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Complete List of Authors:	Keyser, Freya; Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada Wringe, Brendan; Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada; Memorial University of Newfoundland Department of Biology Jeffery, Nicholas; Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada; Dalhousie University Department of Biology Dempson, J. Brian; Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada Duffy, Steven; Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada Bradbury, Ian; Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada; Memorial University of Newfoundland Department of Biology; Dalhousie University Department of Biology
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PREDICTING THE IMPACTS OF ESCAPED FARMED ATLANTIC SALMON ON WILD SALMON POPULATIONS

Freya Keyser¹, Brendan F. Wringe^{1,2}, Nicholas W. Jeffery^{1,3}, J. Brian Dempson¹, Steven Duffy¹, and Ian R. Bradbury^{*1,2,3}

¹ Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada, St. John's, NL A1C 5X1
² Memorial University of Newfoundland, St. John's, NL A1C 5S7
³ Dalhousie University, Halifax, NS B3H 4R2

Emails:
freya.keyser@dfo-mpo.gc.ca
brendan.wringe@dfo-mpo.gc.ca
nick.jeffery@dfo-mpo.gca.ca
brian.dempson@dfo-mpo.gc.ca
steven.duffy@dfo-mpo.gc.ca
ian.bradbury@dfo-mpo.gc.ca

*Corresponding author: Ian Bradbury, Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada, St. John's, NL A1C 5X1. Phone: 709-772-3869, fax: 709-772-4188

Abstract

The escape of Atlantic Salmon (*Salmo salar*) from aquaculture facilities can result in both negative genetic and ecological interactions with wild populations, yet the ability to predict the associated risk to wild populations has remained elusive. Here we assess the potential of a spatiotemporal database of aquaculture facility locations, production estimates and escape events to predict the distribution of escaped farmed salmon and genetic impacts on wild populations in the Northwest Atlantic. Industry production data, reported escape events, and in-river detections of escaped farmed salmon were collected from across the Northwest Atlantic. Genetic estimates of impact were obtained using single nucleotide polymorphisms (95 loci) representing aquaculture and wild salmon throughout the region (30 populations, 3048 individuals). Both the number of escaped farmed salmon detected at counting facilities and the magnitude of genetic impacts were positively correlated with a cumulative spatial measure of aquaculture production. Our results suggest that the risk of escapees and genetic introgression from wild-farmed salmon interactions can be assessed using information on farm production characteristics. This represents a first step in predicting the impact of existing cage-based farms on wild Atlantic salmon.

Draft

Introduction

The Atlantic Salmon (*Salmo salar*) is one of the most valuable aquaculture fish species and the salmon aquaculture industry is expanding worldwide (FAO 2016). Despite continued refinement of production methods and technology, the escape of aquaculture individuals into the wild environment occurs regularly due to weather events, predator attacks, or losses during regular operations (Bentsen and Thodesen 2005; Glover et al. 2017; Jensen et al. 2010). Estimates suggest two million farmed Atlantic Salmon escape into the North Atlantic Ocean every year (Schiermeier 2003) with negative evolutionary and ecological impacts on wild salmon populations (Bolstad et al. 2017; Fleming et al. 2000; McGinnity et al. 2003; Skaala et al. 2012; Weir et al. 2005). Farmed salmon are both genetically distinct (reviewed by Glover et al. 2017), and phenotypically divergent from wild salmon, with differences such as accelerated growth rates, delayed maturity, behavioural differences and reduced immunity (Fleming et al. 1996; Jonsson and Jonsson 2006), contributing to reduced survival in the wild (Fleming et al. 2000; McGinnity et al. 2003; McGinnity et al. 1997; Skaala et al. 2012). Interbreeding between escaped farmed salmon and wild salmon has been widely documented (Bourret et al. 2011; Clifford et al. 1998; Crozier 1993; Glover et al. 2013; Glover et al. 2012; Karlsson et al. 2016; Skaala et al. 2006) and can lead to phenotypic changes in native wild populations (Bolstad et al. 2017; Fraser et al. 2010), which in turn can reduce the fitness of wild populations (Fraser et al. 2008; McGinnity et al. 2003; Verspoor et al. 2015). Although interactions between wild and escaped domestic Atlantic Salmon have repeatedly been identified as significant threats to the persistence of wild populations (COSEWIC 2010; DFO 2008; DFO 2013; Forseth et al. 2017; USASAC 2016), methods to predict and mitigate impacts remain lacking.

Wild Atlantic Salmon populations in the Northwest Atlantic have declined in recent decades (Chaput 2012; ICES 2016) resulting in the closure of both commercial and recreational fisheries (COSEWIC 2010; DFO 2008; DFO 2013; USASAC 2016). The factors responsible for these declines likely vary across regions (COSEWIC 2010; Parrish et al. 1998), however, genetic interaction with escaped farmed salmon has been identified as a major concern (DFO 2013; Ford and Myers 2008; Verspoor et al. 2015). Previous attempts to quantify the frequency of escape events and distribution of escaped farmed

salmon in rivers have been limited, but available data suggest they are present in the majority of rivers near (<300 km) the industry in Atlantic Canada (Morris et al. 2008). Moreover, recent genomic evidence indicates significant hybridization between wild and farmed salmon has occurred in southern Newfoundland (Wringe et al. in review), which may further negatively affect threatened wild salmon populations (COSEWIC 2010; DFO 2013). Despite continued evidence of genetic impacts on wild salmon populations (Bolstad et al. 2017; Bourret et al. 2011; Clifford et al. 1998; Crozier 1993; Fraser et al. 2008; Fraser et al. 2010; Glover et al. 2013; Glover et al. 2012; Karlsson et al. 2016; McGinnity et al. 2003; Skaala et al. 2006; Verspoor et al. 2015) the ability to predict population-level risks to inform management efforts remains limited. Predictive models linking aquaculture industry production characteristics, the distribution of escaped farmed salmon, and impacts on wild populations are ultimately needed to assess and manage risks to wild Atlantic Salmon. A first step in developing these predictive models is to identify variables that are associated with the distribution of escaped farmed salmon or associated impacts on wild populations.

The main objective of this study is to identify factors associated with distribution of escaped farmed salmon in Atlantic Canada and the genetic risk from escaped farmed salmon to wild salmon populations. Results could be used to inform aquaculture management regarding the conservation of wild populations. Specifically, we explored the ability of a spatiotemporal database of aquaculture facility and production data to predict (1) the distribution of escaped farmed salmon and (2) the genetic impacts on wild populations in Atlantic Canada. This work builds on the database and analysis published by Morris et al. (2008) of escaped farmed salmon detections from Maine and Atlantic Canada and extends previous analyses with the inclusion of industry production data and new genetic measures of impact on wild populations.

Methods

Data retrieval and sampling

Data compiled for this study were of three types: (1) aquaculture site production and location data for the Northwest Atlantic region; (2) reports of escaped farmed salmon in Northwest Atlantic rivers (Table S1); (3) population genetic estimates of introgression

between wild and farmed Atlantic Salmon from southern Newfoundland. For the aquaculture site locations and production data, the resolution of available information differed by jurisdiction, so analyses were designed to address any associated assumptions and limitations due to these differences. The numbers listed below represent all licensed aquaculture sites in Newfoundland, New Brunswick, Nova Scotia and Maine for the period of 2005-2015. Inventory data were obtained for a total of 78 aquaculture sites in Newfoundland from the period of 2005-2015 (provided by G. Perry, Aquaculture Management, Newfoundland Region, Fisheries and Oceans Canada (DFO); Fig S1A). Using the reported information on farm-stocked fish (e.g. introductions, transfers, harvest numbers, and mortalities), an annual estimate of the average number of fish per site was calculated. For the province of New Brunswick, data were obtained during annual provincial monitoring activities (i.e. actual counts of fish in cages on the day of monitoring) for 89 sites during the years 2012 to 2015, while annual stocking license data (i.e. the maximum number a site is permitted to stock annually) were available for 2005-2012 (provided by T. Lyons, New Brunswick Department of Environment and Local Government, and G. Cline, Aquaculture Management, Maritimes Region, DFO). Detailed inventory data for Nova Scotia were not available at the time of analysis so annual stocking license information for 18 sites during the years 2005-2015 were used (provided by E. Parker, Aquaculture Management, Maritimes Region, DFO). Annual stocking license data from 17 sites in Maine were also available for 2005-2015 (provided by D. Bean, National Marine Fisheries Service, National Oceanic and Atmospheric Administration).

Due to discrepancies in aquaculture production monitoring between provinces/states, for all region-wide analyses, specific production numbers were not involved. Instead, each aquaculture site was assigned a value depending on whether it was fallow (0) or stocked (1) for each year. For analyses including genetic data (see below), the spatial extent was restricted to Newfoundland so that annual estimates of fish per site could be used. Further details are provided in analysis section below.

Reports of escaped farmed salmon detected in rivers throughout the study region were obtained via opportunistic surveys and dedicated sampling and monitoring programmes (Table S1; Fig S1B), as in Morris et al. (2008). Academic and governmental

Atlantic Salmon research programmes, aquaculture management authorities, and conservation organizations (e.g., Atlantic Salmon Federation) were asked to provide information on any escaped farmed salmon encountered. New reports of river-specific presence (with counts when available) or absence were added to the database compiled by Morris et al. (2008). Additional records were obtained from monitoring facilities (counting fences) in the Magaguadavic River in NB (since 2002), and Garnish River (since 2015) and Conne River in NL (since 1987). Scientific gillnetting and angling were also conducted in several Newfoundland rivers during the fall seasons of 2015 and 2016.

Genetic analysis

Genetic analysis was used to quantify the degree of introgression between escaped farmed salmon and wild populations in southern Newfoundland. Data examined included a combination of previously published sources including: (1) single nucleotide polymorphism (SNP) array data (Bradbury et al. 2015; Moore et al. 2014; Wringe et al. in review), and (2) targeted genotyping of a subset of this larger array using a 96 SNP assay (Wringe et al. in review). The 5568 SNP array developed by the Centre for Integrative Genomics (CIGENE, Norway) (Bourret et al. 2013a; Bourret et al. 2013b) was used for 611 individuals from the Maritimes and southern Newfoundland. For the targeted genotyping of 96 SNPs (Wringe et al. in review) a further 2703 individuals from southern Newfoundland were genotyped. The total SNP data set was comprised of a total of 3314 individuals, including 156 individuals (adults) of known aquaculture ancestry, and from 47 Atlantic Salmon populations wild Maritimes and southern Newfoundland, plus two aquaculture populations. 15 rivers from southern Newfoundland had data for two years (2014 and 2015), providing a total of 64 populations spanning the Maritimes and southern Newfoundland. Results presented here are limited to southern Newfoundland populations (n=26).

The methodology for the 5568 SNP array is described in detail in Bradbury et al. (2015). In summary, only SNP genotypes with a call rate greater than 0.95 were retained and SNPs were removed where minor allele frequencies were <0.05 , or were missing in >0.15 of individuals. For the targeted genotyping of the subset of 95 SNPs, SNP Type assays (Fluidigm) per the manufacturer's protocols were used (Wringe et al., in review).

This SNP genotyping was performed using 96.96 genotyping Integrated Fluidic Circuits, read on an EP1 (Fluidigm) and analyzed using SNP Genotyping Analysis software (Fluidigm). Based on reanalysis of samples and following Pompanon et al. (2005), the genotype error rate was calculated to be 0.01% and consistent with other studies (0 - 0.2%, see Hess et al. (2015), Jones et al. (2015), Larson et al. (2014), Petrou et al. (2014), Seeb et al. (2009)).

We used the Bayesian clustering software STRUCTURE (Pritchard et al. 2000) to estimate population admixture values between domestic and wild populations (i.e. Q-values ranging from 0 to 1 respectively). The R package *ParallelStructure* (Besnier and Glover 2013) was used to estimate K, the putative number of populations, from 1-30, replicated three times each. Each run had a burn-in of 100,000 Markov chain iterations, followed by 500,000 iterations. STRUCTURE was run with the admixture model and without population location priors. The degree of introgression with escaped farmed salmon was then calculated based on the mean proportion of each population's genome that could be attributed to the domestic baseline population.

Spatial analysis: Propagule pressure

The river-specific risk from escaped farmed salmon was spatially quantified using propagule pressure, adapted from invasive species research where it represents the intensity of anthropogenic introductions of non-native species (Colautti 2005; Consuegra et al. 2011; Copp et al. 2007). In this study, propagule pressure was calculated for each river as either the presence of fish at an aquaculture site each year (from 2005-2015), or the number of fish stocked each year, divided by the distance to that site, and summed across all sites and years. For fine-scale analyses involving only Newfoundland, annual estimates of the number of fish per aquaculture site were divided by their respective distances to each river. That is:

$$\text{Propagule pressure for a given river } (R) = \sum_{i,y=1}^S \frac{F_{i,y}}{LCD(S_{i,y} \text{ to } R)}$$

where $S_{i,y}$ represents an aquaculture site in a given year (y), R each river, $F_{i,y}$ the number of fish at site S_i each year OR presence of fish in aquaculture site S_i each year (0 or 1), and LCD represents the least-cost distance function.

For region-wide analyses, as numbers of stocked fish were not available for all jurisdictions, a value of one was assigned to aquaculture sites that were stocked in a given year, and unstocked sites were excluded ($0/LCD = 0$). The propagule pressure variable allows a river-specific measurement of aquaculture intensity that relates to both the proximity of aquaculture sites and the production levels of the sites. As such, rivers that were close to many aquaculture sites or close to a few very high-production sites would have stronger propagule pressures than rivers that were far from aquaculture sites or only near smaller-scale production sites. Propagule pressures were calculated for all rivers in the compiled escaped farmed salmon database, and any rivers with genetic data, including Maritimes rivers reported in Moore et al. (2014) that were not included in this study's analyses (Fig S3).

To explore the utility of propagule pressure in predicting aquaculture impacts, three measures of impact were derived from the collated data: the presence of escaped farmed salmon in rivers, the number of escaped farmed salmon in rivers, and the amount of introgression observed in populations as reflected by the population mean Q-values resulting from the Newfoundland STRUCTURE analysis (see above). Log-log regression models and GLMs (using Poisson and binomial distributions) were applied to examine the relationship between propagule pressure and these measures of impact. T-tests and/or Chi-square tests were used to assess the significant effects of explanatory variables on the measures of impact. AIC (Akaike information criterion), or corrected Akaike information criterion, (AICc), where appropriate were used to compare and weight models according to their fit to the data and complexity. River size (measured as axial distance in kilometres) was also used as an explanatory variable to address the influence of population size. The R statistical environment (version 3.3.2; R Development Core Team 2016) and RStudio (version 1.0.136; RStudio Team 2015) were used for all visual and statistical analyses. Maps were produced using shapefiles downloaded from GADM.org via package *raster*. A package containing a function to calculate propagule pressure was written to facilitate this analysis and is available for download from GitHub via GNU General Public License (Package *AQpress*: <https://github.com/freyakeyser/AQpress>).

Results

In total, an additional 217 records were added (annual river reports of escaped farmed salmon presence or absence) to the Morris et al. (2008) adult farmed escapee database, for a grand total of 467 records from the 1980s to 2016 (Table S1). New records of presence or absence of escaped farmed salmon for 17 rivers for which no information previously existed were collected (mostly in Newfoundland; Morris et al. (2008) only presented records for two rivers in NL), as well as new records of presence or absence for a separate 17 rivers already in the Morris et al. (2008) database. These additions bring the total number of rivers in the database to 112 rivers (Table S1). A total of 1091 escaped farmed salmon have been detected since the publication of the (Morris et al. 2008) database, and our updated database contains detections of 9236 escaped farmed salmon in Northwest Atlantic rivers. Rivers in the Passamaquoddy Bay (outer Bay of Fundy) area had higher numbers of escaped farmed salmon reported than other areas (Fig S1B). In addition to the database of escaped farmed salmon reports, this study also incorporated genetic information for 29 Newfoundland rivers (Fig S1A; Fig S3; Table S2).

The presence of escaped farmed salmon in each river based on all data including opportunistic, standardized sampling data, and reports of escaped farmed salmon in each river (p -value = 0.717, AIC = 35.7, null deviance = 31.8) were not significantly correlated with region-wide propagule pressures (Table S3; Fig S4; Table S4; Fig S5). The total number of escaped farmed salmon detected using all data was significantly related to propagule pressure (p -value < 0.05, Table S4), although very few data points fell within the model's 95% confidence interval (Fig S5, AIC = 3671.024, null deviance = 3687.0). However, when only counting fence or fishway counts were used in the GLM (Poisson distribution), there was a significant positive exponential effect of propagule pressure (p -value < 0.001) on the number of escaped farmed salmon in rivers, and the model fit was improved with AIC of 1064.5 and null deviance of 1941.7 (Fig 1A; Table S5). The removal of the reports from Magaguadavic River (an outlier value with 379 escaped farmed salmon detected) did not change this effect, though the AIC decreased to 213.5 (Fig 1B; Table S5).

Individual Q-values for wild-collected Newfoundland samples ranged from 0.006 to 0.990 (Fig S6), representing both pure wild individuals (0) and pure aquaculture

individuals (1). Population mean Q-values ranged from 0.028 to 0.737, and rivers in Bay d'Espoir and Fortune Bay generally had higher Q-values than other rivers. Most (70 %) of the wild Newfoundland individuals had Q-values < 0.5 (Fig S6). There was a significant positive log-log relationship ($p\text{-value} < 0.01$, $R^2 = 0.279$) between population mean Q-value and localized propagule pressures in Newfoundland (Fig 2A; Table S6), and a weak negative log-log relationship ($p\text{-value} > 0.05$, $R^2 = 0.071$) between population mean Q-value and river size, measured as axial distance in kilometres (Fig 2B; Table S6). A GLM using both propagule pressure and river size ($AIC = 73.1$, null deviance = 26.8) identified a significant log-log effect of propagule pressure ($p\text{-value} = 0.018$) on population mean Q-value, but no significant effect of river size ($p\text{-value} = 0.274$, Table S7), and model comparisons using AICc determined that the strongest model for population mean Q-value did not include river size as an explanatory variable (Table S6).

Discussion

Escaped farmed Atlantic Salmon have been associated with both negative genetic and ecological interactions with wild populations (Bolstad et al. 2017; Glover et al. 2017; Verspoor et al. 2015). Accordingly, the ability to predict risk to wild populations is central to wild salmon conservation and aquaculture management. We evaluated the utility of industry production data and reported escape events to predict the distribution of escaped farmed salmon and the genetic impacts on wild populations in the Northwest Atlantic. Results indicated that increased numbers of escaped farmed salmon detected at counting facilities and an increased magnitude of genetic impacts in wild populations were both positively correlated with increased propagule pressure. These relationships suggest that the location and size of aquaculture facilities directly affects the magnitude of risk to wild salmon populations from escaped farmed salmon. Characterizing this risk represents an important step toward predicting the impact of cage-based Atlantic Salmon aquaculture on wild Northwest Atlantic Salmon populations. Relationships between the magnitude of aquaculture production and escaped farmed salmon occurrence and impacts have been previously observed in the Northeast (Fiske et al. 2006; Hansen et al. 1999), and Northwest Atlantic (Carr et al. 1997), but spatial variables have not been considered. This work extends previous studies that have documented the distribution of escaped

farmed salmon in Atlantic Canada (Morris et al. 2008) and provided evidence of hybridization and introgression among wild and farmed salmon following escape events (Wringe et al., in review) by using these data sources to test a novel approach to predict future impacts.

Escaped farmed salmon have been detected everywhere net-pen salmon aquaculture occurs with reports of millions of individuals escaping net-pens each year (Glover et al. 2017). In eastern North America, our analysis includes a total of 9236 escaped farmed salmon that have been detected since the 1980s, primarily in rivers proximate to the aquaculture industry. This is likely to represent a minimum number of detections since identifying and quantifying escapes will depend on the location, timing, frequency and extent of surveys carried out for this purpose. Information on escaped farmed salmon in eastern North America indicated that rivers in Passamaquoddy Bay, New Brunswick, and southern Newfoundland generally had the highest numbers of escaped farmed salmon. Both areas have high aquaculture production, and high numbers of escaped farmed salmon were previously reported by Morris et al. (2008). The distribution of escaped farmed salmon detected in freshwater has been correlated with the distribution of the industry in both Norway and Scotland (Glover et al. 2017). However, escaped European farmed salmon have also been reported as far away as the Arctic Ocean (Jensen et al. 2013), the Faroe Islands area (Hansen and Jacobsen 2003), and waters of Greenland (Hansen et al. 1997). The distribution of rivers in which escaped farmed salmon were detected here is well within the reported dispersal distance of escaped farmed salmon (Hansen and Youngson 2010) and consistent with previous analyses (i.e. Morris et al. 2008).

Admittedly, reports of the presence of escaped farmed salmon are often not an unbiased sample, with opportunistic or targeted non-random sampling the norm. Research and monitoring has also focused primarily on Passamaquoddy Bay (Carr and Whoriskey 2006; O'Reilly et al. 2006; Whoriskey and Carr 2001) and southern Newfoundland due to the concentration of the aquaculture industry and consequent prevalence of escaped farmed salmon in these areas (DFO 2014; Verspoor et al. 2015). For this reason, the current dataset for eastern North America likely lacks sufficient power to detect escaped farmed salmon at low prevalence outside these core areas.

Furthermore, differences in data types and how they were collected means directly comparing counts obtained at the targeted monitoring facilities with intermittent or anecdotal counts (such as those obtained by angling, gillnetting, snorkel counts, etc.) may be difficult. As such, this underscores the need for standardized reporting and sampling methods, such as occurs in Norway (Anon 2017), to better quantify the distribution of escaped farmed salmon across the Northwest Atlantic region. This explains why we reported associations between propagule pressure and reports of escaped farmed salmon at fixed monitoring locations and excluded other data sources. This observation also supports the continued use of genetic monitoring (Bourret et al. 2011; Glover et al. 2017; Wringe et al. in review; Mjølnerod et al. 1997) for impacts to gain a complete understanding of escaped farmed salmon distribution and the consequences for wild populations.

Accordingly, evidence of genetic impacts due to interbreeding with escaped farmed salmon continues to accumulate on both sides of the North Atlantic (Bourret et al. 2011; Glover et al. 2017; Wringe et al. in review; Mjølnerod et al. 1997; Muhlfeld et al. 2017). Our observation of a significant association between propagule pressure and the amount of introgression between farmed and wild salmon present supports the use of propagule pressure as a predictor of genetic impacts in wild populations. Similar links between propagule pressure and genetic introgression have been detected elsewhere (Bennett et al. 2010; Consuegra et al. 2011; Lamaze et al. 2012; Marie et al. 2012), supporting the use of propagule pressure as a management tool (Pritchard et al. 2007). Moreover, associations between the incidence of escaped farmed salmon in rivers, and river-specific estimates of temporal genetic change (Glover et al. 2012) and admixture (Glover et al. 2013; Heino et al. 2015; Karlsson et al. 2016) have been reported. Our relationship between the number of escapees detected at counting fences and propagule pressure supports these findings.

Interestingly, although evidence of a relationship between the magnitude of genetic introgression and river size has been reported elsewhere (Glover et al. 2012; Glover et al. 2017; Heino et al. 2015; Wringe et al. in review) it was not significant in this study. Glover et al. (2012 and 2013) suggested that this relationship may be explained by larger (more robust) wild populations resisting introgression via increased competition on

the spawning grounds and at juvenile stages. In contrast, our analysis suggests propagule pressure was the dominant factor influencing the amount of introgression detected. We restricted this analysis to the southern Newfoundland region because (1) the availability of industry production data allowed direct inclusion in our calculation of propagule pressure (i.e. rather than presence/absence of stocked sites) and (2) increased divergence between wild and aquaculture salmon associated with a New Brunswick origin of the current domestic line increased the power to quantify levels of introgression with the marker panel used. Extension of the analysis to other regions may be possible in the future with improved industry data and targeted genomic panels (Wringe et al. in review).

We suggest that propagule pressure could be a useful step toward the prediction of escaped farmed salmon impacts in Canada. The relationships with propagule pressure would likely benefit from the inclusion of more data, and/or data collected specifically to measure range-wide impacts, as has occurred with success in Norway through a standardized monitoring program and formal risk assessment (Taranger et al. 2015). Error in our study may also be attributed to the geographic distances measured, better resolution of the distances involved or the incorporation of behavioural data on the movements of escaped farmed salmon may improve these relationships. The lack of consistency among available provincial/state inventory and/or stocking license records prevented an in-depth region-wide analysis of aquaculture production in this study. Furthermore, we identified significant differences in escape event reporting requirements across the region. Improved data collection on inventory and reporting requirements for escape events should improve understanding of escaped farmed salmon distribution, behaviour and impacts (Jensen et al. 2010). Despite these challenges, we were able to calculate the propagule pressure variable successfully across the study region. Accurate estimates of propagule pressure could potentially be directly incorporated into spatial planning as a way to mitigate impacts of escaped farmed salmon on wild salmon populations.

This study has updated the current knowledge on the distribution and prevalence of escaped farmed salmon in rivers of the Northwest Atlantic, and represents the initial steps in testing the use of a cumulative spatial measure of aquaculture production to predict the impacts of escaped farmed salmon on wild populations. The propagule

pressure associations with impact identified here provide a useful metric that could be integrated into a risk management framework to inform aquaculture management activities and identify mitigation strategies.

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Figure captions

Fig 1. The numbers of escaped farmed salmon detected at counting fences and fishways and propagule pressures in Atlantic rivers. Solid curves show predicted relationships using generalized linear models with Poisson distribution including and excluding the Magaguadavic River (panels A and B respectively). Dashed curves represent 95% confidence intervals of models. Both models are statistically significant (p-values <0.001). Point shapes correspond to the province or state of detections. Propagule pressures were calculated region-wide using active (stocked) aquaculture sites from 2005-2015.

Fig 2. Propagule pressures, river sizes, and mean population Q values for Newfoundland rivers (points). Panel A shows log-log regression between propagule pressure and mean Q value (p-value < 0.01). Panel B shows log-log regression between river size and mean Q value (p-value > 0.05). Propagule pressures were calculated using Newfoundland inventory data (2005-2015) and river sizes are axial lengths (km). Solid curves show predicted effects on response; dashed curves show 95% confidence intervals.

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