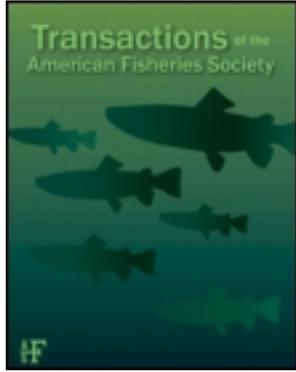


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Intracoelomic Acoustic Tagging of Juvenile Sockeye Salmon: Swimming Performance, Survival, and Postsurgical Wound Healing in Freshwater and during a Transition to Seawater

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ARTICLE

Intracoelomic Acoustic Tagging of Juvenile Sockeye Salmon: Swimming Performance, Survival, and Postsurgical Wound Healing in Freshwater and during a Transition to Seawater

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Abstract

Juvenile hatchery-reared Sockeye Salmon *Oncorhynchus nerka* from Cultus Lake, British Columbia, were implanted during their smolt phase with one of three sizes of dummy acoustic tags to assess how tag burden (tag mass: body mass ratios ranging from 1.3% to 13.6% in air) influenced prolonged swimming performance, survival, and postsurgical wound healing in freshwater for up to 16.5 d and following a transition to seawater for 9 d. Tagged fish were compared with surgical shams and control fish (no tag, no surgery). Fish subjected to sham surgery treatments had mean swim times similar to those of control fish; however, tagged fish had a significantly lower probability of swimming the mean time of nontagged control fish. In addition, we found that the effect of tagging on swimming performance was exacerbated by tag burden and that higher tag burdens decreased the swimming performance of tagged individuals. Fish with tag burdens $\geq 8\%$ had shorter swimming durations than fish with tag burdens $< 8\%$. The incisions of fish implanted with smaller tags healed more quickly than those of fish implanted with the largest tag. Overall, survival was high ($\geq 95\%$) and in freshwater mortalities only occurred in fish that had tag burdens greater than 6%. These findings have important implications for studies using tagging technologies to examine the behavior and survival of migrating salmon smolts.

Juvenile Pacific salmon undertake large-scale migrations from freshwater rearing sites to the North Pacific Ocean to feed. The down stream migration and the transition from freshwater to seawater are life history stages with considerable mortality

(Quinn 2005). Telemetry tracking using intracoelomically implanted acoustic and radio transmitters has been used in recent years to study juvenile salmonid movement and survival during these periods of migration (Chittenden et al. 2009a; Jepsen

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et al. 1998; Welch et al. 2009). Such studies have enhanced the capacity of researchers to understand movement and survival patterns in juvenile Pacific salmon. However, an underlying but often untested assumption with all tagging studies is that the tagging procedure or presence of the tag does not alter the behavior, physiology, health or survival of tagged fish relative to nontagged conspecifics (Cooke et al. 2011).

Most studies examining the effects of intracoelomic tagging on fish have focused largely on assessing the effects of tag size, generally mass, rather than examining the effects of tag burden (the ratio of tag mass in air to fish mass), which represents the mass in relation to the fish's own body (Cooke et al. 2011). Tag burden is an important consideration for assessing the effects of tagging because lower tag burdens may have little to no effect on fish while larger burdens may greatly alter fish behavior or survival. However, if and where these limits in tag burden lie remain undetermined for most species and situations. Historically, a general rule of thumb has been that tag burdens should not exceed 2% of a fish's body mass (Winter 1983). However, several studies have challenged this, and recommend that maximum burdens should be flexible for each species studied and take into account the study's objectives (Cooke et al. 2011; Jepsen et al. 2005). Some studies have suggested that burdens <2% could be a concern because they may have negative effects on fish growth and swimming performance (Zale et al. 2005). Other studies have suggested that tag burdens up to 12% have no negative effects on fish survival or swimming performance (Brown et al. 1999). However, most studies have not explicitly examined ranges of tag burdens.

In addition, there are several knowledge gaps in tagging effect studies that complicate our understanding of tagging effects. The majority of studies that have examined tagging effects failed to include a sham surgery treatment (i.e., surgery is performed but a tag is not implanted) in their study design, which is critical for assessing the effects of surgery relative to effects of the actual tag (Cooke et al. 2011). The most common metrics to assess potential effects of intracoelomic tagging in laboratory studies include mortality, growth, postsurgical wound healing, and tag retention. Less is known about intracoelomic tagging effects on swimming performance, predator avoidance, or physiological characteristics (Cooke et al. 2011). However, all of these factors influence survival of fish in the wild (Plaut 2001; Quinn 2005) and are of direct relevance to studies of tag effects. For example, despite the increasing number of juvenile salmonids that are tagged in freshwater and tracked as they migrate downriver to the ocean as smolts (Chittenden et al. 2009a; Rechisky et al. 2009; Welch et al. 2009), there are only two studies on juvenile salmonids that have examined how tagged fish cope in seawater (Hall et al. 2009), even though this transition is regarded as a stressful period of the life history that can significantly affect survival of fish (Folmar and Dickhoff 1980; Quinn 2005).

To address some of these knowledge gaps, we surgically implanted 255 hatchery-reared Sockeye Salmon *Oncorhynchus nerka* from Cultus Lake, British Columbia, with one of three

types of dummy acoustic tags, reflecting a range of some commercially available acoustic tags: 6 mm (diameter) weighing 1.1 g in air, 7 mm (1.6 g), or 9 mm (2.9 g). We assessed how control and sham surgery groups compared with tagged groups of different tag burdens (1.3% to 13.6% in air, 0.8–7.9% in water) in terms of prolonged swimming performance, survival, and postsurgical wound healing in freshwater and shortly after seawater transition. Cultus Lake Sockeye Salmon were chosen as the model organisms as this population has been the focus of recent telemetry studies (Welch et al. 2009) and is imperiled and thus of particular interest to managers. Furthermore, there is a general lack of knowledge on the implications of tagging procedures on Sockeye Salmon.

METHODS

The Cultus Lake population from the Fraser River watershed is listed as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2003) and a recovery program is in place where broodstock are annually caught as they return to Cultus Lake from the Pacific Ocean and subsequently spawned in captivity. The juveniles are raised for ≥ 1 year at the Department of Fisheries and Oceans Canada (DFO) Inch Creek Hatchery, British Columbia. In March 2010, juveniles used in this study were transported to the University of British Columbia, Forest Sciences Aquatic Laboratory, where they were held indoor in four large fiberglass tanks (0.5 × 4.87 m, 0.5 m deep) supplied with dechlorinated city water (7.8°C in April increasing to 11.7°C in June). Fish were fed to satiation with fish 2.0-mm pellets (Bio-Vita Fry from Bio-Oregon) three times a day from March to May 2010 and a range of fish sizes resulted: fork length (FL) = 100–204 mm, mass = 10.7–86.8 g (Table 1). In general, the fish were similar in length but had a higher condition factor than wild Cultus Lake smolts (80–136 mm and 5.40–24.54 g; Al Stobbart, Department of Fisheries and Oceans, personal communication). All protocols were approved by the University of British Columbia Animal Care Committee (protocol A08-0388).

Tagging treatments and surgical procedures.—Experiments were conducted from May to June 2010, which closely matched the period when wild Cultus Lake Sockeye Salmon migrate to sea (April through the end of May). Fish were assigned to one of five tagging treatments: (1) control group (received anesthetic but no surgery or tag implantation), (2) sham surgery (received surgery but no tag implantation), or implantation with (3) a 6-mm dummy tag, (4) a 7-mm dummy tag, or (5) a 9-mm dummy tag (Table 1). All dummy tags contained a passive integrated transponder (PIT) tags but no additional electronics and were manufactured to mimic the construction, weight, and size range of tags currently produced by VEMCO (www.vemco.com). The PIT tag in each dummy tag enabled the identification of individual fish. Tag burdens used to evaluate swimming performance and survival are based on tag weights in air, which is the commonly adopted practice.

TABLE 1. Weight and length data for Sockeye Salmon used to determine the effects of acoustic tag implantation on swimming performance, survival, and postsurgical wound healing.

Treatment	n	FL (mm)		Mass (g)	
		Mean \pm SD	Range	Mean \pm SD	Range
Control	94	139 \pm 15	111–200	25.8 \pm 9.9	11.7–73.3
Sham	94	139 \pm 12	120–190	25.6 \pm 8.4	14.0–64.2
Tag: 6 mm ^a	102	141 \pm 20	100–204	27.9 \pm 13.9	10.7–86.8
Tag: 7 mm ^b	106	142 \pm 18	100–199	28.0 \pm 13.2	10.7–79.9
Tag: 9 mm ^c	47	154 \pm 18	129–200	35.6 \pm 14.8	20.6–79.8

^aDiameter = 6 mm, length = 16.81 mm (SD = 1.39), mass = 1.15 g (SD = 0.02) in air or about 0.68 g in water, volume = 4.75 mm³.

^bDiameter = 7 mm, length = 19.52 mm (SD = 1.72), mass = 1.49 g (SD = 0.09) in air or about 0.74 g in water, volume = 7.51 mm³.

^cDiameter = 9 mm, length = 21.76 mm (SD = 0.47), mass = 2.95 g (SD = 0.18) in air or about 1.57 g in water, volume = 13.85 mm³.

Fish were assigned to groups (tagged, sham, or control) in two different processing events, one on May 25 (110 tagged) and May 26, 2010 (114 tagged) and the other on June 2 (107 tagged) and June 3, 2010 (112 tagged). The surgical procedure involved indiscriminately netting five to eight fish out of a large tank and placing them into a sedation bath of MS-222 at 10 mg/L of water for 5–7 min to calm individuals before further handling. The fork length of each fish was measured before assigning it to a group to ensure each treatment group would contain a similar range of fish sizes.

Fish were moved to an induction bath of MS-222 (100 mg/L of water buffered (with sodium bicarbonate) for 3–5 min to induce stage-4 anesthesia. Fish were placed supine in a small V-shaped trough and a small hose was positioned in the mouth to facilitate irrigation of gills with a maintenance dose of buffered anesthetic (MS-222 at 50 mg/L). To reduce visual stimuli a wet paper towel was draped over the head of the fish. Tags were implanted into the coelom of the fish (incision made with a number-12 curve blade along ventral linea alba of the fish) and incisions were closed with two simple interrupted sutures (4–0 nonabsorbable Ethicon Monocryl V with a PS-2, cutting-edge needle). Approximate incision lengths were 7 mm for the 6-mm tag, 8 mm for the 7-mm tag, and 10.5 mm for the 9-mm tag. Sutures were placed at one-third and two-thirds of the length of the incision, so that no suture was <2 mm from the end of the incision. The 9-mm tag was only implanted into fish \geq 129 mm because it was considered excessive for fish smaller than this. Sham surgery fish received the same surgical procedure but a tag was not inserted into the body cavity. Control fish did not undergo surgery, but were anesthetized and measured. Fish mass and fork length were recorded for all individuals before surgery and efforts were made to ensure fish in all treatments overlapped in size (Table 1). All water baths were aerated, and water temperatures were maintained between 8.6°C and 11.4°C. The complete surgical procedure took no longer than 3 min per fish.

Following handling, fish were placed indiscriminately into four tanks. Tanks 1 and 2 housed fish tagged on May 25–26 and tanks 3 and 4 housed fish tagged on June 2–3. Each tank was divided into two holding areas by a plastic mesh divider

that allowed water to pass through. Tagged fish were alternately placed into two different tanks and fish placement was as follows: one side of tanks 1 and 2 housed fish tagged May 25, the other side of tanks 1 and 2 housed fish tagged May 26; one side of tanks 3 and 4 housed fish tagged June 2, and the other side of tanks 3 and 4 housed fish tagged June 3. All tanks had the same dimensions and water temperature.

Swimming performance.—To compare the swimming abilities of tagged and nontagged control fish, we used flume experiments to estimate the mean swim time of fish in all five treatments. To evaluate swimming performance, fish were subjected to a prolonged swimming test at a high water velocity. Beamish (1978) defines three categories of swimming: sustained swimming can be maintained for >200 min, prolonged swimming is of shorter duration (20 s to 200 min), and burst swimming is maintained for short periods of time (<20 s). The ecological relevance of swimming has since been discussed (Plaut 2001). For this study we used prolonged swimming as a measure to compare fish, prolonged swimming being defined as swimming that could be sustained for \leq 1,200 s against a constant water velocity. While difficult to measure in the field, prolonged swimming can be measured in the laboratory using fatigue as a proxy (Beamish 1978) and is regarded as an integrative assessment of fish behavior, physiology and health.

Swimming performance trials were conducted in an open water-flow-through swim flume with a water depth of about 15 cm. A pump connected to a 3-m-long hose directed water into the swim flume. A perforated Plexiglas plate with holes drilled 1-cm apart was inserted into the flume to decrease water flow, reduce turbulence, and maximize laminar flow through the swimming section. Fish were restricted to the swimming section using mesh grids anteriorly and posteriorly. Two inserts were used to restrict fish to the swimming section to ensure fish were able to swim unimpeded by the wall but not able to turn around. Based on results of preliminary swim trials, fish <150 mm FL were swum in the small insert (3 cm wide \times 80 cm long) and fish \geq 150 mm FL were swum in the large insert (4 \times 80 cm). Velocity was measured in the swimming section for each insert at the midsection at 100 mm from the water

surface via a flow meter (Hontzsch Instruments, HFA serial no. 363). When using the smaller insert, velocities in the swimming section were slightly higher (about 0.72 m/s) than when using the larger insert (about 0.64 m/s), yet these velocities remained constant through each insert during all swim trials. The absolute swimming speed, measured in body lengths (BL), for fish in the small insert ranged from 4.8 to 7.4 BL/s, while the absolute swimming speed for fish in the large insert ranged from 3.1 to 4.3 BL/s. A light was placed at the back of the swimming section and a black cover at the front to motivate fish to swim towards the front mesh grid.

Swimming performance of all fish was evaluated 4–7 days postsurgery, which is approximately the time required for Cultus Lake sockeye salmon smolts to migrate from their natal rearing lake to the ocean (Welch et al. 2009). Thus, an assumption was made that if fish were recovered to the point that they could make a freshwater migration and seawater transition, then they could be challenged in a prolonged swimming challenge. Within a tagging cohort, fish were randomly chosen to be swum. Individual fish were placed into the water filled flume at zero water velocity for 2 min, and then the water pump was started causing water to flow at maximum velocity: small insert velocity at 0.72 m/s and larger insert velocity at 0.64 m/s. If an individual's tail touched the back of the grid during a trial, a rubber stopper attached to a metal rod was used to gently prod the fish, encouraging swimming. The trial ended when the fish fatigued (defined as when a fish was unable to move off the back grid following three prods in a row) or when swimming duration reached 1,200 s. At the end of the trial, fish were removed from the flume and fork length was measured; fish mass was not measured to avoid anesthetizing the fish. Fish were placed back into the tank they came from (described above) that was filled with fresh aerated water to facilitate recovery. An additional temporary divider was used to separate fish that had completed the trial versus fish that still needed to be swum.

Survival.—Survival of fish in freshwater was calculated at 8.5 and 16.5 d postsurgery, and these values combined to yield overall freshwater survival. Surviving fish were then transitioned into one of four water-recirculating seawater tanks (each 0.91 m diameter by 1.25 m deep) at a salinity of 5‰ (Instant Ocean salt; Dynamic Aqua, Canada). Fish that were housed together after surgery remained together through the study. The seawater tank set up was similar to the freshwater tank set up (four tanks each with a divider) and fish were moved from a divided tank section in freshwater to a divided section in seawater. Every 24 h for 4 days, seawater concentration was increased by 7‰ to simulate a transition from freshwater to seawater through an estuary. By day four, seawater concentration had increased to 30‰, similar to salinity concentrations in the Strait of Georgia into which these fish would have migrated from their natal Cultus Lake. Fish were held at this salinity for an additional 5 d at which time the experiment concluded and necropsies were performed.

Assessments of fish condition.—Tanks were checked three times per day throughout the duration of the study for dead (or

moribund) fish and expelled tags. After the freshwater exposure period (prior to seawater exposure) fish in all treatment groups were anesthetized on the same day to measure fork length and mass. The control treatment group was anesthetized for the same amount of time (approximately 2 min) as the sham surgery and tagged treatment groups to keep the handling time among all treatment groups consistent. Because fish were tagged 7.5 d apart, the first group of tagged fish spent 16.5 d in freshwater and the second group of tagged fish spent 8.5 d in freshwater. In addition, postsurgical wound healing of the incision and the area around the sutures was assessed in the sham surgery and tagged treatment groups and again after 9 d of seawater exposure. Postsurgical wound healing was scored based on a scale from 1 to 4 modified from (Wagner et al. 2000). The postsurgical wound healing scores were 1 = incision and area around sutures closed with no inflammation, incision scar still visible, 2 = inflammation around incision or sutures and (or) sutures pulling skin abnormally tight, 3 = fungus and inflammation around incision or suture points and (or) incision not fully closed, and 4 = open and inflamed incision with tag showing either through the incision or visible through the body wall (indicating pressure necrosis). Inflammation was categorized as skin that was red and puffy around the incision or sutures. Fish fork length and mass (excluding tag mass) were measured a final time at the end of the experiments.

Statistical analysis.—To determine effects of tag burden, a logistic regression analysis was used to compare the swimming duration of tagged and sham surgery fish to the mean swimming duration of the nontagged control group (253.7 s). Mean swimming duration of the control group was used as the baseline time against which to compare tagged and sham tagged treatments since results of achieved swimming durations were variable due to uncontrollable differences in the individual fish performance (e.g., angle and position of fish in the water column and inherent variability of individuals' swimming strength). All swimming durations were included in the data set, including fish that failed to swim (0 s) and fish that swam for the maximum period (1,200 s). A binary response of swimming duration was derived by binning fish from all five treatment groups into two categories of positive and negative outcomes. Specifically, fish that swam ≥ 253.7 s (mean swim time for nontagged control fish) were assigned a positive outcome (i.e., 1), and fish that swam < 253.7 s were assigned a negative outcome (i.e., 0). These binary data were analyzed using a generalized linear model (GLM) with a logit link function (i.e., logistic regression) to answer two questions: (1) do tagged fish (6 mm, 7 mm and 9 mm tagging treatments) have a lower probability of swimming the mean duration of nontagged control fish, and (2) is there a correlation between tag burden and the probability of a tagged fish swimming the mean duration of nontagged control fish?

The two different flume sizes used to swim fish were included as a "flume factor" in the analysis to test for direct and interactive effects of the different flume conditions on swimming duration. The two flumes did not have a significant direct or interactive

effect on swimming performance of tagged or nontagged control fish ($P < 0.05$), thus flume was excluded as a factor from further analysis. Interaction of sham surgery fish and flume size was not significant ($P = 0.98$), and the interaction of tagged treatments (6-mm, 7-mm, and 9-mm tagging treatments) was not significant ($P = 0.33$).

To determine how much of the observed mortality was due to surgery versus tag presence, a proportions test was used between different groups (control and sham surgery, control and the 6-mm tag group, control and the 7-mm tag group, and control and then 9-mm tag group). One-way analysis of variance (ANOVA) was used to compare postsurgical wound healing scores among treatment groups (sham surgery, 6-mm, 7-mm and 9-mm tag groups) in freshwater after 8.5 and 16.5 d and then after 9 d of exposure in seawater. Any fish that died for reasons unrelated to the tagging study (e.g., jumped from tank) were excluded from the analysis (three in freshwater and nine in seawater). A single fish from the sham surgery treatment group was not included in the 16.5-d data analysis, as the postsurgical wound score was not recorded; a single fish from the 6-mm treatment group was not included in the seawater analysis, as the suture score was not recorded. Bonferroni corrections were used for all analyses. The significance level for all statistical procedures was set at $\alpha = 0.05$.

RESULTS

Swimming Performance

Fish in the tagged treatment groups had a significantly lower probability of swimming ≥ 253.7 s (mean swimming duration for nontagged control fish), while fish in the sham surgery group did not ($P = 0.32$). Tag burden was significantly ($P = 0.01$) and negatively correlated with the probability of a tagged fish swimming ≥ 253.7 s (Figure 1). When tag burden was $\geq 8\%$, fish were unable to swim the maximum time (1,200 s, Figure 2); thus an a posteriori analysis was conducted to compare the swimming duration of fish with tag burdens $< 8\%$ and fish with tag burdens $> 8\%$. Excluding fish that swam for 1,200 s, swimming duration of fish with tag burdens $< 8\%$ was significantly longer (mean = 125.3 s, SD = 136.6) than fish with tag burdens $\geq 8\%$ (mean = 93.1 s, SD = 78.0; Mann-Whitney $U = 0$, $Z = -11.04$, $P < 0.05$). The analysis did not show a tag burden threshold where swimming duration begins to significantly decrease; however, as tag burden increased the probability of swimming ≥ 253.7 s decreased (Figure 1).

Survival

There were a total of 17 mortalities throughout the experiment; 5 fish died the day they were tagged, 9 fish died between 7 and 15 d postsurgery, and 3 fish died between 20 and 25 d post surgery. Freshwater survival of fish in the four surgery groups (sham surgery, 6-mm, 7-mm, and 9-mm tags) was not significantly different from survival of fish in the nontagged

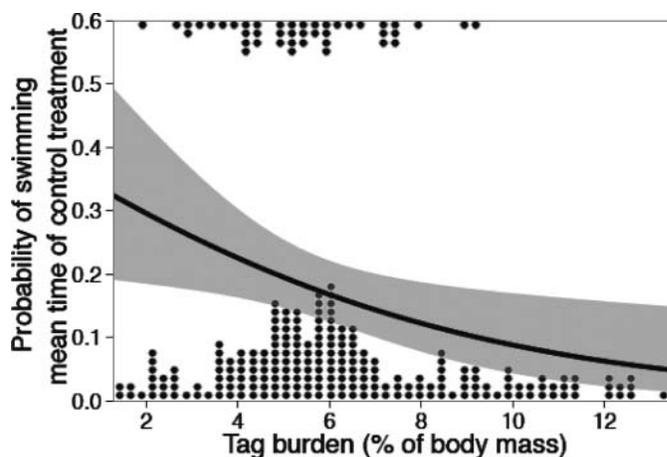


FIGURE 1. Predicted probability that tagged Sockeye Salmon will swim the mean time of those in the nontagged control treatment group (253.7 s) as a function of tag burden. The logistic regression is indicated by the solid line, and the gray polygons are the predicted 95% confidence intervals. The black circles at the bottom of the figure show the numbers of tagged fish that failed to swim 253.7 s, those at the top of the figure the numbers of fish that swam ≥ 253.7 s. Each black circle equals one fish. The data from the 6-, 7-, and 9-mm tagging treatments are combined into 0.4% tag-burden bins.

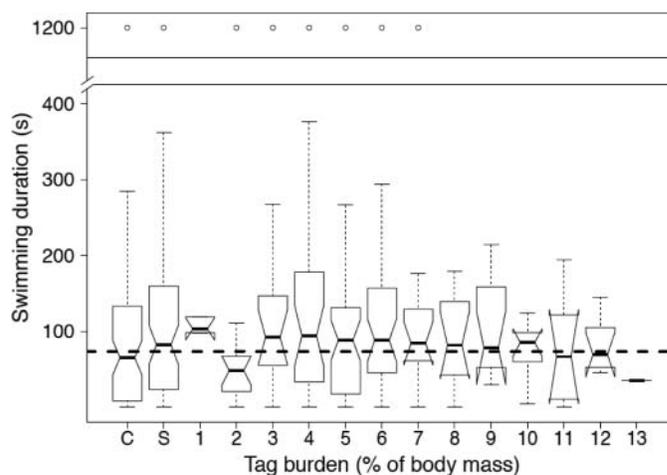


FIGURE 2. Notch box-and-whisker plots of freshwater swimming duration (< 400 s) of Sockeye Salmon relative to tag burden, pooling the 6-, 7-, and 9-mm tagging treatments into 1% bin groups. The numbers of fish by bin group were as follows: 1% = 5, 2% = 15, 3% = 16, 4% = 33, 5% = 46, 6% = 39, 7% = 14, 8% = 12, 9% = 12, 10% = 11, 11% = 8, 12% = 8, and 13% = 1; the nontagged control treatment (C) had 77 fish and the sham surgery treatment (S) had 71 fish. The boxes represent the interquartile ranges (25th to 75th percentiles of the data), the dashed vertical lines show two standard deviations of the mean, and the horizontal bar in each box represents the median of the data. The dashed black line ($y = 73$ s) displays the median swimming duration for the nontagged control treatment group. Fish that swam for the maximum duration during swim trials are represented by open symbols at $y = 1,200$ s. The number (proportion) of fish that swam for 1,200 s in each treatment is as follows: 12 fish (0.13) in the control treatment, 14 fish (0.15) in the sham surgery treatment, 12 fish (0.12) in the 6-mm treatment, 5 fish (0.05) in the 7-mm treatment, and 2 fish (0.04) in the 9-mm treatment.

TABLE 2. Freshwater and seawater survival (mean proportion \pm SE) of five tagging treatments of Sockeye Salmon. Freshwater survival was calculated at 8.5 and 16.5 d postsurgery, and these values were combined to yield overall freshwater survival. Standard errors were calculated using the equation: $SE = \sqrt{(s(1-s)/n)}$, where s = the proportion (0–1, where 1 = 100% fish survival) and n = sample size.

Treatment	Freshwater		Seawater	
	<i>n</i>	Proportion survived \pm SE	<i>n</i>	Proportion survived \pm SE
Control	94	1.00	91	1.00
Sham	94	0.99 \pm 0.010	92	1.00
Tag: 6 mm	102	0.98 \pm 0.014	98	1.00
Tag: 7 mm	106	0.97 \pm 0.017	101	0.97 \pm 0.020
Tag: 9 mm	47	0.97 \pm 0.025	43	0.95 \pm 0.033

control group. There were no mortalities in the control group in freshwater or in seawater (Table 2). In freshwater, of the 349 sham and tagged fish, there were nine deaths (2.6% mortality), one in the sham surgery group (tag burden = 0%), two in the 6-mm group (tag burdens of 6.7% and 9.3%), three in the 7-mm tag group (tag burdens of 6.3, 6.6, and 11.9%), and three in the 9-mm group (tag burdens of 7.9, 10.7, and 11.0%). The range of tag burdens of surviving fish in freshwater was 1.3% to 12.3%. Seawater survival of fish in the three tagging groups and the sham surgery group was not significantly different from survival of fish in the control group. In seawater, of the 334 sham surgery and tagged fish, there were eight deaths in seawater (2.4% mortality, all in the 7-mm or 9-mm tag group); however, tag burden could only be calculated for six of these mortalities. There were three mortalities (3%) in the 7-mm tag group; two of these mortalities had tag burdens of 11.5% and 9.3%. There were five mortalities in the 9-mm tag group (11.5%); four of these mortalities had tag burdens of 13.5, 14.0, 14.0, and 14.9%. Due to logistical constraints the range of tag burdens of surviving fish could not be calculated. We conducted necropsies on all dead fish (in freshwater and seawater) and found no evidence of mortality related to surgical causes (e.g., accidental cuts or punctures to internal organs from the scalpel or suture needle, suturing of intestines to body wall, or infection around incision or sutures).

Assessments of Fish Condition

No tag expulsion occurred during the experiment. Postsurgical wound healing differed among tagging groups in freshwater after 8.5 d ($F_{3,169} = 6.00$, $P < 0.01$) and after 16.5 d ($F_{3,162} = 5.07$, $P < 0.01$). For each elapsed period, the 9-mm tag group had significantly less postsurgical wound healing (higher scores) than the other tagging treatment groups ($P < 0.02$ and $P < 0.01$; Table 3). At 9 d after transfer to seawater, postsurgical wound healing differed among all tagging groups ($F_{3,321} = 1.19$, $P < 0.01$); post hoc analyses indicated that groups with progressively larger tag burdens had higher postsurgical wound scores.

DISCUSSION

Results of this study suggest tagging and tag burden have a negative effect on swimming duration while surgery does not. While visually the mean swimming duration was similar among tag burdens (Figure 2), our findings suggest that increasing tag burdens result in the decreased probability of a tagged fish swimming the mean time of a nontagged control fish (Figure 1). Mortalities only occurred with tag burdens $\geq 6\%$. Overall, it is difficult to relate our seawater survival rates, tag expulsion, and postsurgical wound healing results to previous literature, given the diversity of species and study designs. However, our findings

TABLE 3. Postsurgical wound healing scores for five tagging treatments of Sockeye Salmon in freshwater and seawater experiments. Postsurgical wound healing scores were as follows: 1 = incision and area around sutures closed with no inflammation, incision scar still visible; 2 = inflammation around incision or sutures and (or) sutures pulling skin abnormally tight; 3 = fungus and inflammation around incision or sutures points and (or) incision not fully closed; and 4 = open, inflamed incision and the tag visible either through the incision or through the body wall, indicating pressure necrosis. Within columns, different letters denote significant differences after post hoc testing using Bonferroni correction.

Treatment	Freshwater at 8.5 d		Freshwater at 16.5 d		Seawater	
	<i>n</i>	Mean \pm SD	<i>n</i>	Mean \pm SD	<i>n</i>	Mean \pm SD
Control	49	NA	45	NA	91	NA
Sham	44	1.7 \pm 0.6 y	48	1.8 \pm 0.5 y	92	1.1 \pm 0.3 w
6 mm	51	1.8 \pm 0.9 y	49	1.8 \pm 0.5 y	97	1.4 \pm 0.6 x
7 mm	56	1.7 \pm 0.6 y	47	1.9 \pm 0.6 y	98	1.7 \pm 0.8 y
9 mm	22	2.4 \pm 0.7 z	22	2.3 \pm 0.8 z	38	2.3 \pm 1.0 z

TABLE 4. Tag burdens from various studies of the effects of acoustic intra-coelomic tagging on juvenile salmonids.

Species	Total <i>n</i>	Tag burden (%)
Chinook Salmon ^a	789	2.6–11.5
Sockeye Salmon ^b	442	1–13.6
Coho Salmon ^c	224	7–8
Sockeye Salmon ^d	196	5.8–8.2
Chinook Salmon ^d	189	3.2–10
Chinook Salmon ^e	190	2.6–8.8
Steelhead ^f	168	3.5–15
Chinook Salmon ^g	156	1.4–6.7
Atlantic Salmon ^h	149	2.41–2.18
Atlantic Salmon ⁱ	96	8.5–10.1

^aRechisky and Welch (2010).^bPresent study.^cChittenden et al. (2009).^dBrown et al. (2006).^eHall et al. (2009).^fWelch et al. (2007).^gAngela et al. (2004).^hMoore et al. (1990), but tag burdens calculated by present authors.ⁱLacroix et al. (2004).

indicated that tagged fish cope extremely well with the surgical process and tag burdens, as indicated by high, short-term freshwater and seawater survival, excellent tag retention, and good postsurgical wound healing of the incision and suture holes.

Tag burden limits likely vary among species. Several studies have shown tag burdens of 1.4–8% did not influence swimming performance of juvenile salmonids; however, these studies did not examine tag burdens >8% (Anglea et al. 2004; Chittenden et al. 2009b; Moore et al. 1990, Table 4). Lacroix et al. (2004) observed that a tag burden of 8.5% resulted in lower U_{crit} values in juvenile Atlantic Salmon *Salmo salar* (Lacroix et al. 2004). Brown et al. (2006) found tag burdens 5.8–8.5% in juvenile Sockeye Salmon decreased U_{crit} values, while juvenile Chinook Salmon *Oncorhynchus tshawytscha* with a similar tag burden range (3.2–10%) did not exhibit a decreased U_{crit} . The majority of studies use PIT-tagged control and sham surgery treatment groups to allow for individual identification, resulting in some level of tag burden (Balfry 2011; Chittenden et al. 2009b; Lacroix et al. 2004; Rechisky and Welch 2010; Welch et al. 2007); however, our study did not tag control or sham surgery treatment groups, allowing for true comparison of swimming performance and survival between nontagged (0% tag burden) and tagged fish.

Mortalities only occurred in fish whose tag burden exceeded 6% (7% mortality; 93% of smolts with tag burden >6% survived). It is important to note that there are conditions encountered by wild fish that cannot be replicated in the laboratory; this study only observed fish for the first few weeks posttagging. However, several other studies have observed that survival of tagged fish is high compared with nontagged control groups (Brown et al. 2006; Chittenden et al. 2009b; Lacroix et al. 2004;

Moore et al. 1990; Rechisky and Welch 2010, Table 4). Contrary to results of those studies, Welch et al. (2007) observed early (<12 weeks) size-dependent mortality for smaller juvenile steelhead *O. mykiss* (anadromous rainbow trout), while larger steelhead (>13 cm FL) had increased survival (>90%; tag burdens in fish that perished were not reported).

Few studies have examined tag expulsion rates, postsurgical wound healing, and how tagged salmonids cope with the transition from freshwater to seawater. Previous literature suggests tag expulsion rates vary with species, tag size, and study design. Rechisky and Welch (2010) observed zero tag expulsion for fish implanted with 7-mm tags (similar dimensions to 7-mm tags we used), while fish implanted with 9-mm tags had 5% tag expulsion (24 weeks postsurgery). In juvenile Atlantic Salmon, Moore et al. (1990) found 20% tag expulsion over a 28-d period, whereas Lacroix et al. (2004) observed 100% tag expulsion by 217 d postsurgery for the larger tag size tested (28 mm long × 8 mm diameter, 3.79 g). Welch et al. (2007) and Hall et al. (2009) observed similar tag expulsion rates for juvenile steelhead held in freshwater for 12 weeks (13%) and juvenile Chinook Salmon held in seawater for 10 d (13%).

Fish implanted with the larger 9-mm tag had relatively less healing of the incision and suture holes than fish implanted with smaller tags. Similarly, Chittenden et al. (2009b) observed that in juvenile Coho Salmon *O. kisutch*, sham surgery fish reached a complete healing status (incision completely fused, no stitches remaining) faster than tagged fish (3–5 months versus 7 months, respectively). In juvenile Chinook Salmon, a variety of methods have been used to assess postsurgical wound healing, including number of sutures retained (Deters et al. 2012; Deters et al. 2010; Panther et al. 2011), present-absent scores for tag retention, wound inflammation and wound ulceration (Deters et al. 2010), incision openness scored of 0–1 (lower values represent less incision openness; Deters et al. 2010), incision closure (defined as when incision edges touch and scale regeneration is nearly complete; Panther et al. 2011), apposition (grade based on the proportion of the incision that was apposed, folding inward, overlapping, gaping, or a combination thereof; Panther et al. 2011), erythema on the incision and wound extent measured as the either the area of visible subepidermal tissues (Panther et al. 2011) or the area of incision openness, wound ulceration, and redness (Deters et al. 2010). Panther et al. (2011) observed that 1-month-old postsurgery incisions made on the linea alba had smaller wound extents, but by 98 d fish tagged through the linea alba had higher transmitter loss than incisions made 3 mm from and parallel to the linea alba and incisions made between the parallel lines of myomeres. Panther et al. (2011) suggests that transmitter weight on the incision may cause suture failure resulting in tag loss. In this study, surgical incisions were made on the linea alba, and there was zero tag expulsion across treatments. However, the poor healing of the incision and suture holes for fish implanted with 9-mm tags may be a result of the heavier tag weight on the incision than for the lighter weights of the smaller tags (6 and 7 mm) we used.

Despite the tagging process and large tag burdens, seawater survival and tag retention were high. Similarly, Balfry (2011) observed no effects of tagging on juvenile Chinook Salmon survival in seawater (30 d) and tag retention was high (98%). That study also found no significant differences in sodium–potassium ATPase enzyme activity, plasma ions, or plasma osmolality between sham surgery, PIT tagged, and dummy acoustic tagged fish in seawater. However, Hall et al. (2009) observed high mortality of tagged (31%) and sham tagged (42%) juvenile Chinook Salmon over 42 d, and 10% mortality in the Floy-tagged control group. Unfortunately, in that study large numbers of fish could not be individually identified due to high Floy tag loss (55%) resulting in small sample sizes.

Results of this study play an important role in understanding short-term mortality rates reported from recent telemetry studies. Welch et al. (2011; 2009) tagged and tracked juvenile Cultus Lake Sockeye Salmon smolts over four consecutive years (2004–2007) during their migration from Cultus Lake into the Fraser River estuary and from there to the northern end of Vancouver Island (total migratory distance of 500 km). Welch et al. (2011; 2009) estimated survival and travel rates using an acoustic telemetry array (POST; Welch et al. 2003) but did not conduct a parallel tagging-effects study. Our study used the same population, age-class of fish, and tag type (9 mm) as the Welch et al. (2009) study; tag burdens in that study (2–9%) were within the range we evaluated, and the duration of our experiments was similar to the time taken for Cultus Lake smolts to migrate from their natal lake to the northern tip of Vancouver Island. Given the high survival of tagged fish in freshwater and seawater, good postsurgical wound healing, and the good level of swimming duration up to about 8% tag burden in our study, it is unlikely that tag burden directly played a significant role in observed mortality patterns of fish in the Welch et al. (2009) study. However, little is known of the interaction between tag burden and additional stressors occurring in natural environments. Welch et al. (2011) evaluated the effect of smolt and tag size on fish survival for 3,500 tagged, freely migrating juvenile Pacific salmon (including Sockeye Salmon) by comparing the size distribution of fish at the time of tagging to surviving fish that were detected at the outer reaches of an acoustic telemetry array monitoring the Salish Sea (British Columbia). They found that the size distribution of fish released and the size distribution of surviving fish detected at acoustic arrays changed little, suggesting that tagging and tag size did not play a large role in fish mortality.

It is important to have an understanding of how tagging may affect fish before large-scale telemetry studies are implemented because tagged individuals are used to make conclusions about the migratory behavior and survival of nontagged conspecifics. With advancing technology, the shapes and dimensions of tags are constantly changing and utilizing tag burden as a metric can be helpful to compare across different types and sizes of tags. Future work to understand tagging effects should focus on the effects that tagging has on growth, survival, and physiology in

both freshwater and seawater. In addition, conducting studies over longer periods, particularly in seawater, would give further insight into how juvenile salmonids cope with intracoelomic tagging and long-term tag presence and have broad applicability to future tagging studies on outmigrating juvenile salmonids.

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