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Behavioral Response of Juvenile Chinook Salmon to Surgical Implantation of Micro-acoustic Transmitters

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Abstract
Acoustic telemetry, a commonly used tool for examining movements and survival of aquatic species, is often applied without a comprehensive understanding of transmitter implantation effects. This can be problematic when the goal of the study is to use telemetry results to make inferences regarding broader populations. Here, we examined juvenile Chinook Salmon Oncorhynchus tshawytscha at varying time intervals after transmitter implantation to assess the behavioral implications of tagging. The following behavioral metrics in response to a novel environment were compared across treatment and control groups: time to emergence from shelter into the open portion of the test arena, rheotactic response, total activity, and rates of exploration. Tagged fish (114–132 mm FL) were tested at 0, 1, or 4 d postsurgery (day-0, day-1, and day-4 groups, respectively), and their behavior was compared to that of similarly handled control fish. Emergence from refuge was the only metric that differed significantly between treatment and control groups. Fish tested on the same day as the surgery were less likely to emerge from the refuge, with only 46% of the day-0 tagged fish emerging compared to 88, 93, and 80% of the day-1, day-4, and control groups, respectively. However, day-0 fish that did emerge from the refuge had rheotactic responses, total activity, and exploration rates similar to those of fish from the other treatment and control groups. This study may have fisheries research and management implications, especially for telemetry studies and monitoring efforts. We encourage researchers using this technology to consider (1) observing a post-transmitter-implantation recovery period of at least 24 h prior to release, adjusting study plans and logistics accordingly; (2) applying sufficient scientific rigor to emerging tagging technology prior to wide-scale adoption; and (3) when possible, conducting concurrent battery life and tag effects studies with any field release of tagged fishes, as differing relationships between fish size, tag size, and tagging techniques may yield variable results.

Acoustic telemetry is a valuable tool for studying the movement, survival rates, and ecology of aquatic organisms (e.g., Eckert and Stewart 2001; Ng et al. 2007; Cooke et al. 2013; Cordoleani et al. 2018). For example, in some contexts, telemetry may represent a significant improvement over a traditional mark–recapture framework, which has long struggled with obtaining statistically significant recapture information (Hilborn et al. 1976; Dudgeon et al. 2015; Hussey et al. 2015). More recently, spatiotemporal movement data have been combined with environmental and physiological data to enhance understanding of the complex array of factors affecting animal movement (reviewed by Alexandre et al. 2013). Perhaps most significantly, telemetry provides opportunities for scientists to study animals in their natural environments, including areas that traditionally were beyond the directly observable realm of the researcher (Chapple et al. 2015; Hussey et al. 2015).

One major methodological concern with telemetry is whether transmitter implantation procedures or the presence of the transmitter itself (hereafter, jointly referred to as “tagging”), alters animal behavior. In cases of altered
behavior, data gained from tagged groups might not be representative of the organism’s ecology and thus not applicable to the population (Peuen et al. 2005). For example, if tagging elicits erratic or impaired swimming behavior, predation risk may be increased, or if tagging-induced behavioral changes result in refuge-seeking behavior, this could impact foraging and eventually growth and survival. Furthermore, changes in tagged-animal behavior may augment survival relative to that of nontagged conspecifics (Brown et al. 2007; Archard and Braithwaite 2011). Survival rates and route transition probabilities of tagged juvenile salmon are often used as management standards (Johnson et al. 2017; Klimley et al. 2017a). If tagging significantly alters behaviors or survival of released fish, then management benchmarks developed from these data could be flawed—or worse, wrong. Although there is a substantial body of tag effects literature as related to survival, it is troubling that relatively few studies explore alterations in behavior (Cooke et al. 2011).

Winter (1996) proposed a 2% transmitter : body weight ratio (tag burden) for telemetry studies, a standard that has been widely accepted throughout the field. However, this standard was conceived at a time when miniature transmitters for juvenile fish were not available. Continual technological advances facilitating the miniaturization of acoustic transmitters have allowed for the tracking of smaller and smaller species and earlier life history stages (McMichael et al. 2010). These advances have led to a proliferation of research investigating freshwater and early marine survival of many species of juvenile salmonids (Michel et al. 2013; Gibson et al. 2015; Deng et al. 2017; Johnson et al. 2017; Klimley et al. 2017b; Notch 2017; Cordoleani et al. 2018). As the size of the transmitters continues to be reduced, guidelines on suitable transmitter : body weight ratios continue to be challenged (Brown et al. 1999).

Results from initial studies utilizing micro-transmitters have been promising, with relatively few adverse effects reported. For example, over the course of a 30 d study no differences in growth (weight or length) were observed in tagged (tag burden = 1.3–2.6%) and control juvenile Siberian Sturgeon Acipenser baeri with total lengths ranging from 14 to 19.1 cm (Carrera-García et al. 2017). Critical swimming speeds ($U_{krit}$) of juvenile American Eels Anguilla rostrata ranging from 113 to 175 mm (tag burden = 1.1–5.2%) did not differ among tagged and control groups (Mueller et al. 2017). Juvenile Chinook Salmon Oncorhynchus tshawytscha over 79 mm FL did not have reduced $U_{krit}$ or compromised predator avoidance when compared to untagged controls, but fish less than 79 mm FL had compromised swimming performance (tag burden = 1.5–4.5%; Walker et al. 2016). These results generally support the use of micro-transmitters for studying the movements and ecology of juvenile salmonids. However, a cautious approach is warranted when pushing tag and fish size boundaries.

Historically, Chinook Salmon were abundant in the rivers of California’s Central Valley, with estimates ranging between 1 and 2 million spawners annually (Yoshiyama et al. 1998). Central Valley Chinook Salmon populations are declining, with two evolutionarily significant units (winter run and spring run) federally listed under the Endangered Species Act. Salmonid persistence in the Central Valley is questionable (Katz et al. 2012; Moyle et al. 2017) due to a plethora of interacting factors, including anthropogenic alterations to the habitat (Nichols et al. 1986; Kondolf et al. 2008), competing demands for water resources (Monsen et al. 2007; Moyle et al. 2018), proliferation of nonnative species (Cohen and Carlton 1998), and extensive hatchery management practices leading to reductions in genetic and phenotypic diversity (Williamson and May 2005; Carlson and Satterthwaite 2011). Significant attrition occurs in the juvenile life stage, and even modest improvements in survival within the first year (~6%) could lead to a reversal of population decline (Kareiva et al. 2000). Thus, telemetric investigations into migratory behavior, survival, and regions of high juvenile mortality are becoming increasingly crucial to understanding and managing declining salmon populations in California.

As biologists strive to apply telemetry tools to progressively smaller and more vulnerable life stages of fish, the potential for adverse tagging effects should be considered. Comparing variation in fish behavioral responses to a novel experience is one approach used to examine behavior along the shy–bold continuum (Wilson et al. 1993; Toms et al. 2010) and has also been used to assess an organism’s stress-coping ability (Overli et al. 2006). Here, we use similar methods to examine the behavioral implications (i.e., sublethal effects) of tagging (relating to effects from the presence of the transmitter and the surgical process jointly) by recording initial emergence time from a latency behavioral box (LBB: an opaque acrylic box that sheltered the fish from the open area of the test arena), activity rates, and exploratory behavior of fish when presented with a novel environment. Evaluating the behavioral responses of juvenile Chinook Salmon to tagging allowed us to infer the efficacy of micro-transmitter (weight in air = 0.30–0.43 g; tag burden = 1.2–2.2%) telemetry technology for studying the movement and survival of these fish. Treatment groups were given variable post-tagging recovery periods (0, 1, and 4 d). We then quantified behavior metrics (i.e., time to initial emergence from the LBB, activity, rheotaxis, and area explored) for tagged and control fish at different time intervals after tagging to represent recovery. We predicted that (1) control fish (those that did not undergo surgery) would emerge from the opaque refuge (i.e., the LBB) within the test arena
faster than tagged fish; (2) initial rheotactic responses (positive rheotaxis: orienting into the current; negative rheotaxis: orienting with the current) after emergence from the LBB would vary depending on treatment group; (3) control fish would be more active and exhibit a higher rate of exploration than tagged fish; and (4) significant behavioral effects would diminish over time as fish recovered from surgical trauma.

**METHODS**

*Fish source and care.*—Adult fall-run Chinook Salmon were spawned at the U.S. Fish and Wildlife Service’s Coleman National Fish Hatchery (Anderson, California) on November 13, 2012. Eggs were hatched, and juveniles were reared at the hatchery until transport to the Center for Aquatic Biology and Aquaculture at the University of California, Davis (UCD), on April 15, 2013. Fish were initially kept in one circular, 1,250-L tank (diameter = 162.5 cm; depth = 60 cm) and held for 6 weeks before experiments began. The fish were then split into two circular, 300-L tanks (diameter = 96.5 cm; depth = 42 cm) from which individuals were randomly selected for placement into control or tagged treatments. Temperature in the tanks was 16°C (range = 15.8–16.2°C), and oxygen was above 95% saturation throughout the acclimation period. Tanks were equipped with air-equilibrated, nonchlorinated well water at a turnover rate of approximately the full tank volume every 45 min. Fish were fed a diet of Rangen Soft Moist pellets (Rangen, Inc., Buhl, Idaho) at 1% of their weight daily.

*Surgical procedures.*—Juvenile Chinook Salmon (n = 48) were selected at random, and a single surgeon performed all surgeries. Tagging procedures were similar to those used by Singer et al. (2013). Briefly, fish were fasted for 48 h prior to the surgical procedure and then were anesthetized with a buffered, 90-mg/L solution of tricaine methanesulfonate (MS-222; Argent Chemical, Redmond, Washington) in accordance with UCD Institutional Animal Care and Use Protocol Number 16819. Fish were anesthetized to stage 4—exhibiting a loss of equilibrium, muscle tone, and spinal reflexes and a decreased rate of opercular movements (Summerfelt and Smith 1990). Once anesthetized, individuals were weighed (wet weight, g) and measured (FL, cm; Table 1), and the condition of the fins, eyes, and scales was recorded. Following Martinelli et al. (1998) and Lacroix et al. (2004), a conservative maximum tag burden of 5% was used as a threshold cutoff; thus, fish with ratios exceeding 5% were not used further. This ratio is widely utilized by field biologists studying the movements of juvenile Chinook Salmon (e.g., Michel et al. 2015; Buchanan et al. 2018). Taggable-sized fish were placed ventral-side up in a surgery cradle, with a buffered, 30-mg/L solution of MS-222 continuously running over the gills to maintain sedation. A 6–9-mm incision was made slightly offset from the midline on the ventral side of each fish, ending approximately 3 mm anterior to the pelvic girdle. A transmitter, previously disinfected with Nolvasan (chlorhexidine diacetate; Zoetis USA, Parsippany, New Jersey), was inserted into the coelomic cavity of each fish through the incision. The incision was then closed using two simple interrupted sutures (50 PDS monofilament sutures; AD Surgical, Sunnyvale, California). Fish remained under observation until they regained equilibrium, generally after 2–3 min. After recovery, tagged fish were transferred into two circular, 284-L tanks filled with freshwater. No mortalities were observed during surgical procedures. Tagged fish were then selected for one of three treatment groups: fish tested the same day as the surgeries were performed (day 0); those tested the day after the surgeries were performed (day 1); and those tested 4 d after surgeries were performed (day 4). The tagged fish ranged in size from 11.4 to 13.2 cm FL; mean FL was 12.2 cm (SD = 0.56) for the day-0 group, 12.4 cm (SD = 0.46) for the day-1 group, and 12.2 cm (SD = 0.45) for the day-4 group (Table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N (emerged)</th>
<th>Mean FL (SD)</th>
<th>Mean weight (SD)</th>
<th>Tag burden (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>13 (6)</td>
<td>12.2 (0.56)</td>
<td>23.7 (2.94)</td>
<td>1.4–2.0</td>
</tr>
<tr>
<td>Day 1</td>
<td>16 (14)</td>
<td>12.4 (0.46)</td>
<td>22.7 (3.08)</td>
<td>1.2–2.1</td>
</tr>
<tr>
<td>Day 4</td>
<td>15 (14)</td>
<td>12.2 (0.45)</td>
<td>21.9 (2.33)</td>
<td>1.3–2.2</td>
</tr>
<tr>
<td>Control</td>
<td>41 (33)</td>
<td>12.4 (0.71)</td>
<td>24.2 (4.39)</td>
<td>NA</td>
</tr>
</tbody>
</table>

**TABLE 1.** Treatment type, number of Chinook Salmon per treatment group (N; number of fish that emerged from the latency behavioral box in the test arena is shown in parentheses), and mean FL (cm; SD in parentheses) and mean weight (g; SD in parentheses) for each treatment group. Fish behavior was tested at 0, 1, or 4 d after acoustic transmitter implantation. The range of tag burdens (expressed as a percentage of the fish’s body weight) for each treatment is also presented.
10.7 × 5 × 4 mm. The two transmitter types were distributed randomly throughout the tagged treatment groups (the proportion of single-battery transmitters in the treatment groups ranged from 23% to 37.5%).

Control group.—Forty-one control fish were used in this study (Table 1). Control fish ranged in size from 11.1 to 13.8 cm FL, with a mean of 12.4 cm (SD = 0.71; Table 1). The control fish were treated in a manner similar to that of the tagged treatment groups, except without being subjected to the procedures related to tagging (i.e., they were not anesthetized, weighed, or measured prior to behavioral testing). We did not include a sham control group in this study, the reasoning being twofold: (1) the intention of the study was not to disentangle the effects of the surgical process from those related to the presence of the transmitter; and (2) the intracoelomic implantation of acoustic transmitters is impossible without performing a surgery, so any results gleaned from the sham control group could not be extrapolated to field releases of tagged fish (Walker et al. 2016). Furthermore, an extensive body of literature examining differences between tagged and sham-tagged fish already exists (e.g., Adams et al. 1998; Anglea et al. 2004; Frost et al. 2010). All control fish were used in the analyses (i.e., no premature exposure to the novel arena or media failure was experienced while testing the control fish).

Experimental procedures.—Experiments were conducted in a 4,000-L indoor flume simulating a river current. The test arena, including the space occupied by the LBB, was 1.80 × 0.90 m, with a water depth of 0.29 m. A portable water velocity meter (Model 201D; Marsh-McBirney, Frederick, Maryland) was used to set the channel sweeping velocity to 0.15 m/s, which was measured using a 2-s time constant, just below the upstream fish screen at the midwater depth. We recorded water velocity at each gridline in the experimental flume (Figure 1). The $U_{crit}$ of juvenile Chinook Salmon (19.9 cm FL) is approximately 0.6 m/s (~3 body lengths/s; Randall et al. 1987); thus, the experimental velocity was well below the fish’s $U_{crit}$ but was high enough to elicit a rheotactic response. The location of the LBB within the experimental arena presented the fish with the option of swimming in the upstream or downstream direction upon emergence.

Fish were transferred individually into a 10-L bucket for transport from the holding tank to the experimental arena. Each fish was then placed into the LBB and covered with an opaque black lid. The LBB was positioned against the flume wall but centered along the length of the gridded test arena (Figure 1). The LBB models an area of refuge for the fish, and it is hypothesized that leaving this “refuge” to complete some life history task (e.g., migration, feeding, etc.) makes a fish more vulnerable to predation and other hazards (Brown et al. 2005, 2007; Näslund et al. 2015). Our LBB was made of black acrylic, measured 31.5 × 30.5 × 32 cm (length × width × height), and was designed to prevent fish from viewing the researcher during the trial or the testing area prior to when the front door was remotely opened (Wilson et al. 1993). The front door of the LBB was perforated with thin slits, allowing it to move freely and to prevent water from rushing in or out when opened.

Before the experimental trial began, each fish was given a 15-min settling period after transfer to the LBB. Brown et al. (2007) used a 2-min settling period prior to a similar behavioral assay, but Weber and Borthwick (2000) observed that juvenile Chinook Salmon showed an unwillingness to move within the first 10 min of transfer into an experimental arena. Therefore, we adopted a more conservative 15-min settling time. After this 15-min period, the sliding front gate of the LBB was remotely opened, and the fish was free to emerge and explore the testing arena. The entire area around the experimental arena was shrouded in black shade cloth, and the sliding gate on the LBB was controlled by a pulley system to reduce the influence of external factors on the behavior of test subjects.

A trial was initiated when the front gate of the LBB was opened. The trial was terminated either 2 min after the fish first exited the LBB or 15 min after the gate was opened (i.e., if the fish did not exit the LBB). At the end of the experiment, fish were euthanized with a concentrated, buffered solution of MS-222 (~400 mg/L) and were measured for FL (cm) and weight (g). Day-0 fish were all tested within 12 h of tagging. The number of fish tested per day ranged from 6 to 16, and all trials took place between 0800 and 1800 hours Pacific Standard Time. Ambient lighting over both the holding tanks and the experimental arena was maintained to mimic the natural photoperiod. Juvenile salmon were tested individually, and all trials were conducted within a 2-week window. The trials were recorded with underwater (Model CVCC320WP; Speco Technologies, Amityville, New York) and overhead (Model CVCC627; Speco Technologies) video cameras. Experiments were recorded to DVD media by using DVR recorders (Model DMR-EA18; Panasonic, Newark, New Jersey).

Behavioral metrics.—Video recordings were analyzed using Behavioral Observation Research Interactive Software (BORIS; Friard and Gamba 2016) to capture emergence time, rheotactic response (positive or negative), and measures of activity and exploration. Initial emergence time was defined as elapsed time from the opening of the gate to the moment when the fish’s body completely crossed the threshold of the opening to the LBB. Activity was quantified as $n_t/l_t$, where $n_t$ is the number of 15- × 15-cm squares in the gridded area encountered by the individual during the test period and $l_t$ is the length of the trial. Exploration was quantified as $u_t/l_t$, where $u_t$ is the number of unique squares visited.

Statistical analyses.—Effects related to the size of the transmitters used were addressed in preliminary analyses.
We conducted a series of three-two-way ANOVAs, with emergence time, activity rate, and exploration rate as the respective independent variables. Tag size and tag treatment group were the independent variables across all three tests. Because no independent variables or interaction terms were significant at an $\alpha \leq 0.05$, tag size was eliminated as a variable in subsequent analyses, allowing us to pool fish by treatment.

Time to initial emergence from the LBB was analyzed under the time-to-event framework. This framework is commonly used in industry (e.g., “time-to-failure” analysis of a product) and in biomedical research (e.g., drug trials and subsequent survival analysis; Muenchow 1986). In fisheries-related studies, this method has been used to examine factors contributing to hooking mortality (Bendock and Alexandersdottir 1993) and delays in migration (Castro-Santos and Haro 2003). Here, a Cox proportional hazards model was used to quantify differences in emergence time between treatment groups. In this time-to-event analysis, fish that emerged from the LBB and those that did not were included; their emergence times were censored (i.e., the experiment was ended) after 15 min (right-censored data). The Cox proportional hazards model equation is as follows:

$$ h(t, X) = h_0(t)e^{\sum X_i \beta_i}. $$

The left side of the equation represents the hazard $h$ for an individual at time $t$ with $X$ characteristics or explanatory variables. The right side of the equation has two parts: $h_0(t)$ is the baseline hazard function, and $e^{\sum X_i \beta_i}$ is the exponential expression relating to the effects of the explanatory variables. Therefore, hazard is the product of the baseline hazard for any individual (accounting for time but not for explanatory variables) and the effects of the variables included in the model. The survival function $S(t)$ allows for a more straightforward interpretation of model results in the form of a survival curve; in the context of this analysis, it can be interpreted as the probability of a fish remaining in the LBB at time $t$. The survival function, has the following relation to the hazard function $h(t)$ (Kleinbaum and Klein 2012):

$$ S(t) = \exp \left[ -\int_0^t h(u)du \right]. $$

Here, the survival function $S(t)$ is equal to the exponential of the negative integral of the hazard function, $-\int_0^t h(u)du$, between the integration limits of 0 and $t$. This analysis was completed in R (R Core Team 2017) with the “survival” package (Therneau 2015).

Rheotactic response data were analyzed using logistic regression. Rheotactic response was the dependent variable, and morphometric data for individual fish, treatment, and time to initial emergence were all explored as predictors of rheotactic response (i.e., independent variables). Reductions in residual deviance were examined for statistical significance at a threshold $\alpha \leq 0.05$.

Only fish that emerged from the LBB were included in the activity analysis. To compare differences in activity of
fish across groups, a Kruskal–Wallis rank-sum test was used. Variance across the treatment groups was homogeneous (Levene’s test: df = 3, F = 0.81, P = 0.49); however, the distribution of residuals did not meet the assumptions of normality (Shapiro–Wilk test: w = 0.94, P = 0.002), thereby excluding a parametric ANOVA approach to analyzing the activity rate of the fish.

Similar to the activity analysis, exploratory behavior was only quantified if fish emerged from the LBB during the experimental period. To compare differences in exploratory behavior of fish across groups, a Kruskal–Wallis rank-sum test was used. Variance across treatment groups was homogeneous (Levene’s test: df = 3, F = 0.45, P = 0.72); however, the distribution of residuals did not meet the assumptions of normality (Shapiro-Wilk test: w = 0.96, P = 0.03), thus excluding a parametric approach. For all statistical procedures, an α ≤ 0.05 was interpreted as a significant result.

RESULTS

Forty-four of 48 tagged fish were included in the analyses. Four were excluded due to human errors or technical difficulties (e.g., fish escaping the LBB into the testing arena, affecting their novel experience; or the digital media failing to record the experiments). Mean fish FL ranged from 12.2 to 12.4 cm, and mean weight ranged from 21.9 to 24.2 g across the groups (Table 1). There were no significant differences between treatment groups or controls for FL or weight (FL: Kruskal–Wallis \( \chi^2 = 1.8458, \) df = 3, \( P = 0.605 \); weight: Kruskal–Wallis \( \chi^2 = 4.6239, \) df = 3, \( P = 0.2015 \)). Mean time to stage 4 anesthesia across all fish was 171 s (SD = 28), and average surgery time for the fish in the treatment groups was 110 s (SD = 10).

There was a significant effect of tag treatment on initial emergence time (z-test: \( z = -2.265, P = 0.02 \)). Only 46% of fish from the day-0 treatment group emerged from the LBB before the 15-min test period had elapsed. In contrast, 88, 93, and 80% of the day-1, day-4, and control fish emerged from the LBB. The probability that a fish from the day-0 treatment group remained in the LBB at any given time \( t \) was significantly greater than the probability for a fish from the day-1, day-4, or control group (Figure 2). Fork length was also included in the proportional hazard model but was not a significant factor (z-test: \( z = 0.897, P = 0.37 \)), indicating that individual variation in fish size did not influence the propensity or timing of emergence from the LBB.

There was no significant effect of tag treatment on rheotactic response (\( \chi^2 \) test: df = 3, \( P = 0.628 \); Figure 3). Upon emerging from the LBB, similar proportions of all groups responded to the flow by exhibiting negative

![Figure 2](image_url)

**FIGURE 2.** Survival curve resulting from the Cox proportional hazards model for juvenile Chinook Salmon. The x-axis shows \( t \) (elapsed time, s), and the survival function is shown on the y-axis; can be interpreted as the probability of a fish remaining in the latency behavioral box at time \( t \). Fish behavior was tested at 0, 1, or 4 d after acoustic transmitter implantation (day-0, day-1, and day-4, respectively).
rheotaxis, orienting head-first downstream. Seventy percent of the control group, 67% of the day-0 group, 86% of the day-1 group, and 79% of the day-4 group displayed negative rheotaxis immediately after leaving the LBB. The inclusion of FL and initial emergence time as predictor variables in the logistic regression model did not significantly reduce residual deviance ($\chi^2$ test: $P = 0.867$).

Median activity level was not significantly different between treatment groups (Kruskal–Wallis $\chi^2 = 2.0341$, df = 3, $P = 0.5654$). Generally, individual activity was high immediately after emergence from the LBB and then reached an asymptote before the 2-min observation period had expired (Figure 4). Although the length of the active period varied by individual, there appeared to be variable patterns of activity within each of the groups tested (Figure 4).

Median rates of exploration were not significantly different between treatment groups (Kruskal–Wallis $\chi^2 = 2.8217$, df = 3, $P = 0.4199$; Figure 5). Regardless of treatment group, fish consistently gravitated toward the same section of the test arena (Figure 5). The fish displayed an affinity for the downstream portion of the test arena opposite the LBB, as evidenced by the warmer coloring in the lower right-hand corner of the test arena schematics shaded by elapsed time per area (Figure 5). The upstream-most portions of the test arena were rarely utilized by the fish in this experiment. With the exception of the day-0 group, at least one individual returned to the refuge offered by the LBB after a brief foray into the open portion of the test arena.

**DISCUSSION**

Our experiments recorded an alteration of Chinook Salmon behavior after a common surgical tagging procedure involving an intracoelomically placed transmitter. The goal of our study was not to disentangle the effects of the surgical procedure from those related to the presence of the transmitter but rather to view them as a singular event given that it is impossible to implant a transmitter into the coelom of a fish without first performing a surgery. Therefore, information gained from the inclusion of a sham control group in an attempt to disentangle the effects of the surgical process from the effect related to the presence of the transmitter would not be applicable to any field studies (Walker et al. 2016). Fish that were tested on the same day as tagging (day-0 group) had a higher probability at any given time of remaining in the shelter than untagged control fish (Figure 2), suggesting that behavioral changes occurred within the first 24 h postsurgery. This resulted in fewer than half of the day-0 fish emerging from the LBB during the 15-min trial, relative to 80–93% of fish from the
other treatments. Critically, after this initial 24-h period, behavioral changes dissipated, and tagged fish acted in a manner similar to that of control fish (as supported by all behavioral metrics assessed). Same-day release of tagged fish is a common practice when fish are collected in the field (e.g., from rotary screw traps), and holding fish for an extended recovery period may be neither feasible nor beneficial (Cooke et al. 2016). In contrast, when study fish are obtained from a hatchery or other breeding stock (e.g., Perry et al. 2010; Singer et al. 2013; Michel et al. 2015), the occurrence of fewer logistical constraints may facilitate extended recovery holding periods. Fish that are tagged and released on the same day may not behave like untagged conspecifics, and it may be beneficial, as we have shown here, to hold fish for at least 24 h before release. Furthermore, inferences regarding broader population and movement dynamics (e.g., transit times and mortality rates) drawn from the first 24 h postrelease should likely be made with caution (Peven et al. 2005).

Orientation in response to flow, activity, and exploration rates of the Chinook Salmon that emerged from the LBB were similar across all groups. A predominantly negative rheotactic response to flow was expected from smolt-aged fish; as they move from freshwater to marine waters, orientating in the direction of the flow facilitates efficient transport toward the ocean (Kemp et al. 2005). The logistic regression model for rheotactic response was only marginally improved by the addition of explanatory variables. In other words, whether or not fish oriented with flow could not be reliably predicted by treatment group, emergence time, or FL. Although exploration and activity measured different characteristics of movement, the two values were similar across all groups, and they were highly correlated (Pearson’s product-moment correlation coefficient ≈ 0.87). This result indicated that more active fish tended to use a larger proportion of the total available area rather than remaining active within a confined portion of the test arena. Collectively, our experimental results suggest that when fish are given more than 24 h to recover from tagging, behavioral differences should not bias the interpretation of movement data when inferences are extrapolated beyond tagged individuals of the population.

FIGURE 4. Total activity (as measured by the number of gridlines crossed over time) across the three treatment groups of juvenile Chinook Salmon as compared to control fish (gray). Fish behavior was tested at 0, 1, or 4 d after acoustic transmitter implantation (day-0 [black symbols and lines], day-1 [gold], and day-4 [red], respectively). The gridlines in the test arena formed cells that measured 15 × 15 cm. The x-axis shows elapsed time, and the y-axis shows total activity. The lower-right plot shows treatment group on the x-axis (c = control) and total activity on the y-axis. Horizontal bars represent median values, boxes display the interquartile range (IQR), the whiskers extend to 1.5× the IQR, and outliers are shown by individual points.
Fish telemetry studies are common; however, studies examining the behavioral implications of tagging on a case-by-case basis are much fewer, yet needed. A literature review of intracoelomic tagging studies of fish indicated that the majority of studies were conducted on adult life history stages ($n = 77$ of 108), and these studies often accounted for tagging by examining the implications of transmitter size in relation to fish size or surgical methods (e.g., incision location, wound closure, etc.; Cooke et al. 2011). In contrast, less than 25% of the reviewed research studies included a behavioral assessment component ($n = 25$ of 108), and just over 25% were conducted with fish belonging to the juvenile life history stage ($n = 30$ of 108). Furthermore, “behavior” was broadly characterized, ranging from field comparison of long-range movements by groups representing two different tagging methods (i.e., it was not possible to have a control group; Cooke and Blunt 2001) to laboratory studies measuring activity (Thoreau and Baras 1997; Jadot et al. 2005).

Several studies that did examine behavioral changes associated with tagging reported minimal effects of tagging relative to control fish (reviewed by Cooke et al. 2011). However, tag types and fish sizes used in those studies were highly divergent. For example, adult Blue Tilapia Oreochromis aureus that were tagged with motion-sensitive transmitters exhibited decreased movement for 12–24 h after tagging but resumed normal behavior after 3–4 d (Thoreau and Baras 1997). Chinook Salmon greater than 79 mm FL (tag burden $= 3.4–4.0\%$) exhibited no significant differences between JSATS acoustic-tagged fish (0.22-g weight in air), double-tagged fish (dummy JSATS acoustic transmitter and PIT tag), and untagged control fish when subjected to swimming ($U_{crit}$) and predation avoidance challenges (Walker et al. 2016). Tagged (tag burdens up to 6.7%) and untagged juvenile Chinook Salmon that were tested at 1 and 21 d posttagging did not differ in swimming performance ($U_{crit}$) or susceptibility to predation (Anglea et al. 2004). Similarly, tagged (tag burden $= 3.1–10.7\%$), sham-tagged (anesthetized, given an incision, and sutured, but no tag was inserted), and control juvenile Chinook Salmon did not vary significantly in $U_{crit}$ (Brown et al. 2006). The $U_{crit}$ of 210–280 mm TL Westslope Cutthroat Trout Oncorhyncus clarkii lewisi with tag burdens similar to those applied in this study ($\leq 4\%$) did not differ significantly from the $U_{crit}$ of control fish (Zale et al. 2005). However, in the same study, a slight decrease in swimming performance was observed as tag burden increased (Zale et al. 2005).
Although the advent of JSATS and other micro-transmitter technology has enabled the tagging and tracking of earlier life stages of fish, it also raises important questions concerning minimum taggable sizes and associated behavioral effects. For example, smaller individuals are often disproportionately affected by tagging (Liss et al. 2016), and clear size thresholds are not always apparent but rather can occur along a continuum (Zale et al. 2005; Perry et al. 2013). Understanding the complexity of tagging effects and periodically re-evaluating the assumption that tagged fish are indeed representative of their untagged counterparts will be central to furthering the management systems that rely on acoustic telemetry data. For example, emerging telemetry technologies are often adopted by biologists and resource management agencies with little scientific evaluation of potential behavioral effects. Our research therefore underscores a major need in fisheries science and management: to pursue an understanding of the behavioral effects of tagging before—or, at a minimum, concurrently with—the collection of acoustic telemetry information.

Our results should also be taken with some caution, as conditions in the field clearly differ from those in the laboratory (Liedtke and Wargo-Rub 2012), and comparisons across species or populations can be difficult due to experimental effects (Perry et al. 2013). Additionally, configuration of the test arena is not trivial and could affect the behavioral responses of subjects. Altering the layout of the arena elicited variable responses in one population of juvenile Brown Trout Salmo trutta (Näslund et al. 2015). Here, we have described the configuration of the test arena in detail, with the intention of providing enough detail for other researchers to draw reasonable comparisons. Fish that exited the LBB spent the majority of time in the bottom righthand corner of the test arena (Figure 5). This observation, along with the fish’s return to the LBB after initial emergence, could be interpreted as refuge-seeking behavior. Spending time adjacent to walls (or “wall-following”) has been observed in other behavioral studies of fish (Gautrais et al. 2009; Hansen et al. 2015) and may be indicative of a desire for safety or protection (Simon et al. 1994). Future research might consider expanding the spatial extent of the experimental arena and testing the tagged fish for longer periods of time and in larger bodies of water to further explore extant hydraulic conditions in rivers. Fish movement behavior might also be assessed in greater spatiotemporal resolution by utilizing automatic tracking software, which is now common in the field of behavioral ecology (Dell et al. 2014). Annual changes in abiotic environmental characteristics are also important considerations. For example, higher water temperatures during certain years may induce thermal stress in fishes (Liedtke et al. 2012) and promote bacterial growth (White et al. 1991), and incisions and sutures provide vectors and media for bacterial growth and infection of tagged fish (Liedtke et al. 2012), which can affect swimming behavior (Kent et al. 1989).

In California’s Central Valley, fall-, spring-, and winter-run Chinook Salmon juveniles have been tagged with JSATS transmitters to identify mechanistic drivers of variable out-migration success (Klimley et al. 2017a; Zeug et al. 2017; Cordoleani et al. 2018). In fisheries studies worldwide, it is relatively common for run-of-the-river fish to be captured in a rotary screw trap, tagged with a transmitter, and subsequently released within 24 h of tagging—and often within 1–4 h of tagging. The results of the present study suggest that this practice should be re-evaluated. Holding times of less than 24 h are clearly the result of important logistical, personnel, and work-planning constraints rather than an intentional decision. However, data pointing to behavioral impacts on juvenile salmon fitted with JSATS tags may represent an emerging fisheries management challenge. Indeed, analyses of telemetry data are increasingly being used to inform fisheries management decisions (e.g., the operation of infrastructure related to the California State Water Project and the Central Valley Project; Heublein et al. 2009), occasionally in “real-time” (Johnson et al. 2017). If behavioral tagging effects are present, the validity of telemetry data in representing the broader population might be called into question. In our experiment using fish with FLs ranging from 11.1 to 13.8 cm, weights ranging from 16.8 to 33.8 g, and tag burdens ranging from 1.2% to 2.2%, behavior of tagged and control fish was indistinguishable 24 h after surgery. The results of this study lead us to suggest the following considerations to other researchers, with the caveat that similar studies conducted with fish of a different size or species may yield different results. First, researchers should observe a minimum 24-h holding period after surgical tag implantation. Thus, accommodation of longer recovery times should be built into various project budgets and work plans. Second, scientific studies should be applied to emerging tagging technologies prior to wide-scale adoption to evaluate and quantify potential tagging effects. Telemetry studies frequently represent the “best available science” and are thus heavily relied upon for decision-making frameworks related to water operations or species recovery plans (Sullivan et al. 2006; Delta Stewardship Council 2016). Finally, with any field release of tagged fishes, researchers should conduct concurrent tag effects studies if possible, as differing experimental conditions may yield variable results.

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