The following supplement accompanies the article

Route-specific movements and survival during early marine migration of hatchery steelhead (*Oncorhynchus mykiss*) smolts in coastal British Columbia


*Corresponding author: steve.healy2@gmail.com

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Acoustic Tagging

Following surgery procedures described in Collins et al. (2013) and Furey et al. (2016), fish were randomly selected from raceways and anaesthetized in a solution of buffered tricane methanesulfonate (MS-222; 100 mg L\(^{-1}\); 200 mg L\(^{-1}\) NaHCO\(_3\)), measured for mass and FL (total air exposure <1 minute), and placed ventral side up on a V-shaped surgery trough. Water from a maintenance bath of MS-222, (50 mg L\(^{-1}\) MS-222, 100 mg L\(^{-1}\) NaHCO\(_3\)) which was oxygenated using air stones and monitored for consistent temperature, was irrigated across the gills for the duration of each surgery. A small ~8-10 mm midventral incision was made just posterior of the pelvic fins. VEMCO V7-2L acoustic transmitters (7 mm x 18 mm, ~0.7 g in water; 69 kHz, VEMCO Ltd., Bedford, NS; www.vemco.com) were inserted through the incision and positioned lengthwise inside the body cavity. The incision was closed using two absorbable monofilament sutures (Ethicon monocryl 5-0 monofilament, www.ethicon.com) then fish were placed in an aerated bucket of ambient river water to monitor recovery prior to returning to hatchery raceways.

Survival Analyses

We used a mark-recapture approach to estimate survival of acoustic-tagged smolts, where detection at each acoustic receiver subarray along the migration path was interpreted as ‘recapture’. Estimates of survival (\(\phi\)), detection probability (\(p\)), and their associated variances were calculated using the Cormack-Jolly-Seber (CJS) model (and special cases of the CJS model) for live recaptured animals (Cormack 1964; Jolly 1965; Seber 1965). This model jointly estimates survival and detection probability within a maximum likelihood framework. We used R (R Core Team 2014) with the package RMark (Laake 2013) to construct CJS models using Program MARK (White and Burnham 1999). CJS model assumptions apply for all analyses: equal survival probability, equal probability of detection, and instantaneous sampling.

Data Screening

Prior to beginning survival analyses, we screened the raw detection data from all 273 tagged smolts for false detections, which could occur because of environmental conditions or collisions between multiple acoustic-tag transmissions. Two or more detections of the same tag along a subarray within 0.5 hours and with more detections spaced with short intervals (<0.5 hour spacing) than with long intervals (>0.5 hours spacing) were considered real. Detections that failed to meet these criteria were assessed individually and were passed if the migration sequence was reasonable and if travel time for the segment was within the 10th -
90th percentiles of either segment or cumulative travel times. Of >12,000 steelhead detections recorded across all acoustic subarrays, only six were considered false and removed from subsequent analyses.

**Capture History Sequencing**

A capture history is a sequence of 1s and 0s that indicates whether an individual smolt was detected at each acoustic sub-array during their migration. The capture history sequence for the river-release group began with release in the Seymour River followed by detection at the subarrays deployed at the Seymour River Mouth, Northern Strait of Georgia (NSOG), Discovery Islands (DI), Johnstone Strait (JS), and Queen Charlotte Strait (QCS). The sequence for the marine-release fish was the same except that they were released in Burrard Inlet so that NSOG was the first detection site. Detections of the marine-release fish at the Seymour River Mouth (n=5 fish), were not included in the capture history, but these fish were otherwise retained in the analysis. Finally, we removed the two fish that migrated south after river exit and were detected on the Juan de Fuca (JDF) subarray since these fish would otherwise appear to have died in the Strait of Georgia.

**Goodness-of-Fit**

We assessed goodness of fit (GOF) with the median ĉ test within Program MARK. The variance inflation factor (ĉ) estimate for the most highly parameterized model in our model set [φ(release x segment) release to QCS] p (release x segment) was 1.64 (SE = 0.03) indicating that there was minor overdispersion. We adjusted the likelihood term for the model and inflated (multiplied) the standard errors on the estimates by the ĉ value to account for overdispersion in the data.

**Effect of Release Location**

We investigated the effect of release location to assess if it was reasonable to pool the two groups in the areas where their migration routes overlapped (NSOG to QCS) to increase sample sizes to the furthest marine subarrays, and to test if release location had an impact on survival post-Northern Strait of Georgia (NSOG). For this test, we modeled survival (φ) and detection probability (p) both with and without effects for release location and used AIC to compare the performance of the resulting model set. Groups were kept separate for migrations from release to NSOG because their migration segments differed. Only smolts released in freshwater had detections on Seymour River estuary receivers prior to NSOG, so only river-released smolts were used to estimate detection probability [i.e. p(site)] for estuary receivers. We hypothesized that φ was the same for both release groups at each of the subarrays in the common migration corridor from NSOG to Queen Charlotte Strait (QCS) [φ (release x segment)Release to NSOG + segmentNSOG to QCS], and φ varied by release group at all subarrays [φ (release x segment)Release to QCS]). We used the same strategy to test if detection probability varied by release location [p(site) versus p(release x segment)] resulting in a set of four models across all combinations of φ and p. Although all tagged fish were implanted with the same model of acoustic tag and all tags were programmed identically, it was reasonable to test if release location affected detection probability because the migration timing between the groups was statistically different at NSOG, Discovery Islands (DI), and Johnstone Strait (JS) (Wilcoxin p ≤ 0.001; differences of ~10 days in mean arrival dates), but not at QCS (Wilcoxin p=0.24; difference of 2.5 days in mean arrival). Migration timing can potentially affect detection probability through temporal changes in the level of background noise (e.g. weather events).

Since there was no evidence that survival or detection probability varied by release group (the summed weight across the models where a single parameter was estimated for both release groups was 95% and 98% for survival and detection probability respectively) we
pooled all tagged smolts in the common migration corridor for all subsequent analyses. This model where the release location groups were pooled in common migration segments was used as the base model for further hypothesis tests described below (base model: $\varphi(\text{release} \times \text{segment}_{\text{Release to NSOG}} + \text{segment}_{\text{NSOG to QCS}}) \ p(\text{site})$; Table 2).

**Effects of fork length, tag burden, and gill sampling on survival**

We assessed if body size, tag burden, and gill sampling affected survival. We hypothesized that these factors might cause a consistent shift in survival without changing the relative mortality between migration segments (i.e. an additive effect). To test these effects, we used AIC to compare the performance of the base model with three other models that were parameterized the same as the base model, but that also included an additive effect for one of the three covariates of interest (Table 2).

**Final estimates of survival and detection probability**

To account for model selection uncertainty in top candidate models including the effects of fork length, tag burden, and gill sampling (i.e. Table 2), we obtained the final estimates of survival and detection probability by model-averaging across the four models. The CJS models return the survivals for each migration segment and detection probabilities for each subarray. To calculate cumulative survival estimates from release to QCS, we multiplied survival probabilities for each consecutive migration segment. The cumulative survivals for the two release location groups differ since their segment survivals for the initial migration segments to NSOG were estimated independently. Beyond NSOG, the release location groups were pooled and only one survival estimate was made for both groups. We derived the variance for the cumulative survival estimates using the Delta Method.

**Survival rates**

To better compare survival between migration segments, we converted the survival estimates to survival rates per day and per km as:

$$S^{1/d}$$

where $S=$ estimated survival and $d=$ the mean travel time (days) or mean distance travelled (km). Segment travel time (days) was calculated for each fish from release to arrival on the first subarray, and then from departure from one subarray until arrival at the next along the migratory path. Distances were measured for each fish as the shortest in-water distance between the central point of each subarray. For the subarrays that spanned multiple channels at NSOG and Discovery Islands, we measured the distance to the central point of each channel.

Since both survival and travel time are random variables with associated error, we used bootstrapping to calculate the variance around the estimates of daily survival rate. We first sampled the fish 1000 times with replacement and calculated survival using the CJS model as described above for each sample. It was not possible to calculate the travel times for all of these samples because not all fish that survive have travel times (fish have to be detected on both sides of the segment in order to have a travel time calculation). Rather than discard samples without travel times, we calculated the travel times for each segment from separate samples that were drawn only from fish with travel times in that segment. To reduce the probability of inappropriate pairings (i.e. fast travel times with poor survivals), we calculated the survival rates by matching each survival sample with all 1000 travel time samples. We used the mean of these estimates as the final estimate of survival rate per day, and the distribution to calculate standard deviations and confidence intervals on the mean.
For consistency, we also used bootstrapping to estimate survival rate per km; however, error in the distance estimates was of less concern because there were only a few alternate routes, and because the actual distance swum is unknown. We adjusted the survival estimates for each sample by the average migration distance in each segment. Although the bootstrapped results accounted for error in both survival and travel time estimates, this method also underestimates the error on survival because it does not include the variance inflation factor (\(\hat{\epsilon}\)). Currently, there is no clear method for handling over dispersed data when employing bootstrap techniques. As the \(\hat{\epsilon}\) was only 1.64 for this data set, the effect was minimal.

As a final step in our assessment of route-specific survival, we calculated survival rates per day and per km for each route through the Discovery Islands because the migration distance was \(\sim 1/3\) longer through SC than through DP. We used the same methods for this calculation as for the segment-specific survival rates described above, but substituting the multi-state model for the CJS model.

**Route-Specific Survival**

We assessed route-based movements and survival through the Discovery Islands region (Fig. 1) using a spatial multi-state mark-recapture model where migration routes functioned as ‘states’. Similar to CJS models, multi-state models estimate survival (defined as \(S\) as opposed to \(\varphi\) for CJS models) and detection probability (\(p\)), but they also estimate the probability of movement between states (migration routes; \(\psi\)). A key assumption of multi-state models is that survival is modeled with the survival probability for the state where the animal was captured, and then movement to a new state takes place (i.e. survival in segment \(i\) to \(i+1\) does not depend on state in segment \(i+1\)). Thus, this model is appropriate to assess route selection and route-specific survival through the Discovery Islands since the DI subarray was placed at the south end (entrance) of this area. In addition to this assumption, CJS model assumptions (see Survival Analysis) also apply to multi-state models.

For this analysis, tagged steelhead were assigned to state A until they reached the DI subarray. At this point, those that migrated through Discovery Passage (DP) remained in state A, but those that used Sutil Channel (SC) transitioned to state B. We could not include Desolation Sound (DS, the third route through the Discovery Islands area) in this assessment because only one smolt was detected using this route. For each fish, we defined the migration route based on the location of its last detection on the Discovery Islands subarray (i.e. a fish that was detected on SC and then DP was assumed to have migrated through DP). At the JS subarray, all fish were assigned to state A and remained there until QCS. Six fish that were detected on DI were removed from the analysis because they were subsequently detected on NSOG (i.e. they probably did not migrate north).

To test if survival to JS varied by route through the Discovery Islands, we parameterized \(S\) with and without a route parameter and compared model performance using AIC [base model: \(S(\text{release x segment}_{\text{release} \rightarrow \text{NSOG}} + \text{segment}_{\text{NSOG} \rightarrow \text{QCS}})\)] versus differing-by-route model: \(S(\text{release x segment}_{\text{Release to NSOG}} + \text{route x segment}_{\text{DI to JS}} + \text{segment}_{\text{NSOG-DI and JS to QCS}})\)]. For these models, we assumed the transition probability would vary by route \(\psi(\text{route})\) since the detection data showed the proportion of fish taking each path was quite different. We were unable to estimate detection probability for each route because we could not determine which route was initially taken by those smolts detected at JS but not DI. Therefore, we assumed detection probability to be the same for each route \(p(\text{site})\), which is reasonable because the receiver configurations were very similar in both channels. Multi-state models don’t perform well near the boundaries of 0 and 1, so we used bootstrapping to gain a more robust estimate of survival though these channels. We were not able to include \(\hat{\epsilon}\) goodness-of-fit with bootstrapped results, though this parameter was low at 1.64, indicating a minor lack of fit.
A similar approach was used to assess whether survival to the DI subarray was influenced by route selection at NSOG. Because the NSOG subarray is located at the north end of Texada Island, route (state) for this analysis was assigned based on the channel where smolts were first detected (east or west of Texada Island). The lack of a subarray at the south end of Texada Island (entrance) prevented us from assessing $S$ and $\psi$ within the actual channels around this island. Models were parameterized as for the test of route selection through the Discovery Islands, but with the route parameter for $S$ shifted to the NSOG-DI segment (Table S1). Additionally, because $S$ was modelled separately by release group until smolts reached the common migration corridor at NSOG, we had to consider if $\psi$ would vary by release group in addition to route. However, we constrained $\psi$ to be the same for both release locations (but allowed them to vary by route) because only two river-release smolts were detected to the east of Texada Island.

As a final step in our assessment of route-specific survival, we calculated survival rates per day and per km for each route through the Discovery Islands because the migration distance was ~1/3 longer through SC than through DP. We used the same methods for this calculation as for the segment-specific survival rates described above (see Survival rates), but substituting the multi-state model for the CJS model.
Table S1: Ranking of multi-state models using QAICc to test if survival to the Discovery Islands subarray was influenced by route selection at the Northern Strait of Georgia subarray (i.e. east or west of Texada Island).

<table>
<thead>
<tr>
<th>Model</th>
<th>No. of parameters</th>
<th>QAICc</th>
<th>ΔQAICc</th>
<th>weight</th>
<th>QDeviance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival the same between routes $S(\text{release} \times \text{segment}<em>{\text{NSOG}} + \text{segment}</em>{\text{QCS}}) \mu (\text{site}) \nu (\text{route})$</td>
<td>11</td>
<td>625.02</td>
<td>9</td>
<td>0.737</td>
<td>13.611</td>
</tr>
<tr>
<td>Survival different between routes $S(\text{release} \times \text{segment}<em>{\text{NSOG}} + \text{route} \times \text{segment}</em>{\text{NSOG}} \times \text{DI} + \text{segment}_{\text{DI to QCS}} \mu (\text{site}) \nu (\text{route})$</td>
<td>12</td>
<td>627.09</td>
<td>3</td>
<td>2.064</td>
<td>0.263</td>
</tr>
</tbody>
</table>

a - Segment length to NSOG differed by release group
b - Only river-released smolts were used to estimate $p$ for the estuary receivers
c - The route parameter was used to provide independent estimates for the channels around Texada Island (Strait of Georgia and Malaspina Strait).

Table S2: Raw detection counts of acoustic tagged steelhead (*Oncorhynchus mykiss*) smolts at each subarray (when applicable) through the study system, as well as number detected based on routes through the Northern Strait of Georgia and Discovery Islands subarrays. ‘NSOG’ = Northern Strait of Georgia subarray, ‘DI’ = Discovery Islands subarray, ‘JS’ = Johnstone Strait subarray, ‘QCS’ = Queen Charlotte Strait subarray. Two smolts were also detected at the Juan De Fuca subarray, and were removed from survival analyses. These counts are not necessarily reflective of those used in route-specific multi-state models.

<table>
<thead>
<tr>
<th>Array</th>
<th>Sub-array (route)</th>
<th>Number Detected (marine-release/river-release)</th>
<th>Number of smolts detected based on routes along NSOG and DI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NSOG</td>
</tr>
<tr>
<td>Seymour River</td>
<td>N/A</td>
<td>5/66</td>
<td></td>
</tr>
<tr>
<td>Northern Strait of Georgia</td>
<td>East of Texada</td>
<td>41/3</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>West of Texada</td>
<td>51/12</td>
<td>48</td>
</tr>
<tr>
<td>Discovery Islands</td>
<td>Discovery Passage</td>
<td>60/12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sutil Channel</td>
<td>34/3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Desolation Sound</td>
<td>2/0</td>
<td></td>
</tr>
<tr>
<td>Johnstone Strait</td>
<td>N/A</td>
<td>51/9</td>
<td></td>
</tr>
<tr>
<td>Queen Charlotte Strait</td>
<td>N/A</td>
<td>33/5</td>
<td></td>
</tr>
</tbody>
</table>

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Fig. S1: Frequency distribution histograms of first (top) and last (bottom) detections of steelhead smolts (*Oncorhynchus mykiss*) on the Seymour River estuary receivers by time of day (in hours). Grey and white regions in the background indicate times of local night and day (sundown and sunrise), respectively. Fish movements in the estuary were predominantly nocturnal.
LITERATURE CITED


