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RESEARCH

Survival, Tag Retention, Growth, and Wound Healing of Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) Surgically Implanted with a Dummy Acoustic Tag

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ABSTRACT

Tag effect studies are paramount in interpreting the results of survival studies. The objective of this study was to analyze the influence of tag implantation and tag burden on the survival, tag retention, growth, and wound healing of juvenile Chinook Salmon 7.8 ± 0.9 g initial weight. Fish were obtained from the Merced River Hatchery, held for 7 d, and then surgically implanted with Juvenile Salmon Acoustic Telemetry System (JSATS) SS300 dummy tags (0.3 g in air). Tag burden ranged from 2.9–4.8% (3.86 ± 0.43%, mean ± standard deviation). Weight and fork length were taken immediately before tag implantation. All fish (i.e., control and dummy-tagged) were also implanted with a visible implant alpha tag next to the dorsal insertion. Control and dummy-tagged fish were held in a single tank for 30 d. Any fish that died during the 30-d period were noted. At the end of the holding period, all fish were euthanized, weighed, measured, and necropsied. All dummy-tagged fish retained their dummy tag, and survival rates between the two groups were similar. Wound healing was also similar across the range of tag burdens analyzed. Specific growth rates, however, differed significantly between the two groups, with control fish growing at a rate of 1.08 ± 0.38% d^{-1} compared to 0.55 ± 0.48 % d^{-1} in dummy-tagged fish ($P<0.001$). Tag burdens and specific growth rates for dummy-tagged fish ($P = 0.961$) did not correlate, nor did initial weight and specific growth rate for control ($P = 0.363$) or dummy-tagged ($P = 0.983$) fish. The cause of the decreased growth rate in dummy-tagged fish remains unknown. Determining the cause of decreased growth in tagged juvenile Chinook Salmon, and how that decreased growth may influence survival in the wild, should be investigated further.

KEY WORDS

Chinook Salmon, *Oncorhynchus tshawytscha*, acoustic tagging, tag effect, tag burden

INTRODUCTION

The Sacramento–San Joaquin River Delta produces the majority of Chinook Salmon (*Oncorhynchus tshawytscha*) caught in California's ocean fisheries (Kano 2006). Nine evolutionarily significant units of Chinook Salmon are currently listed under the Endangered Species Act. Various habitat-restoration efforts are being implemented to improve survival during juvenile migration, with varying degrees of

success (Williams 2006). Survival during the juvenile stage has been suggested to be the most critical time for the species' recovery (Kareiva et al. 2000; Zabel et al. 2006). Juvenile migration routes and survival rates are commonly tracked using surgically- implanted acoustic transmitters and an array of acoustic receivers (Welch et al. 2008; Perry et al. 2010; Harnish et al. 2012; Michel et al. 2015; Buchanan et al. 2016; Deng et al. 2017).

Juvenile salmon survival in the Delta has been estimated for several years using acoustic telemetry, and, like most other industries, the technology has improved over time. Specifically, manufacturers are now able to design tags that are smaller than ever before, allowing smaller fish to be tracked and tagged (McMichael et al. 2010; Crossin et al. 2017). One potential challenge associated with surgically-implanted tags is the risk of tag expulsion, where either the tag is lost through the incision or through the body wall (Welch et al. 2007). Study fish that are released and then expel their tag may introduce bias to survival estimates because their lack of movement may cause them to be falsely classified as mortalities. Additionally, any inferences made to the population of interest may be invalid if significant tagging effects exist, such as reduced swimming performance, growth rate, or foraging capability (Bridger and Booth 2003; Brown et al. 2011; Cooke et al. 2011; Liedtke and Wargo–Rub 2012; Cooke et al. 2013). These problems have been remedied in other studies, in part by limiting the tag burden (i.e., the ratio of tag weight in air-to-body weight; Brown et al. 2010; Ammann et al. 2013), since small tags and low burden rates are less likely to cause tag effects (Barnard and Brandes, unpublished data, see "Notes"; Brown et al. 2010).

Although it is widely accepted that a high tag burden influences fish survival, growth, and tag retention, the wide variety of results in the current literature indicate that these negative effects may be compounded by other characteristics (e.g., species, smoltification, surgical technique, and water temperature). For instance, tag implantation has been shown to have no influence on mortality in yearling Chinook Salmon at tag burdens less than 6.7% (initial fork length [FL] 80–109 mm; Brown et al. 2010), from 2.6% to 5.6% (initial FL 138–190 mm; Ammann et al. 2013), and from 6.1% to 9.3% (initial FL 68–85 mm; Barnard and Brandes, unpublished data, see "Notes"). Similarly, Steelhead (*Oncorhynchus mykiss*) 180–225 mm FL have shown no differences in survival with a 1.3% to 4.4% tag burden (Sandstrom et al. 2013). Alternatively, higher mortality rates have been reported in Chinook Salmon 93–116 mm FL (24% mortality at a 5.6% mean tag burden; Brown et al. 2006), Steelhead 120–130 mm FL (33% mortality at a 6.5% mean tag burden; Welch et al. 2007), and Atlantic Salmon (*Salmo salar*) 136–155 mm FL (60%, 40%, and 80% mortality at 8.5%, 9.9%, and 10.1% tag burdens, respectively; Lacroix et al. 2004).

The influence of tag burden on growth has also varied in previous studies. Tag burdens up to 8.2% (Brown et al. 2010) and 5.6% (Ammann et al. 2013) have been reported as resulting in no significant influence on growth rate in Chinook Salmon. However, Frost et al. (2010) reported a significant growth reduction in Chinook Salmon 124 ± 6.4 mm FL (mean ± standard deviation) at a tag burden of 2.6% to 5.9%. Lacroix et al. (2004) found similar results in Atlantic Salmon 136–155 mm FL, although these fish exhibited compensatory growth, and were no longer significantly different from controls beginning 7 months after surgery.

Tag retention is a vital area of concern in survival studies. A study of tag retention rates in juvenile Chinook Salmon found an increase in tag expulsion beginning at a 7% tag burden (initial FL 68–85 mm; Barnard and Brandes, unpublished data, see "Notes"), whereas Brown et al. (2010) reported some degree of tag expulsion in this species at all tag burdens analyzed (4.5% to 15.7%; initial FL 80–109 mm). Other studies have suggested tag burden to be negligible or nonexistent (with regard to tag expulsion) in Chinook Salmon at tag burdens up to 5.6% (initial FL 138–190 mm; Ammann et al. 2013) and 11.5% (initial FL 142–169 mm; Rechisky and Welch 2010).

With the increased ability to tag and track smaller fish with newer, smaller tags, tag effect studies on small fish are paramount in assessing the validity of survival estimates of wild populations (Bridger and Booth 2003; Brown et al. 2011; Cooke et al. 2011; Liedtke and Wargo–Rub 2012; Cooke et al. 2013). Our objective in this study was to assess the influence of tag burden/implantation on growth, survival, tag

retention, and wound healing in juvenile Chinook Salmon.

METHODS

Study Fish

We obtained juvenile Chinook Salmon from a single spawning group from the Merced River Hatchery (California Department of Fish and Wildlife, Snelling, CA) on April 3 (n=400) and 4 (n=725), 2017. Fish were netted from the raceway and individually weighed; those above 7.0 g were transferred into a 300-gal (1,135-L) tank held in the bed of a pick-up truck and transported to the Tracy Fish Collection Facility (TFCF; U.S. Bureau of Reclamation, Tracy, CA) with supplemental oxygen. The size limit was imposed to limit tag burden to ≤5% while still allowing for some weight loss before tagging.

The trip took approximately 2 hours. Once at the TFCF, we distributed fish to four 770-L (1.22-m diameter, 0.66-m height of water) cylindrical indoor tanks that received cooled (13 °C), ozone-treated Delta water, one of which was used for the fish in the present study. TFCF staff cared for fish, so water temperature and flow rate were not recorded daily, but they angled inflow to produce a circular current to ensure fish were not sedentary. Photo-period was 11 h light/13 h dark. Fish were fed at a rate of 3.1% body weight d^{-1} starting the day after transport. Fish were fasted the day immediately before being tagged on April 10.

Tagging

We dummy-tagged fish (i.e., no active components) with Juvenile Salmon Acoustic Telemetry System (JSATS) tags (SS300 Acoustic Transmitter, Advanced Telemetry Systems, Inc., Isanti, MN). Dummy tags were individually weighed to the nearest milligram; and length, width, and height were measured to the nearest millimeter: tag length was 10 ± 1 mm (mean ± standard deviation), width was 5 ± 0 mm, height was 3 ± 0 mm, and weight was 307 ± 4 mg. All tags were labeled with a 4-digit alpha-numeric code and were visually indistinguishable from active SS300 tags. In addition to the dummy acoustic tags, all fish (i.e., dummy-tagged and control) were given a visible implant alpha (VIA) tag (Northwest Marine Technology, Inc., Shaw Island, WA) next to the dorsal insertion, allowing for individual identification (and therefore growth calculations) of control fish and fish that might have expelled the dummy tag. The VIA tags were 1.2 mm in width, 2.7 mm in length, and were printed with a 3-digit alpha-numeric code. Although little information exists on how VIA tags influence growth in Chinook Salmon, they have been shown not to impede growth in Brook Trout (*Salvelinus fontinalis*) 130–160 mm total length in a stream (Bryan and Ney 1994). Nevertheless, we will refer to control fish as "reference" fish to reflect the presence of the VIA tag.

We dummy-tagged 51 juvenile Chinook Salmon (three surgeons, 17 fish/surgeon) and held them with 50 reference fish in a 500-L cylindrical indoor tank that received recirculated, cooled (13°C), treated Delta water for 30 d. We based the methods for our study on the standard operating procedure presented by Liedtke et al. (2012). We disinfected acoustic tags and surgical instruments for a minimum of 20 min in 3% chlorhexidine solution (Nolvasan® Solution, Pfizer Inc., New York, NY) and thoroughly rinsed them with distilled water before use. The VIA tag injectors (Northwest Marine Technology, Inc., Shaw Island, WA) were not allowed to have the full suggested 20-min contact time with the 3% chlorhexidine solution because of an injector shortage. Instead, they were soaked in the disinfectant between uses (approximately 2 min), rinsed, and reused.

To begin the tag insertion process, we anesthetized fish in 70 mg L^{-1} MS-222 (Pentair Aquatic Ecosystems, Inc., Cary, NC) buffered with 70 mg L^{-1} sodium bicarbonate (Arm & Hammer Baking Soda, Church & Dwight Co., Inc., Princeton, NJ). Dummy-tagged fish were required to reach a deeper stage of anesthesia than reference fish, and therefore remained in the anesthesia bath for a longer period of time. We did not record anesthesia times. In accordance with the standard operating procedure (Liedtke et al. 2012), we rejected fish that reached deep anesthesia in less than 1 minute and fish that did not reach a sufficiently deep anesthesia level within 5 minutes.

Once anesthetized, we weighed fish to the nearest 0.1 g, measured (FL) them to the nearest mm, and assessed the level of descaling on the most compromised side of the body. We rejected fish if

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they displayed abnormalities such as bulging eyes, ≥20% de-scaling on either side, non-normal color, gross anatomical deformations, damaged opercula with exposed gill filaments, gross scarring, bleeding scratches, gross signs of disease, any fungal infection, any fin hemorrhaging, or if they weighed <6.0 g.

After taking weight and length measurements, we implanted VIA tags into fish that were to be dummy-tagged. We inserted the needle (with a loaded tag) just below the skin next to the dorsal insertion, advanced the needle shim to expel the tag, and then withdrew the needle. We then placed the fish onto the surgical platform with the ventral surface facing up, made a 5-mm incision with a Sharpoint® microsurgical knife (15-degree, straight-stab, 3-mm blade) anterior to the pelvic girdle next to the mid-line, inserted the tag, and closed the incision with two interrupted stitches (Ethicon, Inc., 5-0 Coated Vicryl Plus suture with an RB-1 needle). The tag was inserted with the transducer pointing anteriorly; the orientation of the tag within the body was consistent for all fish. At the end of the surgery, we placed fish in a 19-L recovery bucket that contained 10 L of water super-saturated to approximately 150% oxygen, with no more than three fish per bucket. Recovery buckets contained water super-saturated with oxygen to help with oxygen uptake after oxygen depletion during anesthesia (Liedtke et al. 2012). We recorded air time (i.e., the time elapsed between removing the fish from anesthesia and placing it in the recovery bucket) for every fish. After a 10-min recovery period, we poured the fish into the holding tank, where they remained for the duration of the holding period (30 d). The water temperature (13°C) during the holding period was identical to that in the tank before tag implantation. Feeding resumed 24 h after tag implantation at a rate of 3.1% body weight d^{-1}.

We anesthetized reference fish in the same concentration of anesthetic and buffer as the dummy-tagged fish, but removed them as soon as they could be handled, so they had a shorter exposure period. Once anesthetized, we weighed, measured, and assessed each fish for scale loss, and then implanted a VIA tag next to the dorsal insertion. We also rejected reference fish if they weighed <6.0 g or displayed any of the anatomical abnormalities that constituted rejection in dummy-tagged fish. A fourth tagger, who did not perform any of the VIA tag implants on dummy-tagged fish, inserted the VIA tags for reference fish. Reference fish were treated the same as the dummy-tagged fish after VIA tagging. We also recorded air time for reference fish. A sham surgery group was not included in the present study because previous studies have found no differences between reference and sham surgery groups, so we deemed it unnecessary (Lacroix et al. 2004; Zale et al. 2005; Brown et al. 2006; Chittenden et al. 2009; Frost et al. 2010; Ammann et al. 2013).

Necropsy Assessments

At the end of the 30-d holding period, we euthanized all fish in 200-mg-L^{-1} MS-222 buffered with 200-mg-L^{-1} sodium bicarbonate. We then weighed, measured, and necropsied the fish. We gave scores for external characteristics (i.e., body color, fin hemorrhaging, eyes, and gill color) to each fish based on the scoring rubric presented in Table 1. We scored dummy-tagged fish on a number of internal necropsy parameters (Table 2). We gave scores of 0 (absent) or 1 (present) to organ damage and fungus presence. We scored (from 0–2 based on severity) signs of tag expulsion, status of sutures (anterior and posterior sutures scored separately), incision apposition, and peritoneal apposition. We then summed these scores to produce a composite score ranging from 0–12, which we used to assess overall wound healing in dummy-tagged fish after surgical tag implantation. Two of the three surgeons (termed "assessors") performed necropsies without their knowing who tagged each fish.

Calculation of Growth Rates and Tag Burden

Specific growth rate (SGR) was defined as

$$Specific\ Growth\ Rate\ (\%\ d^{-1}) = \frac{\ln(Weight_f) - \ln(Weight_i)}{30\,d} \times 100\%$$

where $Weight_i$ is the Day 0 weight and $Weight_f$ is the Day 30 weight (Hopkins 1992).

Tag burden was defined as

$$Tag\ Burden\ (\%) = \frac{TW}{Weight_i} \times 100\%$$

where TW is the tag weight in air (Brown et al. 2010; Ammann et al. 2013).

Table 1 Reference and dummy-tagged fish were scored on external characteristics at the end of the 30-d holding period. Body color, fin hemorrhaging, eyes, and gill color were either given scores of 0 ("Normal") or 1 ("Abnormal"). Percent scale loss on the most compromised side of the fish was also recorded. T. Liedtke, USGS, provided parameters.

Characteristic	Normal	Abnormal
Body color	High-contrast dark dorsal surfaces and light sides	Low contrast dorsal surfaces and coppery-colored sides
Fin hemorrhaging	No bleeding at base of fins	Blood present at base of fins
Eyes	Normally shaped	Bulging or with hemorrhaging
Gill color	Dark-beet-red- to cherry-red-colored gill filaments	Grey- to light-red-colored gill filaments
Vigor	Active swimming (before anesthesia)	Lethargic or motionless (before anesthesia)

Table 2 Necropsy parameters were scored on dummy-tagged fish after the 30-d holding period. Scores from each of the parameters were added together to produce a composite score (0–12) for each fish. Anterior and posterior sutures were scored separately, with each score contributing to the composite score. T. Liedtke, USGS, provided parameters and scoring systems.

Composite score parameter	Score	Score definition
Signs of tag expulsion	0	No signs of tag expulsion (i.e., no signs that the tag is being forced out through the incision or the lateral body wall). Simple encapsulation may be present.
	1	Some bulging or lateral pressure (i.e., some evidence that the tag is causing some pressure on the incision or the lateral body wall)
	2	Expulsion process is obvious or complete (i.e., the tag is obviously being forced out through the incision or the lateral body wall, or the tag is already out)
Suture present?	0	Yes
	1	Yes, but untied or becoming untied
	2	No
Incision apposition	0	Completely closed, perfect apposition
	1	Incision partially open resulting from gape or overlap
	2	Incision completely open (>75%)
Peritoneal apposition	0	Peritoneum completely closed, perfect apposition
	1	Peritoneum partially closed
	2	Peritoneum completely open (>75%)
Organ damage	0	No organ damage present (i.e., no signs of damage either from the surgery or the presence of the tag). Tags can be adhered to organs as part of the encapsulation process, but that does not constitute damage.
	1	Some organ damage present (i.e., the suture captures, punctures, or entangles, the pyloric caeca, stomach, spleen, or intestine).
Fungus present?	0	No fungus present
	1	Fungus present
Fungus location	Suture	Fungus on the suture material
	Incision	Fungus on the skin in/around the incision
	Tail	Fungus on the skin on the tail
	Body	Fungus on the skin on the body

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Statistical Methods

We analyzed differences in SGR between dummy-tagged and reference fish using a t-test. We analyzed the mortality difference between these two groups using a z-test. The differences in air time, initial weight, and final weight between the dummy and reference fish failed the Shapiro–Wilk normality test, so we analyzed these differences with a Mann–Whitney rank sum test.

We analyzed differences in initial weight, initial FL, SGR, and tag burden among the three surgeons using a one-way ANOVA and, if necessary, a Tukey Honest Significant Difference (HSD) test. The difference in air time among the three surgeons failed the test for equal variance, and we therefore analyzed it using the Kruskal–Wallis one-way ANOVA on ranks. All scoring results for necropsy parameters (i.e., fungus presence, organ damage, body color, signs of tag expulsion, gill color, eyes, fin hemorrhaging, anterior and posterior sutures, incision apposition, peritoneal apposition, and composite score) failed the Shapiro–Wilk normality test, and therefore we also analyzed them using a Kruskal–Wallis one-way ANOVA on ranks.

The scoring results for all necropsy parameters between the two assessors failed the Shapiro–Wilk normality test, and were therefore assessed using a Mann–Whitney rank sum test. We evaluated the influence of tag burden on SGR for dummy-tagged fish using a linear regression. Larger fish may possibly have a slower growth rate (Jobling 1983), so we analyzed the influence of the Day 0 weight on SGR of the reference and dummy-tagged groups with a linear regression, as well. Fish might possibly be negatively affected by the amount of time spent out of the water (i.e. air time) they experienced during tagging (Ferguson and Tufts 1992; Cooke and Suski 2005; Thompson et al. 2008). To assess this possibility, we also analyzed the influence of air time on SGR for both reference and dummy-tagged fish with linear regressions.

We performed all statistical tests in SigmaPlot (Version 12.3). Significance in all tests was accepted if $P<0.05$.

RESULTS

Tag Effect on Survival, Tag Retention, and Growth

Two of 51 (3.9%) dummy-tagged fish died during the 30-d holding period, and were discarded before morphometric data could be recorded or a necropsy could be performed. One of these was found severely decomposed on the floor of the tank; it is unclear when or why this fish died. The other mortality was never found, and may have gotten caught in the drain. All remaining fish were swimming vigorously at the time of necropsy. Zero of 50 reference fish died during the holding period; this difference in survival was not significant (Table A-1). Dummy-tagged fish had a tag burden of 3.86% ± 0.43% (mean ± standard deviation; Table 3) and exhibited 100% tag retention.

Over the course of the 30-d holding period, FL increased from 85 ± 3 mm at Day 0 to 93 ± 5 mm at Day 30 for dummy-tagged fish; reference fish

Table 3 Results of external measurements and calculations taken during tagging and necropsies. Values are presented as mean (standard deviation). Different letters between the compared groups in a single column indicate a significant difference ($P < 0.05$).

Treatment group	Sample size	Weight (g)		Tag burden (%)	SGR (% d-1)	Fork length (mm)		Air time (mm:ss)	Scale loss (%)
		Day 0	Day 30			Day 0	Day 30		
Reference	50	7.7 (1.0)	10.6 (1.7)a	---	1.08 (0.38)a	85 (4)	96 (5)	00:40 (00:13)a	21 (12)
Pooled Dummy	51	7.8 (0.9)	9.3 (1.7)b	3.86 (0.43)	0.55 (0.48)b	85 (3)	93 (5)	02:36 (00:23)b	22 (10)
Surgeon A	17	7.94 (0.84)	8.80 (1.34)	3.82 (0.41)	0.36 (0.39)	86 (4)	92 (5)	02:48 (00:14)a	21 (8)
Surgeon B	17	7.99 (1.11)	9.69 (2.10)	3.82 (0.50)	0.60 (0.56)	85 (4)	93 (6)	02:40 (00:27)a	19 (10)
Surgeon C	17	7.66 (0.70)	9.46 (1.68)	3.95 (0.38)	0.67 (0.44)	85 (4)	93 (5)	02:19 (00:13)b	26 (10)

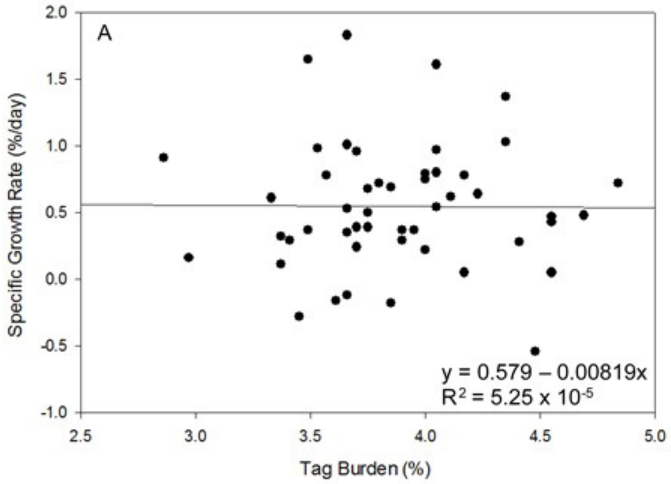

Figure 1 Relationship between tag burden and specific growth rate of dummy-tagged fish. A linear regression analysis found no significant relationship ($P = 0.961$).

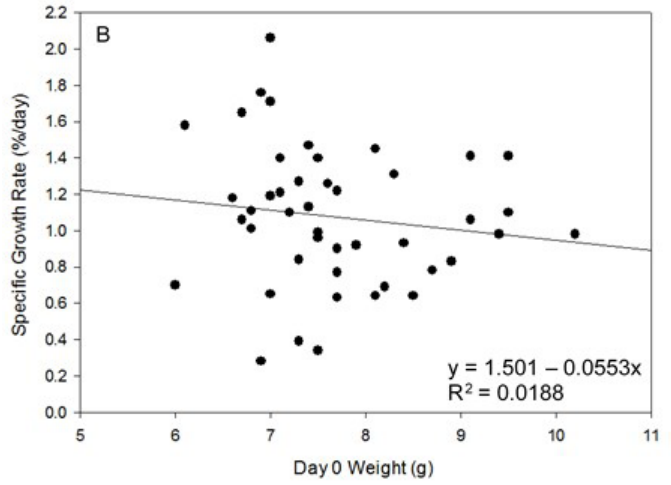

Figure 2 Relationship between Day 0 weight and specific growth rate of reference fish. A linear regression analysis found no significant relationship ($P = 0.363$).

increased from 85 ± 4 mm at Day 0 to 96 ± 5 mm at Day 30 (Table 3). The initial weight of fish in the two groups did not significantly differ, but the final weight did (Table A-1). The weight of dummy-tagged fish increased during this time from 7.9 ± 0.9 g to 9.8 ± 1.8 g, but reference fish exhibited a greater weight increase: 7.7 ± 0.9 g on Day 0 to 10.6 ± 1.6 g on Day 30 (Table 3). This weight increase translates to significantly different SGRs of 0.55% ± 0.48% d^{-1} and 1.08% ± 0.38% d^{-1} for dummy-tagged and

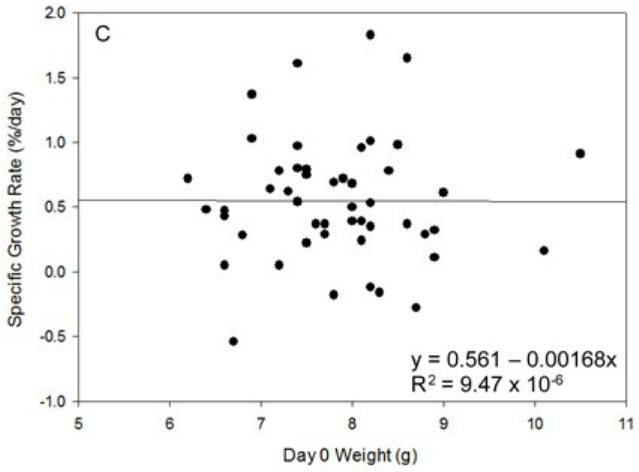

Figure 3 Relationship between Day 0 weight and specific growth rate of dummy-tagged fish. A linear regression analysis found no significant relationship ($P = 0.983$).

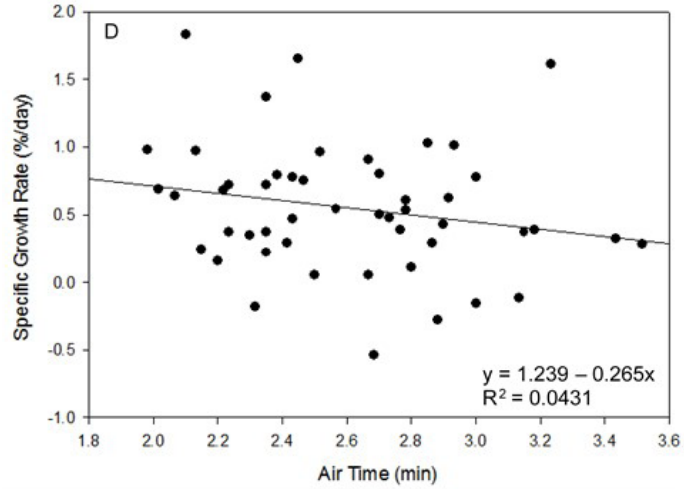

Figure 4 Relationship between air time (i.e., the time elapsed between removing the fish from anesthesia and placing it in the recovery bucket) and specific growth rate of dummy-tagged fish. A linear regression analysis found no significant relationship ($P = 0.152$).

reference fish, respectively (Table 3; Table A-1). There was no correlation between tag burden and SGR of the dummy-tagged fish (Figure 1; Table A-2), between initial weight and SGR of reference fish (Figure 2; Table A-2), between initial weight and SGR of dummy-tagged fish (Figure 3; Table A-2), between air time and SGR of dummy-tagged fish (Figure 4; Table A-2), or between air time and SGR of reference

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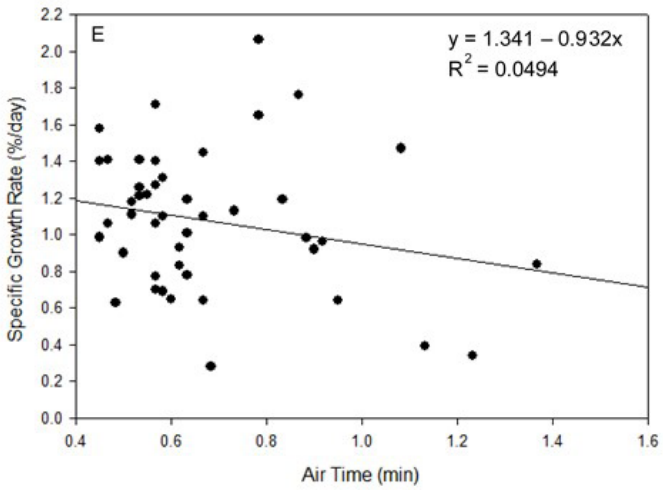

Figure 5 Relationship between air time and specific growth rate of reference fish. A linear regression analysis found no significant relationship (P = 0.137).

fish (Figure 5; Table A-2). Additionally, there was no relationship between air time and SGR when control and dummy-tagged fish were combined into one group ($R^2 = 0.0431$, $P = 0.152$; Table A-2).

Wound Healing

No dummy-tagged fish exhibited poor body color, poor gill color, or bulging eyes, but three displayed some mild fin hemorrhaging. In addition, 81% of dummy-tagged fish developed fungus, all of which was on the suture material (Table 4). All dummy-tagged fish retained their sutures throughout the holding period, but one fish had both anterior and posterior sutures that were coming untied; this fish had been tagged by Surgeon B. No reference fish displayed any signs of poor body color, pale gills, fin hemorrhaging, fungus, or bulging eyes (Table 4).

Surgeon Effect

There were no significant differences among the three surgeons in Day 0 weight, Day 0 FL, tag burden (Table 3), Day 30 survival, tag retention, SGR, or any of the parameters assessed during necropsies (Table 4; Table 5; Table A-3). However, air time did differ significantly among the surgeons (Table A-3; Table A-4). Surgeon A displayed an air time of 02:48 ± 00:14 (mm:ss); Surgeon C had a significantly lower air time of 02:19 ± 00:13 (Table 3). Surgeon B's air time (02:40 ± 00:27) was also significantly higher than that of Surgeon C. The air times of Surgeons A and B did not differ.

Assessor Effect

Two of the three surgeons performed necropsies and scored fish, leading to potential differences in the scoring. The two assessors differed significantly in their scores for fin hemorrhaging; their scores for all other parameters assessed were similar (Table 3; Table 4; Table A-5). Composite scores assigned by the two assessors were 1.17 ± 0.62 and 0.97 ± 0.68.

DISCUSSION

There was no significant difference in mortality between reference and dummy-tagged fish at the tag burdens analyzed (2.9% to 4.8%). This follows with the findings of Brown et al. (2010), which reported no effect on survival of juvenile Chinook Salmon 80–109 mm until 6.7% tag burden, as well as the findings of Ammann et al. (2013), who reported no change in survival at tag burdens up to 5.6% in salmon 139–190 mm. Chittenden et al. (2009) reported only a limited decrease in survival at a tag burden of 7% in Coho Salmon (*Oncorhynchus kisutch*) of 9.5–10 cm and 12–12.5 cm. When kept in seawater, Chinook Salmon 110–170 mm FL were shown to exhibit an 11.5% mortality rate when tag burden was < 5.8%, compared to a 100% mortality at higher tag burdens (Hall et al. 2009). Other authors have reported higher mortality rates than the ones we found:

- 8.2% mortality in Steelhead at a mean tag burden of only 3.9% (Welch et al. 2007)
- 24% mortality in Chinook Salmon at a mean tag burden of 5.6% (Brown et al. 2006)
- 40% mortality in Atlantic Salmon at a mean tag burden of 9.5% (Lacroix et al. 2004)

However, these studies were likely influenced by low sample sizes (Lacroix et al. 2004; Welch et al. 2007), longer durations (Welch et al. 2007), and poor fish health (Brown et al. 2006). JSATS tags at higher tag burdens than we used here may possibly affect survival. Two fish in the dummy-tagged group died during the 30-d holding period, but this proportion

Table 4 Proportion of fish in each group scoring 1 (i.e., a score of "Abnormal") in the external characteristics assessed during necropsies after the 30-d holding period. Different letters between the two compared groups in a single column indicate a significant difference ($P<0.05$).

Treatment Group	Body Color	Gill Color	Eyes	Fin Hemorrhaging	Fungus Presence
Reference	0.00	0.00	0.00	0.00	0.00 [a]
Pooled Dummy	0.00	0.00	0.00	0.06	0.81 [b]
Surgeon A	0.00	0.00	0.00	0.13	0.81
Surgeon B	0.00	0.00	0.00	0.06	0.88
Surgeon C	0.00	0.00	0.00	0.00	0.75

Table 5 Scores of wound healing assessment at the end of the 30-d holding period. Values are presented as mean (standard deviation). Parameter definitions and scoring criteria are defined in Table 2.

Treatment Group	Signs of Tag Expulsion	Suture		Incision Apposition	Peritoneal Apposition	Organ Damage	Fungus Presence	Composite Score
		Anterior	Posterior					
Surgeon A	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.13 (0.34)	0.07 (0.26)	0.19 (0.40)	0.81 (0.40)	1.19 (0.66)
Surgeon B	0.00 (0.00)	0.06 (0.24)	0.06 (0.24)	0.06 (0.24)	0.00 (0.00)	0.12 (0.33)	0.88 (0.33)	1.18 (0.73)
Surgeon C	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.06 (0.24)	0.00 (0.00)	0.00 (0.00)	0.75 (0.45)	0.81 (0.54)

was not large enough to result in a significant difference. Larger sample sizes may reveal effects on survival.

We also found no occurrences of tag expulsion at the tag burdens studied, nor were there any signs of tag expulsion at the end of the holding period. This contrasts with the findings of Brown et al. (2010); they reported tag expulsion in Chinook Salmon in all size classes studied, including tag burdens smaller (i.e., less likely to expel) than those we tested here. This discrepancy is likely not the result of different initial sizes because those authors presented the smallest size groups (i.e., most likely to expel the tag) as 80–89 mm FL, which were similar to those presented here at the time of tagging (85.2 ± 3.5 mm FL, mean ± standard deviation). However, fish in the Brown et al. study were kept in higher water temperatures (17–18.5 °C) than we used, which has been shown to increase tag expulsion rates (Deters et al. 2010). Other authors have reported greater tag expulsion than our results, but only at higher tag burdens (7%: Barnard and Brandes, unpublished data, see "Notes"; 8%: Lacroix et al. 2004). In cases of tag burden similar to our study, a difference in surgical technique, water temperature, smoltification state, or tag shape may have resulted in different tag retention rates.

Reference and dummy-tagged fish did not differ in their health characteristics at the end of the 30-d period by any parameter we assessed, except for fungus presence. The sutures of 81% of dummy-tagged fish showed some fungus growth. However, it is unlikely this contributed to the depressed growth rate observed in this group (2018 email from T.L. Liedtke, USGS, to K.M. Towne, unreferenced, see "Notes"). Although the severity of the fungus growth was not scored, dummy-tagged fish with no fungus present ($n=9$) exhibited a SGR of 0.56% ± 0.70% d^{-1} compared to a SGR of 0.54% ± 0.43% d^{-1} in those with fungus ($n=40$). All fish retained their sutures, and although we did not quantify healing, we noted no fish as having open wounds.

In our study, three surgeons implanted dummy tags into fish, potentially allowing for differences based

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on surgeon. Although air time differed significantly among surgeons, this did not translate to differences in survival, or in any of the parameters assessed during necropsies at the end of the holding period. Fish from Surgeon A exhibited higher incidences of incision apposition, peritoneal apposition, and organ damage, but these rates were not significantly different from those of the other two surgeons. Therefore, it is likely that the surgeon did not influence survival, tag retention, growth, or overall health of the dummy-tagged fish. Additionally, the presence of multiple people conducting necropsies allowed for potentially skewed scoring, based on assessor. Even though the scoring of one necropsy parameter—fin hemorrhaging—differed significantly based on assessor, that difference was not reflected in the composite scores, which are the scores of interest for overall fish health. These scores can, therefore, be reliably used to elucidate differences among surgeons or between reference and dummy-tagged groups.

The dummy-tagged fish in the present study exhibited a significantly lower mean SGR of 0.55% d^{-1} compared to a mean SGR of 1.08% d^{-1} in reference fish. This finding contrasts with those of Ammann et al. (2013) and Brown et al. (2010), who found no effect on growth rate for tag burdens up to 5.6% and 8.2%, respectively. Alternatively, Smircich and Kelly (2014) reported a decrease in SGR for Brook Trout with a 9.3% ± 1.1% tag burden for the first 14 days post-surgery. However, these fish exceeded the growth rate of control fish in the next 19 days (i.e., they exhibited compensatory growth), seemingly because they had either expelled the tag by this time or acclimated to the tag weight. Additionally, these authors found no difference in SGR for fish with a 4.7% ± 1.1% tag burden compared to controls, indicating that the heavier tag resulted in a decreased initial growth rate. Similar results were found in Atlantic Salmon, which exhibited a lower mean weight 4 to 6 months after surgery, but had caught up to control fish by 7 months after surgery (Lacroix et al. 2004).

Tag burden and SGR in dummy-tagged fish revealed no correlation. This is dissimilar to the response of adult Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*) 81.3–206.9 g, which displayed a significant correlation between tag burden and growth rate (g d^{-1}) (Zale et al. 2005). This difference may result from the disparity in size and maturity at the time of tagging. It is reasonable to believe that larger fish (i.e., those with a smaller tag burden) would have a different growth rate than smaller fish. However, linear regression analyses of the influence of initial weight of both dummy-tagged and reference fish on their SGR found no correlation between the two. Although, given the narrow size range of fish used in our study, the influence of tag burden on SGR may not have been apparent. Further study is necessary to elucidate this relationship.

Another possibility was that air time affected growth rate, but a linear regression analysis of air time and SGR in dummy-tagged fish revealed no correlation. A linear regression analysis of air time and SGR in reference fish resulted in a similar equation, poor correlation, and an insignificant relationship. Although we found no significant relationship between tag burden and SGR, the potential still exists, perhaps across a larger range of tag burdens. Another point of interest is the larger standard deviation of SGR between tagged (SD=0.48%) and reference (SD=0.38%) fish. Some tagged fish grew at a rate similar to reference fish, while others with a similar tag burden grew significantly less, or even lost weight. This indicates some other underlying cause of the decreased growth for some tagged fish. The decreased growth rate may have resulted from reduced feeding (Makiguchi and Kojima 2017), which could be caused by surgical wounds, the presence of the tag, reduced swimming performance, social interactions, or a combination of these factors. Surgical technique may also have influenced the growth rate of dummy-tagged fish; the fish tagged by Surgeon A had a lower SGR, poorer incision and peritoneal apposition, and a greater incidence of organ damage compared to fish tagged by the other two surgeons. However, none of these differences were statistically significant, so we cannot conclusively say poor surgical technique resulted in decreased growth rate.

The fish in this study were held at a water temperature of 13 °C for the duration of the holding period, which is lower than the fish would likely have encountered in the wild at this time of year. We used the lower temperature to minimize the risk of proliferative kidney disease, which has plagued fish from this hatchery in the past (Ferguson 1981; Foott et al. 2007; Bruneaux et al. 2017). However, this may have contributed to the differences in growth rates

we observed compared to other literature (Brown et al. 2010), because decreased temperatures reduce fish metabolism, and therefore fish growth. The lower temperature would have reduced growth in both reference and tagged fish, so it is unlikely this caused the growth difference. In addition, Deters et al. 2010 demonstrated that juvenile Chinook Salmon 95–121 mm FL held in cooler water temperatures (i.e., 12 °C) performed better in tag and suture retention, incision openness, inflammation, and ulceration than those held in warmer temperatures (i.e., 17 °C).

We did not record the amount of time spent in anesthesia, but it was likely significantly different between reference and dummy-tagged groups because of the deeper level of anesthesia required for surgery. Zebrafish (*Danio rerio*) have demonstrated a behavioral aversion to MS-222 by avoiding it (Readman et al. 2013), but it has been used as a fish anesthetic in countless studies with seemingly no lasting effects. In fact, MS-222 rose to such prominence as a fish anesthetic because it allows for a full recovery, including an unaffected growth rate (Readman et al. 2013). Exposure to MS-222 has been shown to increase cortisol levels in Rainbow Trout, which can subsequently block oxygen uptake, potentially leading to decreased growth (Iwama et al. 1989). However, Strange and Schreck (1978) demonstrated that short induction times and short overall exposure cause no change in plasma cortisol concentrations in Chinook Salmon compared to control fish. Nonetheless, a second reference group that was exposed to MS-222 for a similar length of time would have been beneficial in this study to determine if the decreased growth rate resulted from the increased anesthesia time or the surgery and the presence of the tag.

Our results indicate tag implantation can result in decreased growth rate in juvenile Chinook Salmon. Although this decreased growth rate did not influence survival over 30 d in the present lab study, we did not include several mortality factors these fish experience in the wild (e.g., predation). A poor understanding of the survival rate in the wild for tagged fish compared to untagged fish could easily lead to a flawed interpretation of results in survival studies. Results such as ours can be useful in determining variables used in survival models so tagged, released fish, and wild fish can be compared. Therefore, further investigation is important to understand the cause of decreased growth rate in tagged fish and how that decreased growth may influence survival in the wild, as well as to develop more robust survival models.

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