1	Movement patterns and predator-prey interactions of domestic Atlantic salmon (Salmo
2	salar) following a mock aquaculture escape event
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5	Lawrence, M.J. <sup>1</sup> *, Wilson, B.M. <sup>1</sup> , Wringe, B.F. <sup>2</sup> , Hawkes, J.P. <sup>3</sup> , Hardie, D.C. <sup>2</sup> , Hamoutene, D. <sup>1</sup> ,
6	Flávio, H. <sup>4</sup> , English, G. <sup>1</sup> , Black, M. <sup>1</sup> , McKindsey, C.W. <sup>5</sup> , Trudel, M. <sup>1</sup>
7	
8	<sup>1</sup> St Andrews Biological Station, Fisheries and Oceans Canada, St Andrews, NB, Canada
9	<sup>2</sup> Bedford Institute of Oceanography, Fisheries and Oceans Canada, Dartmouth, NS, Canada
10	<sup>3</sup> Northeast Fisheries Science Center, National Oceanic and Atmospheric Administration, Orono
11	ME, USA
12	<sup>4</sup> Department of Biology, Wilfrid Laurier University, Waterloo, ON, Canada
13	<sup>5</sup> Maurice Lamontagne Institute, Fisheries and Oceans Canada, Mont-Joli, QC, Canada
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25	*Corresponding author: Michael.lawrence@dfo-mpo.gc.ca
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## **Study overview and purpose:**

Aquaculture has become increasingly important to meet global demand for seafood products (Gephart et al., 2020; Subasinghe et al., 2009). However, such systems could induce adverse consequences for local, native communities through altered nutrient flow, disease transfer, and shifts in ecological processes, among others (Diana, 2009). Globally, Atlantic salmon (*Salmo salar*) aquaculture represents a multi-billion dollar industry (Grand View Research, 2023) and is a major commercial enterprise in Southwestern New Brunswick, comprising 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 coincides in regions where wild, native conspecific populations coexist (e.g., Atlantic Canada, UK, Norway). In North America, many of these native populations are listed as endangered or threatened (COSEWIC, 2010; Fay et al., 2006). As wild salmon are generally considered to be genetically distinct from cultured salmon, escapes could pose a risk to the genetic/population structure of native populations, especially if they interbreed with these wild conspecifics (Bradbury et al., 2022; 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, salmon escapes from net pens do occur in both large scale events and smaller, gradual leaks (Sepúlveda et al., 2013) with data pertaining to the behavioural patterns and ultimate fate of escaped cultured Atlantic salmon being relatively scant. We do know that farmed salmon have been shown to disperse rather quickly throughout the farm-containing bays with some fish reaching nearby estuaries (Bungay et al., 2021; Hamoutene et al., 2018; Skilbrei, 2010a; Skilbrei, 2010b; Skilbrei et al., 2015). Recapture rates of escaped salmon also appear to be quite low (Skilbrei *et al.*, 2015; Skilbrei, 2010a).

The purpose of this work was to establish the behavioural patterns and fate of farmed Atlantic salmon from a mock release event in Southwestern New Brunswick, specifically Passamaquoddy Bay (45.093, -66.964); a region of concentrated salmon aquaculture (Chang et al., 2014). Using Atlantic salmon post-smolts, fish were acoustically tagged and released from a

57 typical aquaculture site. Movement patterns, interaction with predators, and fate was determined

for individuals using a large-scale acoustic array situated in the surrounding area.

## **Materials and methods:**

Fish collection and tagging procedures

Farmed Atlantic salmon were acquired from an aquaculture site in Passamaquoddy Bay, at Fairhaven, Deer Island (44.9641, -67.0117; Cooke Aquaculture Inc., Black's Harbour, NB, Canada). Following land-based hatchery grow-out, these fish were raised from post-smolts in large, at-sea net pens (polar circle; ~33 m diameter, ~10 m depth), as per industry standard. On August 30, 2021, a total of 125 salmon were moved from a single polar circle (of 10 pens at the farm site) to a smaller, sentinel net pen (9 x 3 m; 54 m³ volume) moored centrally. The transfer was undertaken to facilitate ease of fish capture for tagging, and where fish could remain for at least 20 days before tagging commenced to allow for the depuration of applied treatments (e.g., chemical sea lice pesticides). All animal care and surgical procedures were conducted under approval from the Fisheries and Oceans Canada (DFO) 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 and fate 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 (V9TP-2x; 31 x 9 mm; 4.9 g in air; InnovaSea Systems, Inc., Bedford, NS, Canada). Tags had both temperature (-5°C to 35°C; ±0.5°C accuracy, 0.15°C resolution) and depth sensors (to 68 m; ±1m accuracy, 0.3m resolution) and were programmed to randomly emit an acoustic signal between 90 and 150 s for an average of 120 s. All surgical procedures were conducted on board a vessel (24-ft Rosborough) that was docked to the sentinel net pen 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 transferred from the net pen and immediately placed into an anaesthetic water bath of tricaine methanesulfonate (100 mg L<sup>-1</sup>; 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 a weaker MS222 solution (50 mg L<sup>-1</sup>) 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 coelomic cavity, and gently pushed ventrally. The incision was then sutured close with two dissolvable stiches (3-0 coated Vicryl braided suture; Ethicon Inc., Raritan, NJ, USA). All tools used in the surgery, and the tag itself, were first disinfected in a diluted povidine-iodine solution (Betadine®, Avrio Health L.P., Stamford, CT, USA).

Following incision closure, the individual was moved to a recovery bath containing raw seawater, which was actively pumped over the gills. Once independent ventilation and self-righting resumed, the fish was transferred to a floating overboard holding crate (50.8 x 80.0 x 40.6 cm; ~165 L) to recover for 1 h. On each tagging day, over a total of four days (September 21, 23, 27, and 29 of 2021), five groups of fish ( $N \le 5$  per group) were tagged. Following recovery, fish were released into the ocean (and at the centre of the farm) representing t = 0 h of the experimental series. As will be discussed later on, the Fairhaven site had a fine-scale array of seven acoustic receivers (VR2W-69 kHz acoustic receivers; InnovaSea Systems, Inc., Bedford, NS, Canada), used in conjunction with a boat-based hydrophone (VR100 receiver; InnovaSea Systems, Inc., Bedford, NS, Canada), to detect fish as they were released.

## Description of the acoustic array

The region-wide acoustic array consisted of 133 acoustic receivers placed in strategic positions throughout Passamaquoddy Bay and the outer Bay of Fundy (Fig. 1). Receivers consisted of a mixture of VR2AR, VR2W, and VR2Tx receivers (InnovaSea Systems, Inc., Bedford, NS, Canada) that were deployed in advance of the release of the tagged salmon, generally between May to July of 2021. Most receivers were retrieved in late November and early December of 2021 for download providing an approximately three-month monitoring duration of the tagged escapees. A few of the receivers remained deployed in nearby rivers over the winter period (retrieved in May 2022) to monitor for fish passage. All raw receiver data were uploaded to the Ocean Tracking Network (OTN) Data Portal and are publicly available (https://members.oceantrack.org/project?ccode=QRET). For the purposes of the downstream analyses, individual receivers were grouped into subsets (array) that were within close proximity to one another. The arrays were then organized into broad geographical regions of the bay (i.e. sections).

Post-release monitoring and statistical analyses

All analyses were conducted using the R programming language (Version 4.1.1) in R Studio (Version 1.4.1717; R Core Team, 2021). Raw datafiles and R scripts can be found in an open repository on GitHub (https://github.com/mlaw27/Salmon\_escapee\_telem\_2021).

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From the raw receiver files, tag codes assigned to escaped salmon were filtered to obtain only detections relevant to this study. Temperature and depth sensor values from the detections were then corrected using factory calibration values. 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.

Parameters concerning predation events constituted the first portion of our analysis. Predation events were recognized by tag temperature data and split into two main predator groups, endothermic or mesothermic, which were delineated by maximum temperature values. In the case of endothermic predators, marine mammals (e.g., seals, whales) and avians (e.g., sea birds) were representative, with a core body temperature beyond 35°C (Note: temperature sensors were capped at 35°C making it difficult to delineate birds from mammals). However, a maximum temperature value above or equal to 30°C was used as a threshold for category recognition in the event that stomach temperatures had not equilibrated at the time of detection. In the case of mesothermic predators, sharks (e.g., lamnids) and large fish (e.g., tuna) were representative, with core body temperatures above ambient (i.e., > ~14°C). Thus, we set the range of maximum temperatures - that constituted consumption by a mesothermic predator equal to or greater than 18°C, but less than 30°C. For salmon preyed upon by mesothermic predators, we verified that temperatures were not a result of 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 scrutinized to discern consumption by an ectothermic predator or from tagging-related mortality. This was accomplished with through comparison with concurrent depth sensor values, where abnormalities in the animals depth profile might indicate predation. In this study, four fish appeared to remain stationary (i.e. depth not actively changing, detected at a single array), which were removed from the dataset as they likely represented tagging-related mortality.

Once salmon had been assigned to their respective predator category (i.e. endothermic, mesothermic, not predated), we determined overall predation rate as a percentage of the total

number of tagged salmon and on 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 thus considered to be alive/free-swimming. Following predation, the fish would have warmed in the stomach of the predator. The time difference between release and the last ambient temperature detection constituted survival time, and served as a proxy for predation event timing. Using the last ambient temperature, we were also able to relate the detection to respective receiver/section to identify where the predation event likely occurred.

Following identification of predation timing, the raw detection file was adjusted to remove all detections associated with the consumption of each fish 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 time 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, 1-2, 2-4 h). For individual fish, the maximum, minimum, and average experienced depth was tabulated. These values were then averaged across all tagged 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 with a maximum interval between discrete events of 15 min. Analyses also incorporated the section order to map fish movement between various arrays/section in the larger receiver network. A jump warning of two sections was used to identify if a fish traversed numerous arrays without detection, highlighting potential receiver detection inefficiencies, that could lead to misinterpreted findings.

The results of the residency computation were used to determine a number of relevant indices for explaining the post-release behavioural of escaped salmon. We first determined the time to leave the release site (Fairhaven), which constituted the difference from the release time to the last detection on the Fairhaven array (applicable only to fish with first receiver detections at Fairhaven). Additionally, fish that were predated upon at the Fairhaven site, without ever leaving the site, were removed from the analysis so as to not inflate leave times. Interestingly, four fish were not initially detected at any of the Fairhaven array receivers (N = 7), but were

rather first detected on arrays which flanked the site (i.e., Western Passage arrays). For these fish, 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 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, where an individual was faced with either heading into Passamaquoddy Bay (north) or out to the Bay of Fundy (south; see Fig. 1 insert). The initial direction of the fish was noted and then we recorded their subsequent section movements to develop rough movement profile following release from the aquaculture site.

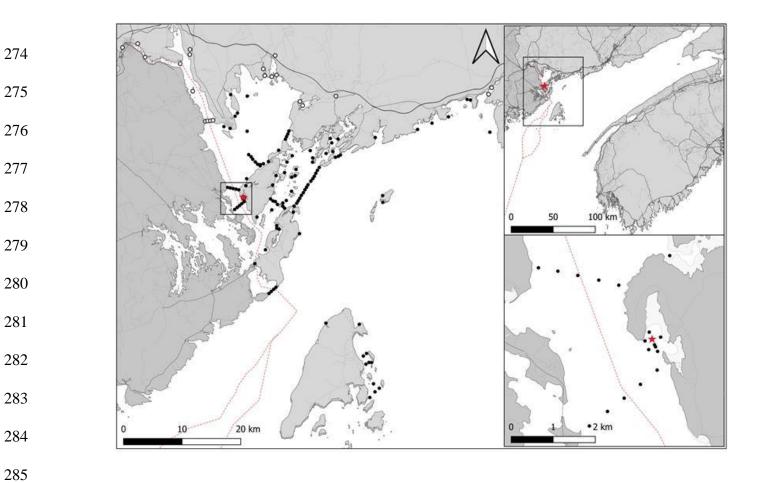
Residency timing within the first 96 h following release was tabulated. Here, residency time values were binned on their duration from release on a 24-, 48-, and 96-h basis. For each time frame, we summed the total amount of resident time at each section in our network. In addition to total time, we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

We were also interested in identifying if any of the escaped salmon reached a nearby river system and, if so, how many frequented and how much time was spent there. To accomplish this, we filtered out section residency times associated with local river systems, which included St Croix, Bocabec, Digdeguash, Magaguadavic, and Lepreau Rivers. All of these systems had receiver arrays placed in the estuary - with downstream and upstream locations to determine directionality. As with the first 96 h residency calculations, we computed both total residency time per section and on a per fish basis. The number of fish detected in rivers was noted and we identified if multiple river systems were visited.

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**Figure 1:** Overview map highlighting the positions of the receivers used in this study and the release site (red star) within the Bay of Fundy/Passamaquoddy Bay. Black circles represent oceanic receiver placements while white circles represent those associated with river systems. The red dotted line represents the Canada-USA boarder.