Movement patterns and predator-prey interactions of domestic Atlantic salmon (*Salmo salar*) following an simulated aquaculture escape event

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Study overview and purpose:

Aquaculture is becoming increasingly important in meeting global demand for seafood products (Gephart *et al.*, 2020; Subasinghe *et al.*, 2009). However, such systems can be could have adverse consequences for local, native communities potentially through altered nutrient flows, disease transfers, and shifts in ecological processes, among others (Diana, 2009). Atlantic salmon (*salmo salar*) aquaculture represents a multibillion dollar industry (Grand View Research, 2023) and is a major commercial enterprise in South Eastern New Brunswick, representing a considerable proportion of the salmon production in Atlantic Canada (Government of Canada, 2023). Salmon aquaculture has a unique concern that is largely absent from other aquaculture systems in that production of this species coincides in regions where wild, native conspecific populations also exist (e.g., Atlantic Canada, UK, Norway). In North America, many of these native populations are listed as endangered/threatened (COSEWIC, 2010; Fay *et al.*, 2006). As cultured salmon are generally considered to be genetically distinct from local salmon populations, the escape of farmed salmon could pose a risk to the genetic/population structure of native salmon populations, especially if they interbreed with these wild conspecifics (Glover *et al.*, 2017; Verspoor *et al.*, 2008). Consequently, ensuring that escape events can be mitigated effectively is an important consideration for fisheries managers in developing sustainable aquaculture practices and in limiting their impact on local species. Unfortunately, data pertaining to the ultimate fate and behavioural patterns of escaped aquacultured Atlantic salmon are relatively scant. We do know that farmed salmon have been shown to disperse rather quickly throughout the bays that farms are situated in with some fish reaching the mouths of nearby rivers (Bungay *et al.*, 2021; Hamoutene *et al.*, 2018; Skilbrei *et al.*, 2015; Skilbrei OT, 2010b, 2010a). Recapture rates of escaped salmon also appear to be quite low (Skilbrei *et al.*, 2015; Skilbrei OT, 2010a). The purpose of this work was to establish the behavioural patterns and fate of farmed Atlantic salmon from a simulated release event in South Eastern New Brunswick, specifically Passamaquoddy Bay (45.093, -66.964); a region of high salmon aquaculture activity (Chang *et al.*, 2014). Using Atlantic salmon post-smolts, fish were acoustically tagged and released from a typical aquaculture site. The animal's movement patterns and interaction with local predators was monitored using a large scale acoustic array situated in the area.

Materials and methods:

Fish collection and tagging procedures

Atlantic salmon were acquired from Cooke Aquaculture's Fairhaven site (44.9641, -67.0117; Cooke Aquaculture Inc., Black's Harbour, NB, Canada). Fish were initially raised to the post-smolt life stage in large aquaculture net pens (~ 33 m diameter, ~10 m depth) as a part of functioning aquaculture operation. On August 30, 2021, a total of 125 of these salmon were moved from the main aquaculture net pen to a smaller, sentinel net pen (9 x 3 m; 54 m^3 volume), which was done to facilitate ease of capture of the fish on the day of the tagging. The fish remained in this net pen for at least 20 days before tagging commenced. All animal care and surgical procedures were done under approval from the Fisheries and Oceans Canada – St Andrews Biological Station's (DFO-SABS) Regional Animal Care Committee (AUP# 21-46) in conjunction with the guidelines and standards set by the Canadian Council on Animal Care (CCAC).

In an effort to monitor the behavioural patterns of salmon escaping from an aquaculture site, we elected to use acoustic telemetry (Crossin *et al.*, 2017) and fit a total of 99 salmon (mean mass = 601.8±118.5 g; mean total length = 370.9±24.71 mm) with acoustic tags (Vemco™ V9TP-2x; 31 x 9 mm; 4.9 g in air; InnovaSea Systems, Inc., Bedford, NS, Canada). All surgical procedures were conducted on board a vessel that was moored to the sentinel cage in an effort to minimize stresses associated with handling, transport, and air exposure (Lawrence *et al.*, 2020). On the day of the tagging, an individual fish was quickly dip netted from the net pen and was immediately placed into a water bath containing a knockdown dose of tricaine methanesulfonate (100 mg L^{-1}; MS222; Syndel Canada, Nanaimo, BC, Canada). Once the fish had lost equilibrium, it was promptly transferred to a wetted V-trough, ventral side up, where the gills were irrigated with weaker MS222 solution (50 mg L^{-1}) by means of a small water pump. A small incision was then made on the ventral midline of the animal (~20 mm) and the tag was inserted into the fish's coelom. The incision was then sutured close with two dissolvable stiches. All tools used in the surgery and the tag itself were disinfected using a diluted povidine-iodine solution (Betadine®, Avrio Health L.P., Stamford, CT, USA).

Following wound closure, the animal was moved to a recovery bath containing MS222-free seawater, which was actively pumped over the fish's gills. Once independent ventilation

resumed, the fish was transferred to a floating holding crate (50.8 x 80.0 x 40.6 cm; ~165 L) attached to the side of the boat (i.e., in the ocean) to recover for 1 h. On a given day, a total of five groups of fish ($N \leq 5$ per group) were tagged in this manner with tagging occurring over a four day period (September 21, 23, 27, and 29 of 2019). Following recovery, fish were released into the ocean representing t = 0 h of the experimental series. As will be discussed later on, the Fairhaven site had an array of seven acoustic receivers (VR2W-69 kHz acoustic receivers; InnovaSea Systems, Inc., Bedford, NS, Canada) in conjunction with a boat-based hydrophone device (VR100 receiver; InnovaSea Systems, Inc., Bedford, NS, Canada) to pick up the initial acoustic signal as the fish were released.

Description of the acoustic array

Our acoustic array consisted of 133 acoustic receivers placed in strategic positions throughout Passamaquoddy Bay and the Bay of Fundy (Fig. 1). Receivers consisted of a mixture of Vemco® VR2AR, VR2W, and VR2Tx receivers (InnovaSea Systems, Inc., Bedford, NS, Canada) that were deployed in advance of the release of the tagged salmon generally being deployed in May and July of 2021. Most receivers were then pulled in late November and early December of 2021 with data being downloaded at this point representing an approximately three month monitoring duration. A few of the receivers remained deployed over the winter period in nearby rivers to monitor fish usage of this habitat type. These receivers were retrieved in May 2022. Receiver detections in this study ranged from 2021-09-21 15:31:31 to 2022-03-15 05:16:45 (UTC). All raw receiver data was uploaded to the Ocean Tracking Network (OTN) database and is publicly available (https://members.oceantrack.org/project?ccode=QRET). For the purposes of the downstream analyses, individual receivers were grouped into smaller array units that were within close geographical position of one another. Arrays were then organized into broad geographical regions of the bay (i.e. sections) that were used to better determine fish movement patterns.

Post-release monitoring and statistical analyses

All analyses were conducted using the R programming language (Version 4.1.1) in the the R Studio interface (Version 1.4.1717; R Core Team, 2021). Raw datafiles and R scripts can be found in an open repository on GitHub.

The initial stages of the analysis used the raw receiver data to compile several metrics of interest to this study namely predation events and depth profiles. Before these metrics were determined, tag codes corresponding to this study's tagged salmon were used to filter out only relevant detection on our receivers. Temperature and depth sensor values from the detections were then corrected using factory calibration values.

Parameters concerning predation events constituted the first portion of our analysis. Predation events were identified using the temperature sensors of the tag and were identified into two main groupings of predator, endothermic or mesothermic, which were delineated by identifying maximum temperature values. In the case of endothermic predators, these were likely resented by marine mammal and avian predators who have a core temperature extending beyond 35°C and, in our study, had a maximum temperature value above 30°C in the event that stomach temperatures had not equilibrated right away while the tag was within range of a receiver. In the case of mesothermic predators, this group include endothermic fishes such as lamnid sharks and tuna, which keep their body temperatures above ambient (i.e., > ~14°C). Specifically, we set the range of maximum temperatures that constituted consumption by a mesothermic predator equal to 18°C but less than 30°C. This was based off of typical body temperatures for these sorts of animals as previously described in the literature. For salmon preyed upon by mesothermic predators, we also verified that temperatures were not a result of being in a warmer water environment, such as a river, that may result in a false positive predation event. Fish that remained at ambient environmental temperatures for the duration of their monitoring period were also visually checked to determine if they were consumed by an ectothermic predator or suffered a natural mortality event. This was done using the tag's depth sensor values and was a qualitative assessment looking for abnormalities in the animals depth profile which might indicate predation or depth. In this instance, we had four fish which appeared to be stationary (i.e. depth not actively changing and being picked up at a single array), which were removed from the dataset as they likely represented natural mortality events.

Once salmon had been assigned to their respective predator category (i.e. endothermic, mesothermic, not predated), we determined both overall predation rate as a percent of the total released population and a per-predator type basis. The timing of the predation event was also determined. Here, we manually identified the timepoint wherein the fish was still at an ambient

temperature and the fish was still considered to be alive/free-swimming. Following this value, the fish would have started warming up inside the stomach of the predator. The time difference between the release time and the last ambient temperature detection constituted the fish's survival time, serving as a proxy for predation event timing. Using the last ambient temperature time, we were also able to determine the receiver/section that this occurred at to identify where the predation event likely happened.

Following identification of predation timing, the raw detection file was adjusted to remove all detections associated with the fish being consumed. Data post-last ambient temperature was removed from that data set to ensure that movement patterns were reflective of the tagged salmon and not the predator. Following this, we then determined depth profiles for salmon following release from the aquaculture site. Depth data was binned at discrete timing intervals (0, 1, 2, 4, 6, 8, 12, 18, 24, 36, 48, 72, and 96 h post-release), bracketed by the proceeding timepoint (e.g. 0-1 h, 1-2 h, 2-4 h, etc.). For each individual fish, the animal's maximum, minimum, and average depth was tabulated. These values were then averaged across all of the study fish to determine mean maximum depth, mean minimum depth, and mean average depth for each of the binned time intervals.

Spatial and residency analyses were conducted using the R package 'actel' (Version 1.2.1.9014; Flávio & Baktoft, 2021). Residency timing and section movements were computed using the 'residency' function using a maximum interval value between new events of 15 min. Analyses also incorporated the section order to map out how fish may travel between the various arrays/section in our receiver array network. A jump warning of two sections was used to check if fish were moving through numerous arrays without detection, which could be an issue in interpreting our findings.

The results of the residency computation were used to determine a number of relevant indices for explaining the salmon's post-release behavioural parameters. We first determined the time to leave the Fairhaven aquaculture site, which constituted the difference from the release time and the last time the animal was detected on the Fairhaven array for fish that had their first detection ping at the Fairhaven array. Additionally, fish that were predated upon at the Fairhaven site without ever leaving it were removed from the analysis so as to not skew average leave times. Interestingly, four fish did not have their initial detection at any of the Fairhaven array

receivers but were rather first identified at arrays just outside of the cove that the Fairhaven site was located (i.e., Western Passage arrays). For this fish only, we tabulated an estimated time to leave the Fairhaven site as the difference between the release time and their first detection at the Western Passage arrays as a surrogate for a time to leave value.

Using this data, we also determined the direction of the fish's travel after leaving the Fairhaven site for the first time. The geography of the waters surrounding Fairhaven allowed us to create a choice flume of sorts in which an individual fish was faced with either heading into Passamaquoddy Bay (relative right direction) or going further out to the Bay of Fundy (relative left direction). The initial direction of the fish was noted and then we recorded their subsequent section movements to develop rough movement profile within the first few steps following the fish's escape.

Residency timing within the first 96 h following release was tabulated. Here, residency time values were binned on their duration from release on a 24, 48, and 96 h basis. For each time frame, we summed the total amount of time spent resident at each section present in our array network. In addition to this total time, we also computed the total residency time on a per fish basis dividing the section's total residency time by the number of individual fish that frequented that section. In this way, we could identify areas where the fish were likely to spend the majority of their time following an escape event with the first 96 h of release.

We were also interested in identifying if any of the salmon reached nearby river system and, if so, how much time did they spend there and how many different systems did they frequent. In order to accomplish this, we first filtered out residency times of sections associated with our major river systems including the St Croix, Bocabec, Digdeguash, Magaguadavic, and Lapreau rivers. All of these systems had receiver arrays in the estuary portions with some, such as the St Croix, having receiver arrays much farther upstream. As with the first 96 h residency calculations, we computed both total residency time per section and this value on a per fish basis. The number of fish reaching rivers was noted and we identified if they had reached multiple river systems.

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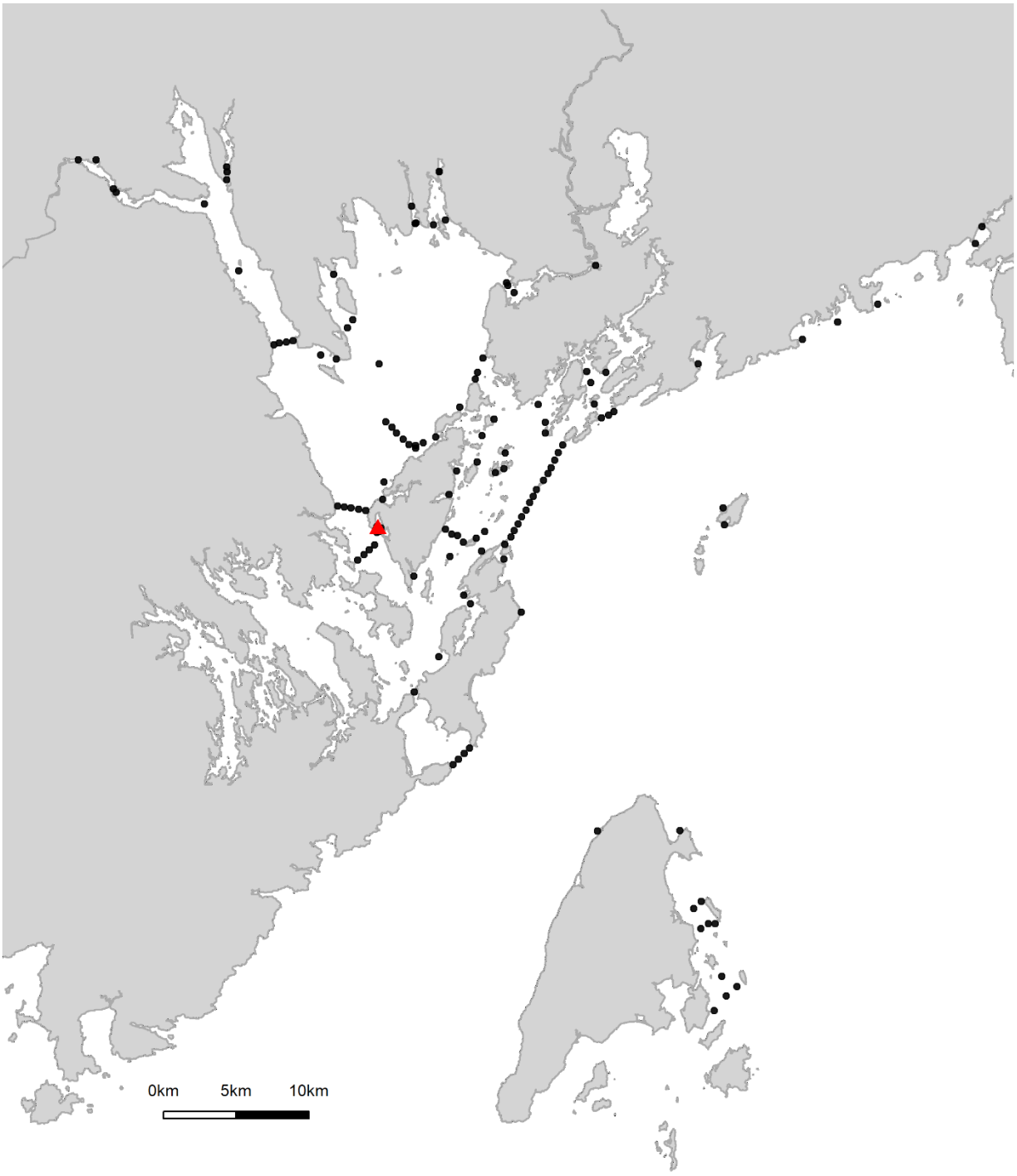

Figure 1: Overview map highlighting the positions of the receivers used in this study (black points) and the release site (red triangle) within the Bay of Fundy/Passamaquoddy Bay.