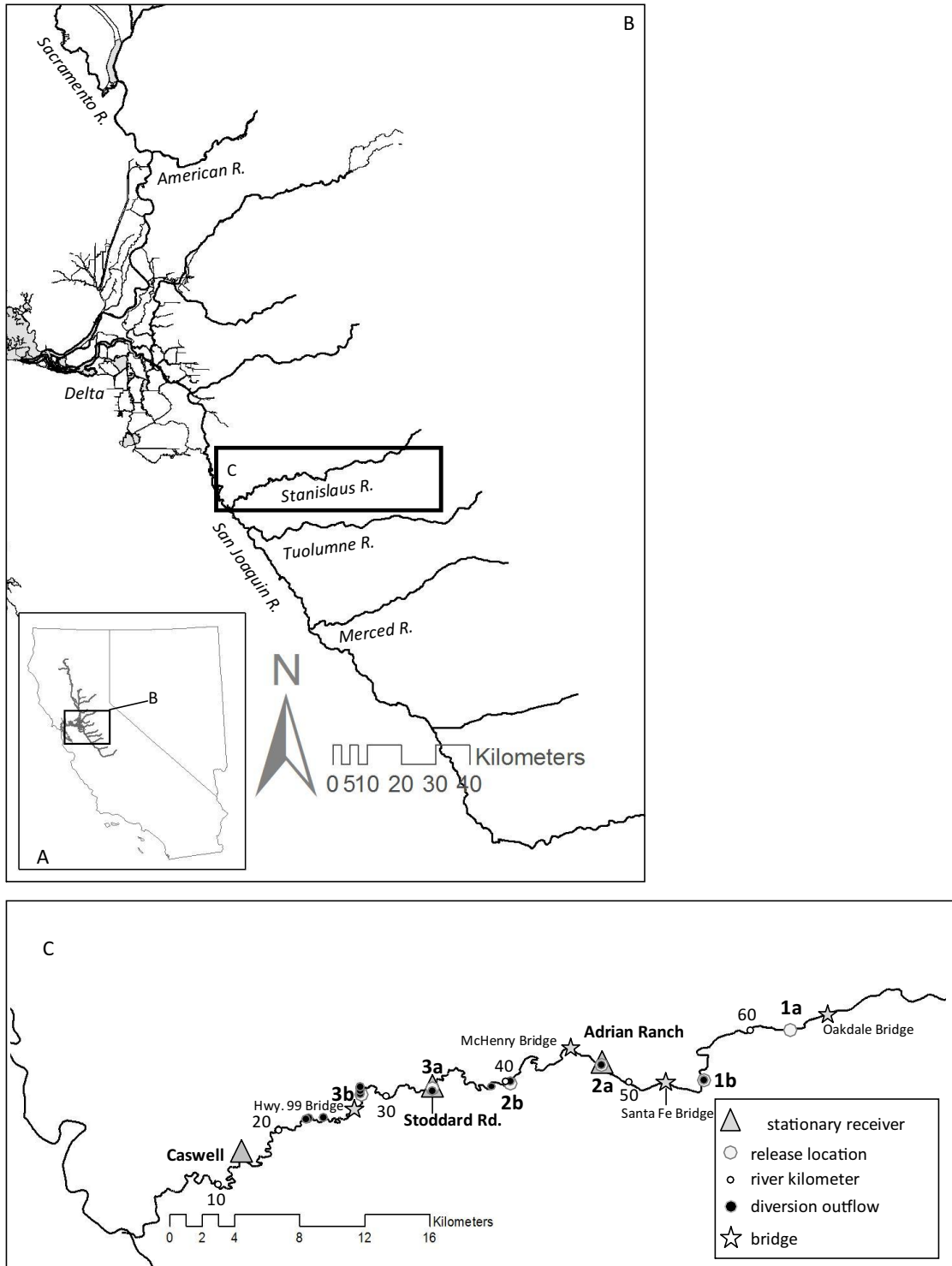


Figure 1. Map of the Stanislaus River within California and the study area in the Lower Stanislaus River. The lower panel shows release locations, stationary receiver locations, diversion outflows, and bridges. Reaches are designated by contrasting light and dark colors.

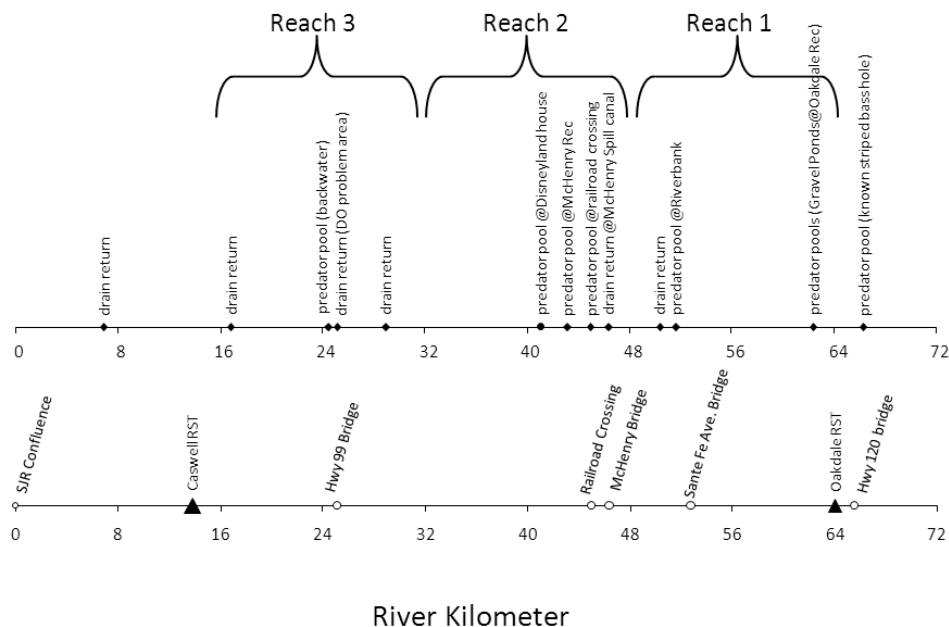


Figure 2. Schematic of three reaches within the study area and potential areas of high salmon mortality.

## Study design

During spring 2012-2014, Cramer Fish Sciences implemented a mark-recapture experiment to estimate survival of radio-tagged fall run Chinook salmon juveniles emigrating through the LSR between the Oakdale and Caswell rotary screw traps (RSTs). There were two release events per year, and within each release event groups of tagged fish were released approximately every 8 km (8.0 – 9.7 rkm) along the 50-km study reach, creating six sub-reaches (Figure 3; Table 1). To correspond with natural periods of juvenile activity, reduce the effects of predation during the initial release into the river, and allow for mixing of release groups during outmigration (Demko *et al.* 1998), the fish were released at night at approximately 2.5 hour intervals starting at the upstream site (1a) at 18:00 and ending at the downstream site (3b) at 06:00 the following morning. Each year, the two experimental releases took place under variable flow conditions, respectively (Table 1). This release design allowed for survival estimates to be derived at multiple scales (i.e., 50, 16, and 8 km) under varying conditions. Mobile telemetry surveys were performed for several days following release events to track the movement and behavior of juveniles between fixed receivers (e.g., directional movement and habitat characteristics at observation locations).

Table 1. Release group dates, times, flows, locations, and number of fish across the three study years.

Year	Release dates	Average streamflow in $m^3 \cdot s^{-1}$ (range) <sup>1,2</sup>	Temperature in °C ( $\pm 1$ SD)			Dissolved oxygen in $mg \cdot l^{-1}$ ( $\pm 1$ SD) <sup>2,3</sup>
			Riverbank (rkm 47.6)	Stoddard Road (rkm 32.7)	Caswell (rkm 14.8)	
2012	April 10-11	44.8 (39.7 - 53.8)	13 $\pm$ 0.8	13 $\pm$ 0.9	14 $\pm$ 1.6	10.1 $\pm$ 0.6
	May 3-4	43.5 (42.9 - 44.6)	14 $\pm$ 0.5	14 $\pm$ 0.5	14 $\pm$ 0.9	10.4 $\pm$ 0.4
2013	March 26-27	11.7 (9.0 - 20.8)	15 $\pm$ 1.2	17 $\pm$ 1.4	17 $\pm$ 1.1	9.2 $\pm$ 0.4
	April 23-24	76.7 (53.3 - 82.8)	13 $\pm$ 0.7	14 $\pm$ 0.5	14 $\pm$ 0.8	10.0 $\pm$ 0.4
2014	April 18-19	65.2 (61.0 - 70.4)	13 $\pm$ 0.5	14 $\pm$ 0.4	14 $\pm$ 0.5	9.6 $\pm$ 0.3
	May 1-2	53.1 (14.2 - 68.1)	15 $\pm$ 1.1	14 $\pm$ 0.9	15 $\pm$ 1.4	9.2 $\pm$ 0.6

<sup>1</sup> USGS stream gage at Ripon (11303000)

<sup>2</sup> Includes beginning of release through the tracking period, 21 days following release.

<sup>3</sup> Dissolved oxygen recorded daily at Caswell rotary screw trap (rkm 13.8).

## Fixed Receiver Station Design and Configuration

Lotek SRX-series datalogging radio receivers (Lotek Wireless, Newmarket, Ontario, Canada) were deployed so each station demarcated the downstream boundary of each 16 km reach (Figure 3). We used SRX-400a receivers for the two upstream stations (R1 and R2) located at the bottom of sub-reaches 1b and 2b, respectively. We used an SRX-600 receiver for the downstream station located at the bottom of Sub-reach 3b (R3.1 and R3.2).

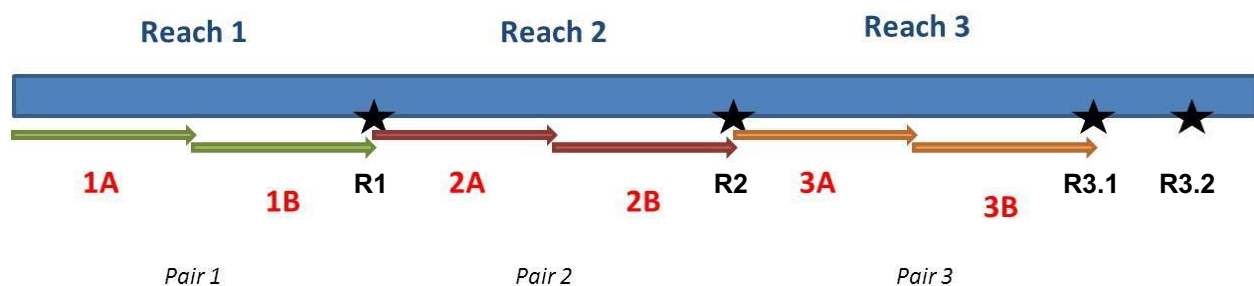


Figure 3. Schematic representation of the study area showing Reaches 1-3, Sub-reaches 1A-3B, and receivers R1-R3.2. Paired releases are made above each sub-reach and survival is assessed by detections at fixed receivers. Flow follows the direction of arrows. Stars represent the relative location of fixed radio receivers. Note: R3.1 and R3.2 represent the two antenna arrays at the Caswell fixed receiver station.

Each fixed station was comprised of a receiver connected to four four-element Yagi antennas (i.e., two oriented upstream and two downstream in an alternating pattern). The downstream-most antenna was labeled A1 and adjacent antennas were spaced from 10–25 m apart depending on local conditions and lines of sight. Antenna arrays were positioned on outside river meanders

with an alternating antenna orientation between downstream (A1 and A3) and upstream (A2 and A4) to better differentiate directional movements (Figure 4). Outside meanders also had elevated cut-banks helping to improve antenna reception; however, encroaching vegetation created some shadows, or interference, which affected the detection radius of those antennas. Antenna bearings were such that the detection trajectory was angled toward the opposite bank to minimize passage windows, or areas where the detection range does not encompass the entire channel cross-section.

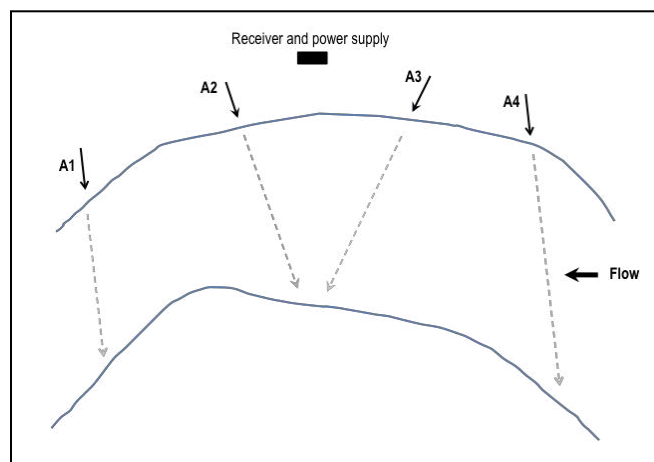


Figure 4. Conceptual diagram of a typical fixed receiver station and antenna array deployed on an outside river meander. Note orientation of antennas (A1–A4) and projected trajectories (dashed grey arrows). Not to scale.

The radio tags were programmed with an individual tag code and set to one of two different frequencies (149.320 and 150.380), and receivers scanned one frequency at a time for a period of 6.5 sec to fully encompass the transmitter pulse rate of 5.0–5.3 sec while not scanning so long that detections on the other frequency could be missed. Receivers were configured to an all-antenna master scan setting and switched between frequencies for a total scan period of 13.0 sec. When a transmission was decoded on the all-antenna master, the receiver switched to antenna A1 and began cycling through both frequencies before switching to the next antenna in the series. Antennas switched sequentially from A1 through A4, performing a sweep in the upstream direction. Since we presume our tagged fish move in a downstream direction, scanning with the downstream-most antenna first generally ensured that transmissions would be received before the transmitter was beyond detection range and outside the array. Once all antennas were scanned for both frequencies (total scan period: 52.0 sec), the receiver automatically switched back to the all-antenna master scan setting and reset the cycle. This design served to increase detection probabilities and determine directional movement.

## Fish Surgery and Transmitter Implantation

Handling protocols were standardized across all experimental groups to reduce potential bias (i.e., fish length, number of times handled, tagging procedures, transport methods, transport time, and release protocol). To be conservative, a tag to body weight ratio of less than 5% was used (Adams *et al.* 1998; Brown *et al.* 1999, 2010), requiring a minimum body weight of 5.0 g, which corresponded to a minimum fork length (FL) of 72 mm. Surgical transmitter implantation

consisted of three steps: (1) pre-operative anesthesia bath; (2) tag implantation; and, (3) post-operative recovery. Post-operative procedures consisted of four steps: (1) post-operative recovery and observation; (2) acclimation and observation prior to release; (3) transport to release location; and, (4) release.

*Preparation* - We used tricaine methanesulfonate (Tricaine-S), buffered with calcium carbonate to pH 7–8, to anesthetize fish prior to surgical procedures. To minimize the chances for pathogen transfer between fish populations, all equipment used for capture, holding, anesthesia, surgery, recovery, and movement of fish during the project was thoroughly cleaned and disinfected before each use. Between surgeries, surgical instruments were placed in a disinfectant bath (e.g., dilute Novalsan S®, chlorhexidine solution, Fort Dodge Inc.) and transferred to a freshwater rinse bath before the next surgery.

As of the 2013-14 field season, CFS was covered under the Investigational New Animal Drug (INAD) Program. In compliance with our INAD permit, we use AQUI-S® 20E (hereafter AQUI-S; AQUI-S New Zealand Ltd.) to anesthetize fish for safe handling. AQUI-S is safe for humans to handle and is an effective anesthetic for fish. The action of AQUI-S is readily reversed when fish are transferred to fresh water. The effectiveness is related to a variety of factors including concentration and fish size.

At the time of tagging, we collected fish individually from a holding tank, weighed them to the nearest 0.1 g, and transferred them to an anesthetic “knockdown” bath containing Tricaine-S solution or AQUI-S for 60-90 seconds. Once a fish was anesthetized, we recorded its condition (e.g., general condition of eyes, scales, and fins) and size (FL to the nearest 1 mm). Following inspection and measurement, we placed fish ventral-side up on a surgery cradle made of Microcell foam with a size-specific mold to hold fish in position. Throughout the procedure, a diffused maintenance anesthesia solution (40-50 mg·l<sup>-1</sup>) was continuously pumped to gently flush the anesthetic solution over gill membranes to maintain a state of anesthesia and remove metabolic wastes away from the gills. Temperature of the maintenance solution was maintained within ±2°C of release water. After surgery, we placed fish in a continuously circulating river water holding tank and monitored them until full recovery was attained. Stress Coat® API Inc., which helps replace slime coat and protect scales on a fish, was added to the knockdown bath, the anesthetic solution, and recovery tanks.

*Surgical Procedures* - The procedure used to implant radio-tags was similar to the shielded-needle technique described by Ross and Kleiner (1982) as modified by Hogen and Scarnecchia (2006) and Watry and Scarnecchia (2008). We used a 5 mm, precision-depth puncture knife to create an 8 mm incision anterior to the pelvic girdle and 1-2 mm off but parallel to the ventral midline. We inserted a thin, grooved instrument into the incision under the pelvic girdle to guide a small-diameter cannula (18 gauge catheter or syringe needle), inserted posterior to the pelvic girdle, to protect the viscera from injury as the cannula was drawn forward to be exposed in the initial incision. We then fed the antenna from a sterilized radio transmitter into the exposed cannula tip. We withdrew the cannula and the antenna extruded out the exit orifice as we carefully inserted the tag into the peritoneal cavity. The tag was generally positioned immediately under the incision. We closed the incision with two simple interrupted sutures using a 16 mm FS-3 reverse-cutting 9.5 mm circle needle with 6/0 monofilament suture material. Finally, we introduced a small amount of slime over the wound from surrounding skin surfaces



before fish were placed into a tank of fresh river water to recover from anesthesia and surgery. Tagged fish were allowed to recover completely before being transferred to an in-river holding tank (containing only tagged fish) to monitor post-surgery recovery and tag retention.

*Recovery and Transport* - We held implanted fish for a minimum of 18 hours for recovery observation before transport. We then transferred healthy tagged fish into large, aerated tanks (segregated by release group) on the release boat. Throughout the transport process, we monitored water temperature and dissolved oxygen levels; we added oxygen to the transport tank when necessary to keep dissolved oxygen levels between  $7 \text{ mg}\cdot\text{l}^{-1}$  and  $12 \text{ mg}\cdot\text{l}^{-1}$ . We used frozen river water as needed to reduce water temperature and acclimate fish to release site temperatures. At release locations, we transferred tagged fish by a dip net in groups of 2–5 fish and released them directly into the channel as the boat traversed from bank to bank, allowing for a diffuse release. This diffused release approach was used to reduce schooling behavior and potential predation, as fish were released over a period of several minutes and distributed laterally across the channel. We performed releases after sunset to reduce potential predation risk immediately after release, when tagged fish were expected to be disoriented and most vulnerable.

## Tag Life Validation

To ensure transmitters operated for the entire recommended manufacturers' calculated 21-day battery life, we purchased an additional 40-60 transmitters during each year of the study to monitor battery life. We activated the test transmitters following identical procedures to those used to activate the experimental tags. We then placed the tags in water, tested them at least once daily, and recorded any dead tags. We were able to detect all test tags throughout the calculated manufacturers' battery life (21 days). As a result, tag life corrections were not made in survival analyses. Instead, we restricted the data used in analysis to detections within 21 days of release.

## Temperature and streamflow

During the first two years of the study, we recorded temperature ( $^{\circ}\text{C}$ ) throughout the study period at each of the three stationary receiver sites at 15-minute intervals using Hobo® temperature loggers (Onset, Bourne, Massachusetts). During the third year, we deployed three additional loggers so that a logger was present at each release site. We used 15 minute interval event and mean daily discharge data obtained from the United States Geological Survey stream gauge on the LSR located near Ripon, CA (USGS gage # 11303000).

## Deep pool habitat

We used bed elevation survey points to identify geomorphic low spots (i.e., deep pool habitat) within the longitudinal profile (slope) of the Stanislaus River channel. We assumed that bed elevations below the channel average slope represented deep pool habitat (i.e., deeper water with lower velocities). To quantify deep pool habitats, a combination of GIS-based mapping and overlay procedures, with post-processing in Microsoft Excel 2010 (Microsoft, Redmond, Washington) was used to calculate reach-specific coefficients of variation (CV) for bed elevation and both stream bed area and percent (%) total stream bed area within pre-defined elevation categories (see below). Reach-specific CV values were used as a surrogate index for the relative

number of potential predatory fish holding pools, with greater CV values assumed to index a greater number of pool-riffle complexes. Streambed area and percent (%) total stream bed area within pre-defined elevation categories were used to index the overall area and relative area of deep pools. We assumed greater area values to index greater total availability of deep pools and greater percent (%) total area values to index greater relative availability of deep pool habitats. To index the relative "depth" of pool habitats, we used pre-defined elevation categories of  $>0.00$  m,  $\geq 1.00$  m,  $\geq 2.00$  m, and  $\geq 3.00$  m below "expected" bed elevation values, with expected values defined as the average longitudinal profile of the river channel. For all calculations, we relied on a modified combination of the "Loess Curves" and "Standard Deviation of Depths" methods described by Bartley and Rutherford (2002) (also see Lisle 1987 and Lisle 1995).

We obtained a GIS file with elevation survey points from the United States Fish and Wildlife Service (USFWS, Sacramento, California, unpublished data). General GIS-based mapping and overlay procedures included placing a uniform  $1.0 \text{ m}^2$  point grid over the entire survey area and then assigning survey bed elevations and 0.01 rkm values to each point in the grid. General post-processing in Microsoft Excel included determining an expected bed elevation for each  $1.0 \text{ m}^2$  grid point based on 0.01 rkm values and then calculating CV and both area and percent (%) total area values based on observed and expected stream bed elevations.

Specific GIS-based mapping and overlay procedures using ArcGIS 10.2 included: 1) developing a polygon shapefile that contained the entire survey area, 2) splitting the polygon shapefile containing the entire survey area into multiple study reach polygons, 3) converting the resulting polygons for each study reach into  $1.0 \text{ m}^2$  raster grid files using the polygon to raster tool, 4) converting the resulting raster grid files for each study reach into point files where each point represented  $1.0 \text{ m}^2$  using the raster to point tool, and 5) assigning bed elevation and 0.01 rkm values to each of the resulting grid points. These geoprocessing procedures were performed to generate evenly spaced grid points throughout the study reach. Evenly spaced grid points were required to provide a uniform sample of the bed elevation in each study reach and served as an effective way to sample bed elevation survey points. Additionally, the number of grid points placed within each study reach polygon provided a relative comparison of the approximate wetted area at the time the bed elevation survey was conducted.

Specific post-processing in Microsoft Excel included: 1) calculating reach-specific CV values, 2) estimating linear relationships for upstream-to-downstream changes in bed elevation within each reach, and 3) applying linear relationships to determine both stream bed area and percent (%) total stream bed area within pre-defined elevation categories (see below) and overall channel gradient. Reach specific CV values were calculated using the standard deviation for bed elevation within a given reach divided by the mean bed elevation within a given reach. Bed elevation and CV (as surrogate for "pool") point topographic features were further processed using the topo-to-raster surface and contour analysis in ArcGIS 3D analyst displaying bathymetric gradient features on the study site.

## **Alcove habitat**

To identify alcove habitat, we created a  $5 \times 5$  m rectangular grid and exported points and polygons using ArcGIS. We clipped this grid to the polygon of the study site. We then created a center line within the polygon feature extent of the study site and split the line into 20-m segments.

Each of these lines was associated with the unique ID and subreach in which it was located. Then, the 20-m lines were spatially joined with the 5x5m grid points so that each grid point had the specific 20-m line id and subreach in its attribute table. The resulting attribute table was exported and summarized using the R statistical package. The number of points belonging to each 20-m line ID was counted and was average by subreach. The average values by subreach were used as threshold for identifying “alcove” habitat; if a channel line has a higher number of points than the average value for that subreach, the area represented by that line is likely to have a wider channel width. After summarizing the number of points for each channel line ID, we imported the results back into ArcGIS, and ran the select query tool by location attributes to select channel lines ID with number of points higher than the threshold values. The selected channel lines were then visually checked to verify that it was an alcove. Alcove area and total area of the study site were then calculated using the ArcGIS geometry.

## Mobile Telemetry

We conducted mobile telemetry surveys in the six sub-reaches to collect data related to fish distribution and movement and identify potential locations of high mortality. Mobile tracking surveys were conducted from a 4.3 m drift boat with a 24.9 kg thrust trolling motor or a 4.9 m jon boat with a 31.8 kg thrust trolling motor and 90-horsepower outboard jet. We used Lotek SRX-400a datalogging receivers connected to a single four-element Yagi antenna (Lotek Wireless, Newmarket, Ontario, Canada). During the first two years of the study, a Trimble GeoXT 6000 series GPS unit was used to record spatial coordinates every second. Time of day was synchronized between the receiver and GPS unit, allowing us to assign a latitude and longitude to each detection based on cross-referencing date and time stamps between the receiver and GPS track data using GPS Pathfinder® Office (Trimble®, Sunnyvale, CA), ArcGIS 10.1 (ESRI; Redlands, CA), and Microsoft Access® 2010 (Microsoft, Redmond, Washington) software programs. During the third year, we recorded the tag code and frequency from the receiver directly in the GPS unit in the field, eliminating the need to sync and merge the spatial and receiver datasets. In all years, we recorded the following information each time a fish was located: time, tag code (unique fish identifier), and other field observations (location in channel, deep water, riffle, etc.). All surveys were conducted during daylight hours. Because it was logistically impossible for a single team to survey the entire study reach in a single day, either 1) only the upstream or downstream portion of the study reach was sampled in a given day, or 2) two separate teams were used to survey the upstream and downstream reach, respectively.

## Movement Patterns

Movement histories or patterns were analyzed to assign each daily detection a directional movement designation according to the criteria listed in Table 2. If tag detections were observed moving in a progressively downstream direction from previous locations (i.e., either release location or previous survey detection) during two or more consecutive survey events with no subsequent upstream or holding behaviors, it was considered a downstream detection string. Tags detected at the Caswell receiver (rkm 14.8; the downstream extent of the study reach) were considered to be moving "downstream". Although downstream detection strings could potentially be attributed to a predator moving downstream with a tagged fish in its stomach, we made the assumption that downstream detection strings were most likely outmigrating juveniles since that is their expected pattern of movement. In contrast, if tag detections were located

greater than 1 rkm upstream from a previous detection location, it was considered an upstream detection string. It is possible that upstream detections could result from tagged juveniles migrating in an upstream direction; however, upstream migration is not a common behavior for juvenile Chinook salmon (Steele *et al.* 2001). As such, we assumed that larger, more mobile fish (e.g., predatory species) were more likely to exhibit upstream movement patterns; therefore, if a predatory fish consumed a tagged juvenile salmon we could expect this pattern of upstream movement.

Table 2. Criteria used to classify directional movement patterns. Each daily detection string for individual tags was assigned a single directional movement pattern and daily data was compiled into a comprehensive detection history, including release location and detections from both mobile units and fixed receiver stations.

Directional movement	Criteria	Possible scenarios
Downstream	One or more detections >1 rkm downstream from previous detection, with no subsequent upstream or holding patterns.	- Outmigrating juvenile - Predator moving downstream
Upstream	One or more observations >1 rkm upstream from previous detection.	- Predator moving upstream - Juvenile migrating upstream

## Data Management, Spatial and Statistical Analyses

### Mobile Telemetry Data

Detection strings recorded during mobile surveys were grouped into two categories based on movement patterns (i.e., downstream, upstream, Table 2). These categories were selected in order to minimize speculation regarding the status of individual detection strings (e.g., whether they were out-migrating juveniles or tagged fish consumed by a predator). However, it is tenuous to designate the status of a detection string based on point observations from mobile surveys, particularly those observations without visual confirmation. As such, analyses were performed using data that were restricted to observations that demonstrated stark contrasts in behavior; analyses of mobile survey data were made based on categories of behavior that appeared counter to what would be expected for an out-migrating juvenile Chinook salmon. For example, while it is relatively common for an out-migrating Chinook salmon to remain in a given location for several days, upstream migration is generally uncommon behavior (Steel *et al.* 2001). However, many common predators (e.g., pikeminnow *Ptychocheilus grandis*, striped bass *Morone saxatilis*) are highly mobile and may move several km upstream or downstream in a single day (Harvey and Nakamoto 1999; Kynard and Warner 1987). Atypical downstream detection strings were scrutinized for timing (days at large), the day after the release date as well the location (in rkm) that a tagged fish was last detected, travel rate, and distance traveled.

### Stationary Receiver Data

Data were downloaded from receivers weekly throughout the study period. Each download consisted of a series of detection data, which were then filtered to eliminate false detections (e.g., detections considered noise or invalid tag codes). Data were entered into MS Access and filtered based on the following criteria: 1) detections that occurred prior to release; 2) detections that occurred after the 21-day tag life; 3) any detections with error codes (e.g., “255” or “999” tag codes); 4) any detections of unknown tag codes (e.g., noise or interference); and, 5) tags with fewer than 2 detections within 30 minutes at a single receiver (Perry *et al.* 2010). After data were filtered, queries were performed to provide the first and last detections (date and time) for each individual study fish at each receiver (i.e., 1, 2, 3.1 and 3.2). These data were then used to

create a database that related these detections to data regarding channel morphometrics, time and location of release, travel rate and detection trajectories for each tagged fish.

## Tag effects

To determine how surgical implantation and presence of radio tags with external antennae impact survival, behavior, and growth of juvenile Chinook salmon *Oncorhynchus tshawytscha* and whether implanted tags are retained, we developed a laboratory study to test the following hypotheses:

H<sub>01</sub>: Tagged fish do not expel their surgically implanted transmitter.

H<sub>02</sub>: Survival rates are equal for tagged and non-tagged fish

H<sub>03</sub>: Behavior of tagged fish is similar to that of non-tagged fish.

H<sub>04</sub>: Growth of tagged fish is similar to that of non-tagged fish.

This study had two components: 1) assessment of tag retention, survival and behavioral recovery, and 2) quantitative evaluation of post-recovery condition. The first component was designed to address questions related to the first three hypotheses, while the second component is designed to address questions related to the fourth hypothesis.

In 2013, for each release event, we selected a representative sample of 40 treatment and 40 control fish. Group 1 fish were tagged on 25 March 2013 (during Release 1 tagging for the field experiment) and Group 2 fish were tagged on 22 April 2013 (during Release 2 fish tagging for the field experiment).

In 2014, for each release event (Group 1 and Group 2), we selected a representative sample of 20-tagged fish, 20 control fish, and 20 fish that had surgery but no implanted tag. This third "surgery only" treatment was added to separate the effects of surgery from the effects of the presence of the tag, and was used for the analysis of survival and tag retention only because it was impossible to distinguish control fish from the surgery-only fish during the behavioral assessments. Group 1 fish were tagged on 18 April 2014 and Group 2 fish were tagged on 30 April 2014. Control fish were selected and transported in a similar manner to study fish except they were not subject to anesthetization, handling, or tag implantation. Treatment fish were anesthetized and tagged following the procedures previously outlined. "Surgery only" fish were anesthetized and surgery was performed without tag implantation following the same procedures. All fish were transported to the NOAA Fisheries Aquaculture Lab in Santa Cruz, CA immediately following the initial post-operative recovery period (minimum 1 hour). A continual flow of oxygenated freshwater pumped through the aquarium system supplied comparable water quality among tanks. All transported fish were acclimated to laboratory filter water by first adjusting their temperature 1°C per hour and then transfer to the laboratory water.

Fish were separated into four 189.3 l tanks with a flow-through circulation system in 2013. In 2014, fish were separated into two 1135.6 l tanks with a flow-through circulation system. Feeding sessions occurred every morning and tanks were siphoned every Monday, Wednesday, and Friday to minimize the accumulation of organic matter and maintain water quality. Siphoning occurred no less than one hour after feeding to allow fish time to consume and digest

rations and to minimize stress following feeding. In 2014, each tank was scrubbed twice a week as needed before siphoning.

### ***Behavioral assessment***

A submersible camera with video capabilities was mounted inside the tanks to passively collect video footage during each of two three-week (2013) or two-week (2014) monitoring periods. In 2013, Group 1 fish were sampled three times during days 9 to 11 following surgery, while Group 2 fish were sampled three times per week during the three-week monitoring period. One video per tank was recorded during the first and last week while two videos per tank were collected during the second week. In 2014, Group 1 and 2 fish were sampled Monday through Friday throughout the two-week monitoring period. For both 2013 and 2014, the start time was recorded as the time the camera was placed in the water and the stop time as when the camera was removed. The filming period lasted 15 minutes.

The first two minutes of each video was removed from analysis to reduce behavioral effects associated with camera placement. Four 5-10 second video segments were randomly selected for viewing to analyze: 1) position in water column; 2) tag presence; and, 3) body orientation (i.e., pitch). We attempted to quantify individual gill and tail beats as a measure of respiration and swim performance, respectively; however, it was too difficult to isolate individual fish to accurately complete this assessment. As such, these data were not included in the final analysis.

Tag retention rates were reported as the percentage of tags retained. Survival was compared among the treatment and control groups with a chi-square test. Data on behavioral recovery was assessed using a chi-square test to compare differences in position in the water column and body orientation among treatment and control groups. Analyses were performed in JMP Pro 11.0.0 (SAS Institute, Inc.) statistical software.

### ***Post-recovery condition***

Following the second three-week monitoring period in 2013, a necropsy was completed for every fish from each tank. After euthanizing fish by immersing them in a lethal-dose solution ( $>150 \text{ mg}\cdot\text{l}^{-1}$ ) of tricaine methanesulfonate (Tricaine-S), we noted whether fish were from the treatment or control group and whether antennas were still attached to the transmitters of tagged fish. We also measured fork length (mm FL) and weight (g) and qualitatively assessed the general condition of eyes, fins, and scales (Table 3). Tagged fish were weighed with the tag; weights were corrected by subtracting the tag weight (0.25 g in water) for those fish. We then qualitatively evaluated incision healing and whether tags were incorporated into tissue (Sandstrom *et al.* 2013).

Table 3. The factors evaluated during the necropsy and the qualitative descriptions given based on a 0-2 scale.

<b>Factor</b>	<b>Range</b>	<b>Description</b>
Eyes	0	Absolute eye clarity
	1	Moderate cloudiness of eye
	2	Extreme cloudiness of eye
Fins	0	No fraying to any fins
	1	Moderate fraying or damage to fins
	2	Extreme fraying or damage to fins
Scales	0	No scale loss
	1	Loss of scales of up to 33% of body coverage
	2	Loss of scales form 33% to 66% of body coverage

In 2014, necropsies could not be performed because all fish contracted the protozoan parasite *Ichthyophthirius multifiliis* (i.e., "Ich") 28 days after the experiment started. Their tags were removed but no data was taken before discarding their bodies.

We analyzed growth in 2013 by comparing fork length and weight of treatment and control groups at the end of the study. We performed a two-sample Kolmogorov-Smirnov test in R with both growth variables to evaluate differences in growth between control and treatment fish. Analysis was performed using the R statistical package (R Core Team 2014).

## Data analysis

### *Survival*

Survival was estimated using stationary and mobile detections to take advantage of the strengths and limit the biases associated with each method of detection. Stationary receivers have the advantage of detecting fish 24 hours a day during the study period and spatially explicit estimates of survival can be obtained. However, stationary receivers are biased against fish that are holding or rearing because they will be considered mortalities if they do not pass a receiver station. Mobile detections are useful because they represent fish that are actively migrating or rearing/holding. Temporally explicit estimates of survival can be produced and there is better spatial resolution of where mortality occurs within a reach. However, detections can only occur during the discrete interval of mobile surveys and fish may be incorrectly classified as mortalities if they move out of the study reach between mobile surveys. Combined, these two types of sampling provide a more complete picture of Chinook salmon survival and behavior in the Stanislaus River.



### ***Mobile detection models***

Mobile detection histories were analyzed with a multi-state model in the program MARK. Multi-state models allow for estimation of fish survival in the study reach between each survey and the probability that a fish may either remain in the study reach or leave the study reach (transition probability). In this way, fish that leave the study reach will not be misidentified as mortalities. The receiver array at Caswell was used to determine if a fish had left the study reach prior to a mobile tracking survey. Detection histories used in multistate models vary from binary responses used in Cormack Jolly-Seber models because the two states must be identified. In the models constructed here we used “U” to represent the state of remaining in the upstream study reach and “D” was used to indicate the fish had transitioned downstream out of the study reach. For example, a detection history of U0D0 would indicate the fish was detected in the upstream study reach on occasion 1, was not detected on occasion 2 and was detected at the downstream receiver (Caswell) on occasion 3. The zero on occasion four does not count as a mortality since the fish had left the study reach. This class of models allows for estimation of survival in both states as well as transition probabilities in both directions. However, there were no surveys downstream of Caswell and upstream migration is an unusual behavior for salmon smolts. Thus, we set to zero estimates of transition from downstream to upstream and survival of fish downstream of Caswell. The two variables estimated from these models were (1) survival of fish in the study reach between each survey and (2) the probability of transitioning out of the study reach between surveys.

Certain aspects of the mobile surveys prevented the data from being combined across years and releases. For example, survey intervals varied among years and releases which could have a large influence on model output because survival is being modeled by time (between survey intervals). Thus, models were constructed for individual releases in each year for a total of six separate analyses. A model selection exercise using  $AIC_c$  was performed to examine spatial differences in survival (1<sup>st</sup> study goal) and model structure. The three models considered were 1) a model where each sub-reach was grouped separately, 2) a model with equal detection probabilities for each release group (equal detection), and 3) a model with no groupings by release group (no groups).

A mobile tracking survey was performed three days after each release in all years. Thus, daily survival values over the first three days were used to compare survival between early and late releases and over different flow levels. Daily survival values for each release group and their 95% confidence intervals were plotted for early and late releases in each year. If the 95% confidence intervals did not overlap, the difference in survival was considered to be significant. To test for an effect of flow, a linear regression was performed where flow was the independent variable and logit transformed survival was the response variable.

### ***Stationary detection models***

Survival was modeled from the stationary detection data using Cormack-Jolly-Seber (CJS) models in the program MARK. These models addressed the first two study goals by estimating reach specific survival rates (1<sup>st</sup> goal) and modeling the effect of biotic and abiotic parameters on survival in the LSR (2<sup>nd</sup> goal). Detection histories were broken out by releases performed in each of the three study reaches. Thus, three separate analyses were performed over all study years including; releases in Reach 1 (1A and 1B), releases in Reach 2 (2A and 2B) and releases in Reach 3 (3A and 3B). For each analysis, a set of candidate survival models was constructed.

Each candidate model was considered a separate hypothesis to explain the detection data with each hypothesis directly related to a study goal. For example, to address differences in survival in space, a variable was added to designate the reach where study fish were released. Model fit was then evaluated using an information theoretic approach. Akaike’s Information Criteria corrected for small sample size ( $AIC_c$ ) was calculated for each candidate model in the set. The  $AIC_c$  value for each candidate model was compared to the value for the best approximating model ( $\Delta AIC_c$ ). Models with a  $\Delta AIC_c$  value  $\leq 2.00$  were considered competing explanations for the detection data (Burnham and Anderson 2002). Predictors used in each model and the hypothesis represented by that model appears in Table 4.

Table 4. Models evaluated to explain detections on stationary receivers and the hypothesis that each model represents.

Model predictor	Hypothesis
Early/Late	Survival varies as a function of release timing (early vs. late).
Flow	Survival varies as a function of flow during the release.
Fork length	Survival varies as a function of fish size at release.
Full model	Survival varies as a function of all potential predictors.
None	No variables improve model fit.
Release group	Survival varies as a function of sub-reach of release.
Temperature	Survival varies as a function of water temperature at the time of release.
Years	Variation in survival is greatest among years.

### *Travel time*

Travel time of study fish was also calculated separately for stationary and mobile detections. Similar to the survival calculations, estimates from the stationary data represent actively migrating fish whereas mobile detections also represent fish displaying holding and rearing behavior. A series of statistical tests were performed to test for differences in travel time between reaches during early and late release and between early and late releases in each reach. Initial screening of the travel time data indicated that the assumption of normality was violated and parametric tests such as analysis of variance could not be used. The nonparametric Kruskal-Wallis test was applied instead to test for differences in travel time. When differences were detected, a Wilcoxon multiple comparison test was performed to determine where the differences occurred. To test for a relationship between travel time and survival, travel time was regressed against logit transformed survival estimates for both stationary and mobile detection datasets.

### *Habitat-mortality relationships*

To identify habitats and spatial locations where mortality of tagged fish occurred, three analyses were performed using the mobile detection data set. These analyses addressed the 3<sup>rd</sup> and 4<sup>th</sup> study goals listed above. First, the river kilometer where the last known detection occurred was plotted on a map of the study reach. For each of the three 10 km sub-reaches random points equal to the number of observed last-detections in that reach were also plotted. We then used nearest neighbor distances to test if observed last-detections were clumped relative to random values using the global Moran’s I tool in ArcGIS. Second, a hot spot analysis was performed in ArcGIS. This analysis identifies areas in the study reach where observed last-detections are

significantly clumped together. Finally, an analysis of covariance (ANCOVA) was employed to test if the relationship between expected and observed bed elevations at locations of last-detection was significantly different from a 1:1 relationship. A relationship with a smaller intercept would indicate last-detection locations were at lower bed elevations than expected (i.e. deeper habitats) whereas a greater intercept would indicate last-detection locations were in shallower areas than expected.

To identify study reaches with features that may negatively impact juvenile salmon, the location of multiple artificial features were plotted on a map of the 50 km study reach. The features identified included water diversions, return drains, and bridges. The proportion of each feature type was calculated for each sub-reach. Additionally, another hot spot analysis was performed to determine if there were significant grouping of these features within the 50 km study reach.

## RESULTS

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### 2012 releases

All Chinook salmon tagged and released in 2012 were obtained from the Merced River hatchery. The early release in 2012 occurred between April 9<sup>th</sup> and 10<sup>th</sup> at a discharge of  $36.1 \text{ m}^3 \cdot \text{s}^{-1}$  ( $1,274 \text{ ft}^3 \cdot \text{s}^{-1}$ ) and a temperature of  $12.0^\circ\text{C}$ . A total of 202 tagged fish were released among the 6 sub-reaches and the mean size of tagged fish was 80.9 mm (SD +/- 2.9). The second release in 2012 occurred between May 2<sup>nd</sup> and May 3<sup>rd</sup> when discharge was  $31.4 \text{ m}^3 \cdot \text{s}^{-1}$  ( $1,109 \text{ ft}^3 \cdot \text{s}^{-1}$ ) and water temperature was  $13.4^\circ\text{C}$ . The 207 juveniles released had a mean fork length of 77.9 mm (+/- 6.1 SD). Tagged fish from both releases had a mean size greater than fish collected in the Caswell screwtrap during the same time period. However, confidence intervals overlapped indicating no statistically significant difference.

### 2013 releases

All Chinook salmon tagged and released in 2013 were obtained from the Merced River hatchery. The first release in 2013 occurred between March 26<sup>th</sup> and March 27<sup>th</sup> at a discharge of  $10.1 \text{ m}^3 \cdot \text{s}^{-1}$  ( $357 \text{ ft}^3 \cdot \text{s}^{-1}$ ) and a water temperature of  $14.2^\circ\text{C}$ . The 210 fish in the first release had a mean fork length of 81.4 mm and a standard deviation of 6.2. The second release occurred on April 23<sup>rd</sup> 2013 when discharge was  $57.5 \text{ m}^3 \cdot \text{s}^{-1}$  ( $2,031 \text{ ft}^3 \cdot \text{s}^{-1}$ ) and water temperature was  $13.8^\circ\text{C}$ . The 206 fish in this release had a mean fork length of 97.8 mm and a standard deviation of 14.3. The mean size of experimental fish was significantly larger than fish captured in the Caswell screwtrap during the same period (Figure 5).

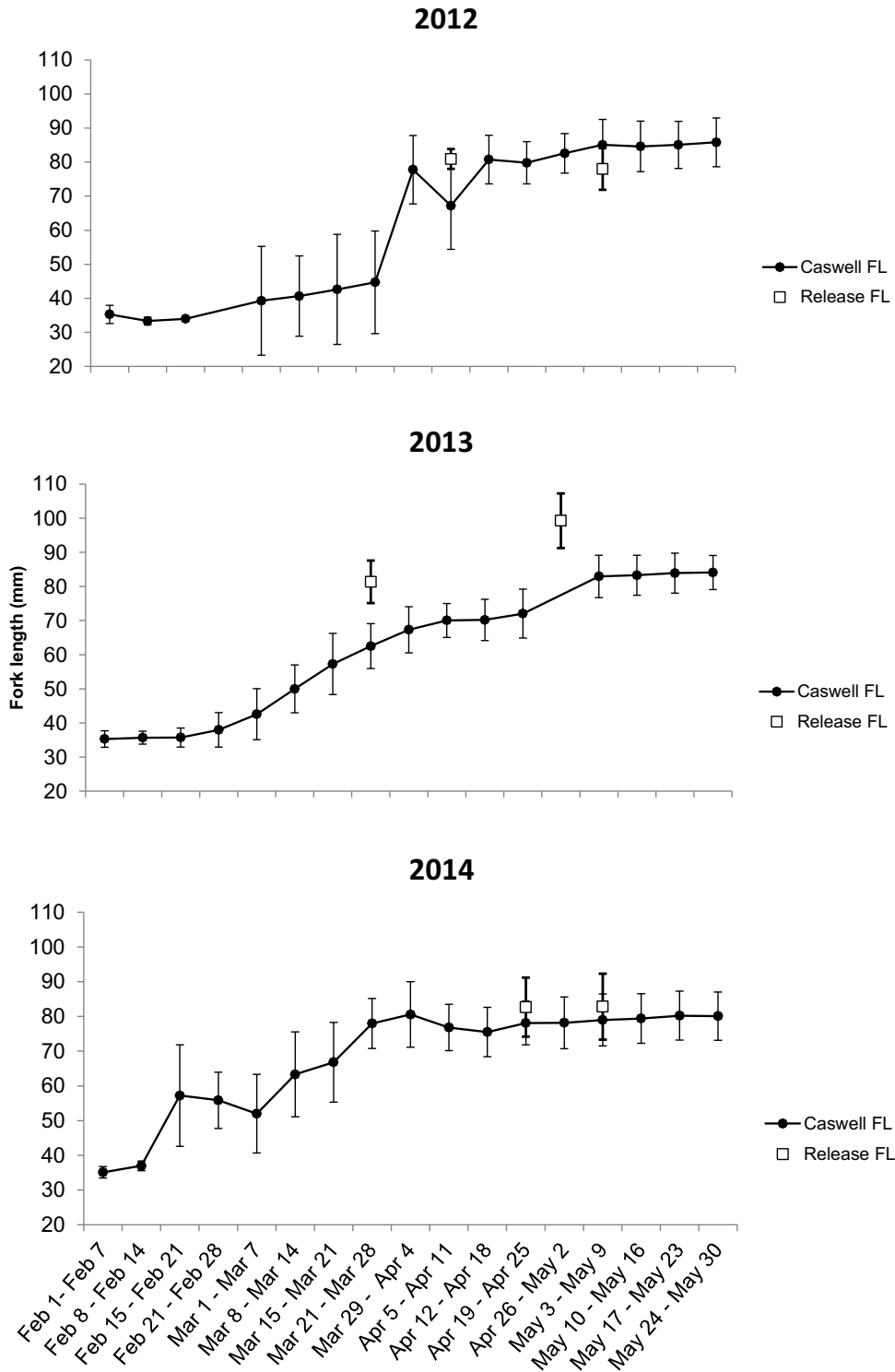


Figure 5. Comparison of the fork lengths of tagged fish (open squares) with natural-origin fish captured in the Caswell screwtrap for each year of the study.

Table 5. Release groups by year and release, including location and time of release, number of fish released, and fork length (mm).

Release group	Total number of fish released Average fork length in mm ( $\pm$ SD)					
	1a	1b	2a	2b	3a	3b
Release location (rkm)	62.5	54.8	47.7	40.1	32.9	27.3
Release time	18:00	20:30	23:00	1:30	4:00	6:30
<b>2012 (Release 1)</b>	50 81( $\pm$ 2.7)	48 81( $\pm$ 2.9)	36 80( $\pm$ 3.2)	28 81( $\pm$ 2.9)	20 81( $\pm$ 3.1)	20 82( $\pm$ 2.5)
<b>2012 (Release 2)</b>	51 79( $\pm$ 2.7)	52 78( $\pm$ 2.7)	33 79( $\pm$ 2.7)	34 78( $\pm$ 3.0)	18 79( $\pm$ 3.0)	19 72( $\pm$ 17.6)
<b>2013 (Release 1)</b>	35 82( $\pm$ 3.0)	35 81( $\pm$ 2.8)	35 81( $\pm$ 2.3)	35 82( $\pm$ 2.6)	35 82( $\pm$ 2.6)	35 82( $\pm$ 2.1)
<b>2013 (Release 2)</b>	39 100( $\pm$ 5.9)	30 96( $\pm$ 18.6)	34 101( $\pm$ 14.8)	34 100( $\pm$ 4.7)	35 96( $\pm$ 7.6)	34 100( $\pm$ 5.2)
<b>2014 (Release 1)</b>	35 83( $\pm$ 2.5)	35 84( $\pm$ 2.5)	35 82( $\pm$ 14.5)	35( $\pm$ 2.3)	35 83( $\pm$ 2.5)	35 84( $\pm$ 2.6)
<b>2014 (Release 2)</b>	33 82( $\pm$ 5.2)	33 84( $\pm$ 3.5)	32 82( $\pm$ 4.0)	32( $\pm$ 3.7)	32 85( $\pm$ 4.8)	31 84( $\pm$ 3.8)

## 2014 releases

Fish released in 2014 were obtained from the Mokelumne River Fish Hatchery due to disease issues at the Merced River Fish Hatchery. During the first release (April 18<sup>th</sup>-19<sup>th</sup>) discharge in the Stanislaus River was  $76.1 \text{ m}^3 \cdot \text{s}^{-1}$  ( $2,687 \text{ ft}^3 \cdot \text{s}^{-1}$ ) and water temperature was  $14.1 \text{ }^\circ\text{C}$ . Mean fork length of the 210 fish in this release was 83.1 mm with a standard deviation of 6.3. The second release in 2014 was performed between May 1<sup>st</sup> and 2<sup>nd</sup> when flow was  $64.6 \text{ m}^3 \cdot \text{s}^{-1}$  ( $2,281 \text{ ft}^3 \cdot \text{s}^{-1}$ ) and water temperature was  $13.8 \text{ }^\circ\text{C}$ . The 193 fish in this release were similar in size to the first release with a mean fork length of 83.6 mm and standard deviation of 4.3. The mean size of tagged fish was greater than fish collected in the Caswell screwtrap during the same period. However, confidence intervals overlapped indicating no significant difference (Figure 5).

## Survival from stationary detections

Multiple survival estimates were derived for reaches 2 and 3 because fish released in Reach 1 also passed through reaches 2 and 3 and releases in Reach 2 also passed through reach 3. Estimating survival separately for releases in each reach provided a way to examine the variability of survival estimates within years. Below are descriptions of the model selection exercise results and survival estimates derived for releases in each reach.

## Reach 1 releases

A total of eight candidate models were evaluated to describe survival of fish between stationary receiver arrays (Table 6). For releases into Reach 1, the model with “Year” as the only predictor variable was selected as the best approximating model with no other model having a  $\Delta AIC_c < 77$ . The model with sub-reach as a variable had a  $\Delta AIC_c$  of 166 indicating variation in survival by sub-reach was not supported by the data. Additionally, the model with release timing (early vs. late) had a  $\Delta AIC_c$  of 154 indicating that this was not a well-supported explanation of the data. None of the models including biotic or abiotic variables had good support in the data as evaluated by  $\Delta AIC_c$  values (Table 6). Across all years, survival was greater in 2013 than in the other two study years. However there was more variation among years in reaches 2 and 3 than Reach 1 (Figure 6). This variation was synchronous in reaches 2 and 3 (i.e. when survival increased in Reach 2 it also increased in Reach 3). Additionally, survival estimates were similar in reaches 2 and 3 in each year. Survival in Reach 1 was generally greater than the other two study reaches and was similar in 2012 and 2013 (Figure 6). In 2014, poor detection probabilities at the Adrian Ranch receiver array resulted in an uncertain estimate of survival in this reach (Table 7).

## Reach 2 releases

Of the eight candidate models evaluated for survival of releases into Reach 2, the model with “Year” as the only predictor variable was selected as the best approximating model with no other model having a  $\Delta AIC_c < 24$ . Similar to releases in Reach 1, the model with a variable identifying early and late releases was not a well-supported explanation of the data ( $\Delta AIC_c = 97$ ) nor was the model that included sub-reach ( $\Delta AIC_c = 81$ ). None of the models including other biotic or abiotic predictors enjoyed support in the data. In Reach 2, the estimates of survival for fish released in this reach had 95% confidence intervals that overlapped with survival estimates of releases in Reach 1 through this reach suggesting little between-release variation (Figure 6). A similar pattern was observed between years where survival was greatest in 2013 and lower yet similar in 2012 and 2014. Survival of these releases through Reach 3 had 95% confidence intervals that overlapped with Reach 1 releases in this reach; however, the pattern of mean estimates was different. Survival was lowest in 2012 and greatest in 2014 (Figure 6).

Table 6. Results of a model selection exercise to elucidate the most likely model to explain patterns of survival derived from stationary receiver detections.

Release group	Model predictors	AIC <sub>c</sub>	ΔAIC <sub>c</sub>
1A, 1B	Years	1310	0
	Full model	1387	77
	None	1399	89
	Fork length	1422	112
	Early vs. late	1464	154
	Temperature	1470	160
	sub-reach	1476	166
	Flow	1480	170
2A, 2B	Years	808	0
	None	832	24
	Fork length	876	68
	Full model	883	75
	sub-reach	889	81
	Flow	898	90
	Early vs. late	905	97
	Temperature	911	103
3A, 3B	Full model	529	0
	Flow	546	17
	Fork length	565	36
	Years	565	36
	Early vs late	575	46
	sub-reach	579	50
	None	581	52
	Temperature	583	54

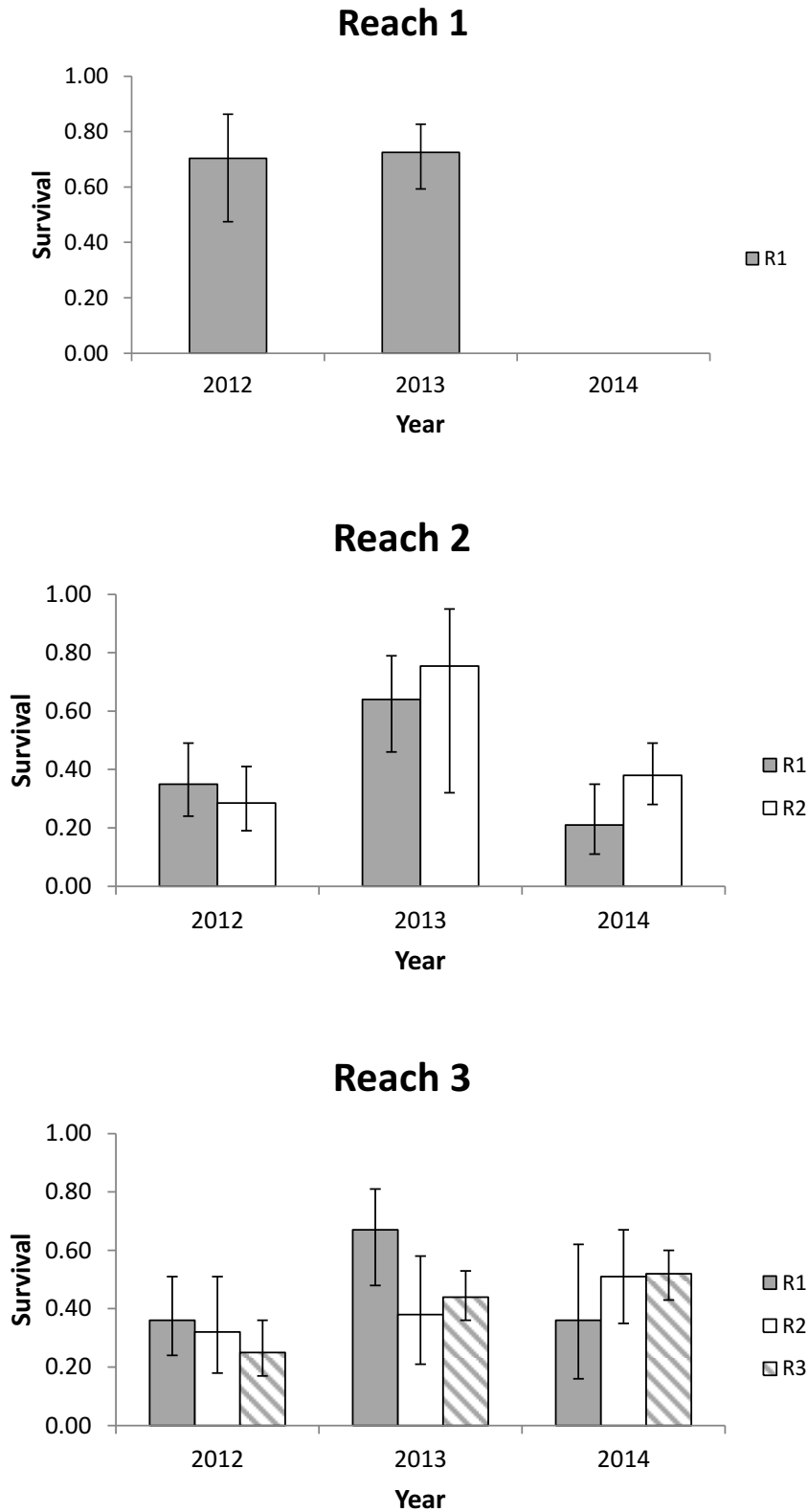


Figure 6. Estimated survival and 95% confidence intervals in each reach from 2012-2014. Survival estimates in reaches 2 and 3 were estimated independently for each release to determine the precision of the estimates.



### **Reach 3 releases**

The full model was selected as the best approximating model for releases in Reach 3 and no other model had a  $\Delta AIC_c < 17$ . When coefficient values for each predictor were examined, only the dummy variable indicating sub-reach was found to be a well-supported predictor of survival for releases in 2014 (Table 8). However, other predictors were significantly related to the combined survival and detection probabilities. This suggests that detection probabilities were influenced by environmental variables. The coefficient for release group was positive indicating that releases made in 3B had a higher survival rate than releases in 3A (farther upstream).

Table 7. Survival estimates and standard errors in the three study reaches in 2012, 2013 and 2014. Model selection indicated there was little support for variation in survival at the sub-reach level. For Reach 3, the full model was the best explanation of survival but estimates by year are provided here for comparison.

Year	Reach	Release group		
		1A, 1B	2A, 2B	3A, 3B*
2012	1	0.70 (0.10)	NA	NA
	2	0.35 (0.07)	0.28 (0.06)	NA
	3	0.36 (0.07)	0.32 (0.09)	0.25 (0.05)
2013	1	0.73 (0.06)	NA	NA
	2	0.64 (0.09)	0.75 (0.18)	NA
	3	0.67 (0.09)	0.38 (0.10)	0.44 (0.04)
2014	1	0.99 (<0.01)	NA	NA
	2	0.21 (0.06)	0.38 (0.05)	NA
	3	0.36 (0.13)	0.51 (0.08)	0.52 (0.04)

Table 8. Parameter estimates for the best fit model describing survival in Reach 3. Only the dummy variable "group" had a 95% confidence interval that did not include zero indicating it was a significant predictor of survival.

Year	2012	2013	2014
Intercept	3.07 (0.00)	-1.65 (954.35)	2.12 (2890.70)
sub-reach	-0.38 (0.58)	-0.36 (0.42)	-0.91 (0.37)*
Early vs. late	-0.86 (0.00)	-1.59 (326.64)	0.64 (117.46)
Fork length	-0.08 (0.11)	0.04 (0.04)	-0.07 (0.05)
Temperature	0.17 (0.00)	-0.10 (63.07)	0.12 (185.62)
Flow	0.02 (40.86)	-0.003 (6.20)	0.03 (10.96)

To compare survival of releases in Reach 3 to survival of other releases through this reach, survival in each year was estimated. The 95% confidence intervals overlapped for releases in each reach (Table 7). However, mean survival estimates were similar for releases made into both Reach 3 and Reach 2 (Figure 6). Additionally, there was a similar pattern to the Reach 2 releases where survival increased each year of the study.

## Survival from mobile detections

For both releases in 2012, the best approximating model included a grouping variable for individual release locations and equal detection probabilities (Table 9). This result indicates that there was significant spatial variation in survival among sub-reaches. No other model had a  $\Delta AIC_c < 7$ . The greatest mortality for each release group occurred between the release and the first mobile survey 2 days later. However, the magnitude of survival over this period varied among release locations from a high of 100% in 3b to a low of 20% in Sub-reach 2b. Survival was less variable among sites over the next 5 days until the final mobile survey 7 days after the

release. Fish released in 3a and 3b transitioned quickly out of the study reach with probabilities of 0.62 and 0.70 in 3a and 3b respectively on day 2. Fish from 1a, 1b and 2a transitioned out of the study reach on day 5; however, probabilities were low (Figure 8). Only fish from 1b transitioned from the study reach on day 7. No fish from 2b were ever observed transitioning out of the study reach and survival was lower for fish released into this reach relative to any other location.

Table 9. Results of a model selection exercise to determine the most likely model to describe survival and transition probabilities as a function of time from mobile detections.

<b>Year</b>	<b>Release</b>	<b>Model</b>	<b>AIC<sub>c</sub></b>	<b>ΔAIC<sub>c</sub></b>
2012	High flow	Equal detection probability	944	0
		Full model	951	7
		No groups	989	45
	Low flow	Equal detection probability	646	0
		Full model	669	23
		No groups	692	46
2013	High flow	Equal detection probability	10696	0
		Full model	10766	70
		No groups	10807	111
	Low flow	Full model	1270	0
		Equal detection probability	1355	84
		No groups	1431	160
2014	High flow	Full model	1463	0
		Equal detection probability	1469	6
		No groups	1619	155
	Low flow	Equal detection probability	1025	0
		Full model	1053	28
		No groups	1079	54

Similar to the first release, the greatest drop in survival of fish in the second release occurred between the release and the first mobile survey two days later. With the exception of the release in 2b survival was lower in all release locations during the second release. Survival remained relatively similar between the mobile survey two days after the release and the last survey four days after the release (Figure 7). Unlike the first release, no fish were observed transitioning out of the study reach until the survey on day three. On day three a large fraction of fish (63%) released in 3b were estimated to have transitioned out of the study reach. Fish released in 2b were also observed transitioning on day three. On day three fish released in 1b, 2a and 2b were observed transitioning out of the study reach in moderate numbers (Figure 7). Fish released in 1a and 3a were not observed leaving the study reach during the period when mobile surveys occurred.

For both releases in 2013, the model that used a grouping variable for each release group and equal detection probabilities was selected as the best approximating model (Table 9). This

indicates spatial variation in survival among sub-reaches was significant. There was considerable variation in survival rate among most release groups between the release and the mobile survey on day 4 with values ranging from 44 to 92% in 3a and 1a respectively (Figure 7). During the first few days, survival was generally higher for releases made farther upstream; however, by day 6, the range of cumulative survival among groups had been reduced to 26% and during the last mobile survey 9 days following the release, there was no consistent spatial patterns in cumulative survival. Fish were observed transitioning out of the study reach between day 2 and day 7 following the release although probabilities were low (5-16%; Figure 8). Between the release and day 4, only fish released in 2b and 3b were observed. On day 6 and 7 fish released in all other reaches were observed transitioning out of the reach (Figure 8).

Following the second release, no fish released at 2b was ever detected again whereas fish at all other release sites experienced relatively high survival rates between the release and the first mobile survey on day 3 (81-100%). Between day 4 and the last survey on day 9, cumulative survival dropped precipitously for fish at most release sites with the exception of fish released in 1b that continued to experience high survival throughout the study period. Fish rapidly transitioned out of the study reach during this release. By day 3, fish from almost all reaches were observed transitioning out of the study reach with greater probabilities for fish released at locations farther downstream (Figure 8). High probabilities of leaving the study reach continued throughout the study period with higher probabilities for upstream releases occurring later (day 6-9; Figure 8).

The best model for the first release in 2014 was the fully time dependent model and no other model had a  $\Delta AIC_c < 6$ . For the second release, the model with equal detection probabilities was selected as the best approximating model. Thus, spatial variation in survival among sub-reaches was significant for both releases. Cumulative survival was greater during this release than during any other release over the three-year study period. Even after 18 days, survival of fish that remained in the study reach was  $\geq 66\%$ . Fish transitioned out of the study reach rapidly during this first release. During the survey one day after the release, fish from all release groups except 1b, were observed transitioning out of the study reach. Fish released at 3a and 3b in particular had high probabilities of transitioning out of the study reach early in the study period. However, there were low transition probabilities for fish released in reaches 1 and 2 even though mobile surveys were conducted up to day 18 following the release.

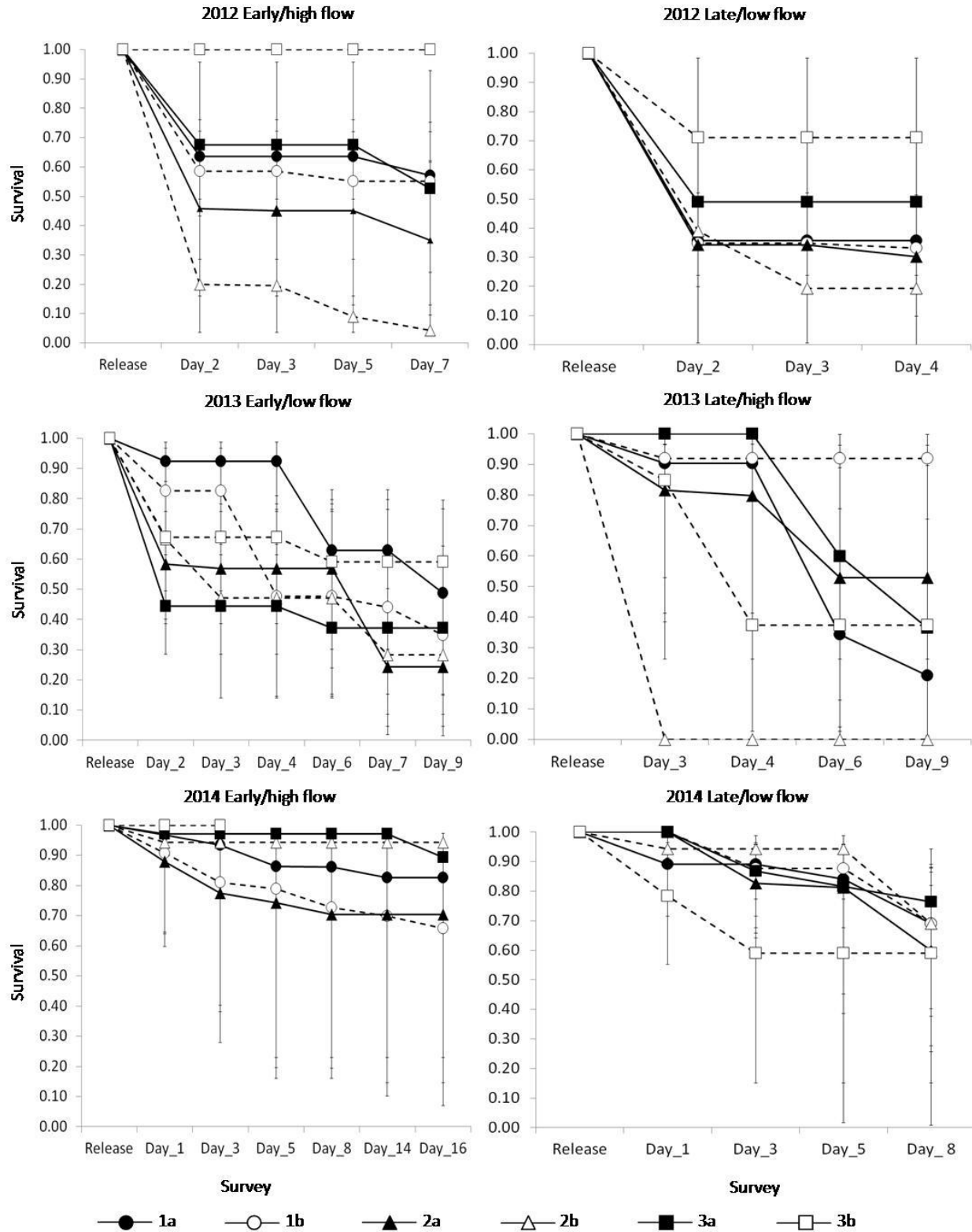


Figure 7. Cumulative survival of tagged Chinook salmon that remained in the 50 km study reach between each mobile survey period.

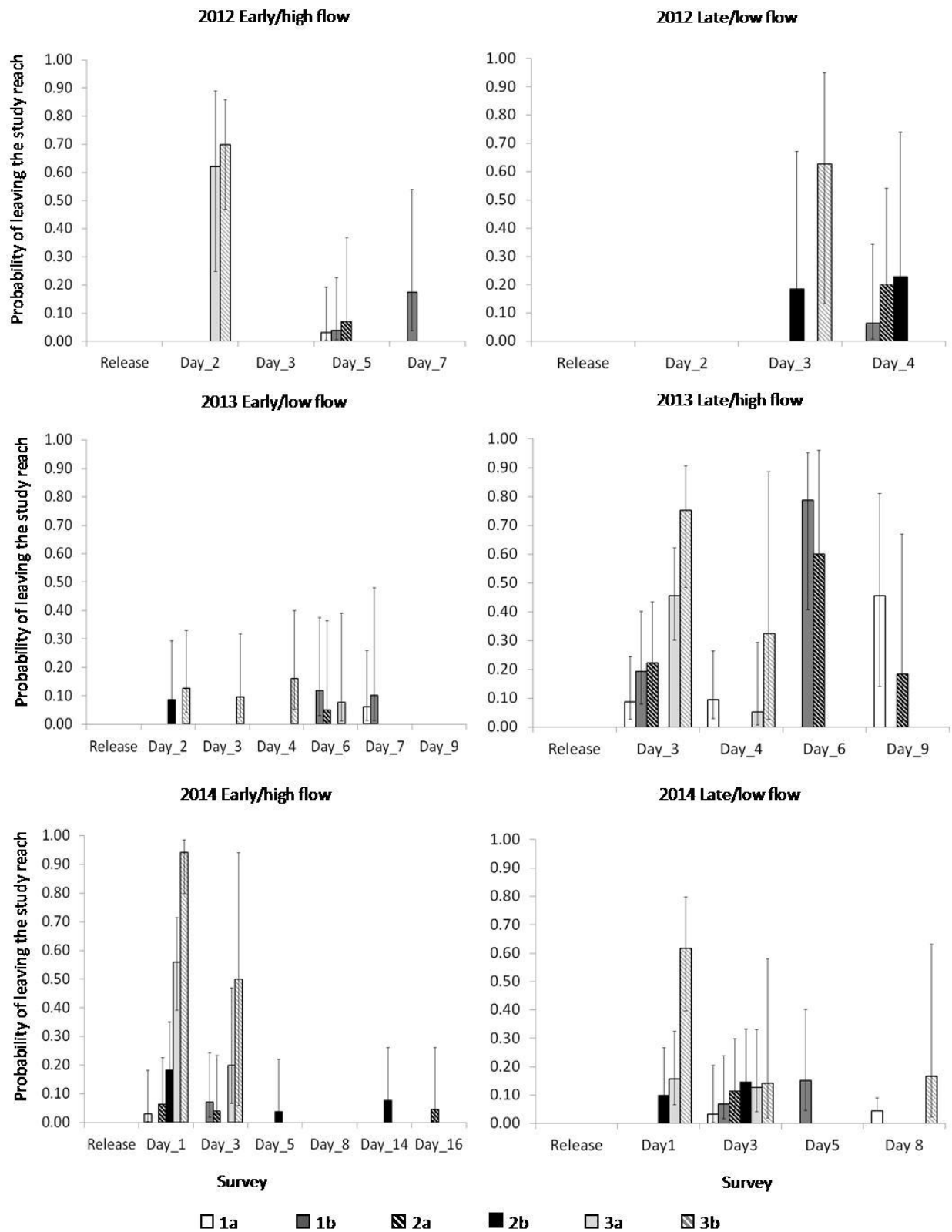


Figure 8. Probability of Chinook salmon released in each sub-reach leaving the 50 km study reach between each mobile survey period.

Cumulative survival in the second release was lower relative to the first release but was higher than releases in other years. By the last survey eight days after the release, cumulative survival ranged from 59 to 76% for releases in 3b and 3a respectively (Figure 7). Fish also transitioned out of the study reach rapidly during the second release though probabilities were lower than during the first release (Figure 8). On the first day after the release, fish released in 2b, 3a and 3b were observed with the greatest probability for fish in 3b (62%) and lowest for fish in 2b (10%). On day 3, fish from all releases were observed transitioning out of the study reach however, probabilities were relatively low (3-15%). Between day 5 and 8 only fish from 1a, 1b and 3b were observed transitioning out of the reach.

The per-day survival rate was calculated over the first three days post-release to facilitate comparison of mobile survival data across releases and years. For releases in 2012, early releases generally had greater survival rates than late releases although 95% confidence intervals overlapped for most releases. Only releases in 1a had non-overlapping confidence intervals (Figure 9). In 2013 daily survival rates were qualitatively greater for late releases however, 95% confidence overlapped for almost all release groups. The exceptions were for the late release into 2b where no fish were observed to survive and the late release in 3a where 100% of fish were observed to survive (Figure 9). Daily survival was qualitatively higher for early releases in 2014 with the exception of the two early releases in reach three where daily survival was observed to be 100% (Figure 9).

Variation in discharge across releases and years explained 2.5- 69.4% of the variation in daily survival rate across all release sites; however, this relationship was only statistically significant for releases in 2a ( $F=9.05$ ,  $p=0.040$ ). . However, the strength of this relationship appeared to be modified by release location.

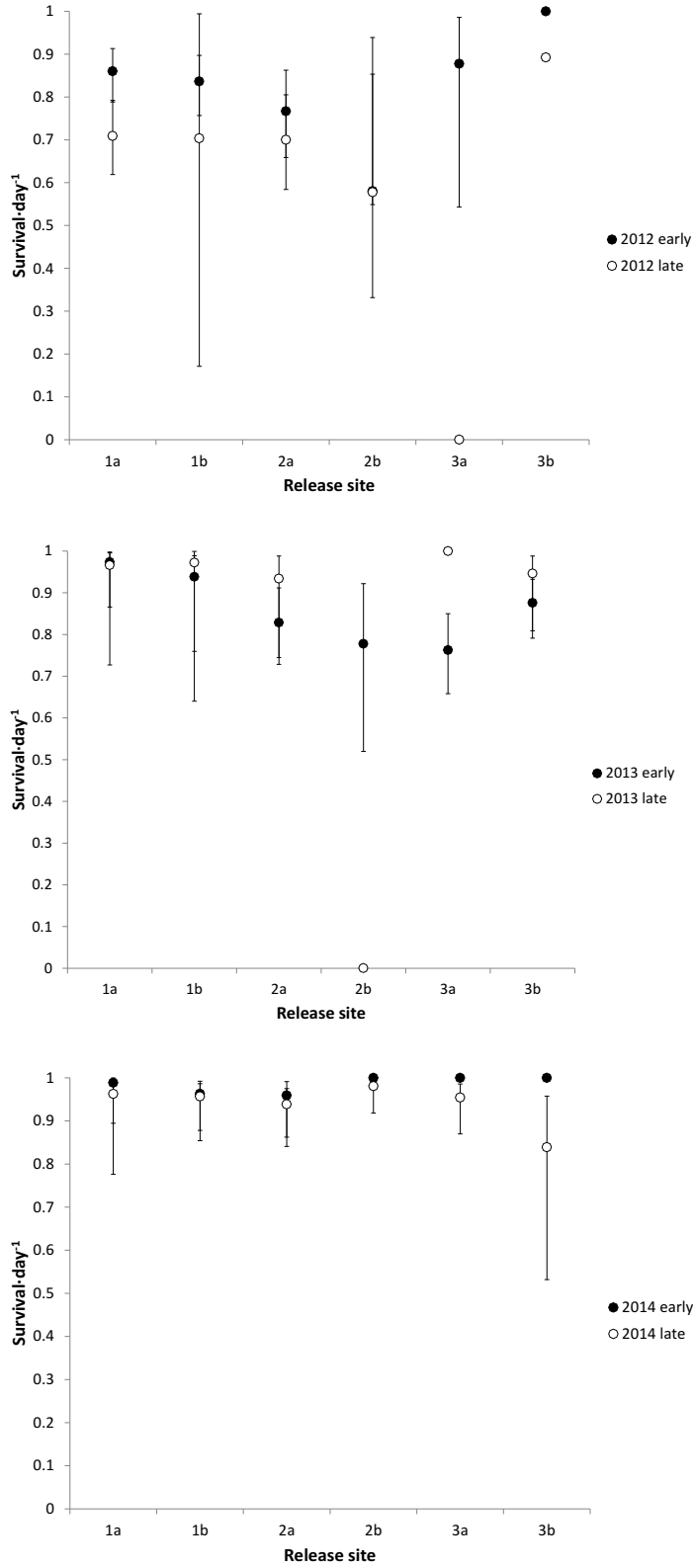


Figure 9. Cumulative survival and 95% confidence intervals on the third day post release for each release group in 2012, 2013, and 2014.



## Travel time

### Stationary detections

During early releases, mean travel time as calculated from stationary detections ranged from a low of 17.8 km·day<sup>-1</sup> in Reach 2, to a high of 27.1 km·day<sup>-1</sup> in Reach 1 (Figure 10). During late releases, mean travel time ranged from a low of 26.0 km·day<sup>-1</sup> in Reach 3 to a high of 51.3 km·day<sup>-1</sup> in Reach 1. A Kruskal-Wallis test yielded a significant difference in travel time between early and late releases in all three reaches with faster rates observed during late releases (Table 10). A significant difference among reaches was detected during the early releases ( $\chi^2 = 30.046$ ,  $p < 0.001$ ). A Wilcoxon multicomparisons test indicated that the significant differences were between reach 1 and the other two reaches. There was no significant difference between reaches 2 and 3. Additionally, no significant difference was detected between reaches during the late releases ( $\chi^2 = 2.621$ ,  $p < 0.270$ ). To determine if there was a relationship between travel time and survival, a linear regression of travel time on logit transformed survival was performed. This model explained 29.5% of the variation in survival; however, the relationship was not statistically significant ( $F = 2.51$ ,  $p = 0.164$ ).

Table 10. Results of Kruskal-Wallis tests to determine differences in travel time between early and late releases in each reach.

Reach	$\chi^2$	<i>p</i> -value
1	27.290	< 0.001
2	14.061	< 0.001
3	11.468	0.001

### Mobile detections

Mean travel times calculated from mobile detections during early releases ranged from a low of 0.88 km·day<sup>-1</sup> in Sub-reach 3b to a high of 9.2 km·day<sup>-1</sup> in 1a (Figure 11). During the late releases, travel time ranged from 2.0 km·day<sup>-1</sup> in Sub-reach 3a to 8.9 km·day<sup>-1</sup> in Sub-reach 1a. A Kruskal-Wallis test was performed to test for differences between early and late releases among all release groups. This test revealed significant differences between release sites in the early period ( $\chi^2 = 261.837$ ,  $p < 0.001$ ) and in the late period ( $\chi^2 = 118.424$ ,  $p < 0.001$ ; Figure 11). A Wilcoxon multicomparisons test was performed to identify which release groups were significantly different (Table 11). A separate Kruskal-Wallis test was performed to test for differences between early and late releases for each release group. The only significant difference between early and late releases was for fish released into 3a and 3b (Table 11). A regression of daily survival rate over the first three days on travel time explained little variation (< 1%) and was not statistically significant ( $F = 0.136$ ,  $p = 0.714$ ).

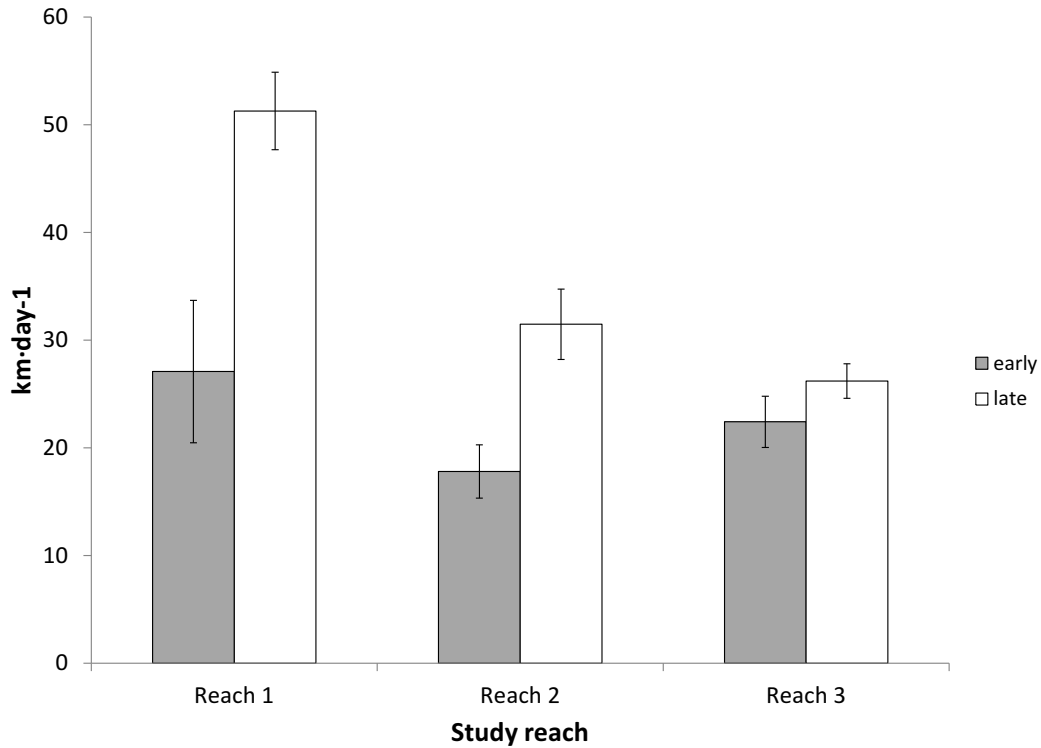


Figure 10. Estimates of travel time (+/- SE) for early and late releases in the three 10km study reaches calculated from stationary detections.

Table 11. Results of Kruskal-Wallis tests to determine differences in travel time between early and late releases in each reach.

Reach	$\chi^2$	<i>p</i> -value
1A	0.162	0.688
1B	1.077	0.300
2A	2.695	0.101
2B	0.328	0.567
3A	11.311	0.001
3B	35.726	< 0.001

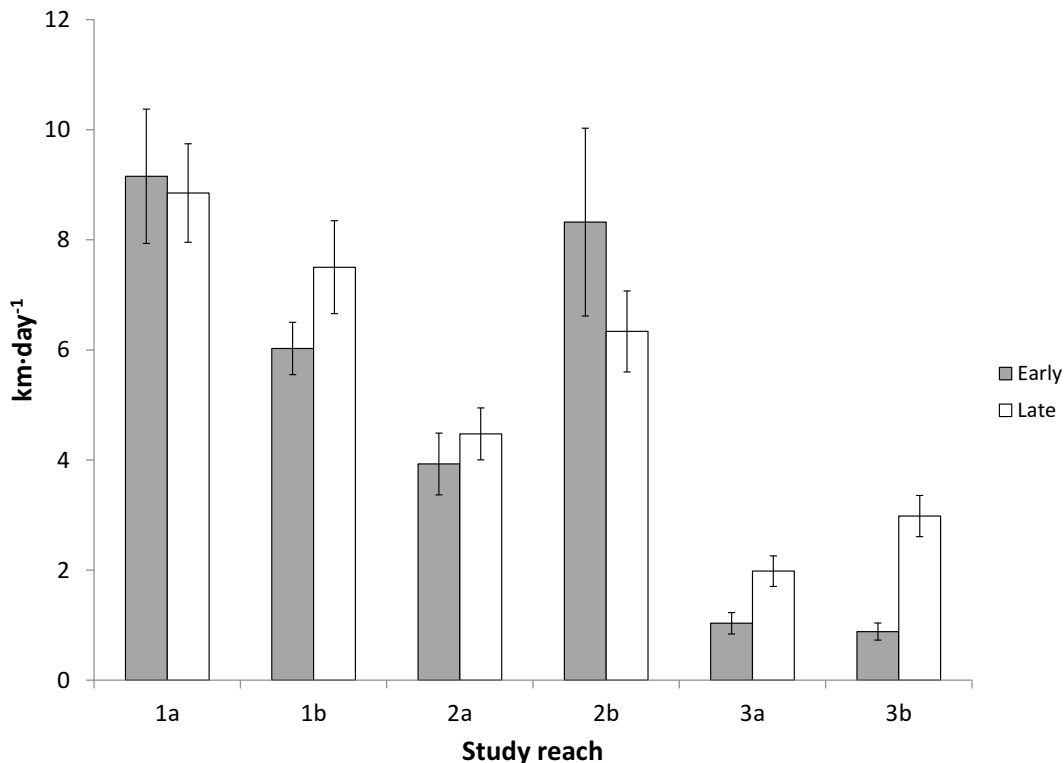


Figure 11. Means and standard errors of travel times for each release group during early and late releases. All values were calculated from mobile detections.

## Spatial analyses

The six sub-reaches exhibited a trend of decreasing gradient from upstream to downstream (Table 12). Channel width was greatest in Sub-reach 1A with the remaining 5 sub-reaches having similar values. Alcove habitat area was greatest in Sub-reach 1A, but was also high in Sub-reach 2a. The most upstream and most downstream sub-reaches had the greatest number of diversions and the greatest percentage of deep pool habitat. The number of drain returns and bridges in each sub-reach was low and variable (Table 12).

Four locations were identified where observed mortality was significantly more clumped than an equal number of randomly distributed points. These four locations were immediately downstream of the release sites in reaches 2 and 3 (Figure 12). No significant clumping of mortalities was observed in Reach 1. The hot spot analysis yielded a single significant clumping of observed mortalities in Sub-reach 2b (Figure 13).

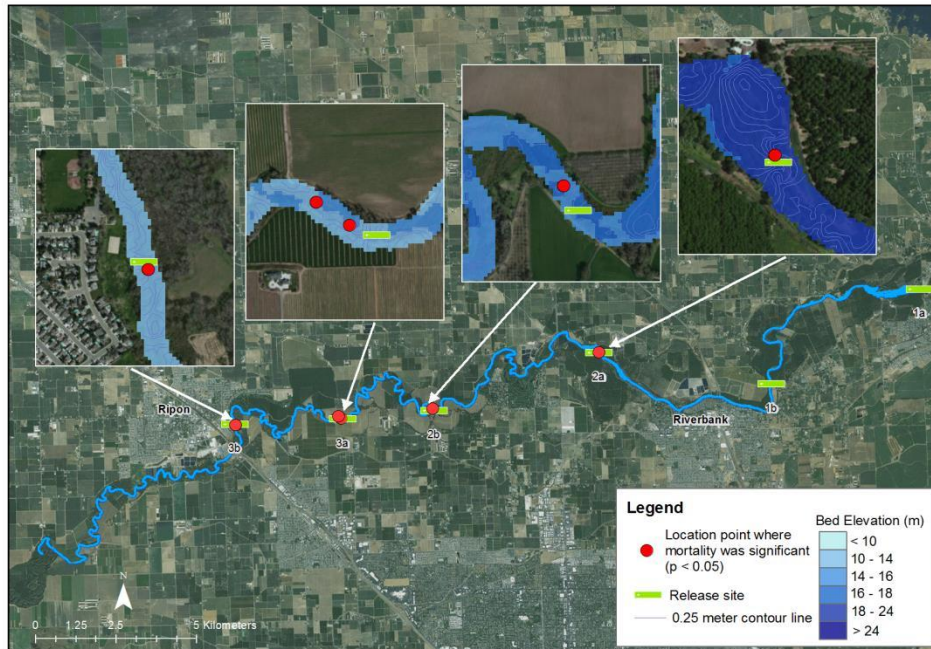


Figure 12. Results of the spatial auto correlation analysis (global Moran's I). Red circles represent locations with significant clumping of last known detections. Green bars are locations where tagged fish were released.

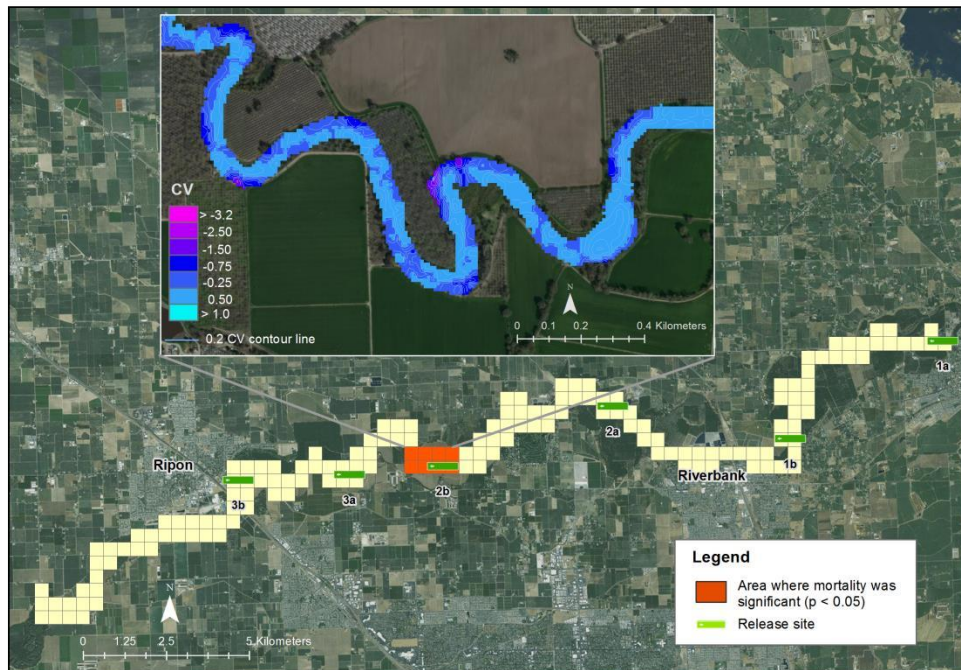


Figure 13. Map of last known detections for fish that never exited the study reach. The orange squares are areas with significant clumping of last known detections.

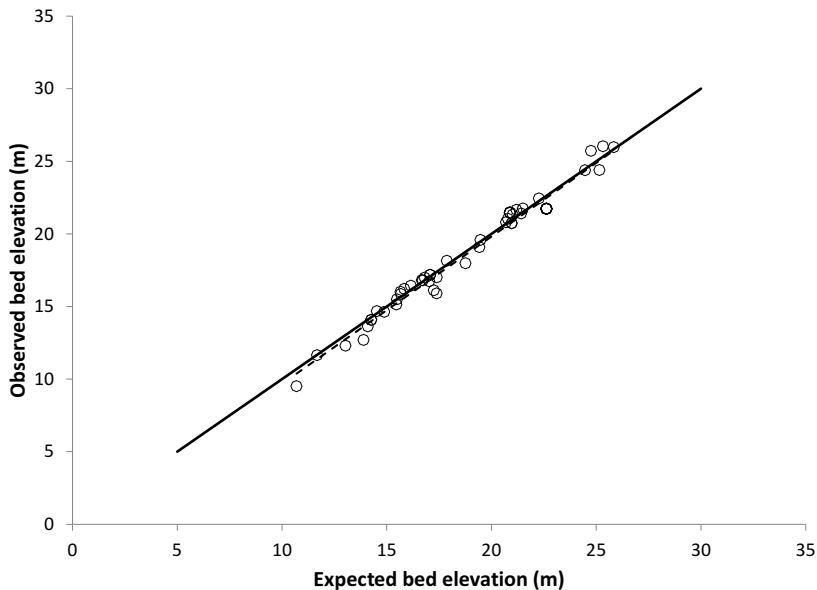


Figure 14. Relationship between expected and observed bed elevations at last known detections in Reach 1. The dashed line is the modeled relationship and the solid line is the 1:1 relationship.

Analysis of covariance in Reach 1 indicated that there was a significant relationship between expected and observed bed elevations at last-detection locations ( $F = 14650$ ,  $p < 0.001$ ) and the interaction between the modeled relationship and a 1:1 relationship was not significant ( $F = 0.53$ ,  $p < 0.469$ ). However, the variable differentiating the study reach from the 1:1 line was not significant indicating that last-detection locations were not deeper or shallower than expected (Figure 14). In Reach 2, ANCOVA indicated there was a significant relationship between expected and observed bed elevations at last-detection sites. However, there was a significant interaction between the relationship in the study reach and the 1:1 line ( $F = 15.438$ ,  $p < 0.001$ ). Thus, the last-detection locations in Reach 2 could be shallower or deeper than expected by chance depending on the expected elevation value. Examination of the plot of the modeled relationship and the 1:1 line indicated that bed elevations at observed last-detection locations were lower than expected at lower bed elevations and more similar to expected at greater bed elevations (Figure 15). In Reach 3, there was a significant relationship between observed and expected bed elevation at the site of last-detections ( $F = 19602$ ,  $p < 0.001$ ) and the interaction between the observed relationship and the 1:1 line was not significant ( $F = 2.772$ ,  $p = 0.100$ ). The reduced model revealed that the modeled relationship had a significantly lower intercept than the 1:1 line indicating that bed elevations at the site of last-detections were significantly lower than expected (i.e. deeper; Figure 16).

 Stanislaus River Juvenile Chinook Salmon Survival Study

Table 12. Physical description of study sub-reaches, including structures (diversions, returns and bridges) and channel geometry.

Sub-reach	Sub-reach location (rkm)	Diversions and returns				Channel dimensions				
		Number of diversions <sup>1</sup>	Total intake size (pipe diameter in cm)	Number of return pipes	Number of bridges	Percent gradient	Average channel width (m)	Total deep pool area (m <sup>2</sup> )	Percent deep pool area	Alcove habitat area (m <sup>2</sup> )
1a	62.5-54.9	6	52	0	0	0.051%	36.1	53,900	18.69%	70,649
1b	54.8-47.8	0	0	3	2	0.040%	23.4	15,073	8.05%	814
2a	47.7-40.2	1	4	0	2	0.040%	22.1	16,372	9.17%	10,806
2b	40.1-33.0	3	34	0	0	0.026%	20.3	5,384	3.27%	1,637
3a	32.9-27.4	3	35	2	1	0.037%	23.9	12,924	5.57%	5,817
3b	27.3-14.8	7	60	3	0	0.031%	21.4	31,073	14.96%	4,438

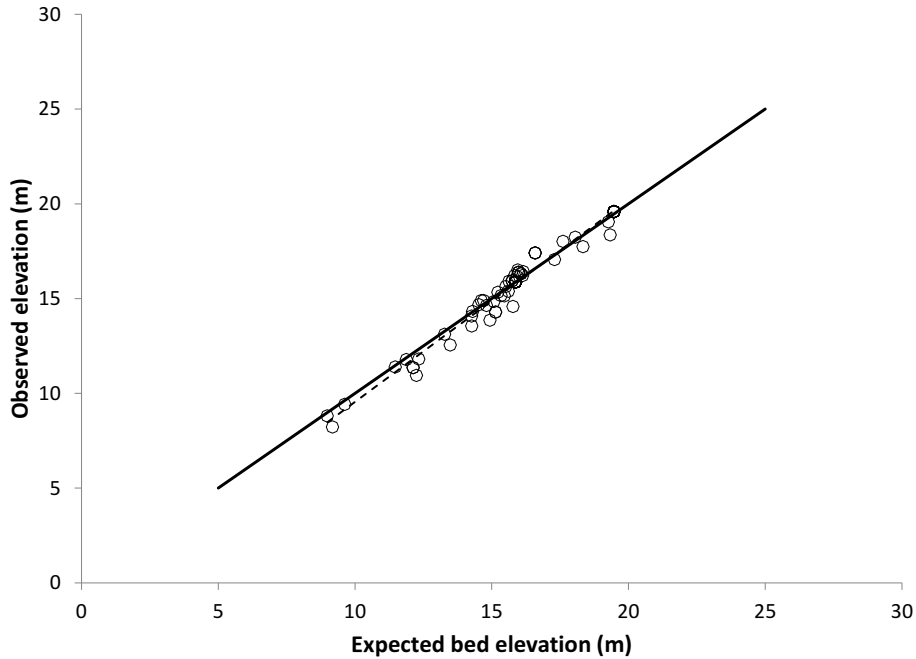


Figure 15. Relationship between expected and observed bed elevations at last known detections in Reach 2. The dashed line is the modeled relationship and the solid line is the 1:1 relationship.

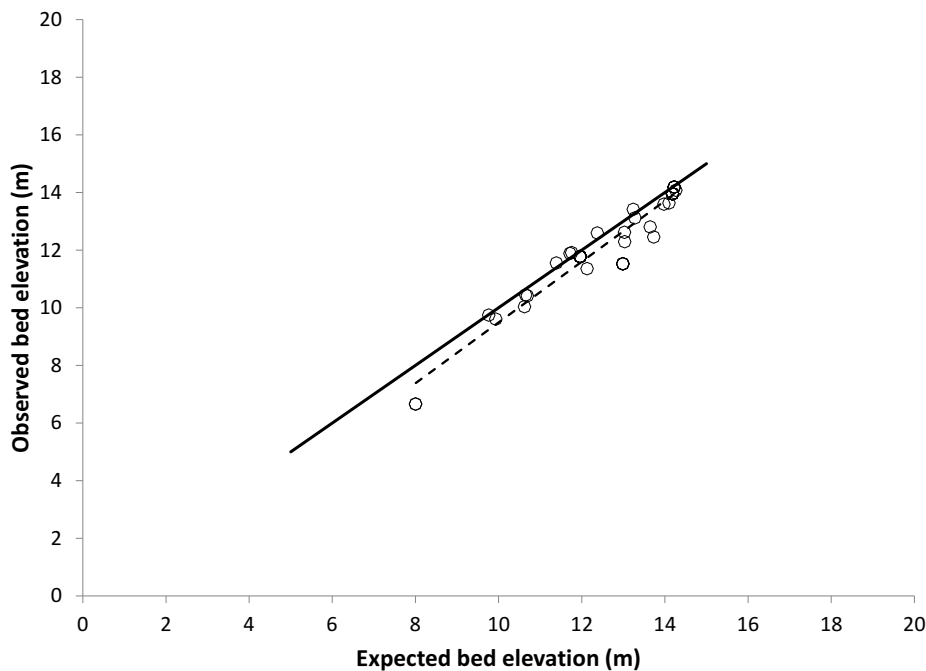


Figure 16. Relationship between expected and observed bed elevations at last known detections in Reach 3. The dashed line is the modeled relationship and the solid line is the 1:1 relationship.

The hot spot analysis of artificial structures in the study reach revealed a single significant clumping at the bottom of Reach 3 (Figure 17). This location was not associated with any of the significant clumping of last known tag detections.

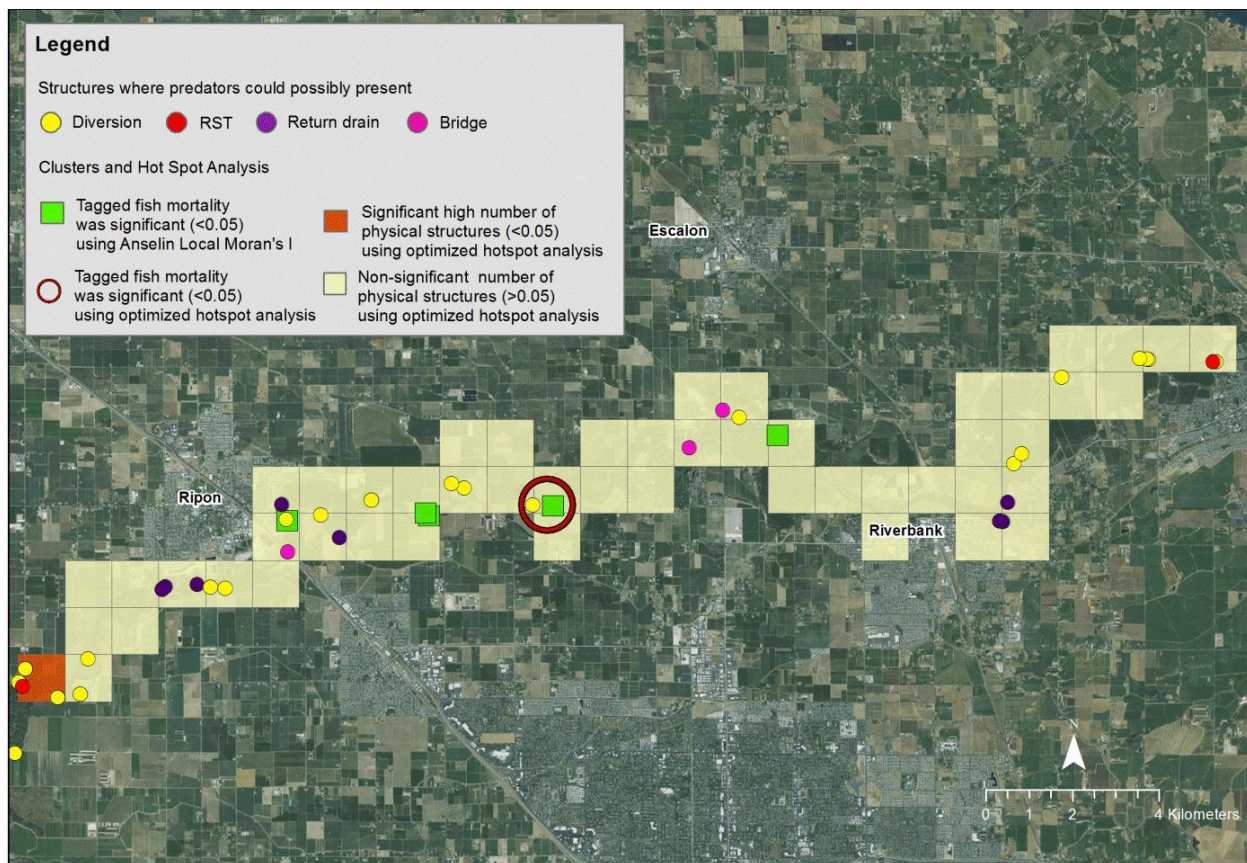


Figure 17. Map indicating the locations of three artificial structure types in the 50 km study reach and significant clumping of both artificial structures and last known tag detections.

## Tag Retention and Survival

In 2013, three experimental fish were lost from Group 1 when tag antennas were entrained by the impeller of a bilge pump used to circulate and maintain water quality during transport. This resulted in two mortalities and a shed tag. Two additional shed tags were recovered within the first 48 hours after arrival at the aquaculture facility. This may have been related to the aforementioned stressors experienced during transport, though this could not be confirmed because the fish that shed their tags survived and could not be recaptured without introducing an additional stressor to the other experimental fish. Of the remaining 15 fish from Group 1, 100% survived and retained their tags for the duration of the entire study (i.e., 50 d). For Group 2, all tagged fish survived and retained their tags for the duration of the monitoring period (i.e., 21 d). All control fish from both groups survived the duration of the study.

In 2014, all fish from Group 1 and Group 2 survived transport. Within 48 hours of arrival, there were two mortalities from the experimental fish of Group 1. Both individuals showed signs of stress upon arrival suggesting potential surgery-related impacts. Overall, experimental fish from Group 1 showed 90% survival and 100% tag retention and experimental fish from Group 2 showed 100% survival and tag retention for the duration of the study (14 days). All of the



surgery- and non-surgery control fish survived through the duration of the experiment.

### Behavioral Tag Effects

In 2013, position in the water column was significantly different for control and treatment fish ( $\chi^2 = 9.282$ ;  $df = 3$ ;  $p = 0.0258$ ; Figure 18). In general, control fish highly favored the bottom of the tank, holding there roughly half of the time. Tagged fish tended to orient in the middle of the water column, with a smaller proportion of fish on the bottom. Few tagged or control fish were seen at the surface. There was a significant difference in body orientation between control and tagged fish ( $\chi^2 = 64.268$ ;  $df = 2$ ;  $p < 0.0001$ ; Figure 17). Control fish exhibited a horizontal body position roughly 85% of the time while tagged fish were seen in a pitched body position with the head up and tail down over half the time (Figure 18).

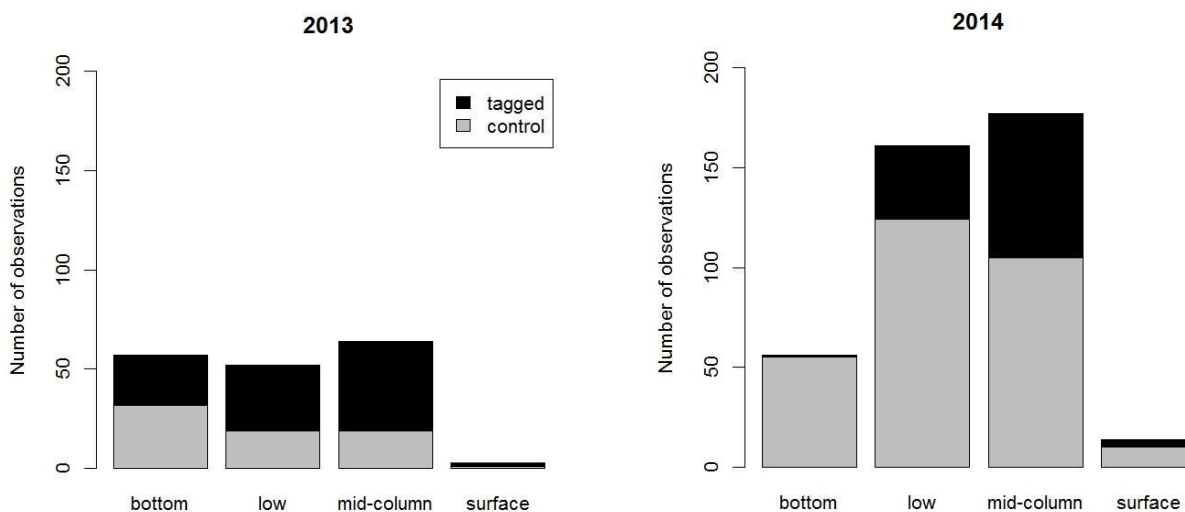


Figure 18. Observations of tagged and control fish in the water column in 2013 (left) and 2014 (right).

In 2014, position in the water column was significantly different for control and treatment groups over the two-week monitoring period ( $\chi^2 = 47.808$ ,  $df = 6$ ,  $p < 0.0001$ ; Figure 18). Tagged fish spent most of their time in the middle and lower portions of the water column, with no individuals on the very bottom. Control fish were evenly distributed among the bottom, lower, and middle portions of the water column. There was also a significant difference in the body orientation of control and tagged fish ( $\chi^2 = 23.867$ ,  $df = 4$ ,  $p < 0.0001$ ; Figure 19). Tagged fish oriented horizontally and with a pitched position (head up and tail dropped) in roughly equal proportion (Figure 20). Control fish oriented roughly 90% of the time with a horizontal body position.

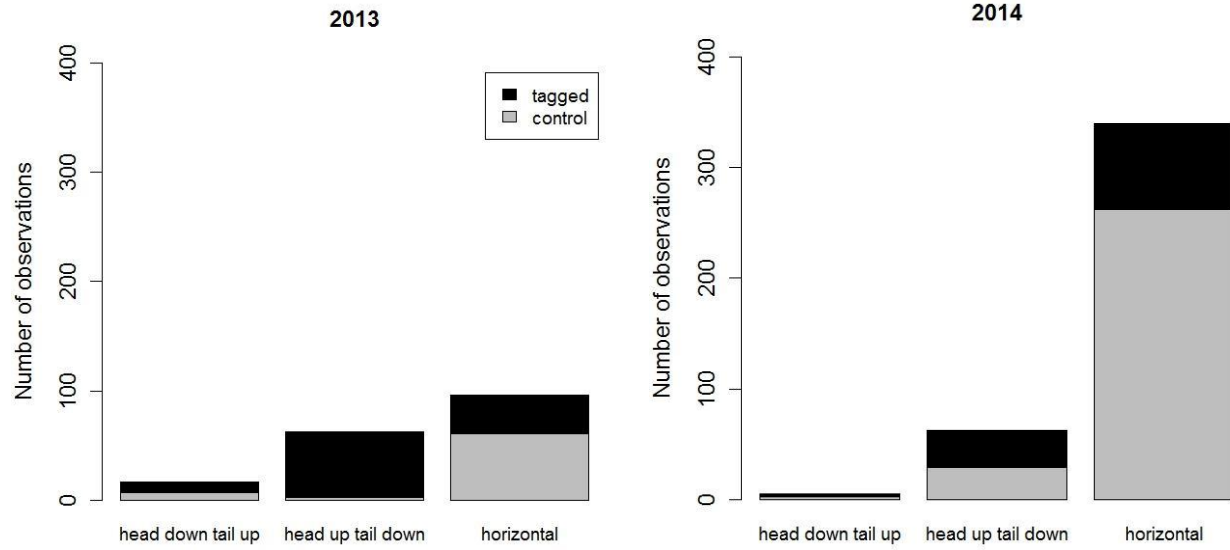


Figure 19. The total number of observations of body position (pitch) for control and tagged fish for 2013 (left) and 2014 (right).



Figure 20. Tagged fish (within black circle) displaying "head up, tail down" body orientation.

## Tag Effects on Growth and Post-Recovery Condition

In 2013 only, final fork length and weight were measured and post-recovery health was assessed in tagged and control fish.

Prior to the study, the population of tagged fish and the entire study population showed no significant difference in their size distribution ( $D=0.149$ ,  $p=0.4079$ ). Because of this, we assumed the tagged fish were representative of the population of tagged fish used in our field study. We also assumed control fish conformed to the same distribution because they were also randomly selected as a subset of the field study population.

After the study, the tagged and control fish showed no significant difference in fork length ( $D = 0.171$ ,  $p = 0.4903$ ; Figure 21). The relationship between fork length and weight, which is considered a metric of body condition, was also similar for control and treatment fish (Figure 22).

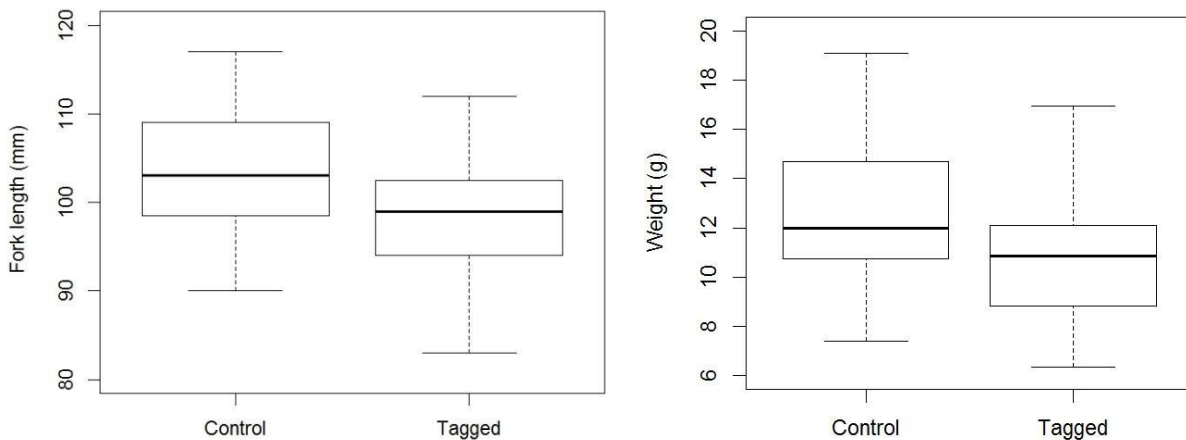


Figure 21. Fork length (left) and weight (right) in the tagged and control groups at the end of the 2013 study.

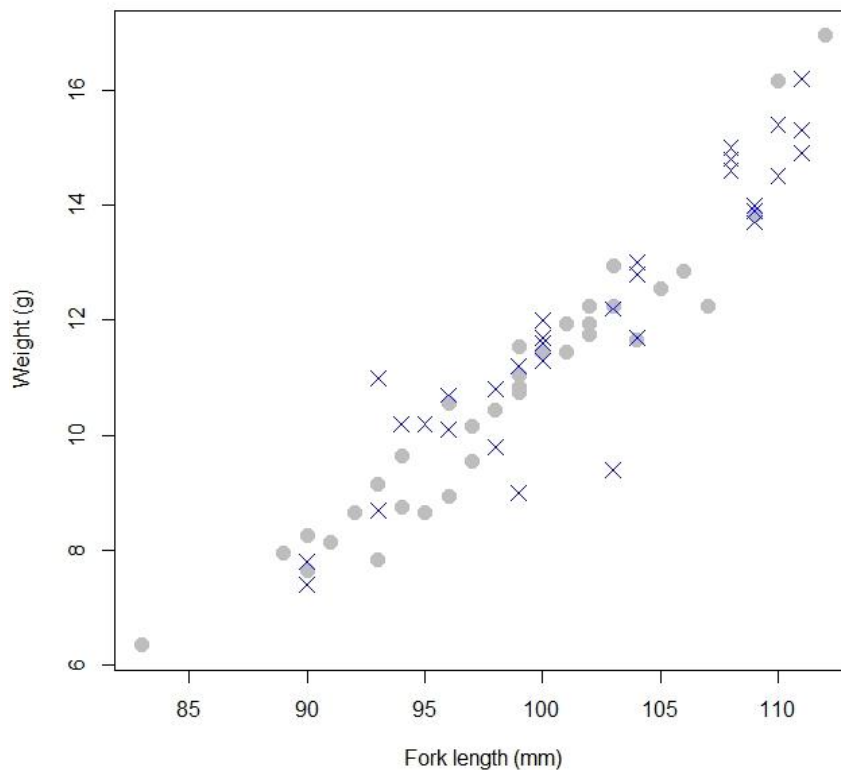


Figure 22. Fork length-to-weight relationship for tagged (blue X) and control (gray circle) fish.

There was no significant difference in the post-recovery condition (eyes, fins, and scales) of control and tagged fish for 2013 (all  $p > 0.05$ ).

## DISCUSSION

The data provided from three years of radio tagged juvenile Chinook salmon releases in the LSR indicated that there was sufficient evidence to reject the first null hypothesis that the survival of Chinook salmon juveniles does not differ among sub-reaches of the LSR. Estimation of survival using both the mobile and stationary detections indicated there were significant differences in survival between different LSR segments and for fish released in different sub-reaches.

However, these differences were not consistent in space and time. At the sub-reach level, the best model of survival calculated with mobile detections for each release included a grouping variable for release site (sub-reach). Yet, the pattern of occurrence of the best and worst survival occurred was variable among releases and years. Fish released into Sub-reach 2b often experienced poor survival rates and the hot spot analysis identified areas in Sub-reach 2b as having significantly more last known detections than any other sub-reach. This suggests that Sub-reach 2b has particularly poor conditions for juvenile salmon survival. Analysis of the stationary detections indicated that including a variable for sub-reach ( $\approx 8$  km) where fish were released was only supported for releases in Reach 3 (3a, 3b). However, there were significant differences in survival at the 16 km reach level (Reach 1, 2, 3). Reach 1 tended to have greater survival than the other two reaches; however, 95% confidence intervals often overlapped.

Estimates in reaches 2 and 3 tended to be similar and confidence intervals almost always overlapped.

There was no conclusive evidence to reject or accept the second hypothesis that survival is constant throughout the migration period. The survival models calculated from stationary detections did not provide support for a significant effect of release timing (i.e., early vs. late in the migration period) on survival. However, daily survival rates calculated from the mobile detections for early and late periods had non-overlapping confidence intervals for some release groups. In general, fish released in sub-reaches located farther downstream had greater probabilities of transitioning out of the study reach. However, these probabilities changed considerably among early and late releases across years. The timing of the release appeared to have less effect on transition probabilities than the flow conditions experienced. During the higher flow release in each year, tagged fish tended to be more likely to transition out of the study reach and also to transition at an earlier date following release. The structure of the data prevented a direct test of this relationship but it appears flow may act as a cue for fish to begin migrating toward the ocean. Flow is a well-known cue used by fishes to initiate migration behavior (Poff *et al.* 1997; Bunn and Arthington 2002) yet individuals must also be physiologically ready to migrate. During early releases, some fish may not have been ready to initiate migration regardless of flow conditions.

There was sufficient evidence to reject the third null hypothesis, that the spatial distribution of last known detections is random throughout the study reach. A test for clumping relative to random point distributions yielded five significant points of high mortality in the study reach. All five of these points were located immediately downstream of the release sites in reaches 2 and 3. Initially, this would appear to be a release effect; however, there was no significant clumping of points at the two release sites in Reach 1. This may suggest that if a sub-reach is poor quality habitat (e.g. high predator abundance, poor abiotic conditions) mortality occurs relatively quickly after release. This hypothesis is supported by the mobile survival models that indicated most of the mortality occurred in the first few days following the release. A significant clumping of observed points was also detected in Sub-reach 2b using hot spot analysis. As described above, survival in this reach was consistently low in most years and releases. Multiple lines of evidence have identified Sub-reach 2b as low-survival habitat for juvenile Chinook salmon survival and restoration efforts may be beneficial in this reach of the LSR.

The fourth hypothesis, that survival estimates are not related to migration speed of juvenile Chinook salmon could not be rejected based on the evidence provided by the tagging data. The regression of survival on travel time was not significant when using the stationary or mobile detection data sets. However, the difference between estimates of travel time using mobile vs. stationary detections yielded insights into the behavior of tagged fish within the study reach. Calculation of travel time using mobile detection data yielded much lower estimates than calculations using stationary data. It was clear from the detection histories that during certain releases, fish held in the study reach prior to initiating migration or held until tag life expired. The lack of significant movement for some fish was reflected in the low estimates of travel time from mobile detections. Even the highest estimates of travel time from mobile detections were relatively low. This may have occurred because fish that move quickly can leave the study reach between mobile survey intervals. The stationary receivers can detect fish 24 hours a day allowing even the fastest-moving fish to be detected leaving the study reach. Thus, estimates

from stationary receivers represent travel times of tagged fish that are actively migrating whereas estimates from mobile detections are more representative of tagged fish that hold for various lengths of time before initiating migration out of the study reach. The estimates of travel time from stationary detections were similar to estimates for Chinook salmon in the Sacramento River (Michel *et al.* 2013) suggesting our estimates for actively migrating fish were accurate.

There was mixed evidence to evaluate the fifth null hypothesis, that survival is not associated with identified biotic or abiotic characteristics. None of the models that included biotic or abiotic predictors were selected as the best explanation of survival in reaches 1 and 2. In Reach 3 the full model was selected as the best yet only the variable for “sub-reach” was a significant predictor of survival. Other predictors were significantly related to the combined survival and detection probabilities. Since there were few significant relationships with survival it is likely that it was detection probabilities that were influenced by environmental conditions. Flow in particular was observed to have an effect on detection probabilities during releases in 2013 and 2014 and radio signals attenuate quickly with water depth. Temperatures were within or slightly above the optimal range for Chinook salmon during all releases and never reached lethal or sub-lethal levels during the study (Piper *et al.* 1982). Thus, it is not surprising that temperature had low predictive power. Fish size has been shown to have a significant effect on survival in previous studies of juvenile Chinook salmon survival (Zabel and Achord 2004; Zeug and Cavallo 2013) but there was no evidence for an effect here. The effect of fish size is usually attributed to gape limitation of predators (Sogard 1997). The range of fish sizes released was limited due to tag burden restrictions and there may not have been enough variation to detect a difference. The lack of a strong flow-survival relationship was surprising because a recent analysis of 14 years of rotary screw trap data in this reach found a significant positive relationship between cumulative discharge and survival (Zeug *et al.* 2014).

A partial explanation for the lack of a flow effect is the use of hatchery fish as a surrogate for wild fish in the current study. Wild juvenile Chinook salmon in the Central Valley display multiple life history types (Miller *et al.* 2010). They may migrate from natal habitats as fry or parr early in the year (peak in February) in response to flow pulses and rear downstream in the tidal delta before entering the ocean or they may rear in the river until they transform into smolts and then initiate migration between March and June. The hatchery fish used in this study only represent the smolt life history and thus the importance of flow to the entire population may be underestimated. Additionally, hatchery fish have been shown to express different behaviors than natural origin fish (Jonsson 1997). Other studies utilizing hatchery origin fish in the Central Valley have yielded mixed results with several finding a positive flow effect (Kjelson and Brandes 1989; Newman 2003; Perry 2010) and others failing to find an effect (Newman and Rice 2002; Newman 2008; Michel 2010; Zeug and Cavallo 2013). As a domesticated stock, hatchery fish may have attenuated responses to environmental cues that could obscure a flow effect.

The spatial analysis of last known detections suggested that there may be habitat characteristics that affected survival but were not easily quantified in the current study. Sub-reach 2b was identified in the hot spot analysis of last known detections and fish released in this reach often experienced poor survival. This suggests that Sub-reach 2b contains habitat features that increase mortality (e.g. predator holding areas, lack of shallow shoal areas) or poor abiotic conditions that were not quantified here and may be discrete events that are not easily captured

without continuous monitoring (e.g. periodic low dissolved oxygen events). Survival was not always poor in this reach and although there is habitat for predators, there may be variation in the frequency and abundance of predators in that habitat; in particular, migratory species such as striped bass that move into freshwater rivers to spawn as juvenile Chinook salmon are moving toward the ocean. Observed locations of last known detections in Reach 3 were significantly deeper than expected. Deep habitats in lotic systems have been shown to be preferred by predatory fishes (Gelwick *et al.* 1997). This suggests that there is an abundance of habitat that is favorable for predators of juvenile Chinook salmon in Reach 3. Additionally, the location of last known detection may not correspond to the exact location where mortality occurred because mobile surveys only occur at discrete intervals. Multidimensional tracking would be required to effectively estimate mortality locations at a finer scale.

The use of both mobile and stationary detections in this study revealed several biases that occur in telemetry studies but are not often quantified. These biases need to be considered carefully when interpreting estimates of survival and travel time and assessing what they mean to the wild Chinook salmon population in the Stanislaus River. First, it was clear during certain releases that significant numbers of tagged fish held in the study reach and some did not initiate migration before tag life expired. These individuals appear as mortalities when estimating survival using stationary receiver detections and Cormack Jolly-Seber statistical models. Thus, although the survival estimates from stationary receiver detections are statistically precise, they are unlikely to be accurate because they would underestimate the true survival values. Holding behavior also influenced travel time estimates with significant differences between estimates produced using stationary and mobile detections. Travel time estimated from stationary detections are accurate and precise but are representative only of actively migrating fish. Travel times calculated with mobile data are likely biased toward fish that hold in the study reach. Fish that move quickly out of the study reach are likely to be missed by mobile surveys that only occur every few days. Additionally, Chinook salmon juveniles tend to migrate between dusk and dawn and even mobile surveys on consecutive days would be likely to miss many fish (Michel *et al.* 2013).

The laboratory tag effects study found that tag retention was nearly 100% suggesting that it would be unlikely that fish would be incorrectly categorized as mortalities due to tag loss. Additionally, survival and growth was not significantly different between experimental and control fish, indicating the presence of an implanted tag did not directly affect survival. However, there were significant differences in the position of fish in the water column in some treatments and significant differences in body orientation for all experimental groups. It is unknown how these differences may influence survival; however, Adams *et al.* (1998) reported that juvenile Chinook salmon that had radio tags surgically implanted were significantly more susceptible to predation relative to untagged controls. The changes in water column position and body orientation observed here may be linked to predation susceptibility although we did not directly link these in our study. Regardless, previous work on mortality of tagged fish suggests that survival may generally be underestimated in these telemetry studies due to tag effects; however, the estimates were precise and comparisons of survival among treatments and modeling of environmental effects is appropriate.

The use of hatchery fish as surrogates for the wild population and the use of different hatchery stocks (Merced River vs. Mokelumne River) is also an important consideration. In a review of relative fitness of hatchery and natural salmon, Berejikian and Ford (2006) found that the relative

fitness of hatchery fish was reduced under many scenarios. It is unknown to what extent behavioral differences may occur in the hatchery stocks used here. This suggests that caution should be used when interpreting the magnitude of survival for hatchery fish in relation to the wild population. In the first two years of the study, fish from the Merced River hatchery were used whereas in the final year, fish from the Mokelumne River hatchery were used. Survival estimates from mobile detections were higher in the final year than the first two years but how much of this effect can be attributed to the stock used is unknown.

Despite the limitations inherent in the use of telemetry, we found strong evidence that survival is not homogeneous through the study reach. The  $\approx 8$ km Sub-reach 2b was found to have especially poor survival and restoration activities in this reach have the potential to benefit migration conditions for juvenile Chinook salmon. Additionally, spatial analyses of last known detections suggest that mortality occurs in habitats that are significantly deeper than expected in Reach 3 and the lower portions of Reach 2. There was little evidence that survival was significantly different between early and late releases; however, during some releases tagged fish held in the river until tag life expired. This suggests that some fish were not physiologically ready to outmigrate; yet the survival analysis using mobile detections indicated survival was not significantly different for these releases. Flow did not have a significant effect on survival; however, because some fish exhibited holding behavior, the stationary detection models were biased toward actively migrating fish. The mobile detection models suggested that there was a greater probability of fish transitioning out of the study reach when discharge was higher, which is supported by previous studies in this reach (Zeug *et al.* 2014). Future studies in the LSR would benefit by focusing on reaches identified in this study to contain a significant clumping of last known detections. Experimental manipulation of environmental drivers and habitat structure would be particularly useful because noise in the data resulting from behavioral differences between hatchery and natural fish and the difficulty in identifying exact locations of mortality in larger study reaches.



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