EXPERIMENTAL AND GENETIC EVALUATION OF THE IMPACTS OF HYBRIDIZATION AND INTROGRESSION OF FARMED ATLANTIC SALMON (*SALMO SALAR*) INTO WILD POPULATIONS

Coral San Roman

A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of

Master of Science, Department of Ocean Sciences, Faculty of Science

Memorial University of Newfoundland

June 14th, 2024

St. John's, Newfoundland and Labrador

Abstract

Marine aquaculture of Atlantic salmon (*Salmo salar*) has been ongoing for several generations, during which farmed fish have experienced different selection pressures relative to their wild counterparts. The number of domesticated individuals exceeds wild individuals, and escape events occur regularly, yet current understanding of the long-term effects of interbreeding on population productivity and life history traits is limited. Therefore, understanding the impacts of farm introgression on relative survival and performance of wild Atlantic salmon is critical to manage the impacts of aquaculture escapees on threatened wild populations. In my first data chapter, I compared cross specific patterns of survival, size, sex ratio, and precocial male maturation over a 28-month period from an experimental release of wild, farm, and reciprocal F1 hybrid fry in southern Newfoundland. Trends in cross type survival changed over the study period, while size trends remained consistent. Parr with wild mothers had the highest recapture at 3-months, while aquaculture offspring had the highest rates of recapture at 15-28 months. Throughout the duration of the study, pure farm and wild-mother hybrids consistently being larger than wild individuals, and rates of parr maturation differed by sex and cross type. In my second data chapter, I evaluated the presence of hybridization and subsequent introgression of escaped farmed salmon into 18 southern Newfoundland rivers and estimated the number of successfully breeding escapees over an 8-year period encompassing three reported escape events. Results from these analyses demonstrated an increasing proportion of backcross-wild hybrid individuals with a consistent occurrence of F1s every year, and most rivers exhibited evidence of farm admixture. Additionally, at least one successfully breeding farm parent was detected annually. Overall, the research presented here provides insight into the consequences of escape events and direct genetic interactions and informs conservation actions for at risk populations.

Acknowledgements

I would first like to thank my supervisors, Dr. Ian Fleming and Dr. Ian Bradbury, for their invaluable advice, continuous support, and guidance during my MSc study. Thank you for allowing me to learn from you and grow as a person and a young scientist; I could not have undertaken this journey without you. I am also grateful to my committee member, Dr. Sarah Lehnert, who generously provided knowledge and expertise on my project and thesis.

I would also like to thank my fellow member of the Fleming Lab, Victoria Heath, for the helpful feedback on my work and moral support, as well as members of the Bradbury Lab for helping me with the genetic data analysis both individually and during lab meetings. Thank you to Steve Duffy for coordinating the field logistics of my project in addition to assisting with field work. Special thanks to Dr. David Schneider for his help with the statistical analyses used in my project and to Danielle Davenport for her assistance running relatedness analyses. Thank you also to my classmates and cohort members at the Ocean Sciences Centre who impacted and inspired me, I am very grateful to have had the chance to form friendships and learn alongside you all. Last, but certainly not least, I would like to thank my close friends Rachael Moran and Jillian McGroarty, as well as my family for the endless advice and support throughout my degree program, their belief in me has kept my spirits and motivation high during this process.

I am grateful to have had this research opportunity through funding provided by the Ocean and Fresh-water Science Contribution Program of Fisheries and Oceans Canada, the Program for Aquaculture Regulatory Research of Fisheries and Oceans Canada, and the Ocean Frontier Institute (OFI) through an award from the Canada First Research Excellent Fund.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations and Symbols

W♀hyb	Wild-mother hybrid
F♀hyb	Farm-mother hybrid
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
glm	Generalized linear model
lm	Linear model
LR	Likelihood Ratio
ANODEV	Analysis of Deviance
SE	Standard error
BCW	Backcross wild
BCF	Backcross farm
BDN	Bay du Nord River
BTB	Bottom Brook
CNR	Conne River
DLR	Dollards Brook
GAR	Garnish River
GBB	Grand Bank Brook
GLP	Grand La Pierre
LMS	Lamaline Salmonier River
LTR	Little River
LHR	Long Harbour River
NEB	Northeast Brook
NWR	Northwest River
OBB	Old Bay Brook
SMB	Simms Brook
SEB	Southeast Brook
TRB	Tailrace Brook
TBB	Taylor Bay Brook
TEB	Terrenceville Brook

Co-Authorship Statement

The work described in this thesis was conducted by Coral San Roman under the supervision of Ian Fleming and Ian Bradbury, who conceptualized and designed the study. Shahin Islam created the cross types of Atlantic Salmon used for the study described in Chapter 2 and reared them to release. Steve Duffy chose field sites and coordinated field work for data collection for Chapters 2 and 3. Melissa Holborn conducted the genetic analysis required for Chapter 3, and Danielle Davenport analyzed these data using COLONY to identify hybrid family structure in Chapter 3. Data analyses were conducted, and manuscripts written by Coral San Roman with assistance from Ian Fleming and Ian Bradbury, with contributions to the editing process from Sarah Lehnert. Current and anticipated publications arising from this thesis are co-authored by Coral San Román, Ian R. Bradbury, Samantha E. Crowley, Steven J. Duffy, Shahinur S. Islam, and Ian A. Fleming1 (Chapter 2); and Coral San Román, Ian A. Fleming, Melissa K. Holborn, Steven J. Duffy, Nicole Smith, Amber Messmer, Danielle Davenport, Ian R. Bradbury (Chapter 3).

Chapter 1: General Introduction

The domestication of animals and plants can be traced back several thousand years (Tang et al. 2010, Zeder 2012). Humans have selected and brought a wide variety of species under domestication for several uses such as food, work, and materials. Therefore, there is an extensive range of pathways that have been taken for different groups to reach domestication, which depended on several species specific biological constraints, as well as the different cultural contexts under which they evolved (Zeder et al. 2006, Larson & Fuller 2014). The process can be considered a unique form of evolution as it is a co-evolutionary interaction that leads to the development of a domesticated species, whose growth and reproduction are heavily controlled by another species that is benefited by this relationship (Hale 1969, Clutton-Brock 1994, 2014). In contrast, some other approaches, most commonly among research focusing on plant domestication, define this as a mutualistic relationship in which both humans and domesticate are benefited by their interaction (Harlan et al. 1973, Blumler 1996, Harris 1996, Smith 2001). However, domestic strains or species often differ substantially from their wild relatives in various aspects. The process of domestication has resulted in a shared set of traits among domestic species, collectively known as the "domesticated phenotype" or "domestication syndrome". For instance, domesticated crops have been observed to show synchronization of flowering time, enlargement of reproductive organs, loss of seed dispersal, and increased apical dominance, among other unique characteristics to domesticated species (Wilkins et al. 2014). Similarly, domestic animals have also been found to display an increased reproductive capacity and earlier sexual maturation (Setchell 1992, Künzl et al. 2003), as well as shorter skulls and reduced relative brain size compared to their wild counterparts (Kruska 1988, Brusini et al. 2018, Katajamaa & Jensen 2020, Katajamaa et al. 2021, Balcarcel et al. 2022). Such distinctive variations demonstrate the substantial impact that artificial selection can exert on shaping the evolution of species.

There is extensive literature regarding domestication of wild animals, more recently using archeological and genetic approaches (Price 2002, Tang et al. 2010). Findings from these studies suggest that farm animals, such as pigs and chickens, were domesticated 9,000-8,000 years ago (Giuffra et al. 2000, Tixier-Boichard et al. 2011), following the development of agriculture. Additionally, further research has demonstrated that domestication may have occurred independently several times. For instance, archeological records have showed that pigs, descending from wild boar (*Sus scrofa*), were first domesticated in the Near East (Tixier-Boichard et al. 2011), while studies relying on molecular data point to independent domestication events in the Far East (Larson et al. 2005) and the possible existence of multiple domestication centers across Eurasia (Dobney & Larson 2006). This suggests that an early domestication event was followed by a major radiation following the enhancement of agriculture, as animals developed traits that were adaptive under these new conditions. Hence, the conditions under which domesticated species are raised, along with anthropogenic artificial selection have caused domestic populations to diverge rapidly from their wild ancestors in appearance, physiology, and behavior (Jensen 2014).

Documenting and detecting domestication requires identifying clear-cut markers that can be linked to a specific aspect of the process (Zeder et al. 2006). These markers can vary depending on the relationship between humans and the domesticate, as selective pressures differ among different species undergoing domestication. As the primary pressures on animals undergoing domestication are often behaviour-related, rather than morphological traits, determining such markers has been challenging (Zeder et al. 2006). For instance, certain behaviours that characterize an effective candidate for domestication are tolerance of penning, sexual precocity, and reduced wariness among others (Albert et al. 2009, 2011). Yet, selection for these behavioral traits may be associated with morphological characters commonly observed in domestic animals, which are genetically driven. Therefore, investigating the microevolutionary processes underlying animal domestication at the molecular level has become a focus for researchers in recent years. This has been made possible using the tools of modern genomics. An example of this was presented by Albert et al. (2009), who identified an epistatic network of genes as well as quantitative trait loci (QTLs) influencing tameness in rats. Two of these QTLs include a gene involved in the synthesis of the neurotransmitter serotonin (*Tph1*), and one that encodes a subunit of the receptor for γ-aminobutyric acid, a fundamental inhibitory neurotransmitter (*Gabra5*) (Carneiro et al. 2014). In addition, the genetic changes shaped by animal domestication have also been explored using population genomics tools to compare genome sequence data from domesticated breeds and wild populations. Though Carneiro et al. (2014) focused their investigations on phenotypically distinct domestic rabbit breeds and wild rabbit populations, they were able to determine specific microevolutionary processes relevant during the early stages of domestication for several vertebrate species. Their findings suggest that tame behavior in domestic animals has evolved by changes in allele frequencies at several loci, rather than by substantial shifts in few domestication loci. Furthermore, domestication of animals has been found to have long-lasting impacts on wild environments, leading to permanent changes, with the alteration of diverse in ecosystems to meet human and animals' needs (Terrell et al. 2003, Vigne 2011).

Farming fish and shellfish is of growing importance for contemporary economies; however, its origins are poorly known. Recent studies have found evidence suggesting that the first trials of farming fish species for human consumption might date back to 8,000 years ago, with the managed aquaculture of common carp (*Cyprinus carpio*) in Henan Province, China (Nakajima et al. 2019). This practice heavily expanded around the early 1980s to meet the growing demand for fish product, and aquaculture production has surpassed capture fisheries in only four decades (Teletchea 2016; FAO, 2019; Houston et al., 2020). The rapid growth of aquaculture has partially relied on the domestication of an increasing number of fish species (FAO, 2019; Teletchea, 2019), resulting in a continuously increasing number of fish that have been exposed to artificial culture conditions, and can be released into the wild, subsequently coming into contact with wild fishes. Current research has heavily focused on the impact of domesticated Atlantic salmon (*Salmon salar*) on wild populations, as it has become evident that these fish differ from wild individuals in various fitness-related traits. Salmon fish farming started on an experimental level in the 1960s, but became an industry in Norway in the 1980s, and in Chile in the 1990s. In Canada, the practice began in the 1970s, using ocean net pens in the northwest Pacific to raise Coho (*Oncorhynchus kisutch*) and Chinook salmon (*Oncorhynchus tshawytscha*). However, because Atlantic salmon are more suitable to domestication, being faster growing and adapting to higher densities in cages, the focus shifted to this species, which was first farmed successfully in Atlantic Canada in 1979 (FAO 2023). The farmed salmon industry has expanded substantially in the past 40 years, and aquaculture worldwide has been the fastest-growing food production sector as it had expanded by 609% between 1990–2020 in annual output with an average growth rate of 6.7% per year (FAO 2022). The expansion of this practice has allowed for a decrease in the world's reliance on wild salmon stocks as a resource, yet new threats to wild salmon have been introduced following the expansion of aquaculture.

Farm Atlantic salmon are considered one of the most domesticated food fish species (Teletchea & Fontaine 2012) having undergone >12 generations of artificial selection for traits desirable by the aquaculture industry. These are generally related to survival in a net pen environment and economic profitability, and are coupled with unintentional domestication selection, and random changes such as genetic drift and founder effects (Glover et al. 2017). Domestication-driven divergence may therefore lead to the expression of traits that are maladaptive to life in the wild (Fleming & Einum 1997, Ferguson et al. 2007) and as such interbreeding between farm and wild salmon can negatively impact adaptation and lead to a reduction in fitness (McGinnity et al. 2003, Skaala et al. 2019, Sylvester et al. 2019). Such interbreeding is driven by escapes of farmed fish from net pen aquaculture. These events are now known to be inevitable and allow for wild-maladapted farm fish to interact genetically and ecologically with wild salmon. Direct genetic interactions occur in instances where farm escapees survive in the wild to breed with local individuals (wild or hybrid), which can result in the introgression of maladapted farmed genetic traits into wild populations. In the wild, Atlantic salmon are highly adapted to their local environments (Garcia de Leaniz et al. 2007, Fraser et al. 2011, Watson et al. 2022), therefore farm introgression can negatively impact wild stocks by altering the frequency of wild genotypes, and eroding this local adaptation (Verspoor et al. 2015, Karlsson et al. 2016, Glover et al. 2017, Wringe et al. 2018, Bolstad et al. 2021). Ultimately, farm introgression may lead to reductions in fitness of entire populations (McGinnity et al. 2003, Skaala et al. 2019, Sylvester et al. 2019) and population decline (Fleming et al. 2000, Bradbury et al. 2020).

Some studies have investigated how genetically driven differences in certain traits impact survival and fitness among juvenile wild, farm, and hybrid Atlantic Salmon in the same environment (Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2012, 2019). Maintaining common-garden conditions during such studies is critical in informing our understanding of real-world impacts of aquaculture and introgression on wild salmon populations. However, these types of wild-environment experiments have largely occurred in Europe and are generally difficult to conduct due to the complexity of environmental and ecological conditions of a wild system. Therefore, extensive work still remains to be done to accurately describe the effects of farm escapees in the wild, particularly for non-European populations in regions with high potential for aquaculture impacts.

Various regions in North America have become areas of high aquaculture activity. It is estimated that 50% of aquaculture production in Canada originates in the four Atlantic provinces of New Brunswick, Newfoundland and Labrador, Nova Scotia, and Prince Edward Island, with room to double or triple its output (Statistics Canada 2010). Two thirds (65%) of the current volume consists of finfish such as salmon and trout (Statistics Canada 2010). However, in Newfoundland and Labrador, wild Atlantic Salmon populations are currently at-risk along the south coast, having been classified as "threatened" by COSEWIC in 2010 (COSEWIC 2010). South coast populations have also undergone an overall decline of 45% from 1996 to 2010, and this trend has persisted since (DFO 2020). Genetic farm introgression is currently a major concern as it has been found to be widespread throughout the region, and wild populations near farms appear to be experiencing larger declines relative to others (DFO 2013).

My study expands on existing research of wild, farm, and hybrid Atlantic salmon, using genetic data to examine their performance in a wild environment as well as trends in hybridization and introgression in southern Newfoundland. In Chapter 2, I expanded on previous work by Crowley et al. (2022) where the authors studied recapture odds of experimentally released wild, farm, and hybrid individuals. In line with previous analyses, they found that wild offspring had higher recapture odds than farm offspring (McGinnity 1997, Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2012, 2019). Here, I examined the differences in survival and size of four cross types (wild, farm, and reciprocal F_1 hybrids) across an environmental gradient of a river on the south coast of Newfoundland for a period of 28 months following release. I hypothesized that: (i) wild fish would have higher survival/recapture odds than farm individuals, with hybrids being intermediate; (ii) farm fish would be larger at recapture than wild fish, with hybrids being intermediate; and (iii) differences in size within cross types would remain constant through time. In Chapter 3, I assessed spatial and temporal trends in hybridization and admixture of farmed and wild Atlantic salmon in southern Newfoundland over an 8-year period. Following a large escape event in 2013 and somewhat smaller escapes in 2015 and 2018 in the region, recent work revealed spatial variation in hybridization (Sylvester et al. 2018) and selection against domestic offspring in the wild (Sylvester et al. 2019). In a more recent study, Holborn et al. (2022) determined that the high rate of precocial male maturation in farmed-wild F1 hybrids can have potentially accelerate the introgression process and associated impacts. I extend this work and provide evidence that the genetic impacts of farm escapes are long-lasting. Overall, studying these trends and differences among cross types is critical to determine, predict, and manage the genetic impacts of farm escapees on wild salmon populations.

References

Albert FW, Carlborg Ö, Plyusnina I, Besnier F, Hedwig D, Lautenschläger S, Lorenz D, McIntosh J, Neumann C, Richter H, Zeising C, Kozhemyakina R, Shchepina O, Kratzsch J, Trut L, Teupser D, Thiery J, Schöneberg T, Andersson L, Pääbo S (2009) Genetic Architecture of Tameness in a Rat Model of Animal Domestication. Genetics 182:541–554.

Albert FW, Hodges E, Jensen JD, Besnier F, Xuan Z, Rooks M, Bhattacharjee A, Brizuela L, Good JM, Green RE, Burbano HA, Plyusnina IZ, Trut L, Andersson L, Schöneberg T, Carlborg Ö, Hannon GJ, Pääbo S (2011) Targeted resequencing of a genomic region influencing tameness and aggression reveals multiple signals of positive selection. Hered 107:205–214.

Balcarcel AM, Geiger M, Clauss M, Sánchez-Villagra MR (2022) The mammalian brain under domestication: Discovering patterns after a century of old and new analyses. J Exp Zool B Mol Dev Evol 338:460–483.

Blumler MA (1996) Ecology, evolutionary theory and agricultural origins. In: *The origins and spread of agriculture and pastoralism in Eurasia*. Washington DC: Smithsonian Institution Press, p 25–50

Bolstad GH, Karlsson S, Hagen IJ, Fiske P, Urdal K, Sægrov H, Florø-Larsen B, Sollien VP, Østborg G, Diserud OH, Jensen AJ, Hindar K (2021) Introgression from farmed escapees affects the full life cycle of wild Atlantic salmon. Sci Adv 7:eabj3397.

Bradbury IR, Burgetz I, Coulson MW, Verspoor E, Gilbey J, Lehnert SJ, Kess T, Cross TF, Vasemägi A, Solberg MF, Fleming IA, McGinnity P (2020) Beyond hybridization: the genetic impacts of nonreproductive ecological interactions of salmon aquaculture on wild populations. Aquacult Environ Interact 12:429–445.

Brusini I, Carneiro M, Wang C, Rubin CJ, Ring H, Afonso S, Blanco-Aguiar JA, Ferrand N, Rafati N, Villafuerte R, Smedby Ö, Damberg P, Hallböök F, Fredrikson M, Andersson L (2018) Changes in brain architecture are consistent with altered fear processing in domestic rabbits. Proc Natl Acad Sci 115:7380–7385.

Carneiro M, Rubin CJ, Di Palma F, Albert FW, Alföldi J, Martinez Barrio A, Pielberg G, Rafati N, Sayyab S, Turner-Maier J, Younis S, Afonso S, Aken B, Alves JM, Barrell D, Bolet G, Boucher S, Burbano HA, Campos R, Chang JL, Duranthon V, Fontanesi L, Garreau H, Heiman D, Johnson J, Mage RG, Peng Z, Queney G, Rogel-Gaillard C, Ruffier M, Searle S, Villafuerte R, Xiong A, Young S, Forsberg-Nilsson K, Good JM, Lander ES, Ferrand N, Lindblad-Toh K, Andersson L (2014) Rabbit genome analysis reveals a polygenic basis for phenotypic change during domestication. Sci 345:1074–1079.

Clutton-Brock J (1994) The unnatural world: Behavioural aspects of humans and animals in the process of domestication. In: *Animals and Human Society*. Routledge

Clutton-Brock J (2014) The Walking Larder: Patterns of Domestication, Pastoralism, and Predation. Routledge.

COSEWIC (2010) COSEWIC assessment and status report on the Atlantic Salmon *Salmo salar* (Nunavik population, Labrador population, Northeast Newfoundland population, South Newfoundland population, Southwest Newfoundland population, Northwest Newfoundland population, Quebec Eastern North Shore population, Quebec Western North Shore population, Anticosti Island population, Inner St. Lawrence population, Lake Ontario population, Gaspé-Southern Gulf of St. Lawrence population, Eastern Cape

Breton population, Nova Scotia Southern Upland population, Inner Bay of Fundy population, Outer Bay of Fundy population) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xlvii + 136 pp.

Crowley SE, Bradbury IR, Messmer AM, Duffy SJ, Islam SS, Fleming IA (2022) Common-garden comparison of relative survival and fitness-related traits of wild, farm, and hybrid Atlantic salmon Salmo salar parr in nature. Aquacult Environ Interact 14:35–52.

DFO (2013) Recovery potential assessment for the South Newfoundland Atlantic Salmon (*Salmo salar*) designatable unit. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2012/007.

DFO (2020) Stock Assessment of Newfoundland and Labrador Atlantic Salmon in 2018. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2020/038.

Dobney K, Larson G (2006) Genetics and animal domestication: new windows on an elusive process. J Zool 269:261–271.

FAO (Food and Agricultural Organization) (2019) The state of the world's aquatic genetic resources for food and agriculture. Rome (Italy): FAO Commission on Genetic Resources for Food and Agriculture assessments

FAO (Food and Agricultural Organization) (2022) The State of World Fisheries and Aquaculture (2022) Towards Blue Transformation. Rome, FAO.

FAO (Food and Agricultural Organization) (2023) Canada. Text by Olin, P. Fisheries and Aquaculture Division [online]. Rome.

Ferguson A, Fleming IA, Hindar K, Skaala O, McGinnity P, Cross T, Prodoehl P (2007) Farm Escapes in The Atlantic Salmon–Genetics, Conservation and Management. Verspoor, E., Stradmeyer, L., and Nielsen, J. L.

Fleming IA, Einum S (1997) Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. ICES J Mar Sci 54:1051–1063.

Fleming IA, Hindar K, Mjølnerød IB, Jonsson B, Balstad T, Lamberg A (2000) Lifetime success and interactions of farm salmon invading a native population. Proc Biol Sci 267:1517–1523.

Fraser DJ, Weir LK, Bernatchez L, Hansen MM, Taylor EB (2011) Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. Hered 106:404–420.

Garcia de Leaniz C, Fleming IA, Einum S, Verspoor E, Jordan WC, Consuegra S, Aubin-Horth N, Lajus D, Letcher BH, Youngson AF, Webb JH, Vøllestad LA, Villanueva B, Ferguson A, Quinn TP (2007) A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biol Rev 82:173–211.

Giuffra E, Kijas JM, Amarger V, Carlborg O, Jeon JT, Andersson L (2000) The origin of the domestic pig: independent domestication and subsequent introgression. Genetics 154:1785–1791.

Glover KA, Solberg MF, McGinnity P, Hindar K, Verspoor E, Coulson MW, Hansen MM, Araki H, Skaala Ø, Svåsand T (2017) Half a century of genetic interaction between farmed and wild Atlantic salmon: Status of knowledge and unanswered questions. Fish Fish (Oxf) 18:890–927.

Hale EB (1969) Domestication and the evolution of behavior. The bevavior of domestic animals:22–42.

Harlan JR, de Wet JMJ, Price EG (1973) Comparative Evolution of Cereals. Evol 27:311–325.

Harris D (1996) Introduction: Themes and concepts in the study of early agriculture. In: *The origins and spread of agriculture and pastoralism in Eurasia*. Washington, DC: Smithsonian Institution Press., p 1–9

Holborn MK, Crowley SE, Duffy SJ, Messmer AM, Kess T, Dempson J, Wringe BF, Fleming IA, Bentzen P, Bradbury IR (2022) Precocial male maturation contributes to the introgression of farmed Atlantic salmon into wild populations. Aquacult Environ Interact 14:205–218.

Houston RD, Bean TP, Macqueen DJ, Gundappa MK, Jin YH, Jenkins TL, Selly SLC, Martin SAM, Stevens JR, Santos EM, Davie A (2020) Harnessing genomics to fast-track genetic improvement in aquaculture. Nat Rev Genet 21:389–409

Jensen P (2014) Behavior Genetics and the Domestication of Animals. Annu Rev Anim Biosci 2:85–104.

Karlsson S, Diserud OH, Fiske P, Hindar K (2016) Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. ICES J Mar Sci 73:2488–2498.

Katajamaa R, Jensen P (2020) Selection for reduced fear in red junglefowl changes brain composition and affects fear memory. R Soc Open Sci 7:200628.

Katajamaa R, Wright D, Henriksen R, Jensen P (2021) Cerebellum size is related to fear memory and domestication of chickens. Biol Lett 17:20200790.

Kruska D (1988) Mammalian Domestication and its Effect on Brain Structure and Behavior. NATO ASI Series G17:211–250.

Künzl C, Kaiser S, Meier E, Sachser N (2003) Is a wild mammal kept and reared in captivity still a wild animal? Horm Behav 43:187–196.

Larson G, Dobney K, Albarella U, Fang M, Matisoo-Smith E, Robins J, Lowden S, Finlayson H, Brand T, Willerslev E, Rowley-Conwy P, Andersson L, Cooper A (2005) Worldwide Phylogeography of Wild Boar Reveals Multiple Centers of Pig Domestication. Sci 307:1618–1621.

Larson G, Fuller DQ (2014) The Evolution of Animal Domestication. Annu Rev Ecol Evol Syst 45:115–136.

McGinnity, P, Stone C, Taggart JB, Cooke D, Cotter D, Hynes R, McCamley C, Cross T, Ferguson A (1997) Genetic impact of escaped farmed Atlantic salmon (Salmo salar L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. ICES J Mar Sci54:998–1008.

McGinnity P, Prodöhl P, Ferguson A, Hynes R, Maoiléidigh NO, Baker N, Cotter D, O'Hea B, Cooke D, Rogan G, Taggart J, Cross T (2003) Fitness reduction and potential extinction of wild populations of Atlantic salmon, Salmo salar, as a result of interactions with escaped farm salmon. Proc Biol Sci 270:2443–2450.

Nakajima T, Hudson MJ, Uchiyama J, Makibayashi K, Zhang J (2019) Common carp aquaculture in Neolithic China dates back 8,000 years. Nat Ecol Evol 3(10):1415-1418.

Price EO (2002) Animal domestication and behavior. CABI Pub, Wallingford, Oxon, UK ; New York.

Setchell BP (1992) Domestication and reproduction. Anim Reprod Sci 28:195–202.

Skaala Ø, Besnier F, Borgstrøm R, Barlaup B, Sørvik AG, Normann E, Østebø BI, Hansen MM, Glover KA (2019) An extensive common-garden study with domesticated and wild Atlantic salmon in the wild reveals impact on smolt production and shifts in fitness traits. Evol Appl 12:1001–1016.

Skaala Ø, Glover KA, Barlaup BT, Svåsand T, Besnier F, Hansen MM, Borgstrøm R (2012) Performance of farmed, hybrid, and wild Atlantic salmon (*Salmo salar*) families in a natural river environment. Can J Fish Aquat Sci 69:1994–2006.

Smith BD (2001) Low-Level Food Production. J Archaeol Res.

Statistics Canada, 2010. Canadian Aquaculture Statistics, Cat. 23-222-XWE, Ottawa, Ont.

Sylvester EVA, Wringe BF, Duffy SJ, Hamilton LC, Fleming IA, Bradbury IR (2018) Migration effort and wild population size influence the prevalence of hybridization between escaped farmed and wild Atlantic salmon. Aquacult Environ Interact 10:401–411.

Sylvester EVA, Wringe BF, Duffy SJ, Hamilton LC, Fleming IA, Castellani M, Bentzen P, Bradbury IR (2019) Estimating the relative fitness of escaped farmed salmon offspring in the wild and modelling the consequences of invasion for wild populations. Evol Appl 12:705–717.

Tang H, Sezen U, Paterson AH (2010) Domestication and plant genomes. Curr Opin Plant Biol 13:160–166.

Teletchea F (2016) Is fish domestication going too fast? Nat Resour 7(6):399– 404.

Teletchea F (2019) Fish domestication in aquaculture: reassessment and emerging questions. Cybium 43:7–15.

Teletchea F, Fontaine P (2012) Levels of domestication in fish: Implications for the sustainable future of aquaculture. Fish Fish (Oxf) 15.

Terrell JE, Hart JP, Barut S, Cellinese N, Curet L, Denham T, Kusimba CM, Latinis K, Oka R, Palka J, Pohl MED, Pope KO, Williams PR, Haines H, Staller JE (2003) Domesticated Landscapes: The Subsistence ecology of plant and animal domestication. jarcmt 10:323.

Tixier-Boichard M, Bed’hom B, Rognon X (2011) Chicken domestication: From archeology to genomics. Comptes Rendus Biologies 334:197–204.

Verspoor E, Mcginnity P, Bradbury I, Glebe B (2015) The potential direct and indirect genetic consequences for native Newfoundland Atlantic Salmon from interbreeding with European-origin farm escapes. DFO Can Sci Advis Sec Res Doc 2015/030:viii + 36.

Vigne J-D (2011) The origins of animal domestication and husbandry: A major change in the history of humanity and the biosphere. Comptes Rendus Biologies 334:171–181.

Watson KB, Lehnert SJ, Bentzen P, Kess T, Einfeldt A, Duffy S, Perriman B, Lien S, Kent M, Bradbury IR (2022) Environmentally associated chromosomal structural variation influences fine-scale population structure of Atlantic Salmon (Salmo salar). Molec Ecol 31:1057–1075.

Wilkins AS, Wrangham RW, Fitch WT (2014) The “Domestication Syndrome” in Mammals: A Unified Explanation Based on Neural Crest Cell Behavior and Genetics. Genetics 197:795–808.

Wringe BF, Jeffery NW, Stanley RRE, Hamilton LC, Anderson EC, Fleming IA, Grant C, Dempson JB, Veinott G, Duffy SJ, Bradbury IR (2018) Extensive hybridization following a large escape of domesticated Atlantic salmon in the Northwest Atlantic. Commun Biol 1:1–9.

Zeder MA (2012) The domestication of animals. Journal of Anthropological Research 68:161–190.

Zeder MA, Bradley DG, Emshwiller E, Smith BD (2006) Documenting Domestication: New Genetic and Archaeological Paradigms. University of California Press.

Chapter 2: Experimental comparison of changes in relative survival and fitness-related traits of wild, farm and hybrid Atlantic salmon (*Salmo salar*) in nature

Abstract

Farming of Atlantic salmon (*Salmo salar*) has resulted in highly domesticated individuals, with notable genetic and phenotypic differences relative to their wild counterparts. Understanding how interbreeding with aquaculture escapees affects wild often at-risk populations is increasingly essential to conservation efforts. Here, we used an experimental release of wild, farm, and reciprocal F_1 hybrid fry at three sites in the Garnish River in Newfoundland, Canada to evaluate family and cross specific patterns of recapture/survival, size, sex ratio, and precocial male maturation over a 28-month period. Trends in cross type recapture changed over the study period with the highest recapture at 3-months in parr with wild mothers, contrasting with that between 15-28 months where aquaculture offspring had overall the highest rates of recapture. Size trends among crosses and sites remained consistent over the study duration with pure farm and wild-mother hybrids consistently being larger than wild individuals and one site displaying elevated sizes in all crosses. Rates of parr maturation differed by sex and cross type, and the family-based analysis indicated family representation and size also remained consistent through time. These results indicate that there is a difference in vital rates such as survival and precocial maturation between farm and wild Atlantic salmon during the freshwater early life history period and this can change significantly over time. As such, an improved understanding of genetic and ecological interactions which takes this ontogenetic variation into account is likely essential to fully understand how hybridization and introgression with farm escapes are affecting wild populations.

Introduction

The domestication of Atlantic salmon (*Salmo salar)* in both North America and Europe, has resulted in notable genetic differences between farm individuals and their wild counterparts (Glover et al. 2017, Wringe et al. 2019). Due to this domestication-driven divergence, introgression of farm Atlantic salmon alleles into wild populations may result in the expression of traits that are maladaptive for life in the wild (Fleming & Einum 1997, Ferguson et al. 2007), and erode local adaptation by altering the frequency of wild genotypes (Verspoor et al. 2015, Karlsson et al. 2016, Glover et al. 2017, Wringe et al. 2018, Bolstad et al. 2021). In the wild, Atlantic salmon are highly adapted to their local environments (Garcia et al. 2007, Fraser et al. 2011, Watson et al. 2022) and as such interbreeding of farm and wild salmon can negatively impact adaptation, leading to a reduction in fitness (McGinnity et al. 2003, Skaala et al. 2019, Sylvester et al. 2019) and population decline (Fleming et al. 2000, Bradbury et al. 2020). For this reason, investigating the different effects of selection on survival among wild, farm, and hybrid salmon in the wild is key to determining and managing the genetic and demographic impacts of escaped farmed salmon on wild salmon populations.

Farm fish differ from wild fish both genetically (Besnier et al. 2015, Wringe et al. 2019), and phenotypically at a variety of traits (Fleming & Einum 1997, Wringe et al. 2016, Skaala et al. 2019, Islam et al. 2020), often through selection for increased aquaculture production (Fleming & Einum 1997, Gjøen & Bentsen 1997, Harvey et al. 2016). For instance, farm Atlantic salmon typically grow faster than their wild counterparts (Fleming et al. 2000, Glover et al. 2009, Solberg et al. 2013a, b, Harvey et al. 2016, Skaala et al. 2019). In the wild, such increased growth may present an advantage in certain aspects of competitive displacement (Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2019), but may also be detrimental with evidence of selection against fast-growing individuals (Biro et al. 2006, Solberg et al. 2015, Glover et al 2018). In association with the growth differences, farm fish also tend to be bolder and therefore more risk-prone than their wild counterparts (Fleming & Einum 1997, Islam et al. 2020, Solberg et al. 2020), and in a pedigree structure study, families of farm origin persistently exhibited poorer survival in their early years of life (Reed et al. 2015). As such, it appears that both, the nature of the interactions between genetics and the environment in which they live can influence the impact of growth on survival (Glover et al. 2018).

However, to date, the few studies that quantified survival and phenotypic differences among wild, farm, and hybrid individuals in the wild have been done in Europe or at limited spatial or temporal scales (e.g., Fleming & Einum 1997, Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2019, Crowley et al. 2022). Such localized experiments may not reflect the conditions of all wild populations and landscapes (Fleming et al. 2000, Skaala et al. 2012), and the true effect of selection on genotypes over time. The goal of this study was to expand on existing research of wild, farm, and hybrid Atlantic salmon performance in the wild in Southern Newfoundland to better inform predictions of population responses to escaped farmed salmon in Atlantic Canada specifically. We built upon the previous findings of Crowley et al. (2022) that explored the growth and survival of wild, farm, and hybrid salmon fry three months after release. Here, we assessed the differences in survival and size of the four cross types (wild, farm, and reciprocal F_1 hybrids) and family representation across an environmental gradient of three tributaries of a River in the south coast of Newfoundland for a period of 28 months following release. By subjecting differing cross types to common environmental conditions in three tributary sites, the overall goal was to isolate the genetic impact on these traits and determine differences among the four cross types and their specific families. We hypothesized that: (i) wild fish would have higher survival/recapture odds than farm individuals throughout the 28-month study period, with hybrids being intermediate; (ii) farm fish would be larger at recapture than wild fish, with hybrids being intermediate; and (iii) family representation/survival and differences in size within cross types would remain constant through time.

Methods

Crosses

In the late fall of 2017, four cross types of Atlantic salmon were produced as outlined in Crowley et al. (2022): 9 families of offspring from wild parents of the Garnish River, 6 families of offspring from parents of the Saint John River farm strain in New Brunswick (the only farm strain used in Atlantic Canada commercial aquaculture operations), 7 families of F_1 hybrids with the mentioned farm mothers and wild fathers (farm-mother hybrids [F♀hyb]), and 7 families of reciprocal F_1 hybrids (wild-mother hybrids [W♀hyb]) (Table 1). The Saint John River farm strain has been domesticated for 8-10 generations, and a multiple trait selection process including parr length, percent yearling smolt, market size and mature size of two-sea-winter broodfish was used to select gamete donors for early generations (Glebe 1998). A low number of farm parents was used reflecting the number of available individuals that matured by the late fall of 2017. Fin-clip samples from parents of each cross were retained in 100% ethanol for later use in parentage assignment of offspring. The Garnish River system is located on the south coast of Newfoundland near an area of intensive Atlantic salmon aquaculture on the Burin Peninsula, emptying into Fortune Bay (Figure 1). Escapees have been previously documented in this system, leading to the presence of F_1 hybrids following escape events (Wringe et al. 2018, Bradbury et al. 2020). For the present experiment, all wild fish used in the crosses were genetically screened to ensure that they were pure (i.e. not introgressed).

Embryo and early fry care are described in Crowley et al. (2022). Briefly, embryos were incubated on ambient water in Heath trays at the Ocean Sciences Centre of Memorial University (St. John's, Newfoundland, Canada), which were monitored daily, and dead embryos were removed every 4-5 days. At first feeding in late May 2018, juveniles were pooled by cross type and transferred to 470-litre flow-through circular holding tanks (0.9 m diameter x 0.5 m high) on ambient water. During the first month they were fed a combination of *Artemia* and salmonid starter dry feed (EWOS-Cargill, BC, Canada), followed by only the dry feed until release.

Field Methods - Release and Recapture

Release occurred on 11 July 2018 at three tributary sites of the Garnish River (Figure 1). To distinguish generated fry from wild fish at recapture, individuals were adipose fin-clipped before release (Crowley et al. 2022). Of the 2000 fin-clipped juveniles to be released per site some died during transport, such that 1932 were released at Site 1, 1980 at Site 2, and 1972 at Site 3. The number of individuals of each cross type released per site was relatively even (roughly 500 of each cross), although the W♀hyb group had approximately 50 more fish, and the wild group roughly 50 fewer fish than the farm and F♀hyb groups at each site. The fish were released at four locations approximately 50 m apart at each site. Animal use was approved by the Memorial University of Newfoundland Institutional Animal Care Committee (IACC) following Canadian Council of Animal Care (CCAC) guidelines, under protocol number 18-01-IF.

Following up on the sampling in October 2018 by Crowley et al. (2022), single pass electrofishing, using a LR-24 Backpack Electrofisher (Smith Root, Vancouver, WA, USA), was undertaken in August 12 and September 17, 19, and 20 of 2019, as well as September 30 of 2020 to recapture juveniles. The units were set at 550 volts and 60 Hz, with a duty cycle of 25%. In sites 1 and 2, recapture began roughly 100 m downstream from the first release points and continued upstream fishing up to 250 m above the release point. In Site 3, electrofishing started at a culvert downstream from the first release point and extended until a natural barrier was reached.

Processing of recaptured fish occurred approximately two hours after concluding electrofishing each day. Following this, fish were euthanized using MS-222 (AQUALIFE TMS, Syndel Laboratories Ltd., Nanaimo, BC, Canada) at a dose of 400 mg L-1 buffered with an equal dose of sodium bicarbonate. To allow for later parentage analysis, caudal fins were clipped, and fin samples were stored in 100% ethanol, and the rest of the fish frozen at -20ºC. All unmarked fish were released back into the stream unharmed.

Genetic Analysis

To assign individuals to a family and their cross type (wild, farm, or one of the two hybrid groups) a panel of 31 microsatellite loci with a total of 277 alleles (multiplex panel 1a from Bradbury et al. 2018) was used, which are a subset of a larger panel of 101 loci previously utilized for the Atlantic salmon in Atlantic Canada. Ultimately, six of these loci were excluded due to either a high percentage of missing offspring genotypes or a high estimated allelic dropout rate. Therefore, of these 31 loci, 25 were used for parentage assignment. Two of these loci included a tetranucleotide repeat sequence and 23 a trinucleotide repeat sequence and 10-13 repeats. All loci had ≥ 4 alleles, with an average of 8.4 alleles/locus over the entire panel (Bradbury et al. 2018). Additional information on locus-specific primers, repeat motifs, and chromosome numbers can be found in Table S1 of Bradbury et al. (2018).

A DNeasy 96 Blood and Tissue Kit (QIAGEN, Hilden, Germany) was used to extract DNA following the manufacturer's protocol for Purification of Total DNA from Animal Tissues. The protocol described by Zhan et al. (2017) was followed to PCR amplify microsatellite loci, and sequencing was performed using an Illumina MiSeq and scored using MEGASAT software. Each fish was assigned back to its cross type and family using COLONY (Jones & Wang 2010). To ensure the assignment sensitivity and accuracy of COLONY given the set of input parameters used, test trials included genotype data for all unique samples, parents, within-plate redundant, and cross-plate controls. Only unique samples were included in the final analysis (i.e. excluding redundant or controls).

Size analysis

Fork length measurements were taken from photos of all recaptured fish at each site. This was done using the *Measuring Distance Between Points* tool of ImageJ software (version 1.52a), diligently following the lateral line of the fish's body to account for body arching when present. Each recaptured sample was also weighed to the nearest hundredth of a gram. Finally, condition factor at recapture was calculated for the samples collected in 2019 and 2020 by dividing the residuals taken from the regression of ln(weight) over ln(recapture length) (Bolger & Connolly 1989, Wootton 1998).

The average growth rate for the different cross types was calculated between the 3-month post-release and 1-year post-release recapture events, the 3-month post-release and 2-year post-release recapture events, and the 1-year post release and 2-year post-release recapture events. This was done using the typical growth rate equation that accounts for allometry (Ostrovsky

1995), where the allometric mass exponent (b) for Atlantic salmon is 0.31(Elliott & Hurley 1997).

Sex ratios and maturity analysis

The proportion of males and females recaptured was calculated for the 2019 and 2020 recaptures based on genetic sexing. Within each category (male and female), mature versus immature individuals were distinguished, and each proportion was also quantified.

Statistical analysis

All statistical analyses were performed in R version 4.1.1 (R Core Team, 2022). The probability of recapture is the product of the probability of survival to time of recapture and the probability of being encountered at time of recapture. However, the design of this experiment did not allow for these probabilities to be separated. Because Atlantic salmon in Newfoundland do not generally migrate downstream and enter the sea until three or four years of age (DFO 2006), odds of recapture alone (proportion of fish recaptured relative to the initial number of fish released) were used as estimates of survival for the cross type and family representation analyses. For our experiment, the population was considered to be open, although no marked fish beyond those added in 2018 could join this population, and we only quantified mortality. Standard assumptions of a mark-recapture experiment were applied here, assuming all fish had an equal probability of being captured, their catchability did not change over time, and there was a 100% probability of detecting genetic markers. The estimates of survival for a given year included not only individuals recaptured that year, but also those recaptured in subsequent years given that they must have been alive in the preceding years. Since the recapture (survival) data are presence/absence in form, a generalized linear model with binomial family and logit link, with odds of recapture as the response variable was used to analyze survival. Cross type, site and year were included as fixed factors in the model in addition to all possible interaction terms.

Assumptions of normality and homoscedasticity of residuals were assessed by visual examination of residual vs. fitted plots and normal Q−Q plots of residuals. Where these assumptions were met, a general linear model was used for analysis, while a generalized linear model was used if one or more assumptions were not met. Length at recapture was examined using a linear model with cross type and site included as factors in the model. An interaction between these two variables was also analyzed. Recapture weight data were analyzed applying a generalized linear model with the Gamma family (identity link) as the Gamma model family is appropriate for positive continuous data (Dunn & Smyth).

We used analysis of deviance (McCullagh and Nelder 1989) to identify the evidential strength of different fixed factors in an experimental design of survival and size, where a normal error structure was not appropriate. We used the change in deviance to calculate a likelihood ratio (LR), a measure of strength of evidence (Royall 1997, Burnham & Anderson 2014). Where residual assumptions were met, the LR was calculated using the sums of squares of all the terms in the model. We chose not to declare decisions at a fixed error rate, consistent with best statistical practice (Läärä 2009, Wasserstein et al. 2019) and consistent with Snedecor and Cochran (1989) in the case of interaction terms in experimental design. In addition, we chose this approach as LRs provide a measure of evidence that is invariant across experimental designs, while the p-value does not share this property (Vieland and Hodge 1998). P-values were also reported in the instance that readers may not be acquainted with analysis of deviance and the evidentialist approach of Royall. However, only likelihood ratios are measure of evidence. The evidence for the data given the models and their parameter estimates was assessed using likelihood ratios (LRs) with assessments of relative magnitude of evidence for an alternative hypothesis given with LR = 10 indicating 'strong' evidence, LR = 32 'substantial' evidence, and LR >100 'decisive' evidence (Jeffreys 1961, Royall 1997). LRs provide the likelihood of the data given a model including certain variables vs. a model lacking them. An intercept-only model was used as the basis for the comparisons we were interested in all the terms included in each model, and the coefficient for each predictor was exponentiated to calculate odds ratios of recapture.

A goodness of fit test of the number of individuals recaptured per family relative to the expected number of recaptures (i.e. all families of all cross types were expected to have an equal probability of being captured) was used to analyze family representation through time, with proportion of individuals recaptured from each family (out of the total fish recaptured at each site each year) as the response variable in the model. Cross type, site, year, and all the possible interaction terms were again included as fixed factors in the model. This same analysis was used to determine whether there was a notable skew in sex ratios of recaptured samples in 2019 and 2020 as well as in the proportion of mature individuals in each site.

Results

In 2018, a total of 1284 fish were recaptured, and 1242 were successfully genotyped and assigned parentage (Table 1). In 2019 and 2020, 407 and 39 individuals were recaptured respectively, and all these were successfully genotyped.

Recapture/Survival

There was evidence for an interaction between cross type x site x year ($p = 0.087$, LR = 13835.6) suggesting that the recapture of each cross type by site differed from year to year (0+ to 1+ to 2+) (Figure 2 & Table S1 in the supplement). Therefore, recapture analyses were performed for each year separately. Overall, the trend of recapture shifted over time, from wild and W♀Hyb having the highest recapture rates at 3 months post-release, to farm and wild individuals having similar recapture odds one year later, and finally farm fish having the highest recapture with wild individuals having the lowest at 2 years post-release. In 2018 and 2019, there was also evidence for an interaction between site x cross type (2018: $p = 0.079$, LR = 289.3; 2019: $p < 0.001$, LR = 5.79×10^5), while in 2020 the evidence was 'strong' based only on the likelihood ratio score ($p = 0.38$, LR = 24.82) (Tables S2, S3, & S4). Given these results, pairwise comparisons for odds of recapture among cross types were subsequently assessed within each site separately. Three months post release, wild and W♀Hyb fish had the highest and nearly identical odds of recapture across all sites (33.8% and 34.3% respectively) (Figures 2A & 3A). Farm and F♀Hyb individuals had overall lower rates of recapture than the other two crosses (26.4% and 17.6% respectively), differing from them in Sites 2 and 3, while only F♀Hyb differed in Site 1 (Figure 3A). Following the first year after release (3-15 months post release), W♀Hyb fish had the highest recapture rates across all sites (8.79%), followed by farm and wild fish with similar proportions recaptured (8.09% and 7.83%, respectively), while F♀Hyb continued to have the lowest percentage recaptured (5.51%). However, the cross types with greater recapture odds at Site 2 were different from those with higher recapture odds at Site 3 (Figure 2B). At Site 2, farm and W♀Hyb had higher odds of recapture than wild individuals, and farm fish also had higher odds than F♀Hyb (Figure 3B). At Site 3, the odds of recapture of wild and farm fish were reversed, with wild and W♀Hyb having higher odds than the other two cross types (Figure 3B). Two years after release (15-28 months post release), in 2020, there were no wild fish recaptured at Sites 1 and 2 (Figure 2C), and no differences in probability of recapture between any other cross type pairs (Figure 3C). However, overall across the three sites, wild fish had substantially lower odds of recapture than farm fish (farm: 1.02%; wild: 0.15%; $p = 0.018$, $LR = 149.9$) (Table S5), with W♀Hyb (0.73%) and F♀Hyb (0.67%) being intermediary.

Size and condition at recapture

Length and weight at recapture varied in a similar manner across the three sites over the 3 sampling years (Figures 4 & 5). Site 3 consistently had the largest sizes at recapture, with Sites 1 and 2 having similar sizes, except in 2018 when fish at Site 2 tended to be larger than those at Site 1. As previously reported by Crowley et al. (2022), individuals recaptured in 2018 (3 months after release) were largest at Site 3, which was also where the greatest pairwise differences in recapture weight and length among cross types occurred (Figures 4A & 5A). Growth patterns also followed this trend, as growth was evidently higher at Site 3 between the 3-month and 1-year post-release period as well as the 3-month to 2-year post-release period (Figure S1). Similarly, all cross types were notably larger at Site 3 than the other two sites in 2019 (length: $p < 0.01$, $LR = 6.41 \times 10^{58}$, weight: $p < 0.01$, $LR = 2.29 \times 10^{8}$) (Figures 4B & 5B, Tables S6 & S7). Furthermore, there was a strong cross type x site interaction for length ($p = 0.002$, $LR = 5.33 \times 10^{4}$) (Table S6), and therefore, it was analyzed at each site separately. Although the small sample size in 2020 reduced the statistical power to detect a difference in size at recapture in 2020 across sites, the trend was similar to that of previous years with fish at Site 3 being larger than those at the other sites (Figures 4C & 5C, Tables S8 & S9 respectively). In terms of cross type differences, farm and W♀Hyb fish tended to be consistently the largest across sites in 2018 and 2019, with wild and F♀Hyb typically being the smallest. Specifically, in 2018, W♀Hyb and farm were the largest at Sites 2 and 3, followed by wild, while at Site 1 farm and F♀Hyb were larger than the other two crosses (Crowley et al. 2022). This was again consistent with growth patterns between the 2018 and 2019 recapture periods, where farm and W♀Hyb had higher growth rates than the other two crosses (Figure S1). In 2019 although farm and W♀Hyb were larger than the other two crosses across all sites, the evidence only pointed to an actual difference in size in Sites 1 and 3, where farm and W♀Hyb were longer and heavier (Figure 6, Tables S10 & S7 respectively). However, in 2020, the differences among cross types were less detectable, which again may in part be due to low statistical power.

In 2018, wild parr had lower condition than all other cross types in Sites 2 and 3, with no differences between any crosses in Site 1 (as reported previously by Crowley et al. 2022). There was, however, little evidence for an effect of cross type, site, or any interaction between these variables in 2019 ($p = 0.77$, $LR = 5.41$), and no notable differences in condition between cross type pairs and across sites. Nonetheless, in 2020, there was a decisive effect of cross type on the condition of recaptured salmon, with wild fish again having a lower condition than the other three cross types (Table S11) (Figure 7).

Sex ratios and maturity

Overall, there was little to no evidence for a deviation from an even sex ratio (ie. 50:50) in both 2019 and 2020 ($p = 0.104$, $LR = 3.74$ & $p = 0.0406$, $LR = 8.13$ respectively). The proportion of mature males in 2019 increased from Site 1 to 3 (30.3% to 69.7% to 81.6%), reflecting differences in size among the sites. Additionally, there was decisive evidence for a difference in maturation rates among cross types ($p = 0.0117$, LR = 245.06, Table S12). W♀Hyb and wild parr had similarly high proportions of mature males (82.1% and 70.2% respectively), while farm males had the lowest (56.1%), with F♀Hyb males being intermediate (65.6%). The only substantial pairwise contrast, however, was between W♀Hyb and pure farm fish. Size also strongly affected the proportion of mature males for W♀Hyb and wild parr ($p < 0.001$, LR = 526.10 & $p < 0.001$, LR = 13725.35 respectively), but not for the other two crosses. In 2020, all recaptured males were mature, while all recaptured females both years remained immature.

Family representation through time and size

There was little evidence for an effect of year of recapture on family representation ($p = 0.992$, LR = 1.01) (Table S13), with no notable effects of selection for or against any specific families (i.e. from 2018 to 2019 and then to 2020) (Figure S2). Even though certain families had substantially higher recaptures rates than others, this difference among families remained constant through the years sampled (2018-2020). Similarly, the effect of family on weight was substantial in the first two sampling periods, also having an interaction with site (2018: $p < 0.001$, LR = 4929.5; 2019: $p < 0.001$, LR = 40.04) (Tables S14 & S15). For this reason, weight was analyzed in each site individually both years. Family and site had a strong effect on length at 3 months post-release ($p < 0.001$, LR = 13.08 and $p < 0.001$, LR = 13.43 respectively), but had no interaction (Table S17). In contrast, there was little evidence for either of these variables influencing weight in 2020, as well as on length in 2019 and 2020 (Tables S16, S18, and S19 respectively). Overall, the differences in weight among families appeared to decrease over time until there was not enough power, due to a small sample size, to detect any difference in 2+ parr.

Certain families tended be larger than others, however, these again, remained constant throughout the sampling periods.

Discussion

Given substantial genetic and phenotypic differences between highly domesticated farm Atlantic salmon and their wild counterparts, understanding how interbreeding can affect local adaptation and fitness of wild, often at-risk populations has become essential to conservation efforts. Here, we found that the odds of recapture of different cross types varied significantly across the study period. There was a transition from pure wild offspring initially having higher recapture rates than farm parr, to farm offspring gradually having the highest recaptures of all crosses during the15-28 months post-release. The proportion of mature individuals differed by cross type, but overall, there was not a difference in sex ratios either year. We observed high rates of precocial male maturation in the dominant hybrid group (W♀Hyb), indicating that the risk of introgression (backcrossing) is high. This, along with the evidence for elevated hybrid survival, suggests that the risk of wild populations being negatively impacted by farm escapees may be high. Overall, our results build on the findings from Crowley et al. (2022) and expand on previous work studying cross type performance in the wild (McGinnity et al. 1997, 2003, Fleming et al. 2000, Skaala et al. 2012, 2019). More specifically, we provide novel insight into the impacts of hybridization and interactions of farmed with wild salmon in southern Newfoundland.

Survival

We observed a notable difference in rates of recapture/survival of Atlantic salmon from year to year. Crowley et al. (2022) studied recapture odds of these experimentally released individuals in 2018 (3-months post-release) and determined that in accordance with previous studies, wild offspring had higher recapture odds than farm offspring (McGinnity et al. 1997, 2003, Fleming et al. 2000, Skaala et al. 2012, 2019). They found an overall survival trend of wild > hybrids > farm, although W♀Hyb, had substantially high survival not too dissimilar to wild. Here we found that in the subsequent year (3-15 months), farm offspring reached overall survival rates that were analogous to those of wild salmon, and only slightly lower than W♀Hyb. Specifically, there were no differences in relative survival at Site 1 among any cross types, while farm and W♀Hyb had higher survival than wild fish at Site 2, and by contrast, wild and W♀Hyb had greater survival than farm and F♀Hyb at Site 3. Two years post-release (15-28 months), few wild individuals were recaptured, and these were only encountered in one site. Farm offspring had higher recapture odds than both wild and W♀Hyb, though this difference was not substantial. This could suggest that selection against farm fish primarily occurs during the first year (0+) where results follow the generally observed trend (wild > hybrids > farm) (McGinnity et al. 1997, 2003, Fleming et al. 2000). Our results not only differ from the three-month post-release sampling, but also from a broad-scale study of the change in proportions of wild, feral, and hybrid offspring following an escape event in southern Newfoundland, where wild salmon were implicated to have the highest annual survival rates over the first 2+ years in the river and pure farm offspring the lowest (Sylvester et al. 2019). However, our findings are in line with previous results from European studies, where evidence of differential survival after the 0+ stage was scarce (McGinnity et al. 1997, 2003, Fleming et al. 2000), and hybridization of domestic salmon decreased the production of wild salmon smolts and therefore wild adult abundance, through resource competition in freshwater (Skaala et al. 2019).

This analysis extends the time scale evaluated in Crowley et al. (2022), where only the three-month post-release period was studied, not accounting for survival during the winter months. Studies have shown that survival during the winter season tends to be lower in several salmonids due to unfavourable conditions and thus may be an important selective event in the salmon life history (Beamish et al. 2004, Finstad et al. 2004, Piou & Prévost 2013). Here, the larger farm salmon did not appear to incur a differential survival cost over the winter relative to wild salmon, and W♀Hyb continued to have the highest overall survival among cross types. Additionally, since selection against farm individuals can be more pronounced during early life (McGinnity et al. 1997, 2003, Fleming et al 2000), as we saw during the first 3 months post release, the weakest individuals may die, leaving the most fit to persist. This could account for the change in survival patterns among cross types over the three-year period. Factors beyond cross type may also substantially influence recapture odds, as, for instance, the role that limiting resources can have on mortality, growth and population dynamics (e.g., Keeley 2001, Finstad et al. 2009). Here, survival trends differed across sites; Site 1 had the fewest recaptures relative to the other two sites all sampling years, and this site appeared the least abundant in resources of the three tributary sites as reflected in fish body size, particularly in 2018 and 2019. Resource availability could therefore also be affecting the survival of all cross types, impacting the smaller wild and F♀Hyb salmon more heavily, which might explain the effects in Site 2 in 2019 and 2020. Furthermore, offspring in this experiment were released into the river sites during early development. However, previous studies found that the difference in survival between wild and farm juveniles is the most notable between eyed-egg stage and the first summer (McGinnity et al.

1997, Fleming et al. 2000). Thus, if farm individuals had experienced natural conditions from hatch, their mortality rates may have been higher in the first year and may not have survived as far as the third summer.

Modelling studies in Atlantic Canada also support the hypothesis that introgression between wild and escaped domestic individuals can reduce population viability and genetically alter wild salmon (e.g., Sylvester et al. 2019, Bradbury et al. 2020). Yet, specifically in North America, studies on the long-term effects of farm escapes on the survival of local populations are still scarce and indefinitive, highlighting the need to further study on this topic. Overall, our results combined with those of Crowley et al. (2022) show that there is, in fact, a difference in survival among crosses of Atlantic salmon, and this effect changes over time and varies spatially. Here, the shifting site-specific pattern of survival over the 3 years following release ultimately differs from previously established general patterns, further emphasizing the contrasts among location and populations, and the need for additional population-focused work through longer time-series.

A major challenge from our experiment was characterizing parr dispersal and individuals learning to avoid recapture based on previous experience. We could not disaggregate the probability of survival to time of recapture and the probability of being encountered and subsequently caught at time of recapture from each other, which means that parr dispersal was unaccounted for in our survival estimates. Therefore, it is also plausible that differences observed in recapture rates among cross types could be a function of and attributed to dispersal. It has been shown that environmental factors such as water velocity can influence dispersal of young salmon, such that higher velocities could lead to an increase in the passive dispersal of parr in the downstream direction (Gowan et al. 1994, Heggenes & Dokk 2001). Furthermore, density-dependent growth and mortality could have also prompted parr dispersal as a way to avoid these costs (Grant et al. 2005, Grossman et al. 2012). It also possible that a few of the largest parr may have smoltified and migrated at age 2+ (O'Connor & Ash 1993), prior to the third recapture event. Thus, it is possible that rates of dispersal were different among crosses and were influenced by environmental variation of traits that were not quantified across our tributary sites. These were not measured as this was not a focus of the study; rather, the interest was in replicability of findings across different environments. Still, parr movement does not discount the possibility that survival can differ substantially among cross type groups, or for an interaction between survival and dispersal that could be further explored.

Size

Our overall results are in line with previous findings from both, the first summer of this experiment (Crowley et al. 2022), and studies throughout Europe (e.g., Einum & Fleming 1997, McGinnity et al. 1997, Fleming et al. 2000), where wild parr tended to be the smallest of the crosses, and farm fish were larger across various environmental conditions. Unlike the changing survival patterns, the size trend remained constant throughout the sampling periods, with Site 3 typically having the largest individuals, and farm and W♀Hyb fish also being generally larger than wild and F♀Hyb. In contrast, recent studies in Newfoundland have failed to find evidence for a consistent growth and size pattern across the freshwater life stages. For instance, Hamoutene et al. (2017) reported that egg size of wild females relative to those of farmed females was notably larger, which likely produced larger wild offspring at hatch. Similarly, Perriman et al. 2022 detected a significant difference in size between pure wild and pure farm individuals, where, under tank and semi-natural conditions, wild fish were larger than both farm and hybrid salmon at first feeding. However, since this difference became absent by Day 80, it was again attributed maternal effects of egg size. These contrasting results could be associated with the different wild populations that were studied and/or differences in rearing experiences of the juveniles. On the other hand, Crowley et al. (2022) reported that there was little difference in average egg size of wild and farmed mothers in the fish used for this experiment, and wild juveniles were the smallest cross type at release.

Although the design of this experiment did not allow for a link between size at recapture and survival within cross type groups to be studied, there appears to be a correlation between these two variables. We only had enough power to detect a difference in size among cross types in 2018 and 2019 due to a small number of samples being recaptured in 2020. Nevertheless, farm and W♀Hyb were generally larger than the other two cross types, and also exhibited overall higher rates of recapture (W♀Hyb) or increasing odds relative to wild individuals through time (farm). However, these patterns of recapture may not completely reflect survival; it is possible that the generally larger farm and W♀Hyb individuals displaced the smaller wild fish from these sites when competing for resources. Offspring of escaped farm salmon have been found to negatively impact the wild population through competition, as as faster-growing farm parr may competitively displace wild parr from suitable environments in the wild (Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2019) and can induce mortality (Roberston et al. 2018). Additionally, increased aggression has been observed in farm salmon (Fleming & Einum 1997, Fleming et al. 2002, Islam et al., in press), which may further be advantageous to these already larger fish in competitive encounters. Yet, the possibilities that wild fish were displaced out of the tributary sites and that their survival is lower over time are not mutually exclusive, as the impact of this displacement on wild individuals is dependent on whether displaced parr can survive downstream or not. Even so, previous research has also indicated that farm Atlantic salmon offspring risk a substantially higher level of predation exposure and that fast-growing salmonids have greater vulnerability to fishing due to their greater appetite and correspondingly, greater foraging activity rates and boldness (Einum & Fleming 1997, Biro & Post 2008, Solberg et al. 2020).

Site had a strong effect on differences in size all years as fish of all cross types were larger in Site 3, relative to individuals of the same cross in Sites 1 and 2. This likely reflects differences in available forage, though this was not quantified. However, this is where wild and W♀Hyb had decisively higher survival than farm and F♀Hyb in 2019, which further suggests that resource availability and competition could affect odds of recapture of smaller cross types over time (Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2019).

Sex ratios and maturity

Although there was no evidence for a biased sex ratio in our recapture samples, the effect of sex on maturation rates was notable. A recent study in southern Newfoundland determined that precocial male maturation may play a key role in the observed increasing presence of backcrossed individuals over time following an escape event and thus genetic introgression (Holborn et al. 2022). Consistent with this observation, we found that the proportion of mature hybrid male parr was high at age 1+ and did not differ from that of wild males under the same environmental conditions, while that of farm parr was lower. Additionally, growth has been previously found to influence the rates of precocial maturation (Letcher & Gries 2003, Jonsson & Jonsson 2011), with some findings suggesting that exceedingly high growth rates can cause individuals to outgrow the size threshold for early maturation (Moreau & Fleming 2012, Harvey et al. 2018). However, here, size had a strong effect on the maturation rates of wild and W♀Hyb parr, even though W♀Hyb were substantially larger than wild individuals suggesting that they might have differing growth rate thresholds for maturation. Moreover, we observed an increase in the proportion of mature males from Site 1 to 3, likely reflecting the differences in size among the sites. Rates of introgression will be further influenced by differences among cross types in the relative reproductive success of males that mature precocially, which has been shown to differ previously with farm and hybrid males having superior success to wild males (Garant et al. 2003). Thus, the high rates of male precocial maturation observed here, particularly among hybrid males, will likely lead to high rates of introgression of farm genotypes into wild Atlantic salmon populations.

Family representation and size

Survival and selection at the family-level have been studied less frequently than cross type survival. Here we found that certain families outperformed others regardless of their body size relative to each other. Moreover, this difference in survival rates remained consistent throughout the three years of sampling, and families that differed in size did not always differ in odds of recapture. This consistent survival pattern could indicate that family-level selection occurred prior to the first recapture event. Results similar to these have been observed previously in a European study, where families that were highly represented at the 0+ parr stage in the experiment were also highly represented at the 1+ parr stage, implying consistent performance differences in the wild (Reed et al. 2015). This was despite the finding that offspring with one or two farm parents exhibited poorer survival in their first and second year of life compared with those with two wild parents. Similarly, Skaala et al. (2019) found that families with high egg to smolt survival also had high smolt to adult survival.

The relatively small family numbers used in this study per cross type was due to the limited number of farmed parents that matured by the time breeding occurred. This could present a challenge in our ability to draw certain conclusions regarding cross type survival as the performance of one or a few families within a cross type could weigh heavily on the average performance of that cross. However, the proportion of individuals of one family would still have to be substantially higher than the rest at each site if it were to disproportionately increase the odds of recapture of an entire cross type group over another. Since we found that the families with greater representation are the same over the three years, but this was not the case for cross types, it is therefore unlikely that the effect of family numbers heavily affects cross type survival results.

Conclusion

Our overall results indicate that the relative differences in survival among wild, farm, and hybrid Atlantic salmon parr change during the freshwater period. Here, selection against farm individuals appears to be stronger during the first summer after release (0+ parr) and shifts over time. Moreover, rates of male precocial maturation can be high already in the second year of life, particularly among hybrid individuals. We show that vital rates such as survival and precocial maturation of wild, hybrid and farm offspring can change during their life span. Therefore, improved understanding of this variation is needed to determine, predict, and manage the genetic and demographic impacts of farm escapees on wild salmon populations.

References

Beamish RJ, Mahnken C, Neville CM (2004) Evidence That Reduced Early Marine Growth is Associated with Lower Marine Survival of Coho Salmon. Trans Am Fish Soc 133:26–33.

Besnier F, Glover KA, Lien S, Kent M, Hansen MM, Shen X, Skaala Ø (2015) Identification of quantitative genetic components of fitness variation in farmed, hybrid and native salmon in the wild. Heredity 115:47–55.

Biro PA, Abrahams MV, Post JR, Parkinson EA (2006) Behavioural trade-offs between growth and mortality explain evolution of submaximal growth rates. J Anim Ecol 75:1165–1171.

Biro PA, Post JR (2008) Rapid depletion of genotypes with fast growth and bold personality traits from harvested fish populations. Proc Nat Acad Sci 105:2919–2922.

Bolger T, Connolly PL (1989) The selection of suitable indices for the measurement and analysis of fish condition. J Fish Biol 34:171–182

Bolstad GH, Karlsson S, Hagen IJ, Fiske P, Urdal K, Sægrov H, Florø-Larsen B, Sollien VP, Østborg G, Diserud OH, Jensen AJ, Hindar K (2021) Introgression from farmed escapees affects the full life cycle of wild Atlantic salmon. Sci Adv 7:eabj3397.

Bradbury I, Duffy S, Lehnert S, Jóhannsson R, Fridriksson J, Castellani M, Burgetz I, Sylvester E, Messmer A, Layton K, Kelly N, Dempson J, Fleming I (2020) Model-based evaluation of the genetic impacts of farm-escaped Atlantic salmon on wild populations. Aquacult Environ Interact 12:45–59.

Bradbury IR, Wringe BF, Watson B, Paterson I, Horne J, Beiko R, Lehnert SJ, Clément M, Anderson EC, Jeffery NW, Duffy S, Sylvester E, Robertson M, Bentzen P (2018) Genotyping-by-sequencing of genome-wide microsatellite loci reveals fine-scale harvest composition in a coastal Atlantic salmon fishery. Evol Appl 11:918–930.

Burnham KP, Anderson DR (2014). P values are only an index to evidence: 20th- vs. 21st-century statistical science. Ecol 95(3): 627-630.

Crowley S, Bradbury IR, Messmer A, Duffy SJ, Islam SS, Fleming IA (2022) Common-garden comparison of relative survival and fitness-related traits of wild, farm, and hybrid Atlantic salmon Salmo salar parr in nature. Aquacult Environ Interact 14:35–52.

DFO (Fisheries and Oceans Canada) (2006) Aspects of the Life History, Biology, and Population Dynamics of Atlantic Salmon (*Salmo salar L.*) in Eastern Canada - 2006. Can Sci Advis Sec Sci Advis Rep 2006/014.

Dunn PK, Smyth GK (2018). Positive Continuous Data: Gamma and Inverse Gaussian GLMs In: Generalized Linear Models With Examples in R. Springer Texts in Statistics Springer, New York, NY.

Einum S, Fleming IA (1997) Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. J Fish Biol 50:634–651.

Elliott JM, Hurley MA (1997) A functional model for maximum growth of Atlantic salmon parr, *Salmo salar*, from two populations in northwest England. Funct Ecol 11(5): 592-603.

Ferguson A, Fleming IA, Hindar K, Skaala O, McGinnity P, Cross T, Prodoehl P, Verspoor E, Stradmeyer L, Nielsen JL (2007) Farm Escapes in The Atlantic Salmon–Genetics, Conservation and Management. Blackwell Publishing Ltd 357-398

Finstad AG, Einum S, Ugedal O, Forseth T (2009) Spatial Distribution of Limited Resources and Local Density Regulation in Juvenile Atlantic Salmon. J Anim Ecol 78:226–235.

Finstad AG, Ugedal O, Forseth T (2004) Energy-related juvenile winter mortality in a northern population of Atlantic salmon. Can J Fish Aquat Sci 61: 2358-2368

Fleming IA, Einum S (1997) Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. ICES J Mar Sci 54:1051–1063.

Fleming IA, Agustsson T, Finstad B, Johnsson JI, Björnsson BT (2002) Effects of domestication on growth physiology and endocrinology of Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 59: 1323–1330.

Fleming IA, Hindar K, Mjølnerød IB, Jonsson B, Balstad T, Lamberg A (2000) Lifetime success and interactions of farm salmon invading a native population. Proc Roy Soc B 267:1517–1523.

Fraser DJ, Weir LK, Bernatchez L, Hansen MM, Taylor EB (2011) Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. Heredity 106:404–420.

Garant D, Fleming IA, Einum S, Bernatchez L (2003) Alternative male life-history tactics as potential vehicles for speeding introgression of farm salmon traits into wild populations. Ecol Lett 6:541–549.

Garcia de Leaniz C, Fleming IA, Einum S, Verspoor E, Jordan WC, Consuegra S, Aubin-Horth N, Lajus D, Letcher BH, Youngson AF, Webb JH, Vøllestad LA, Villanueva B, Ferguson A, Quinn TP (2007) A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biol Rev Camb Philos Soc 82:173−211.

Gjøen HM, Bentsen HB (1997) Past, present, and future of genetic improvement in salmon aquaculture. ICES J Mar Sci 54:1009–1014.

Glebe BD (1998) East coast salmon aquaculture breeding programs:history and future. Can Stock Assess Sec Res Doc 1998/157.

Glover KA, Otterå H, Olsen RE, Slinde E, Taranger GL, Skaala Ø (2009) A comparison of farmed, wild and hybrid Atlantic salmon (*Salmo salar* L.) reared under farming conditions. Aquaculture 286:203–210.

Glover KA, Solberg MF, Besnier F, Skaala Ø (2018) Cryptic introgression: evidence that selection and plasticity mask the full phenotypic potential of domesticated Atlantic salmon in the wild. Sci Rep 8:13966.

Glover KA, Solberg MF, McGinnity P, Hindar K, Verspoor E, Coulson MW, Hansen MM, Araki H, Skaala Ø, Svåsand T (2017) Half a century of genetic interaction between farmed and wild Atlantic salmon: Status of knowledge and unanswered questions. Fish Fish 18:890–927.

Gowan C, Young MK, Fausch KD, Riley SC (1994) Restricted movement in resident stream salmonids: a paradigm lost? Can J Fish Aquat Sci 51(11): 2626-2637.

Grant JWA, Imre I (2005) Patterns of density-dependent growth in juvenile stream dwelling salmonids. J Fish Biol 67(SB):100-110.

Grossman GD, Nuhfer A, Zorn T, Sundin G, Alexander G (2012) Population regulation of brook trout (*Salvelinus fontinalis*) in hunt creek, Michigan: A 50-year study. Freshw Biol 57(7):1434-1448.

Hamoutene D, Perez-Casanova J, Burt K, Lush L, Caines J, Collier C, Hinks R (2017) Early life traits of farm and wild Atlantic salmon Salmo salar and first generation hybrids in the south coast of Newfoundland. J Fish Biol 90:2271–2288.

Harvey AC, Skilbrei OT, Besnier F, Solberg MF, Sørvik A-GE, Glover KA (2018) Implications for introgression: has selection for fast growth altered the size threshold for precocious male maturation in domesticated Atlantic salmon? BMC Evol Biol 18:1-13.

Harvey AC, Solberg MF, Troianou E, Carvalho GR, Taylor MI, Creer S, Dyrhovden L, Matre IH, Glover KA (2016) Plasticity in growth of farmed and wild Atlantic salmon: is the increased growth rate of farmed salmon caused by evolutionary adaptations to the commercial diet? BMC Evol Biol 16:1-13.

Heggenes J (1990) Habitat utilization and preferences in juvenile Atlantic salmon (*Salmo salar*) in streams. Regul Rivers Res Manage 5(4): 341-354.

Holborn M, Crowley S, Duffy S, Messmer A, Kess T, Dempson J, Wringe B, Fleming I, Bentzen P, Bradbury I (2022) Precocial male maturation contributes to the introgression of farmed Atlantic salmon into wild populations. Aquacult Environ Interact 14:205–218.

Islam SS, Wringe BF, Bradbury IR, Fleming IA (2020) Behavioural variation among divergent European and North American farmed and wild Atlantic salmon (*Salmo salar*) populations. Appl Anim Behav Sci 230:105029.

Islam SS, Wringe BF, Conway C, Bradbury IR, Fleming IA (2022) Fitness consequences of hybridization between wild Newfoundland and farmed European and North American Atlantic salmon. Aquacult Environ Interact. Aquacult Environ Interact 14: 243-262.

Jeffreys H (1961) Theory of Probability, 3rd ed. Oxford University Press.

Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551–555.

Jonsson B, Jonsson N (2011) Ecology of Atlantic Salmon and Brown Trout: Habitat as a template for life histories. Springer, London.

Karlsson S, Diserud OH, Fiske P, Hindar K, Handling editor: W. Stewart Grant (2016) Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. ICES J Mar Sci 73:2488–2498.

Keeley ER (2001) Demographic Responses to Food and Space Competition by Juvenile Steelhead Trout. Ecology 82:1247–1259.

Läärä E (2009) Statistics: reasoning on uncertainty, and the insignificance of testing null. Ann Zool Fennici 46: 138–157.

Letcher BH, Gries G (2003) Effects of life history variation on size and growth in stream-dwelling Atlantic salmon. J Fish Biol 62:97–114

McCullagh P, Nelder JA (1989) Generalized linear models 2nd edition chapman and hall. London, UK.

McGinnity P, Stone C, Taggart JB, Cooke D, Cotter D, Hynes R, McCamley C, Cross T, Ferguson A (1997) Genetic impact of escaped farmed Atlantic salmon (Salmo salar L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. ICES J Mar Sci 54:998–1008.

McGinnity P, Prodöhl P, Ferguson A, Hynes R, Maoiléidigh NO, Baker N, Cotter D, O'Hea B, Cooke D, Rogan G, Taggart J, Cross T (2003) Fitness reduction and potential extinction of wild populations of Atlantic salmon, Salmo salar, as a result of interactions with escaped farm salmon. Proc Roy Soc B 270:2443–2450.

Moreau DTR, Fleming IA (2012) Enhanced growth reduces precocial male maturation in Atlantic salmon. Funct Ecol 26:399–405.

O'Connor MF, Ash EGM (1993) Smolt size in relation to age at first maturity of Atlantic salmon (*Salmo salar*): the role of lacustrine habitat. J Fish Biol 43: 551-569.

Ostrovsky I (1995) The parabolic pattern of animal growth: determination of equation parameters and their temperature dependencies. Freshw Biol 33(3): 357-371.

Perriman BM, Bentzen P, Wringe BF, Duffy SJ, Islam SS, Fleming IA, Solberg M, Bradbury IR (2022) Morphological consequences of hybridization between farm and wild Atlantic salmon Salmo salar under both wild and experimental conditions. Aquacult Environ Interact 14:85–96.

Piou C, Prévost E (2013) Contrasting effects of climate change in continental vs. oceanic environments on population persistence and microevolution of Atlantic salmon. Glob Change Biol 19:711–723.

R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reed, TE, Prodöhl P, Hynes R, Cross T, Ferguson A, McGinnity P (2015) Quantifying heritable variation in fitness-related traits of wild, farmed and hybrid Atlantic salmon families in a wild river environment. Heredity 115:173-184.

Robertson G, Reid D, Einum S, Aronsen T, Fleming IA, Sundt-Hansen L, Karlsson S, Kvingedal E, Hindar K (2019) Can variation in standard metabolic rate explain context-dependent performance of farmed salmon offspring? Ecol Evol 9: 212-222.

Royall R (1997) Statistical evidence: a likelihood paradigm. CRC Press, Boca Raton, FL

Skaala Ø, Besnier F, Borgstrøm R, Barlaup B, Sørvik AG, Normann E, Østebø BI, Hansen MM, Glover KA (2019) An extensive common-garden study with domesticated and wild Atlantic salmon in the wild reveals impact on smolt production and shifts in fitness traits. Evol Appl 12:1001–1016.

Skaala Ø, Glover KA, Barlaup BT, Svåsand T, Besnier F, Hansen MM, Borgstrøm R (2012) Performance of farmed, hybrid, and wild Atlantic salmon (*Salmo salar*) families in a natural river environment. Can J Fish Aquat Sci 69:1994–2006.

Snedecor GW, Cochran WG (1989) Statistical Methods. 8th Edition, Iowa State University Press, Ames: 305.

Solberg MF, Robertsen G, Sundt-Hansen LE, Hindar K, Glover KA (2020) Domestication leads to increased predation susceptibility. Sci Rep 10:1929.

Solberg MF, Skaala Ø, Nilsen F, Glover KA (2013a) Does Domestication Cause Changes in Growth Reaction Norms? A Study of Farmed, Wild and Hybrid Atlantic Salmon Families Exposed to Environmental Stress. PLOS ONE 8:e54469.

Solberg MF, Zhang Z, Glover KA (2015) Are farmed salmon more prone to risk than wild salmon? Susceptibility of juvenile farm, hybrid and wild Atlantic salmon Salmo salar L. to an artificial predator. Appl Anim Behav Sci 162:67–80.

Solberg MF, Zhang Z, Nilsen F, Glover KA (2013b) Growth reaction norms of domesticated, wild and hybrid Atlantic salmon families in response to differing social and physical environments. BMC Evol Biol 13:1-23.

Sylvester EVA, Wringe BF, Duffy SJ, Hamilton LC, Fleming IA, Castellani M, Bentzen P, Bradbury IR (2019) Estimating the relative fitness of escaped farmed salmon offspring in the wild and modelling the consequences of invasion for wild populations. Evol Appl 12:705–717.

Verspoor E, Mcginnity P, Bradbury I, Glebe B (2015) The potential direct and indirect genetic consequences for native Newfoundland Atlantic Salmon from interbreeding with European-origin farm escapes. DFO Can Sci Advis Sec Res Doc 2015/030:viii + 36.

Vieland VJ, S.E. Hodge (1998) Review of Statistical Evidence: A Likelihood Paradigm. Am J Hum Genet 63(1): 283–289.

Wasserstein RL, Schirm, AL, Lazar NA (2019). Moving to a world beyond “p< 0.05”. The American Statistician 73(sup1): 1-19.

Watson KB, Lehnert SJ, Bentzen P, Kess T, Einfeldt A, Duffy S, Perriman B, Lien S, Kent M, Bradbury IR (2022) Environmentally associated chromosomal structural variation influences fine-scale population structure of Atlantic Salmon (*Salmo salar*). Mol Ecol 31:1057–1075.

Wootton RJ (1998) Ecology of teleost fishes. Kluwer Academic, Boston, MA

Wringe BF, Anderson EC, Jeffery NW, Stanley RRE, Bradbury IR (2019) Development and evaluation of SNP panels for the detection of hybridization between wild and escaped Atlantic salmon (*Salmo salar*) in the western Atlantic. Can J Fish Aquat Sci 76:695–704.

Wringe BF, Purchase CF, Fleming IA (2016) In search of a "cultured fish phenotype": a systematic review, meta-analysis and vote-counting analysis. Rev Fish Biol Fish 26:351–373.

Wringe BF, Jeffery NW, Stanley RRE, Hamilton LC, Anderson EC, Fleming IA, Grant C, Dempson JB, Veinott G, Duffy SJ, Bradbury IR (2018) Extensive hybridization following a large escape of domesticated Atlantic salmon in the Northwest Atlantic. Commun Biol 1:1–9.

Zhan L, Paterson IG, Fraser BA, Watson B, Bradbury IR, Nadukkalam Ravindran P, Reznick D, Beiko RG, Bentzen P (2017) Megasat: automated inference of microsatellite genotypes from sequence data. Mol Ecol Resour 17:247–256.

Figures

Table 2.1. Families produced of each cross type with specific mother and father ID and number of individuals released and recaptured of each cross type and family at each site. Wild parents originated from the Garnish River in Southern Newfoundland, while farm parents were from the Saint John River, New Brunswick, farm strain (the only farmed strain used in Atlantic Canada commercial aquaculture operations). Release of all crosses occurred on 11 July 2018. Specific numbers of each family released at each site could not be quantified.

	Families		Number of individuals released (2018)				Number of individuals recaptured (2018-2020)			
Cross type	Mother ID	Father ID	Site 1	Site 2	Site 3	Total	Site 1	Site 2	Site 3	Total
Wild			427	439	437	1303	83	164	195	442
	W11	*W08*					11	24	6	41
	W13	*W01*					23	30	66	119
	W17	*W22*					5	12	16	33
	W19	*W03*					5	6	13	24
	W20	*W04*					10	32	38	80
	W23	*W07*					8	15	15	38
	W24	*W08*					5	22	13	40
	W26	*W22*					6	12	10	28
	W28	*W16*					10	11	18	39
Farm			483	495	493	1471	74	164	165	403
	F01	*F02*					30	56	50	136
	F01	*F03*					10	19	12	41
	F01	*F06*					17	46	34	97
	F04	*F02*					17	42	66	125

	F05 F06					0	1	3	4
F♀hyb		483	495	493	1471	47	129	93	269
	F01 W01					13	24	34	71
	F01 W02					15	39	42	96
	F04 W08					17	58	12	87
	F05 W07					2	8	5	15
W♀hyb		539	551	549	1639	103	244	227	574
	W11 F02					16	36	41	93
	W17 F02					9	26	16	51
	W19 F06					17	53	49	119
	W23 F06					13	45	36	94
	W24 F03					19	29	25	73
	W26 F02					8	19	16	43
	W28 F02					21	36	44	101

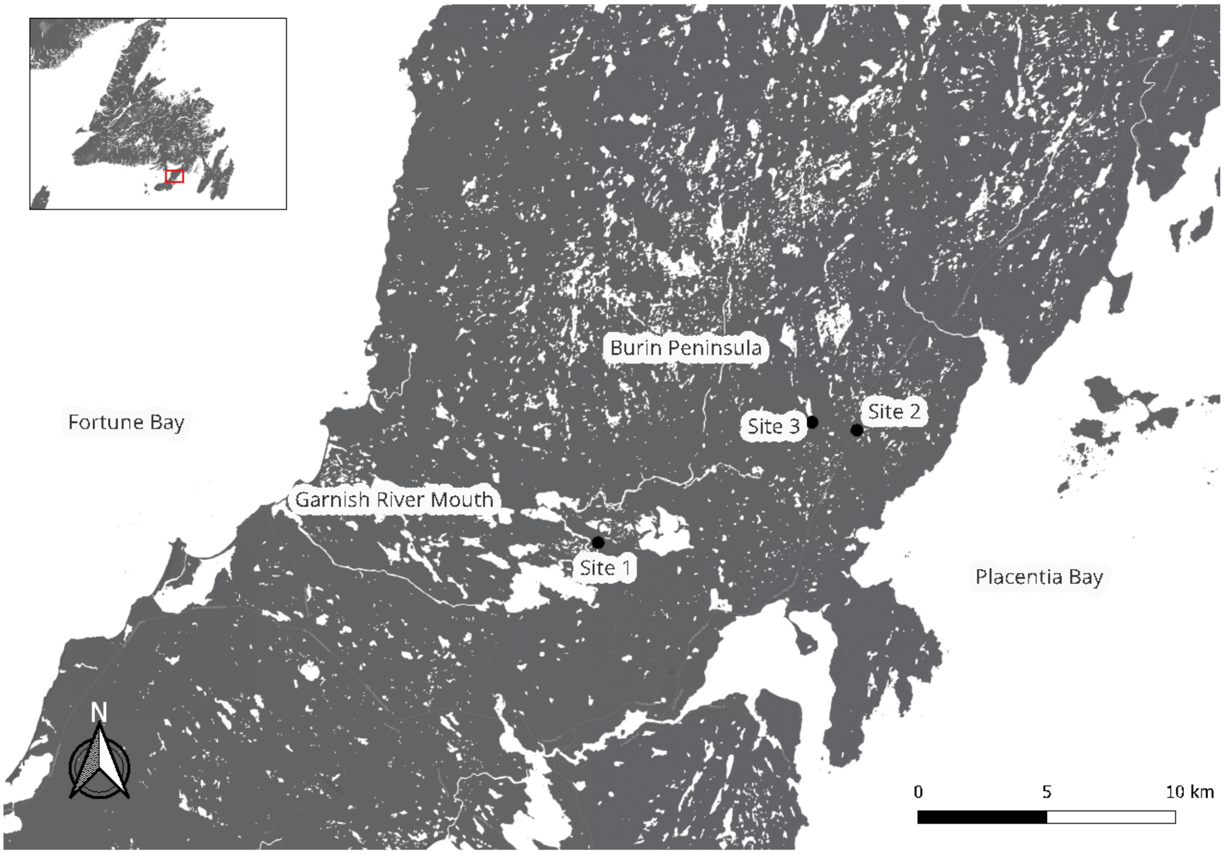


Figure 2.1. The three tributary study sites of the Garnish River used for the release and recapture experiment with Atlantic salmon. These are located in the Burin Peninsula in Newfoundland, Canada. Inset: the island of Newfoundland; box: general study area. Inset: the island of Newfoundland; box: general study area. Map created in QGIS, map style created in Mapbox, data by OpenStreetMap under ODbL.

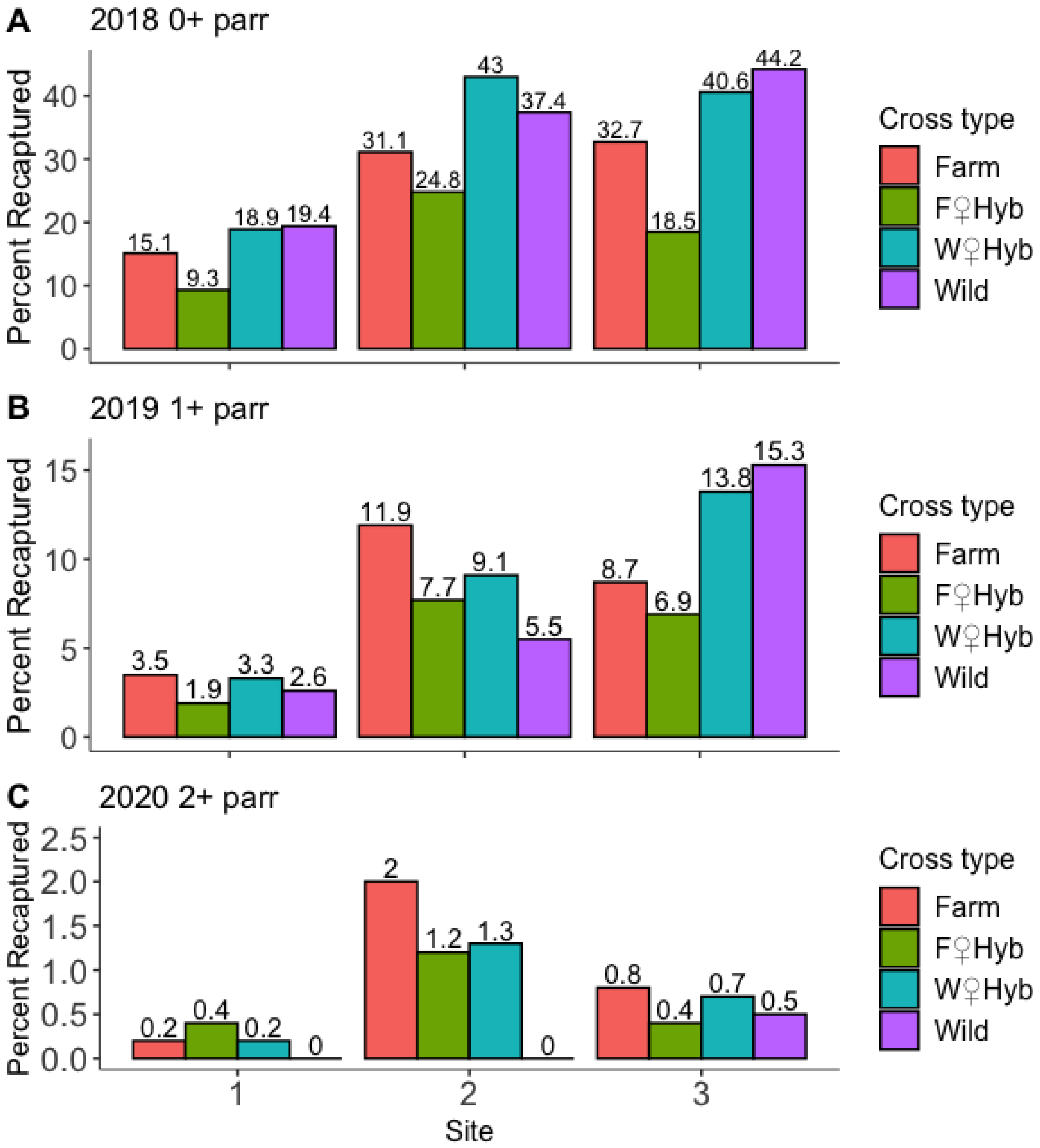


Figure 2.2. Percentages of Atlantic salmon recaptured by cross type and site that were present in (A) 2018, (B) 2019, and (C) 2020. F♀hyb: farm-mother hybrid; W♀hyb: wild-mother hybrid.

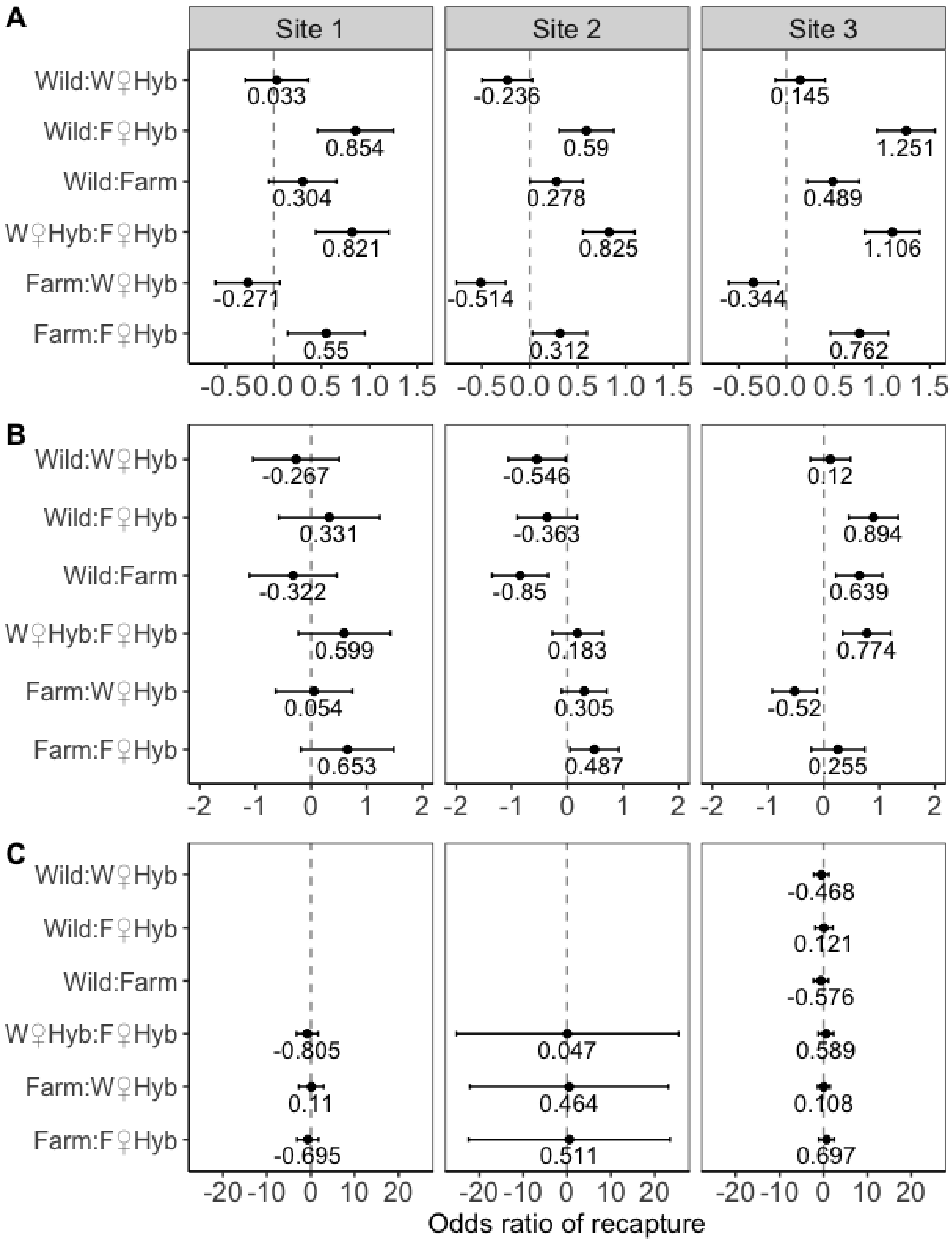


Figure 2.3. Odds ratios of recapture for pairwise cross type comparisons at each site in (A) 2018, (B) 2019, and (C) 2020. Error bars represent ±2 SE of the odds ratio estimates. Odds ratios and SE were back- transformed from the logit scale. There were zero recaptures of the wild cross type in Sites 1 and 2 in 2020, therefore pair wise comparisons relative to wild were not possible here.

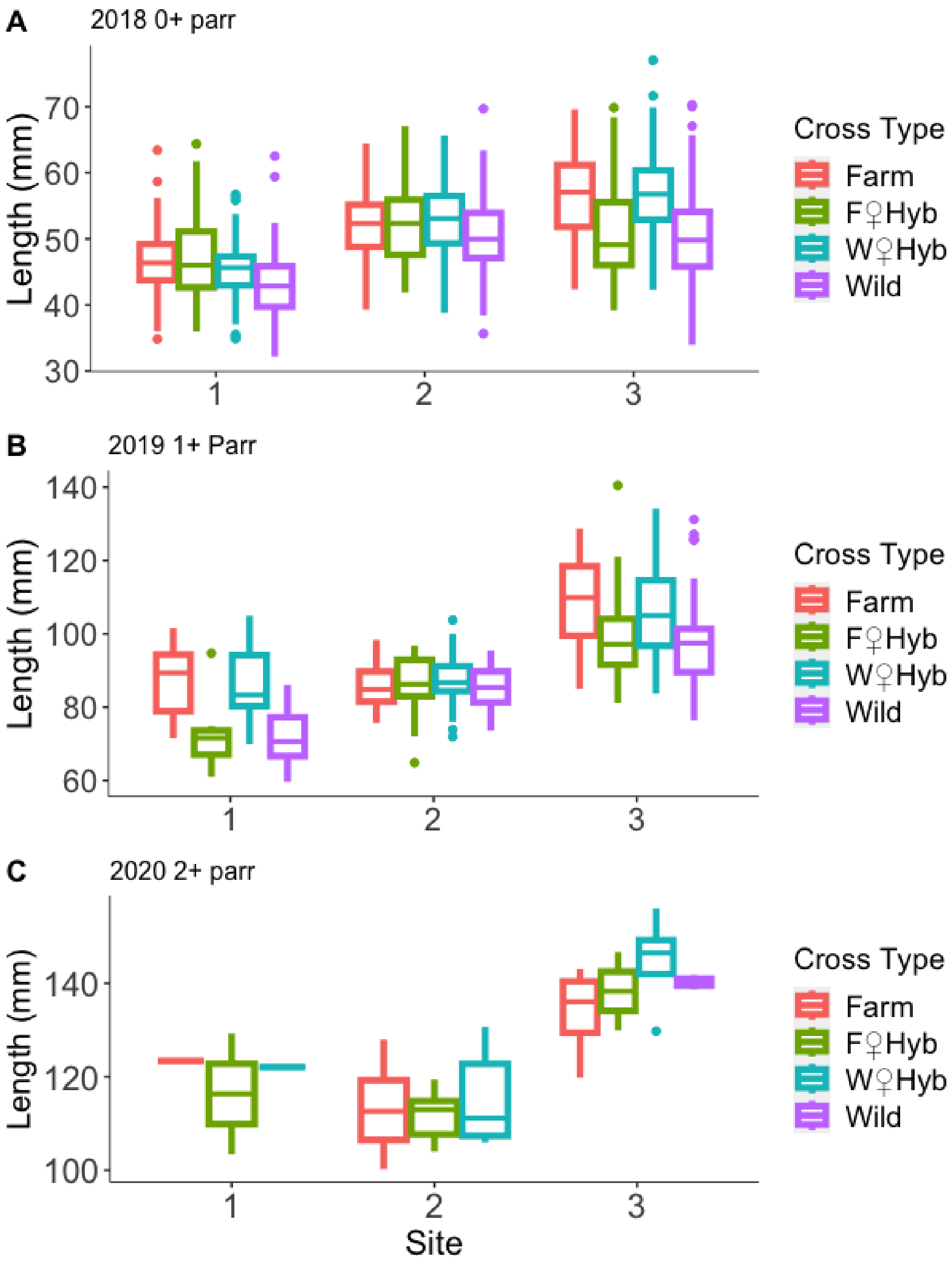


Figure 2.4. Lengths of Atlantic salmon by cross type at recapture in each site at (A) 3-months post-release (2018), (B) one-year post-release (2019), and (C) two years post-release (2020). Bold lines represent median values, boxes 25% and 75% quartiles, whiskers 1.5 times the inter-quartile range, and dots outliers.

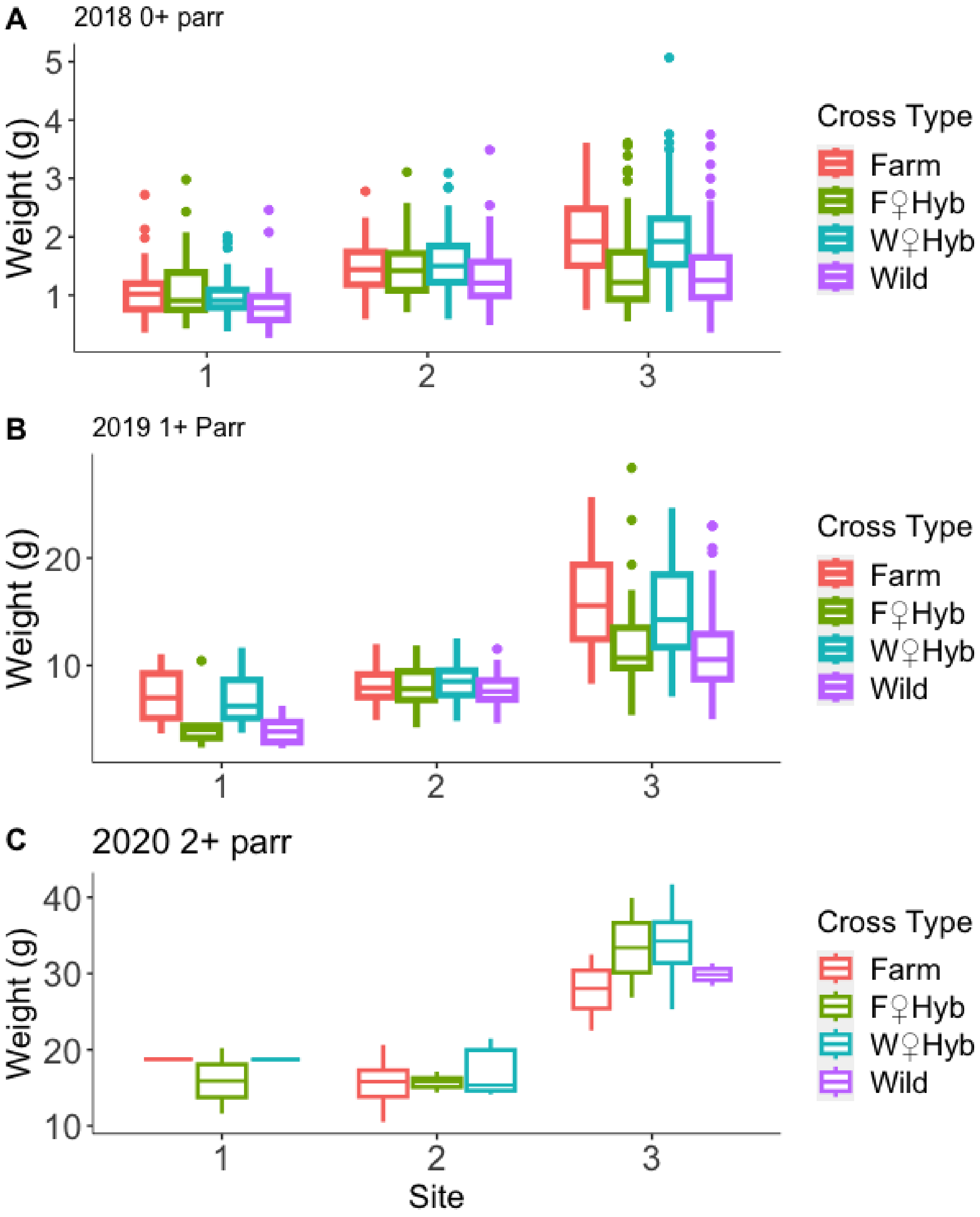


Figure 2.5. Weights of Atlantic salmon by cross type at recapture in each site at (A) 3-months post-release (2018), (B) one year post-release (2019), and (C) two years post-release (2020). Bold lines represent median values, boxes 25% and 75% quartiles, whiskers 1.5 times the inter-quartile range, and dots outliers.

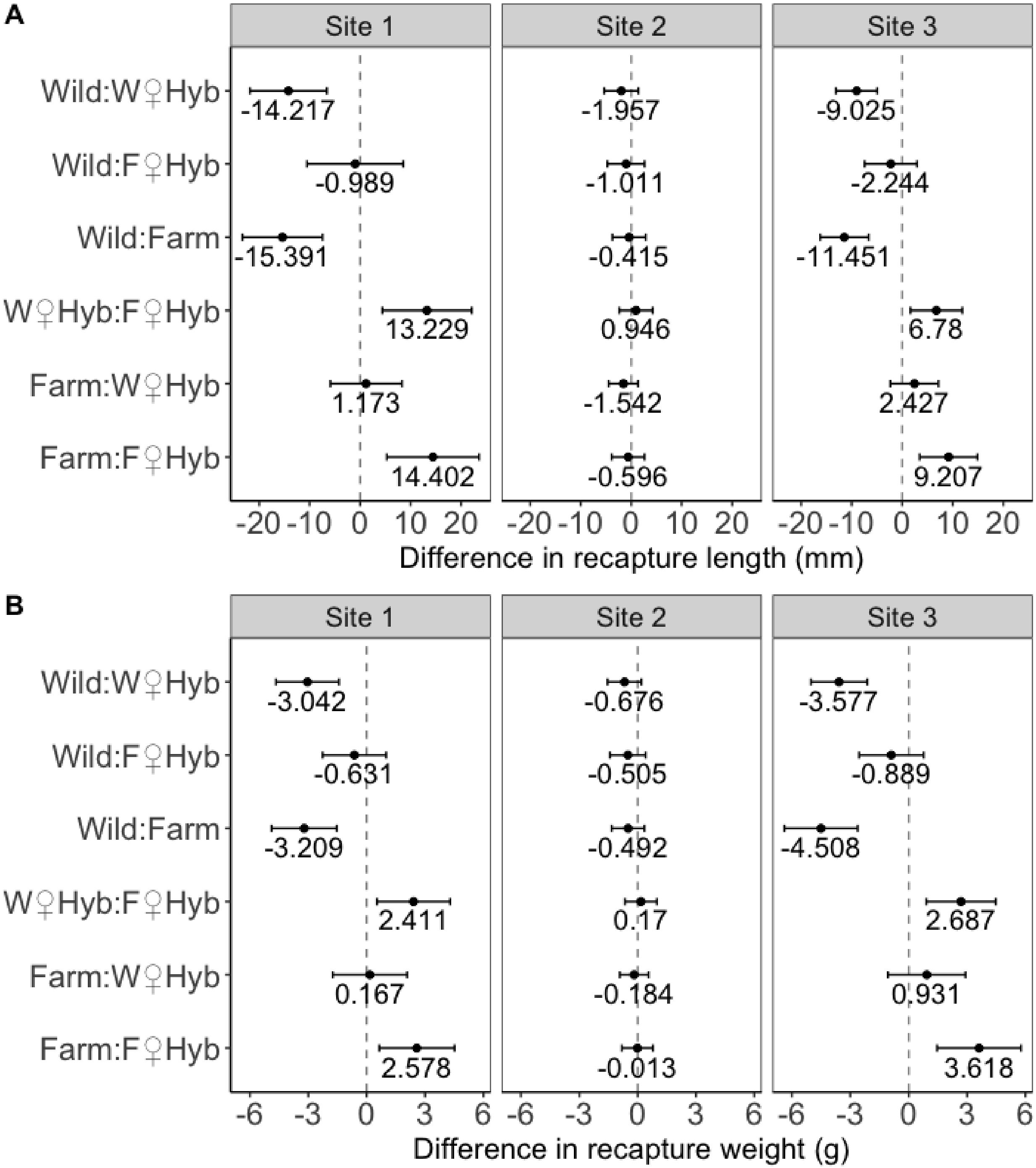


Figure 2.6. Pairwise differences in mean (A) length and (B) weight at recapture in each site one-year post-release (2019). Error bars represent ±2 SE of the difference estimate.

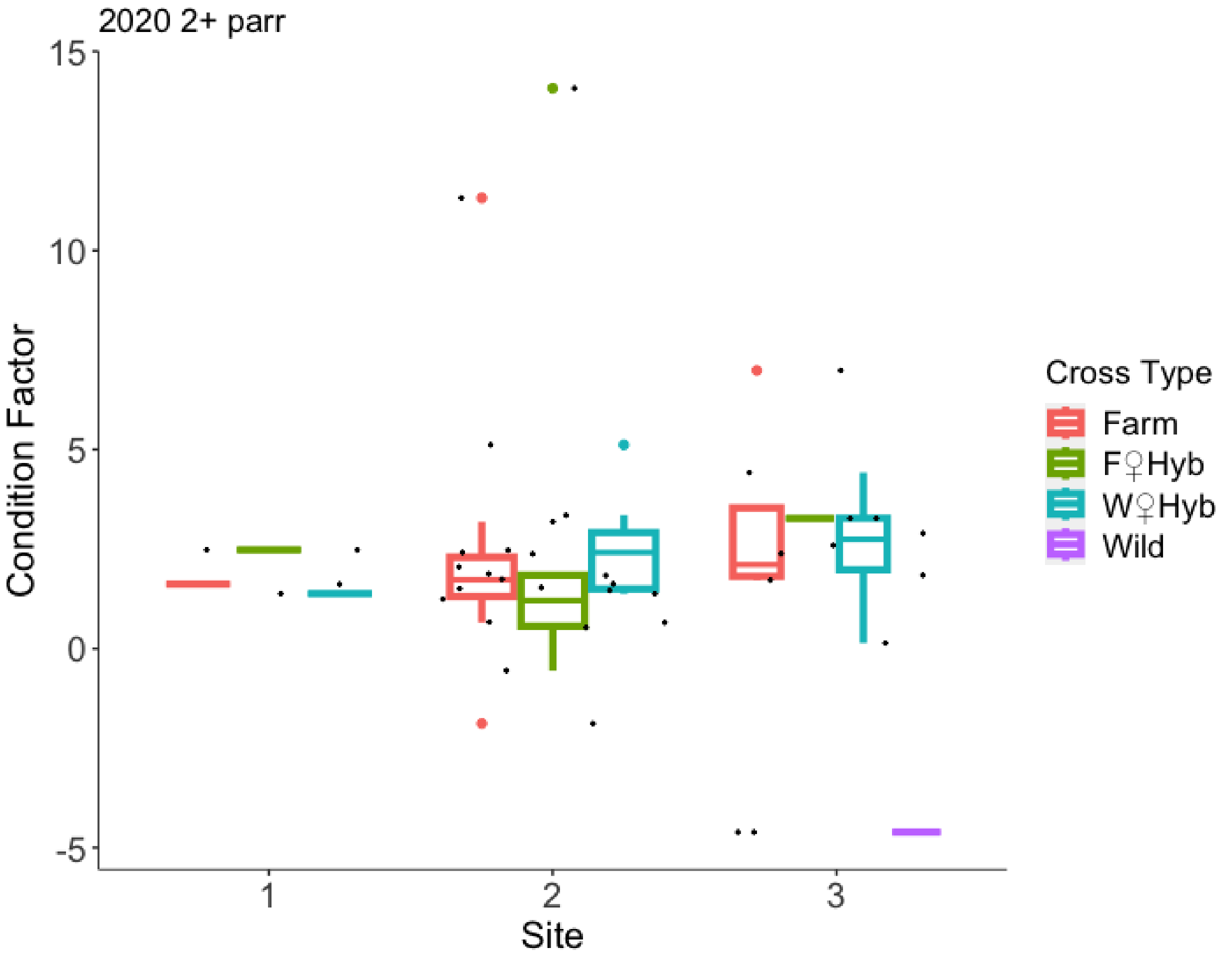


Figure 2.7. Condition of each Atlantic salmon at recapture in 2020 for each cross type by site pairing. Two wild samples recaptured only in Site 3. Bold lines represent median values, boxes 25% and 75% quartiles, whiskers 1.5 times the inter-quartile range, and dots outliers.

Chapter 3: Genetic monitoring suggests ongoing genetic change in wild salmon populations due to hybridization with aquaculture escapees

Abstract

Substantial domestication associated divergence of farmed from wild Atlantic salmon has raised concerns regarding the negative impacts of farm escapes on the genetic integrity and adaptability of wild salmon populations. Studies from across the North Atlantic have continually demonstrated the presence of hybridization and introgression from escapees into wild salmon populations, however longer-term genetic monitoring has rarely been conducted. Here we use targeted SNP panels to evaluate the presence of hybridization and subsequent introgression of escaped farmed salmon into 18 southern Newfoundland rivers and reconstruct the number of escapees contributing to hybrids sampled over an 8-year period encompassing three reported escape events. Ongoing hybridization was observed throughout the entire time series, with F1 young-of-the-year individuals detected every sampling year, as well as a notable increase in the proportion of backcross wild fish over the 8-year period. Population estimates of domestic admixture were highest in smaller rivers reaching up to 78%, and the presence of aquaculture associated European ancestry within individuals ranged as high as 39%. Spatial patterns of hybridization were closely associated with proxies of wild population size based on habitat area. Using genetic estimates of relatedness, farm escapee parents were predicted to be present each year preceding sampling, and this estimate was elevated following two escape events. This 8-year genetic time series presents the longest existing time-period for monitoring the impacts associated with farm escapees in North America and provides valuable insight into the ongoing genetic impacts of escaped farmed salmon on wild salmon populations.

Introduction

Atlantic salmon (*Salmo salar*) have been domesticated for ≥12-15 generations (Glover et al. 2017, Coulson et al., *in press*), resulting in considerable genetic differences in farm individuals relative to their wild counterparts (Karlsson et al. 2011, Glover et al. 2017, Bradbury et al. 2020a, Diserud et al. 2023). As a result, farmed salmon generally exhibit reduced levels of adaptation to the wild environment, and the introduction of farm alleles into wild populations can compromise genetic integrity and negatively affect wild population viability (Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2012, Diserud et al. 2019). Furthermore, the number of farm escapees can frequently match or even exceed that of wild individuals (Morris et al. 2008, Diserud et al. 2019), and the consequences of interbreeding are known to be long-lasting and heritable, resulting in reduced in fitness for wild populations (McGinnity et al. 2003, Skaala et al. 2019, Sylvester et al. 2019). As such, hybridization and genetic introgression between wild and farmed salmon has been widely documented (Bourret et al. 2011, Karlsson et al. 2016, Glover et al. 2017, Wringe et al. 2018, Harvey et al. 2018) and escapees from net-pen farming operations have been identified as a major threat to wild Atlantic salmon populations (e.g., Forseth et al. 2017).

Despite the potential risk of significant impacts of aquaculture escapees on wild salmon populations, the long-term consequences remain difficult to predict. The impact of escapees on wild populations likely depends on a variety of factors including the extent of maladaptation of cultured individuals to a given natural environment (Fleming 1995, Baskett et al. 2013). Various experimental and observational studies have revealed that introgression of domesticated individuals can have negative effects on fitness (Fleming et al. 2000, McGinnity et al. 2003, Skaala et al 2012, 2019, Sylvester 2019) and ultimately the phenotype of wild stocks (Bolstad et al. 2017, 2021, Besnier et al. 2022). Furthermore, modelling studies support the hypothesis that such introgression can translate into reduced population fitness and threaten persistence (Hindar et al. 2006, Castellani et al. 2018, Sylvester et al. 2019, Bradbury et al. 2020b). The prevalence of escaped farmed salmon and their introgression into wild populations have been associated with several factors in both Norwegian (Fiske et al. 2006) and North American rivers (Keyser et al. 2018) including proximity to aquaculture facilities (Fiske et al. 2006), farm production estimates (Keyser et al. 2018), wild salmon abundance in a stream, and mean yearly discharge (Mahlum et al. 2021). More recently, Holborn et al. (2022) documented that the high rate of precocial male maturation in farmed-wild- F1 hybrids can potentially accelerate the introgression process and associated impacts. However, estimating the long-term effects of these complex genetic interactions remains challenging as there is variation in levels of hybridization, and introgression in space and time in the wild and small escape events are often undetected.

Here our main goal is to use genetic monitoring data to examine spatial and temporal trends in hybridization and introgression of farmed and wild Atlantic salmon in southern Newfoundland over an 8-year period. Specifically our objectives were to (1) use genetic estimates of domestic admixture and assignment to hybrid class to assess temporal and spatial changes in hybrid class composition in young-of-the-year salmon, (2) identify potential environmental and spatial drivers of hybrid abundance, and estimated levels of domestic admixture in wild populations; and finally (3) estimate the number of farm escapee parents contributing to our samples per year in each river by exploring family structure and relatedness patterns among F1 hybrids. This study provides further evidence that the genetic impacts of farm escapes are long-lasting and can be exacerbated by factors such as precocial maturation and the size of a river, and we demonstrate that smaller farm escapes are constantly occurring. It directly extends previous work on genetic interactions between escapees in eastern Canada focusing on southern Newfoundland following a large escape event (Wringe et al. 2017), and extensive hybridization and selection against domestic offspring in the wild (Sylvester et al. 2019).

Methods

River sampling and genotyping

Atlantic Salmon parr were collected from 18 rivers in southern Newfoundland during the summer months between 2014 and 2021 via electrofishing (Wringe et al. 2018, Holborn et al. 2022) (Table 1, Figure 1). Captured fish were euthanized either by a lethal dose of MS-222 or brain pithing, and a fin clip was collected from all sampled individuals and preserved in 95% ethanol for later DNA extraction. Fork length was measured in samples collected and used as in Sylvester et al. (2019) to assign individuals to the young-of-the-year (YoY) age class. An allele frequency was determined for pure wild and domestic populations as described in Wringe et al. (2018). Early studies genotyped fish from the YoY age class with Fluidigm SNP Type assays for 96 diagnostic SNP markers (primary panel) previously shown to assign individuals to a hybrid class (Wringe et al. 2018; Sylvester et al. 2019, Holborn et al. 2022). Here, we followed these methods to continue the collection and genotyping of samples from 2019–2021. Following quality control filtering, individuals with a sample call rate ≥0.9 were retained for subsequent analysis. Samples were assigned to one of six genetic classes: 1) pure wild, 2) pure farmed (indicating feral offspring), 3) F1 hybrid, or one of several second-generation hybrids that are further introgressed, 4) F2, 5) backcross wild (BCW), and 6) backcross farm/feral (BCF). Individuals assigned to any one of the four hybrid genetic classes based on the primary panel were selected for genotyping on an additional 95 SNP panel for fish collected in 2014 - 2016 (Wringe et al. 2019) or a 96 SNP panel for 2017 - 2021 fish (Holborn et al. 2022). Both panels have an overlap of 83 SNPs, however, the second 96 SNP panel was redeveloped to reflect population changes over the time series by incorporating several more informative SNP loci. A combination of these additional panels and the primary panel (herein called the secondary panel) allowed for confirmation of hybrid genetic class and improvement of assignment power.

Genetic class analysis

The R packages *genepopedit* (Stanley et al. 2017) and plink v1.9 (Purcell et al. 2007, Chang et al. 2015) were used to perform genetic data manipulation, while the program NEWHYBRIDS (Anderson & Thompson 2002) and the R package *parallelnewhybrid* (Wringe et al. 2017b) ran simultaneously to categorize samples into one of the 6 genetic classes (see previous section). NEWHYBRIDS employs a Bayesian model-based clustering analysis for each individual, utilizing Markov Chain Monte Carlo sampling to calculate the posterior probability. To minimize the risk of misinterpreting inter-river genetic differences as admixture, individual analyses were performed for each river. In this process, we exclusively considered pure wild individuals with a posterior probability of assignment ≥0.995, specifically tailored to singular rivers in the baseline.

For the baseline sample analysis and to determine the posterior probability of assignment for each genetic class we followed the methods outlined in Holborn et al. (2022), Based on which genetic class had the highest posterior probability, each individual was then classified as pure wild, pure farmed/feral, or hybrid, where hybrid is represented by the sum of the F1, F2, BCW, and BCF posterior probabilities. A posterior probability threshold of 0.8 was set as a minimum for pure wild or pure farmed/feral genetic classes, and individuals that did not pass this threshold were removed from the dataset. Further analysis using SNPs from the primary and secondary SNP panels were performed on fish initially identified as a hybrid genetic class as described in Holborn et al. (2022). To determine the ability of the secondary panel to appropriately assign individuals to a hybrid genetic class, its efficiency and accuracy were estimated and described in Holborn et al. (2022) and Wringe et al. (2017a).

Admixture Estimates

Levels of domestic admixture were estimated for each river based on the genetic class each sample was assigned to, so that F1 and F2 hybrids had an admixture value of 0.5, BCF hybrids had a value of 0.75, BCW hybrids were considered to be 0.25 admixed, and pure wild and farm were 0 and 1 respectively**.** The domestic admixture values were averaged across all the samples collected from each river each year. To avoid elevated estimates in sites that were only sampled up to 2018, we split our analysis into two different groups, 2014-2018, which included all rivers, and 2014-2021, which only included rivers sampled through the entire time series. This allowed us to make more accurate comparisons across sites. Recent work has also identified traces of European ancestry in escaped North American farmed salmon and escape-associated European ancestry in the wild (Bradbury et al. 2022). As such, we also estimated the individual European admixture for any individuals identified as aquaculture offspring (see above) using a targeted subset of 301 SNPs genotyped with a custom amplicon panel using the PYTHON package SALMONEUADMIX (Nugent et al. 2023), which uses a custom SNP amplicon panel and deep neural network to make de novo estimates of European ancestry (see Nugent et al. 2023 for details).

Spatial drivers of hybrid abundance and domestic admixture

Accessible habitat, estimated wild population size, and propagule pressure were analyzed as potential drivers of hybrid YOY abundance and domestic admixture in each river. These estimates were taken from previous work by Keyser et al. (2018), Wringe et al. (2018), and

Bradbury et al. (2020). Axial length (i.e. accessible habitat) was measured as the straight line distance along the longest axis of a river to complete obstruction using data from Porter et al. (1974) (as in Bradbury et al. 2020). Estimates of population size were derived using a previously established relationship between river size and wild population size for Newfoundland following Wringe et al. (2018). These values were then corrected for population declines experienced over recent decades (I.R. Bradbury, pers. communication, 2023). Propagule pressure is a composite measure referring to the intensity of introductions of non-native species into a region (Colautti 2005, Copp et al. 2007). In Bradbury et al. (2020), propagule pressure was calculated by dividing the maximum stocking allowable at an aquaculture site, by the distance from each river to that site, and summed across all aquaculture sites for a given river. The same estimates of propagule pressure were used here.

To account for the fact that only certain rivers being sampled after 2018, we divided the analysis into two sampling periods. The first period, from 2014 to 2018, included data from all rivers, and the second period, from 2014 to 2021, focused only on rivers sampled during these years. This division allowed us to mitigate potential bias in the average proportion of hybrids, particularly in rivers that were only sampled in the earlier years.

Hybrid family structure and escapee parent estimates

COLONY v.2.0.6.8 (Jones & Wang 2010) was used to identify related individuals, including full-sibs (FS), half-sibs (HS), and family clusters in all samples collected from the 18 rivers between 2014-2021. Each river was analyzed individually using the same SNP panels that were used in the genetic class analysis (see Genetic class analysis section). COLONY calculated the likelihood that each pair of individuals were full-sib, half-sib, non-sib, or identical twins, and the relationship with the maximum likelihood was returned as the best estimate. Since F1 and BCF hybrids require a single feral parent, and pure farm individuals have two feral parents, we calculated the range of unique pure farm parents by considering the number of family clusters observed in each river and year. The number of families represented the minimum count of wild or farm parents involved in hybrid crosses per river. Additionally, half of the unique parents of F1 hybrids in each river was taken as a middle estimate for the number of breeding aquaculture escapees.

Statistical analysis

To identify river or population specific factors which may be important for hybrid abundance and domestic admixture, we used the proportion of hybrid salmon in wild populations as described in Diserud et al. (2022) as the response variable, and investigated its association with accessible habitat, estimated wild population size, and propagule pressure. This was logit-transformed to stabilize the variance and allowed for traditional linear regression models to be used for the transformed responses. Normality and homoscedasticity of residuals were assessed by visual examination of residual vs. fitted plots and normal Q−Q plots to validate the assumptions of linear modelling. Where these assumptions were met, a general linear model was used for analyses of the transformed variables, while a generalized linear model was used when residuals did not meet assumptions. For generalized linear models, we employed analysis of deviance (McCullagh and Nelder 1989) to identify the evidential strength of different fixed factors as a normal error structure was not appropriate.

To study changes in genetic class composition over time, temporal trend analyses were conducted with general linear mixed models, using the logit transformed proportions of each genetic class as a response variable, year and genetic class as regression variables, and river as a random effect, as not all rivers were sampled every year. Similarly, temporal changes in domestic admixture estimates were analyzed using general linear mixed models using logit transformed admixture estimates as response variables, year as a regression variable, and river as a random effect. The spatial analysis of hybrid abundance and domestic admixture in rivers was conducted using the logit transformed average proportion of hybrids as a response, and accessible habitat, propagule pressure, and estimated wild population size as regression variables. Due to an interaction among all the regression variables, these were then analyzed individually, using the same method where possible, and a generalized linear model when residuals did not meet assumptions.

All statistical analyses were performed in R version 4.3.0.

Results

Genetic class analysis

A total of 5,845 YoY fish over the 8-year period were successfully genotyped and exceeded the posterior probability threshold of 0.8 for assignment to one of the 6 genetic classes on either the primary or secondary SNP panel. Temporal changes in genetic class composition following the escape events (2013, 2015, and 2018) indicated an overall increasing trend in the proportion of pure wild YoY throughout the region with slight decreases in 2017, 2019, and 2021 coupled with a decrease in the proportion of hybrid and farm YoY (Figure 2A). The highest proportion of pure wild individuals across all rivers (0.96) was found in 2020, as this value increased from 0.52 in 2014, the year following the first escape event of the time series. This same year (2014), the highest proportion of pure feral fish were sampled (0.13), decreasing to 0.02 the following year, and absent in 2016. However, a small proportion of pure farm individuals was again encountered in 2017 and

2018 throughout the area (0.02 and 0.01 respectively) though these were not found again until 2021, when 6 of the 784 fish sampled were of pure farm origin. The largest proportion of hybrids was encountered in 2014 and 2017 with hybrid YoY making up 0.35 of the total sample both years. In 2017, the proportion of hybrids increased from 0.23 in 2016, and the proportion of pure wild YoY dipped from 0.77 to 0.63. There was a large decrease in the overall proportion of hybrids from 2019 to 2020, at which point hybrid individuals only made up 0.04 of the sample. During the final year of sampling, the proportion of hybrid YoY again rose from previous years, and this was paired with the lowest proportion of pure wild fish since 2017.

Of the four hybrid classes, BCW was dominant every year except in 2014 (Figure 2B). There was a consistent increase in the overall proportion of BCW individuals of the hybrids detected, starting at 0.1 in 2014 and reaching a peak of 0.91 in 2021. F1 was the dominant class among the hybrid sample in 2014, making up 0.66 of it. However, a general decrease in this proportion was observed over the sampling period, with 2021 being the lowest at 0.007 and mirroring the peak in BCW that same year. There was evidence for an interaction between year of sampling and genetic class in our model ($p < 0.01$, Supplementary Table 1). Temporal analysis applied to each genetic class separately, using sampling year as a regression variable, provided evidence for a significant effect of year on the proportion of individuals per genetic class for all classes except F2 (F1, BCW, and BCF $p < 0.001$; pure farm $p = 0.00127$; pure wild $p = 0.0347$; F2 $p = 0.867$; Figure 2B, Supplementary Table 2).

Admixture Estimates

The average estimate of domestic admixture varied significantly over the course of 8 years as levels ranged from 1.2% up to 78% among rivers, with the annual average peaking in 2014 following the large escape event, and then decreasing throughout the sampling period (p <

0.001, Figure 3, Supplementary Table 3). Certain sites had a slight increase in levels of domestic admixture between 2016-2018 relative to previous years, however, admixture was overall lower in the later years of the study compared to the first year of sampling. Some of the smallest rivers had the highest levels of domestic admixture, upwards of 60%; specifically, Grand LaPierre (GLP) was the most highly admixed river during 2014-2018, having an admixture estimate close to 70% (Figure 4A). Tailrace Brook (TRB) and Terrenceville Brook (TEB) also had high overall domestic admixture, both higher than 40%, and up to 61%, while Taylor Bay Brook (TBB) and Northwest River (NWR) showed the greatest variation in admixture levels. Of those rivers sampled over the entire period (2014-2021), TEB was the most highly admixed river, having roughly 50% domestic admixture, followed by TRB and Southeast Brook (SEB) (Figure 4B). Populations from most rivers had some level of domestic admixture by the end of the sampling period (2014-2021).

Estimates of individual European admixture ranged from 1% up to 39%, with the highest levels frequently being in those rivers where high levels of domestic admixture were also detected (Figure 4C). It has been previously established that there is a degree of neutral European ancestry in North American wild Atlantic salmon, which does not exceed 10% (Bradbury et al. 2022). There were 7 rivers where individuals exceeded this threshold, Bay du Nord River (BDN), Conne River (CNR), Garnish River (GAR), Long Harbour River (LHR), SEB, TEB, and TRB. Of these rivers, all but TEB and TRB had 5 or fewer individuals that had a European admixture estimate above 10%, while TEB and TRB had 22 and 46 individuals, respectively, surpassing the 10% threshold. The highest individual admixture estimate was 0.388 for a fish sampled in TRB.

Spatial drivers of hybrid abundance and domestic admixture

The site-specific variables, accessible habitat, wild population size, and propagule pressure, were evaluated independently from each other as their effect on the average proportion of hybrids during 2014-2018 when all populations were sampled had a significant interaction with one another ($p = 0.0112$, Supplementary Table 4). Even with the logit transformation, none of the residuals from the individual models met both the homoscedasticity and normality assumptions, therefore, they were analyzed using generalized linear models. There was an effect of wild population size ($p = 0.0172$) and accessible habitat ($p = 0.0186$) on the average proportion of hybrid YoY per river (Figure 5, Supplementary Table 5A, B). The average proportion of hybrid YoY was negatively correlated with both of these factors, as larger rivers with higher numbers of wild salmon consistently had lower proportions of hybrids than several of the smaller rivers. The effect of propagule pressure on average proportion of hybrids was weak but had an overall positive trend ($p = 0.387$, Supplementary Table 5C).

A significant interaction between estimated wild population size and propagule pressure was observed in the admixture spatial analysis ($p = 0.0101$, Supplementary Table 6), therefore, these two factors were again evaluated independently. Accessible habitat, however, did not interact with any other variable and did not have an effect on the degree of domestic admixture in the rivers sampled ($p = 0.111$, Supplementary Table 6). Although the relationship between the three site-specific variables and the degree of domestic admixture per river followed the same pattern observed in the hybrid abundance analyses, none of these relationships were statistically significant ($p \geq 0.0648$, Supplementary Tables 6 and 7). This suggests that wild population size and propagule pressure likely interact with each other to influence the levels of admixture per river, but individually, they do not exert a substantial effect on domestic admixture.

When considering just the 12 populations sampled throughout 2014-2021, the smaller sample size reduced the statistical power to detect any effects of wild population size, accessible habitat, and propagule pressure on the proportion of hybrid YoY as well as domestic admixture (Supplementary Tables 8 and 9 respectively). However, the overall relationship between these variables and both the proportion of hybrids and degree of admixture, followed the same trend to that of previous years, with wild population size and accessible habitat having a negative association with the average proportion of hybrid YoY.

Hybrid family structure and escapee parent estimates

The number of pure farm parents in all 18 southern Newfoundland rivers peaked the same year as the largest escape event (2013) and had a subsequent slight increase in 2016, following a second escape (Figure 6). In 2013, the possible number of successfully breeding escapees ranged from 108-264 and was followed by a substantial decline the following years. After the slight increase in 2016, the decline continued. However, at least one farm parent was detected every year between 2013 and 2020 (Figure 6). There was one more substantial escape in 2018, however, fewer domestic parents were detected immediately following this event relative to previous escapes. Still, the occurrence of breeding farm individuals was consistent during the years subsequent to this event, with up to 4 domestic parents identified from the last year of sampling.

Discussion

The genetic impacts of escaped farmed salmon represent a substantial threat to the persistence and stability of wild salmon populations (Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2012, 2019, Sylvester et al. 2019), yet managing these interactions remains challenging due a general lack of monitoring data on the prevalence of escapees, and levels of hybridization. Here our study builds upon previous research conducted over an 8-year period in southern Newfoundland. In this study, we provide the most extensive monitoring period to date on the impact of farm salmon escapees in North America. Our results suggest the continued presence of escapees in the wild, ongoing hybridization of farmed and escapee salmon with wild individuals, and elevated introgression in smaller wild populations. Significant genetic changes appear to have occurred due to introgression in some smaller populations which display high domestic admixture (up to 78%), and estimates of aquaculture associated individual European ancestry within individuals ranged as high as 39%. This work directly builds on previous studies documenting introgression between wild and farm escaped salmon both in North America and Europe and reinforces the potential for genetic impacts in wild populations due to interbreeding with escaped farmed salmon. Estimating the genetic impacts of farm escapes over longer time periods such as accomplished here and genetic reconstructions of escapees contributing to hybrids detected, provides a novel time series and unprecedented insight into the consequences of escape events and direct genetic interactions, which can directly inform conservation actions for at risk populations and aquaculture management decisions in the future.

Genetic class analysis

Our observations of persistent wild-farm hybrids within the southern Newfoundland population is consistent with both the continued presence of escapees in the wild and ongoing introgression as has been reported elsewhere (Bourret et al. 2011, Karlsson et al. 2016, Glover et al. 2017, Gilbey et al. 2021, Palm et al. 2021). Evidence of hybridization was present in the detection of F1 hybrids which were the most prevalent YoY hybrid class in 2014 (Wringe et al. 2018), the year immediately following the largest reported escape event during this period. This is consistent with a substantial inflow of mature farm escapees as the overall proportion of F1s decreased over the following years and was the lowest during our last sampling year. However, we observed F1 YoY every year over our 8-year monitoring period, even though only two other escape events were reported (2015 and 2018). This indicates the likelihood of smaller, ongoing escapes and is in line with reports from counting fences in southern Newfoundland, where mature farm escapees have been noted entering the rivers (Bradbury et al. 2020b). These observations are consistent with previous work in Norway suggesting reported farm escapes represent an underestimate of escapees present (Skilbrei et al. 2015), and support the hypothesis that escapes in southern Newfoundland could be occurring without detection.

Interestingly, the hybrid class composition changed significantly over the 8-year sampling period, with a continuous increase in the proportion of BCW individuals expanding on the observations of Wringe et al. (2018), Sylvester et al. (2019), and Holborn et al. (2022). This provides further evidence that hybrid individuals can successfully breed likely as precocial males in the wild, despite lower survival rates compared with wild fish (McGinnity 1997, Fleming et al. 2000, Skaala et al. 2012, 2019). Since most of the sampled fish included contributions from wild Newfoundland Atlantic salmon, which have a high likelihood of male parr maturation (Dalley et al. 1983), the precocial maturation observed in our sample may be due to a genetic predisposition for precocial hybrid male maturation or the availability of food resources, as growth has been shown to influence the incidence of precocial maturation (Saunders et al. 1982, Letcher & Gries 2003). There was a notable increase in BCW hybrids between 2016-2019, suggesting that F1s from the first large escape likely started spawning between 2015 and 2017. Given that Atlantic salmon juveniles in southern Newfoundland typically spend 3-4 years in freshwater prior to smoltifying and migrating downstream (DFO 2006), followed by one winter in the ocean (DFO

2022), it is unlikely that F1 hybrids would have successfully returned from sea during this timeframe. This observation therefore supports the contention that hybrid males, which can mature precocially at high rates in rivers (San Román et al. 2023) and reproduce without undergoing certain selective events, such as marine migration, may expedite introgression through interbreeding (Garant et al. 2003, Holborn et al. 2022). Moreover, this trend continues following smaller escape events, the number of BCW individuals peaked in 2021, 3 years after the third escape event when resulting F1s could have begun spawning as 2-year-olds, in addition to farm individuals from the possible ongoing small escapes. While it remains possible that some breeding F1s in these later years could be adults from previous escapes returning from a winter at sea, the peak in BCW hybrids during the years closely following escape events points to a greater role of F1 males maturing precocially in the introgression observed.

Admixture estimates

Our results suggest both domestic and associated European admixture were widespread and elevated in smaller populations. In line with previous findings, domestic admixture varied temporally, but overall decreased as more time elapsed since the major escape event (Wringe et al. 2018, Sylvester et al. 2019, Wacker et al. 2021). Various rivers within our sample showed high levels of domestic admixture, suggesting that the genetic impacts of farm escapees are substantial (Glover et al. 2013), and some of these exhibit instances of hybrid swarms, characterized by the extensive introgression of farm genotypes into the wild population, potentially resulting in the loss of pure genotypes over time (Allendorf et al. 2001). This can be detrimental for wild Atlantic salmon as reduced survival of hybrid and introgressed parr has been observed resulting from moderate levels of admixture (Fleming et al. 2000, McGinnity et al. 2003, Wringe et al. 2018, Wacker et al. 2021). Therefore, the persistence of genotypes associated with domesticated individuals can contribute to a decline in the overall viability of already endangered wild Atlantic salmon populations

We also detected significant levels of European admixture, which again varied by river, where smaller sites with higher domestic admixture were more heavily affected by European admixture as well. Genomic studies have recently reported substantial levels of European admixture in farm and escaped individuals (Liu et al. 2017, Bradbury et al. 2022). This can be detrimental for local wild populations as there is substantial genome-wide genetic differentiation between European and North American Atlantic salmon (Bourret et al. 2013, Jeffery et al. 2017, Lehnert et al. 2020), and salmon in Europe have been subjected to domestication selection for a few more generations than individuals in North America (Glover et al. 2017). Given that this divergence has been previously associated with genomic regions relating to metabolic, developmental, immune, and neural processes (Lehnert et al. 2020), there is potential for substantial maladaptation resulting from interbreeding between wild and farm Atlantic salmon (Islam et al. 2022).

Spatial drivers of hybrid abundance and domestic admixture

We found that the average proportion of hybrid individuals in rivers was associated with river and population specific features. The proportion of hybrids decreased with increasing river and population size, though this negative relationship was only significant between 2014-2018, the five years directly following the first major escape event. Since the largest escape event occurred in 2013, causing the highest influx of farm escapees into the rivers throughout the first few years directly following the escape, this could have resulted in a diluted effect of the spatial factors on proportion of hybrids in later years, when the influx of farm fish was smaller.

Wild population size has been previously identified as a potential predictor for the proportion of escaped farm salmon in a river. In Norway, Diserud et al. (2022) determined that large population size had a negative effect on the proportion of escapees in the river, and Mahlum et al. (2021) suggested that farm escapees may follow wild salmon to their native rivers, therefore influencing the abundance of farm fish per river. Our results more closely align with Diserud et al. (2022) as the proportion of hybrids present per river decreased with increasing population size. Rivers with smaller populations potentially more easily saturated by the influx of farm escapees (Glover et al. 2013, Heino et al. 2015). Furthermore, previous research has suggested that the size of a native population may affect the relative success of escaped farm individuals, by increasing competition between wild and feral fish both on the spawning grounds and at juvenile stages (Fleming et al. 2000, Glover et al. 2012, 2013, Skaala et al. 2012). Even though the spatial factors studied here only had a significant effect on the degree of domestic admixture when analyzed together, these followed the same trend as the hybrid analysis. Smaller rivers with lower population sizes have a higher likelihood of being more heavily admixed when compared to larger nearby sites with larger populations. The data available to estimate wild population size in each river, however, was relatively limited, and the estimates relied heavily on river size. Therefore, this could have impacted our ability to detect more modest associations. Yet, our results overall suggest that smaller Atlantic salmon populations are at greater risk of hybridization with, and genetic introgression from farm escapees, and continuous monitoring of aquaculture-affected sites is warranted.

Hybrid family structure and escapee parent estimates

Estimation of family structure among the aquaculture escapee offspring represents a novel approach to monitor the minimal number of escapees present in a region contributing to successful reproduction. Our results suggest there was a clear peak in the number of estimated domestic salmon interbreeding with wild individuals in 2013, the same year as the largest escape event. As previously discussed, this was followed by the highest proportions of feral and F1 YoY in 2014. There was also a smaller, yet notable increase in the number of successfully breeding farm individuals the year directly following a second escape in 2015. This peak could again be linked to the increase in hybrid and pure farm YoY observed from 2016 to 2017, where we also detected an increase in the proportion of BCF hybrids. Even though the number of successfully breeding farm individuals generally decreased over our sampling period, we identified at least one feral parent every year. These observations indicate that escapees are a regular occurrence from salmon aquaculture in this region despite a lack of reported escape events and that the effects of large escapes can persist for several years after the event leading to substantial genetic changes in wild salmon populations. The results support observations in other regions where salmon farming and wild populations also overlap which have reported population-level genetic changes in Atlantic salmon populations associated with aquaculture escapees and introgression (e.g., Clifford et al. 1998a, b, Skaala et al. 2006, Bourret et al. 2011, Glover et al. 2012, Karlsson et al. 2016).

Conclusions

Here we present the longest time series study on the effects of farm salmon entering wild ecosystems in the Northwest Atlantic encompassing several escape events. Our overall results indicate that aquaculture escapees are a persistent threat to wild Atlantic salmon and can genetically alter wild populations for extended periods following escape events. The hybrid class makeup of most of the rivers analyzed here changed over our study period as we identified a substantial increase in the proportion of BCW hybrids throughout the eight years. We detected

F1 YoY in rivers, suggesting that escapes from farms occur constantly even in the absence of reported escape events, and feral individuals are able to successfully interbreed with wild salmon. These results clearly demonstrate that escapees and subsequent introgression of domestic salmon into wild populations can modify the genetic make-up of these populations, which can be both an immediate and long-term threat to their persistence. As such consistent genetic monitoring of wild populations and escapees is critical to quantify the genetic impacts of farm escapes on wild salmon populations and to inform appropriate conservation and mitigation actions.

References

Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. Trends Ecol Evol 16:613–622.

Anderson EC, Thompson EA (2002) A Model-Based Method for Identifying Species Hybrids Using Multilocus Genetic Data. Genetics 160:1217–1229.

Baskett ML, Burgess SC, Waples RS (2013) Assessing strategies to minimize unintended fitness consequences of aquaculture on wild populations. Evol Appl 6:1090–1108.

Besnier F, Ayllon F, Skaala Ø, Solberg MF, Fjeldheim PT, Anderson K, Knutar S, Glover KA (2022) Introgression of domesticated salmon changes life history and phenology of a wild salmon population. Evol Appl 15:853–864.

Bolstad GH, Kjetil H, Grethe R, Bror J, Harald S, Diserud OH, Peder F, Jensen AJ, Kurt U, Næsje TF, Barlaup BT, Bjørn F-L, Lo H, Eero N, Sten K (2017) Gene flow from domesticated escapes alters the life history of wild Atlantic salmon. Nat Ecol Evol 1(5):0124.

Bolstad GH, Karlsson S, Hagen IJ, Fiske P, Urdal K, Sægrov H, Florø-Larsen B, Sollien VP, Østborg G, Diserud OH, Jensen AJ, Hindar K (2021) Introgression from farmed escapees affects the full life cycle of wild Atlantic salmon. Sci Adv 7:eabj3397.

Bourret V, O'Reilly PT, Carr JW, Berg PR, Bernatchez L (2011) Temporal change in genetic integrity suggests loss of local adaptation in a wild Atlantic salmon (*Salmo salar*) population following introgression by farmed escapees. Heredity 106:500–510.

Bourret V, Dionne M, Kent MP, Lien S, Bernatchez L (2013) Landscape Genomics in Atlantic Salmon (*Salmo salar*): Searching for Gene–Environment Interactions Driving Local Adaptation. Evol 67:3469–3487.

Bradbury IR, Burgetz I, Coulson M, Verspoor E, Gilbey J, Lehnert SJ, Kess T, Cross T, Vasemägi A, Solberg MS, Fleming IA, McGinnity P (2020a) Beyond hybridization: the genetic impacts of nonreproductive ecological interactions of salmon aquaculture on wild populations. Aquacult Environ Interact 12:429–445.

Bradbury IR, Duffy SJ, Lehnert SJ, Jóhannsson R, Fridriksson JH, Castellani M, Burgetz I, Sylvester EVA, Messmer AM, Layton K, Kelly NI, Dempson JB, Fleming IA (2020b) Model-based evaluation of the genetic impacts of farm-escaped Atlantic salmon on wild populations. Aquacult Environ Interact 12:45–59.

Bradbury IR, Lehnert SJ, Kess T, Van Wyngaarden M, Duffy S, Messmer AM, Wringe B, Karoliussen S, Dempson JB, Fleming IA, Solberg MF, Glover KA, Bentzen P (2022) Genomic evidence of recent European introgression into North American farmed and wild Atlantic salmon. Evol Appl 15:1436–1448.

Castellani M, Heino M, Gilbey J, Araki H, Svåsand T, Glover KA (2018) Modeling fitness changes in wild Atlantic salmon populations faced by spawning intrusion of domesticated escapees. Evol Appl 11:1010–1025.

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaSci 4:7.

Clifford SL, McGinnity P, Ferguson A (1998a) Genetic changes in an Atlantic salmon population resulting from escaped juvenile farm salmon. J Fish Biol 52:118–127.

Clifford SL, McGinnity P, Ferguson A (1998b) Genetic changes in Atlantic salmon (*Salmo salar*) populations of Northwest Irish rivers resulting from escapes of adult farm salmon. Can J Fish Aquat Sci 55(2):358-363.

Colautti RI (2005) Are characteristics of introduced salmonid fishes biased by propagule pressure? Can J Fish Aquat Sci 62:950–959.

Copp GH, Templeton M, Gozlan RE (2007) Propagule pressure and the invasion risks of non-native freshwater fishes: a case study in England. J Fish Biol 71:148–159.

Coulson MW, Bradbury IR, Wringe BF, Burgetz I, McGowan C, Mimeault C, Parsons GJ. Assessment of the risk posed to wild Atlantic salmon population abundance and diversity by direct genetic interaction with escapes from East Coast Atlantic Salmon aquaculture. Can Sci Advis Sec Sci Advis Rep, in press.

Dalley EL, Andrews CW, Green JM (1983) Precocious male Atlantic salmon parr (*Salmo salar*) in insular Newfoundland. Can J Fish Aquat Sci 40:647-652.

DFO (Fisheries and Oceans Canada) (2006) Aspects of the Life History, Biology, and Population Dynamics of Atlantic Salmon (*Salmo salar* L.) in Eastern Canada - 2006. Can Sci Advis Sec Sci Advis Rep 2006/014.

DFO (Fisheries and Oceans Canada) (2022) Stock Assessment of Newfoundland and Labrador Atlantic Salmon in 2020. Can Sci Advis Sec Sci Advis Rep 2022/031.

Diserud OH, Fiske P, Sægrov H, Urdal K, Aronsen T, Lo H, Barlaup BT, Niemelä E, Orell P, Erkinaro J, Lund RA, Økland F, Østborg GM, Hansen LP, Hindar K (2019) Escaped farmed Atlantic salmon in Norwegian rivers during 1989–2013. ICES J Mar Sci 76:1140–1150.

Diserud OH, Fiske P, Karlsson S, Glover KA, Næsje T, Aronsen T, Bakke G, Barlaup BT, Erkinaro J, Florø-Larsen B, Foldvik A, Heino M, Kanstad-Hanssen Ø, Lo H, Lund RA, Muladal R, Niemelä E, Økland F, Østborg GM, Otterå H, Skaala Ø, Skoglund H, Solberg I, Solberg MF, Sollien VP, Sægrov H, Urdal K, Wennevik V, Hindar K (2022) Natural and anthropogenic drivers of escaped farmed salmon occurrence and introgression into wild Norwegian Atlantic salmon populations. ICES J Mar Sci 79:1363–1379.

Diserud OH, Hindar K, Karlsson S, Glover KA, Skaala Ø (2023). Genetisk påvirkning av rømt oppdrettslaks på ville laksebestander–oppdatert status 2023.

Fiske P, Lund RA, Hansen LP (2006) Relationships between the frequency of farmed Atlantic salmon, *Salmo salar* L., in wild salmon populations and fish farming activity in Norway, 1989–2004. ICES J Mar Sci 63:1182–1189.

Fleming IA (1995) Reproductive success and the genetic threat of cultured fish to wild populations. Protection of aquatic biodiversity 117–135.

Fleming IA, Hindar K, Mjølnerød IB, Jonsson B, Balstad T, Lamberg A (2000) Lifetime success and interactions of farm salmon invading a native population. Proc Biol Sci 267:1517–1523.

Forseth T, Barlaup BT, Finstad B, Fiske P, Gjøsæter H, Falkegård M, Hindar A, Mo TA, Rikardsen AH, Thorstad EB, Vøllestad LA, Wennevik V (2017) The major threats to Atlantic salmon in Norway. ICES J Mar Sci 74:1496–1513.

Garant D, Fleming IA, Einum S, Bernatchez L (2003) Alternative male life-history tactics as potential vehicles for speeding introgression of farm salmon traits into wild populations. Ecology Letters 6:541–549.

Gilbey J, Sampayo J, Cauwelier E, Malcolm I, Millidine K, Jackson F, Morris D (2021) A national assessment of the influence of farmed salmon escapes on the genetic integrity of wild Scottish Atlantic salmon populations. Scottish Marine and Freshwater Science 12(12):1-70.

Glover KA, Quintela M, Wennevik V, Besnier F, Sørvik AGE, Skaala Ø (2012) Three Decades of Farmed Escapees in the Wild: A Spatio-Temporal Analysis of Atlantic Salmon Population Genetic Structure throughout Norway. PLOS ONE 7:e43129.

Glover KA, Pertoldi C, Besnier F, Wennevik V, Kent M, Skaala Ø (2013) Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNPs. BMC Genetics 14:74.

Glover KA, Solberg MF, McGinnity P, Hindar K, Verspoor E, Coulson MW, Hansen MM, Araki H, Skaala Ø, Svåsand T (2017) Half a century of genetic interaction between farmed and wild Atlantic salmon: Status of knowledge and unanswered questions. Fish Fish 18:890–927.

Harvey AC, Skilbrei OT, Besnier F, Solberg MF, Sørvik A-GE, Glover KA (2018) Implications for introgression: has selection for fast growth altered the size threshold for precocious male maturation in domesticated Atlantic salmon? BMC Evol Biol 18:188.

Heino M, Svåsand T, Wennevik V, Glover K (2015) Genetic introgression of farmed salmon in native populations: quantifying the relative influence of population size and frequency of escapees. Aquacult Environ Interact 6:185–190.

Hindar K, Fleming IA, McGinnity P, Diserud O (2006) Genetic and ecological effects of salmon farming on wild salmon: modelling from experimental results. ICES J Mar Sci 63:1234–1247.

Holborn MK, Crowley SE, Duffy SJ, Messmer AM, Kess T, Dempson JB, Wringe BF, Fleming IA, Bentzen P, Bradbury IR (2022) Precocial male maturation contributes to the introgression of farmed Atlantic salmon into wild populations. Aquacult Environ Interact 14:205–218.

Islam SS, Xue X, Caballero-Solares A, Bradbury IR, Rise ML, Fleming IA (2022) Distinct early life stage gene expression effects of hybridization among European and North American farmed and wild Atlantic salmon populations. Mol Ecol 31:2712–2729.

Jeffery NW, Stanley RRE, Wringe BF, Guijarro-Sabaniel J, Bourret V, Bernatchez L, Bentzen P, Beiko RG, Gilbey J, Clément M, Bradbury IR (2017) Range-wide parallel climate-associated genomic clines in Atlantic salmon. R Soc open sci 4:171394.

Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551–555.

Karlsson S, Moen T, Lien S, Glover KA, Hindar K (2011) Generic genetic differences between farmed and wild Atlantic salmon identified from a 7K SNP-chip. Mol Ecol Resour 11:247–253.

Karlsson S, Diserud OH, Moen T, Hindar K (2014) A standardized method for quantifying unidirectional genetic introgression. Ecol Evol 4:3256–3263.

Karlsson S, Diserud OH, Fiske P, Hindar K (2016) Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. ICES J Mar Sci 73:2488–2498.

Keyser F, Wringe BF, Jeffery NW, Dempson JB, Duffy S, Bradbury IR (2018) Predicting the impacts of escaped farmed Atlantic salmon on wild salmon populations. Can J Fish Aquat Sci 75:506–512.

Liu L, Ang KP, Elliott J a. K, Kent MP, Lien S, MacDonald D, Boulding EG (2017) A genome scan for selection signatures comparing farmed Atlantic salmon with two wild populations: Testing colocalization among outlier markers, candidate genes, and quantitative trait loci for production traits. Evol Appl 10:276–296.

Lehnert SJ, Kess T, Bentzen P, Clément M, Bradbury IR (2020). Divergent and linked selection shape patterns of genomic differentiation between European and North American Atlantic salmon (*Salmo salar*). Mol Ecol 29(12):2160-2175.

Mahlum S, Vollset KW, Barlaup BT, Skoglund H, Velle G (2021) Salmon on the lam: Drivers of escaped farmed fish abundance in rivers. Journal of Applied Ecology 58:550–561.

McCullagh P, Nelder JA (1989) Generalized linear models 2nd edition chapman and hall. London, UK.

McGinnity P, Stone C, Taggart JB, Cooke D, Cotter D, Hynes R, McCamley C, Cross T, Ferguson A (1997) Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. ICES J Mar Sci 54:998–1008.

McGinnity P, Prodöhl P, Ferguson A, Hynes R, Maoiléidigh NO, Baker N, Cotter D, O'Hea B, Cooke D, Rogan G, Taggart J, Cross T (2003) Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. Proc Biol Sci 270:2443–2450.

Morris MRJ, Fraser DJ, Heggelin AJ, Whoriskey FG, Carr JW, O'Neil SF, Hutchings JA (2008) Prevalence and recurrence of escaped farmed Atlantic salmon (*Salmo salar*) in eastern North American rivers. Can J Fish Aquat Sci 65:2807–2826.

Nugent CM, Kess T, Brachmann MK, Langille BL, Holborn MK, Beck SV, Smith N, Duffy SJ, Lehnert SJ, Wringe BF, Bentzen P, Bradbury IR (2023) Genomic and machine learning-based screening of aquaculture-associated introgression into at-risk wild North American Atlantic salmon (*Salmo salar*) populations. Mol Ecol Resour.

Palm S, Karlsson S, Diserud OH (2021) Genetic evidence of farmed straying and introgression in Swedish wild salmon populations. Aquacult Environ Interact 13:505–513.

Porter T, Riche L, Traverse G (1974) Porter TR, Riche LG, Traverse GR (1974) Catalogue of rivers in insular Newfoundland, Vol D. Data Record Series No. NEW/D-74-9. Resource Development Branch, Fisheries and Marine Service, Newfoundland Region, St John's.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, De Bakker PIW, Daly MJ, Sham PC (2007) PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. AJHG 81:559–575.

San Román I, Bradbury I, Crowley S, Duffy S, Islam S, Fleming I (2023) Experimental comparison of changes in relative survival and fitness-related traits of wild, farm, and hybrid Atlantic salmon *Salmo salar* in nature. Aquacult Environ Interact 15:323–337.

Saunders RL, Henderson EB, Glebe BD (1982) Precocious sexual maturation and smoltification in male Atlantic salmon (*Salmo salar)*. Aquaculture 28:211−229

Skaala Ø, Wennevik V, Glover KA (2006) Evidence of temporal genetic change in wild Atlantic salmon, *Salmo salar* L., populations affected by farm escapees. ICES J Mar Sci63:1224–1233.

Skaala Ø, Glover KA, Barlaup BT, Svåsand T, Besnier F, Hansen MM, Borgstrøm R (2012) Performance of farmed, hybrid, and wild Atlantic salmon (*Salmo salar*) families in a natural river environment. Can J Fish Aquat Sci 69:1994–2006.

Skaala Ø, Besnier F, Borgstrøm R, Barlaup B, Sørvik AG, Normann E, Østebø BI, Hansen MM, Glover KA (2019) An extensive common-garden study with domesticated and wild Atlantic salmon in the wild reveals impact on smolt production and shifts in fitness traits. Evol Appl 12:1001–1016.

Skilbrei OT, Heino M, Svåsand T (2015) Using simulated escape events to assess the annual numbers and destinies of escaped farmed Atlantic salmon of different life stages from farm sites in Norway. ICES Journal of Marine Science 72:670–685.

Stanley RRE, Jeffery NW, Wringe BF, DiBacco C, Bradbury IR (2017) Genepopedit: a simple and flexible tool for manipulating multilocus molecular data in R. Mol Ecol Resour 17:12–18.

Sylvester EVA, Wringe BF, Duffy SJ, Hamilton LC, Fleming IA, Castellani M, Bentzen P, Bradbury IR (2019) Estimating the relative fitness of escaped farmed salmon offspring in the wild and modelling the consequences of invasion for wild populations. Evol Appl 12:705–717.

Wacker S, Aronsen T, Karlsson S, Ugedal O, Diserud OH, Ulvan EM, Hindar K, Næsje TF (2021) Selection against individuals from genetic introgression of escaped farmed salmon in a natural population of Atlantic salmon. Evol Appl 14:1450–1460.

Wringe BF, Stanley RRE, Jeffery NW, Anderson EC, Bradbury IR (2017a) Hybriddetective: A workflow and package to facilitate the detection of hybridization using genomic data in r. Mol Ecol Resour 17:e275–e284.

Wringe BF, Stanley RRE, Jeffery NW, Anderson EC, Bradbury IR (2017b) Parallelnewhybrid: an R package for the parallelization of hybrid detection using newhybrids. Mol Ecol Resour 17:91–95.

Wringe BF, Jeffery NW, Stanley RRE, Hamilton LC, Anderson EC, Fleming IA, Grant C, Dempson JB, Veinott G, Duffy SJ, Bradbury IR (2018) Extensive hybridization following a large escape of domesticated Atlantic salmon in the Northwest Atlantic. Commun Biol 1:1–9.

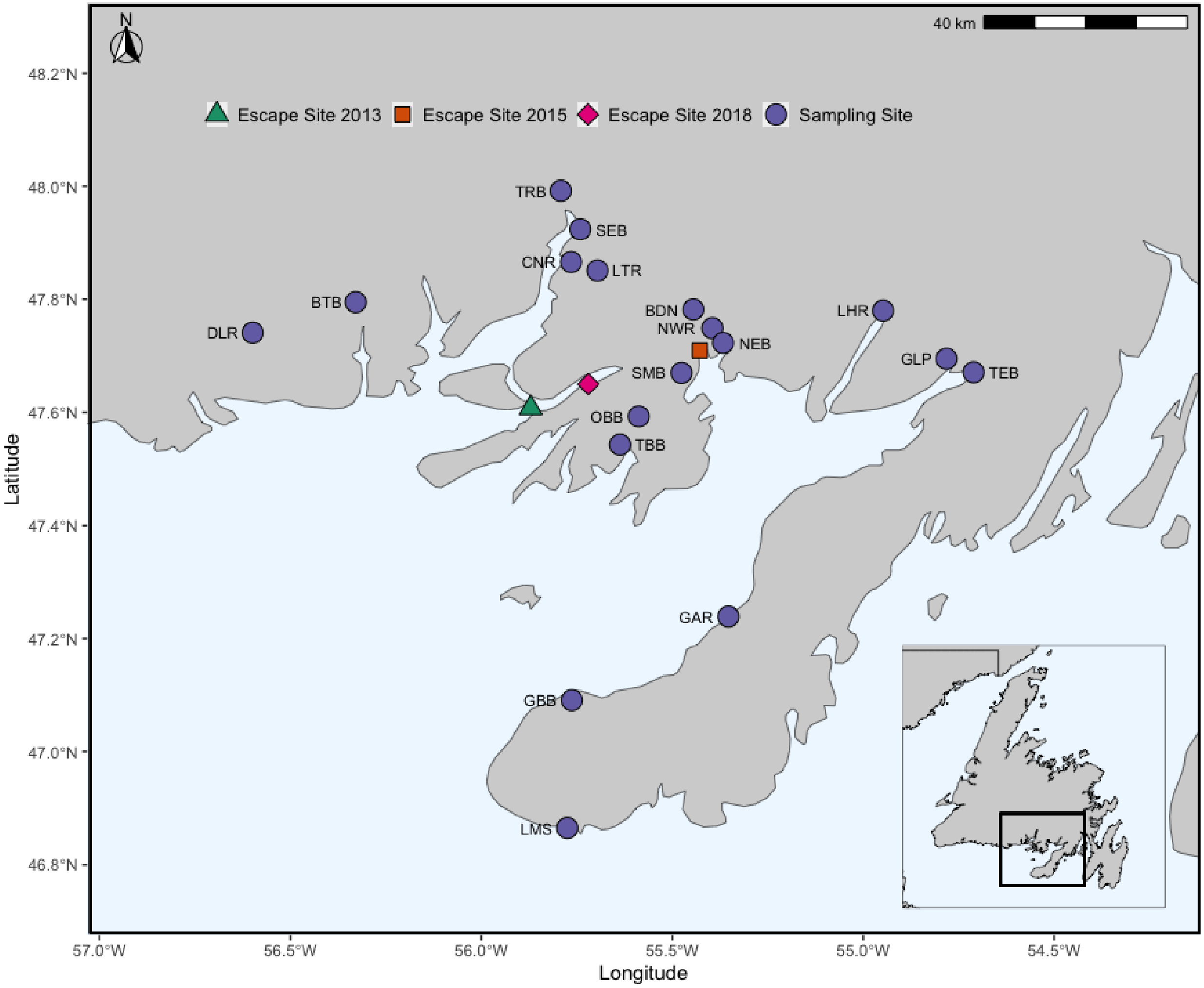
Wringe BF, Anderson EC, Jeffery NW, Stanley RRE, Bradbury IR (2019) Development and evaluation of SNP panels for the detection of hybridization between wild and escaped Atlantic salmon (*Salmo salar*) in the western Atlantic. Can J Fish Aquat Sci 76:695–704.

Figures

Table 3.1. Eighteen southern Newfoundland rivers sampled for Atlantic salmon and associated river codes, latitudinal and longitudinal coordinates, and number of sampled and successfully genotyped young-of-the-year parr from 2014-2021.

River name	River code	Latitude	Longitude	2014	2015	2016	2017	2018	2019	2020	2021
Bay du Nord River	BDN	47.782	-55.445	-	55	50	33	13	12	38	35
Bottom Brook	BTB	47.795	-56.329	32	3	46	8	-	-	-	-
Conne River	CNR	47.866	-55.765	370	20	81	53	71	6	37	103
Dollards Brook	DLR	47.741	-56.599	24	22	67	61	10	-	-	-
Garnish River	GAR	47.239	-55.353	199	56	102	41	79	55	89	100
Grand Bank Brook	GBB	47.091	-55.763	42	15	3	39	4	49	87	13
Grand La Pierre	GLP	47.695	-54.782	118	14	7	6	-	-	-	-
Lamaline Salmonier River	LMS	46.865	-55.775	40	89	59	54	41	51	79	3
Little River	LTR	47.851	-55.696	130	-	86	56	40	8	80	120
Long Harbour River	LHR	47.78	-54.948	137	49	49	41	8	-	-	-
Northeast Brook	NEB	47.723	-55.367	115	1	50	67	3	33	54	18
Northwest River	NWR	47.749	-55.395	41	-	80	52	29	1	2	64
Old Bay Brook	OBB	47.593	-55.588	18	-	34	66	73	-	-	-
Simms Brook	SMB	47.67	-55.476	69	20	87	55	27	7	85	96
Southeast Brook	SEB	47.924	-55.741	31	-	-	16	10	19	-	99
Tailrace Brook	TRB	47.992	-55.792	79	9	50	52	25	39	27	98
Taylor Bay Brook	TBB	47.543	-55.637	120	-	4	16	1	-	-	-
Terrenceville Brook	TEB	47.671	-54.711	120	9	48	45	5	49	4	35

A.


N
40 km
Escape Site 2013
Escape Site 2015
Escape Site 2018
Sampling Site
TRB
SEB
CNR
LTR
BTB
DLR
BDN
NWR
NEB
LHR
GLP
TEB
SMB
OBB
TBB
GAR
GBB
LMS
48.2°N
48.0°N
47.8°N
47.6°N
47.4°N
47.2°N
47.0°N
46.8°N
Latitude
57.0°W
56.5°W
56.0°W
55.5°W
55.0°W
54.5°W
Longitude

B.

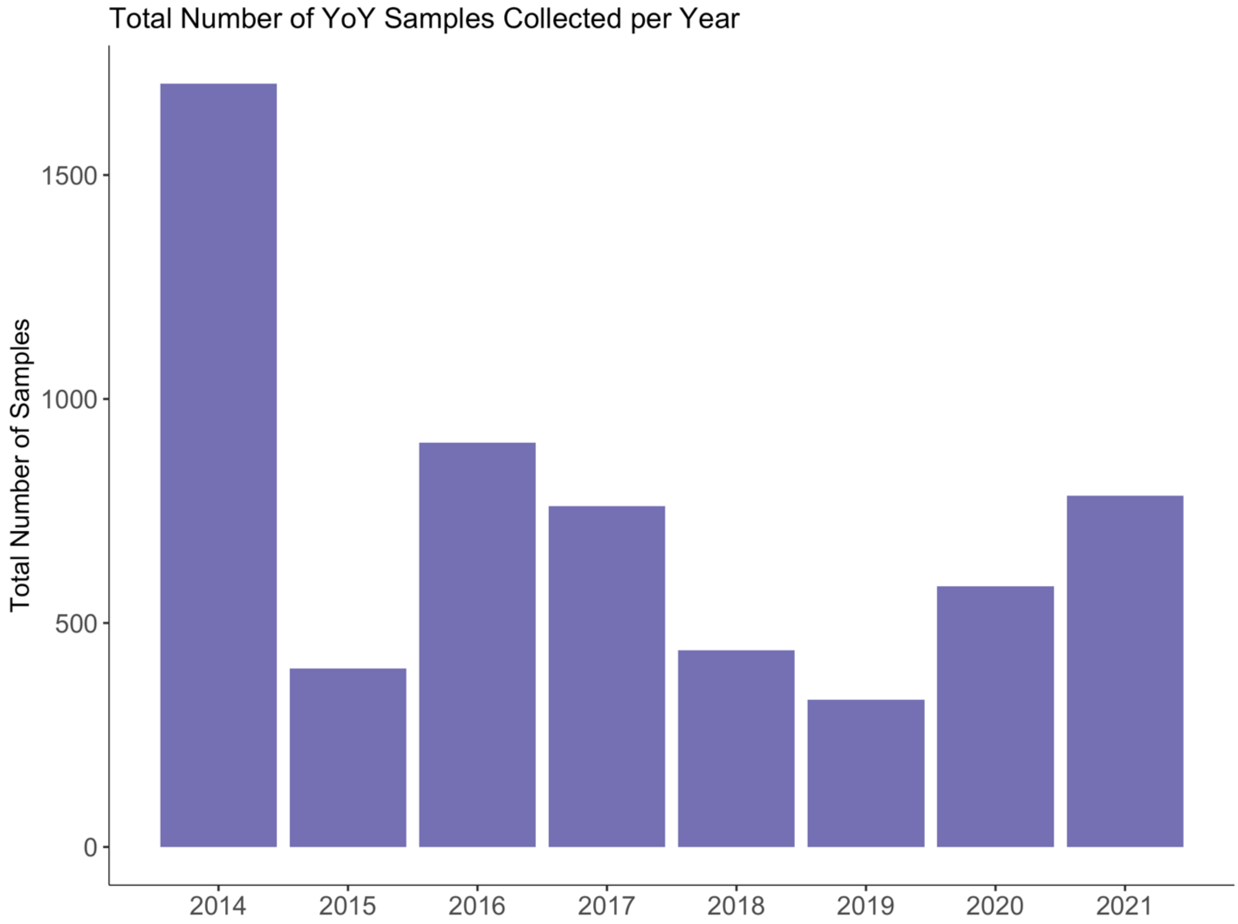


Figure 3.1. (A) Southern Newfoundland Atlantic salmon parr sampling sites relative to the sites of the large escape event in 2013, and the smaller escape events in 2015 and 2018. For river codes see Table 1. (B) Total number of young-of-the-year (YoY) samples collected each sampling year across all rivers.

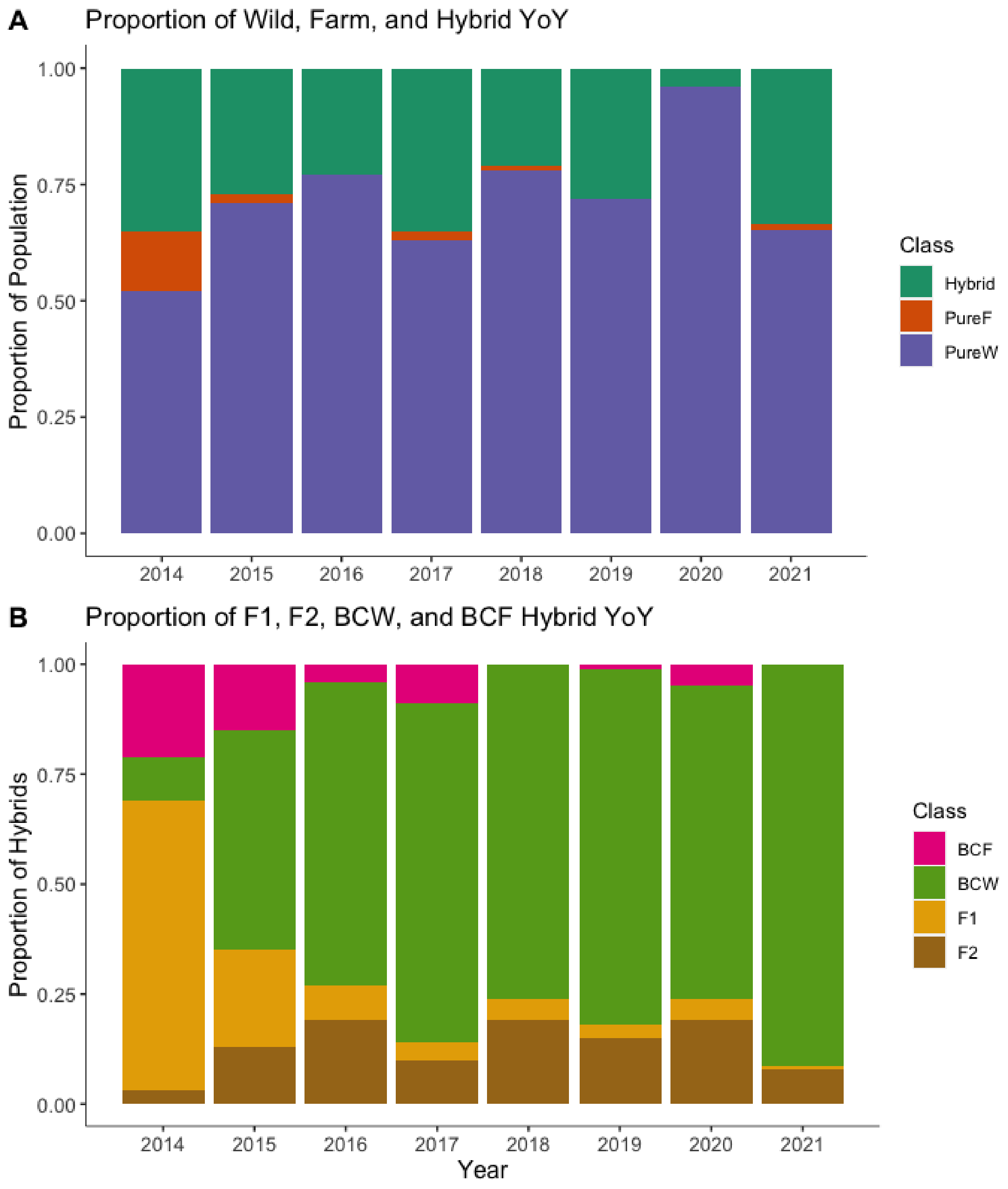


Figure 3.2. Annual proportions of each genetic class for the overall southern Newfoundland population of Atlantic salmon throughout the sampling period (2014-2021). (A) Proportion of pure feral/farm (PureF), pure wild (PureW), and hybrid young-of-the-year (YoY) individuals, where hybrid represents the 4 hybrid classes combined. (B) Proportion of each of the 4 hybrid classes (BCF = backcross farm, BCW = backcross wild, F1, and F2) within the hybrid proportion indicated in (A).

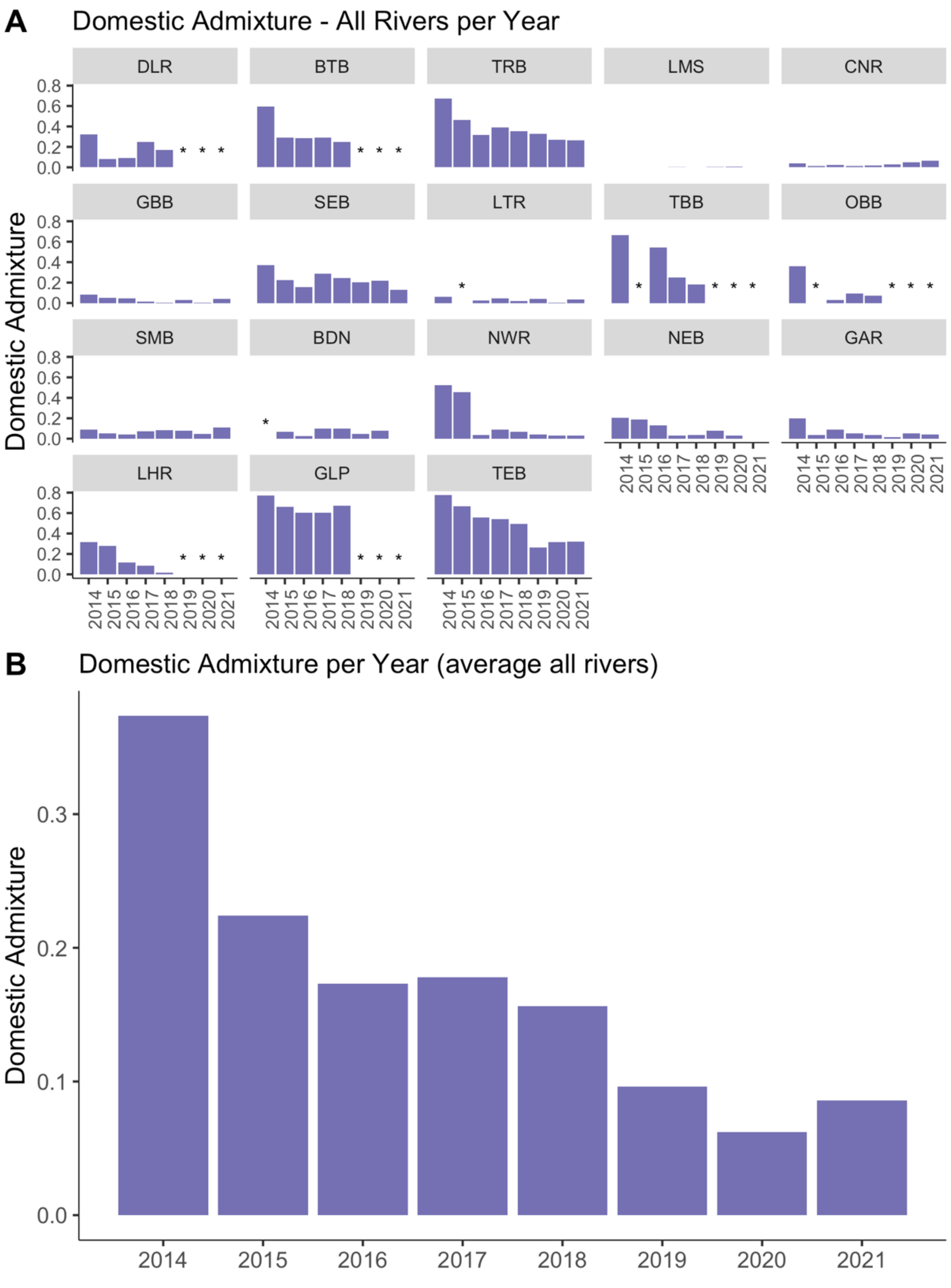


Figure 3.3. Annual domestic admixture estimates for (A) each individual southern Newfoundland river and (B) for the overall southern Newfoundland population of Atlantic salmon throughout the sampling period (2014-2021). An asterisk (*) in panel A indicates years during which a river was not sampled.

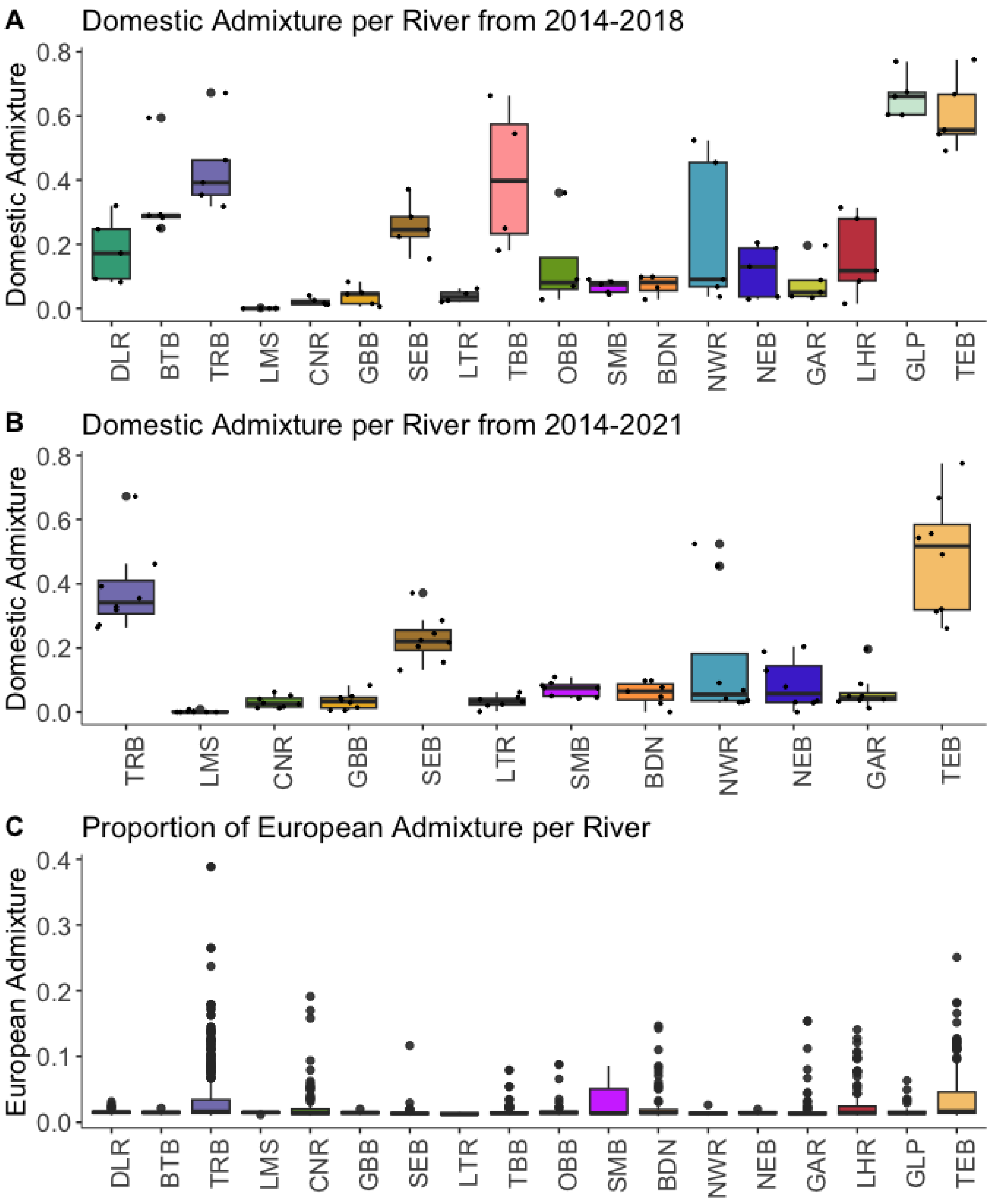


Figure 3.4. Degree of farm and European admixture per river throughout the 8 years following

a large aquaculture escape in 2013, followed by smaller escapes in 2015 and 2018. (A) Farm admixture estimates from 2014-2018 of all rivers sampled. (B) Farm admixture estimates from 2014-2021 of rivers that were sampled during the entire time period. (C) European admixture per river, using hybrid samples collected throughout the entire time series. Bold lines represent median values, boxes 25% and 75% quartiles, whiskers 1.5 times the inter-quartile range, and bold dots outliers. Smaller dots represent admixture estimates each year of sampling in each river.

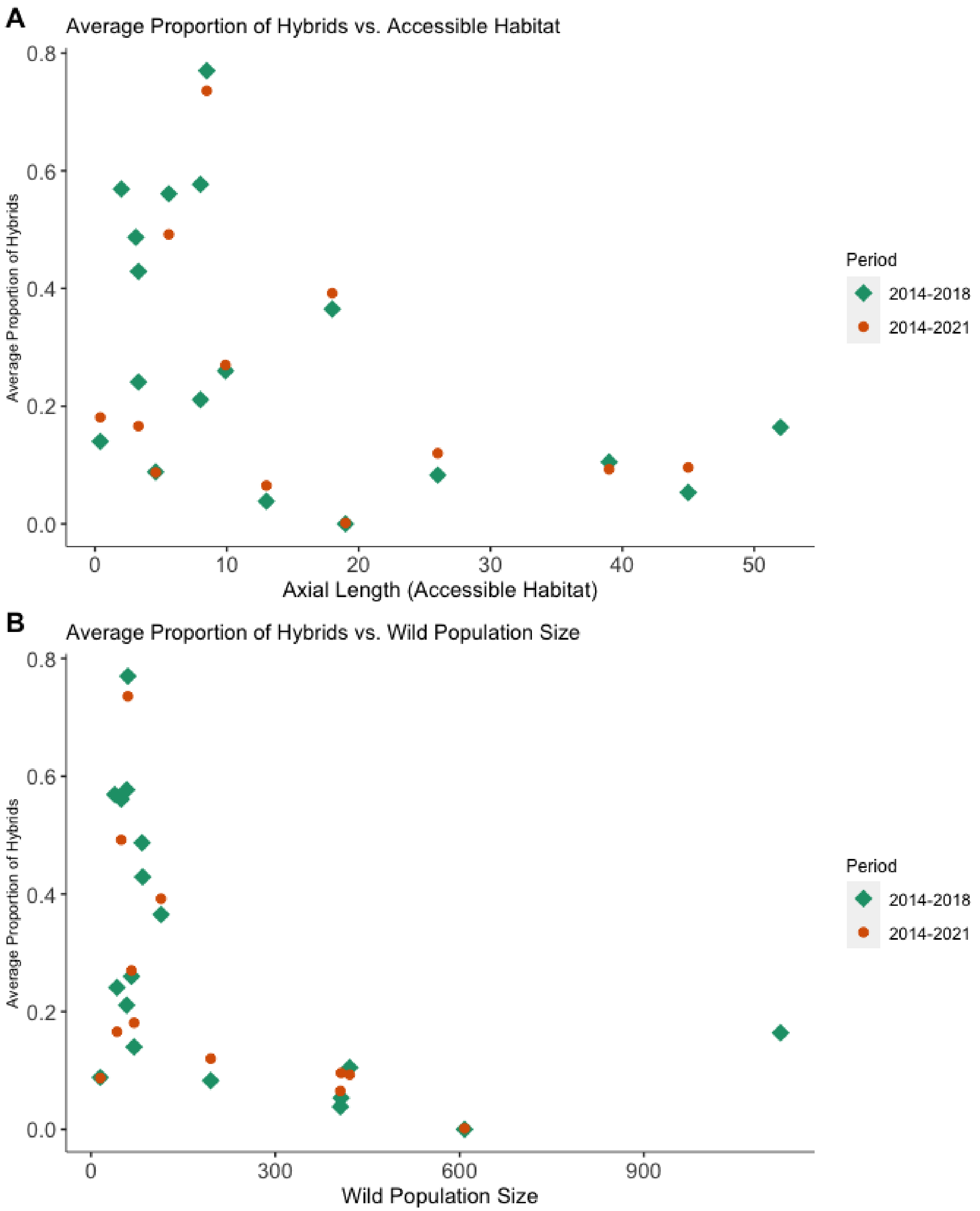


Figure 3.5. Association between the average proportion of young-of-the-year YoY hybrids and (A) wild population size and (B) river size, measured as axial length. Diamonds represent the average hybrids in all rivers from 2014-2018. Circles represent the average hybrids only in rivers sampled from 2014-2021.

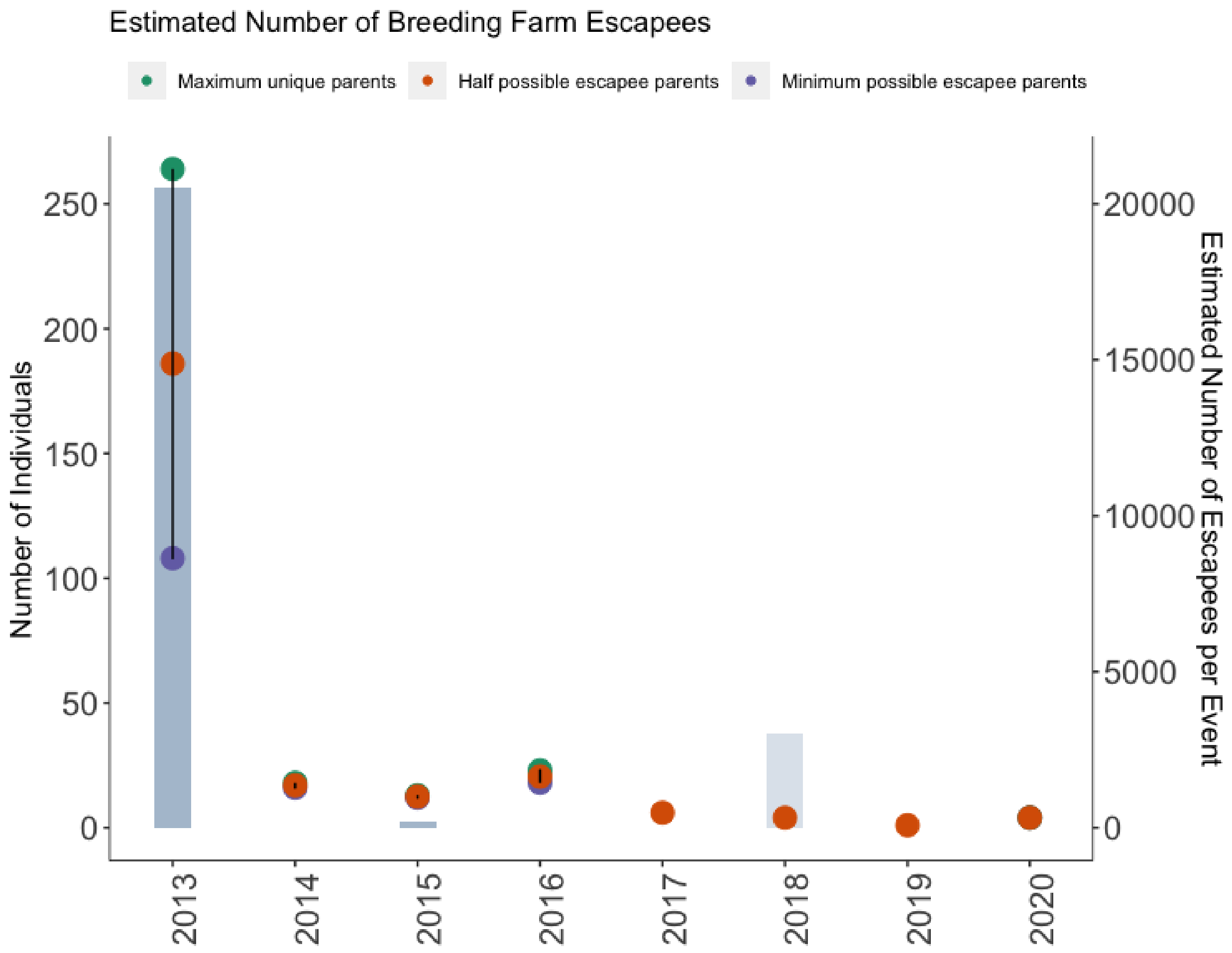


Figure 3.6. Total maximum and minimum possible escapee parents, and half of the unique breeding parents in 18 southern Newfoundland rivers throughout the 8 years following a large aquaculture escape in 2013 and smaller escapes in 2015 and 2018. The points on the plot represent the number of escapee parents, calculated based on the number of pure farm, F1, and BCF families at each site. Bars represent the total number of aquaculture escapees recorded from the escape events. The x-axis shows the year the parents would have been present in the systems and breeding.

Chapter 4: General Conclusion

Atlantic salmon farming was developed approximately fifty years ago, and, although technical standards for the production of aquaculture infrastructure have improved during recent decades (Jensen et al. 2010), the primary methods for cultivation have remained similar, with the size of sea cages and the number of stocked smolt continuously increasing. The growth of this industry has allowed for a reduction in fishing pressure on wild salmon populations, however, it has also faced several challenges such as environmental concerns, disease management, and farm escapes. Domestic escapees entering wild environments has become a critical area of research for scientists and managers, as it is known that farm genotypes introduce maladaptive traits into wild populations (Fleming & Einum 1997, Ferguson et al. 2007). Atlantic salmon have been domesticated for ≥12-15 generations, during which traits that are favorable for the aquaculture industry and a net pen environment have been more heavily selected (Glover et al. 2017). Changes in life history, such as growth rate and age at maturity (Fleming et al. 2000, Glover et al. 2009, Solberg et al. 2013a b b, Skaala et al. 2019, Holborn et al. 2022), can have demographic consequences for wild populations, yet has been difficult to make quantitative assessments on these effects and current estimates are uncertain and conservative (Bolstad et al. 2021). Therefore, coupling empirical results on survival and life history with additional molecular markers and a diverse range of populations can provide a more in depth understanding of the ecological and genetic impacts of introgression. North American populations remain under-represented in current studies that isolate the influence of genetics on survival and fitness-related traits in admixed Atlantic Salmon populations (Einum & Fleming 1997, McGinnity 1997, Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2012, 2019). In this study, I aimed to expand on existing research of wild, farm, and hybrid Atlantic salmon performance in nature and address a portion of this research gap by analyzing field data on several North American populations of wild Atlantic Salmon and the farmed populations they interact with after escape events of various magnitudes.

In Newfoundland, Salmon populations on the south coast are considered threatened (COSEWIC 2010), therefore the threat of invasion of farm escapees into wild populations is of growing concern to management and conservation efforts (Clifford et al. 1998, Glover et al. 2012, Forseth et al. 2017). Previous studies have determined that offspring of pure wild descent often have higher survival rates than pure farm offspring, with the most commonly observed survival trend being wild > hybrids > farm (McGinnity 1997, Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2012, 2019, Crowley et al. 2022). In Chapter 2, I compared survival and fitness-related traits among wild, farm, and hybrid fry released into the wild, and found that this trend is not consistent in the population studied. Crowley et al. (2022) focused on recapture 3-months post-release and determined that, in accordance with previous studies, wild offspring had higher recapture odds than farm offspring. My results, which extend to the two following years, are noteworthy, because during the 3-15 month period, farm offspring reached overall recapture rates that were analogous to those of wild salmon, and at two years post-release (15-28 months), farm offspring had the highest recapture odds. Saloniemi et al. (2004), have found that, although survival of reared salmon is generally lower than that of wild fish, survival of larger smolts is higher in individuals of both origins during favourable stocking years. Although I did not study the relationship between recapture odds and size, I observed that farm individuals were consistently larger than wild parr. This could have compensated for poorer survival in farm salmon, thus substantially reducing the difference in survival rates between the two groups. In future work, it could be relevant to also include an environmental analysis, studying factors such as temperature, quality and amount of food, and precipitation, as these are known to influence the growth rate, final length, physiological state, and overall survival of fish. This would allow researchers to more clearly determine whether environmental factors interact with size to influence survival odds of farm, wild, and hybrid salmon. In addition, studying this relationship can also provide valuable insight on how wild populations can be affected by changing environmental conditions and whether this shift could exacerbate the negative impacts of farm escapes.

I also observed high rates of precocial male maturation by the second and third years of my study, which is consistent with the demonstrated ability for 1+ aged parr to mature (Dalley et al. 1983, Myers 1984). There were some differences in the proportion of mature parr across sites, indicating that rates of precocial maturation can differ within areas of the same river, possibly due to differences in food resources, as size also significantly differed among sites. Though not quantified here, Site 3 appeared to have the highest resource availability, and this is where parr of all cross types were consistently larger and matured at the highest rate at age 1+. In previous research, the occurrence of precocial maturation has been demonstrated to be affected by growth (Saunders et al. 1982, Letcher & Gries 2003), therefore, future work in southern Newfoundland could also explore this relationship, as high rates of precocial male parr have been documented throughout the region (Myers et al 1986). Early introgression work has also suggested that mature farm escapee parr can successfully spawn (Clifford et al. 1998), and more recently, it has been shown that F1 hybrids can mature precocially in wild environments at a similar rate as wild salmon (Holborn et al. 2022). This indicates that considering the reproductive contribution of F1 hybrid precocial male parr may be a critical component of monitoring introgression and predicting farm escapee impacts. Future studies could explore the potential that hybrids may fast-track introgression by maturing precocially, and continuous monitoring of both farm escapes and affected wild populations.

My findings from this chapter highlight the distinct survival, growth and maturation trends inherent to specific populations and how these can shift over time. Based on these results, I concluded that localized studies, that span over several years are critical to fully understand the effects of farm introgression into wild salmon populations. An escape event in 2013 of ~20,000 sexually mature, domestic Atlantic salmon in southern Newfoundland, which occurred just prior to the natural spawning period for salmon in this region, provided a unique opportunity to analyze the longest existing time series genetic for monitoring the impacts associated with farm escapees in North America. Previous studies following this escape have found spatial variation in hybridization (Sylvester et al. 2018), selection against domestic offspring in the wild (Sylvester et al. 2019), and high rates of precocial male maturation in F1 hybrids (Holborn et al. 2022).In my analysis, I demonstrated that there is an increasing proportion of backcross-wild hybrid individuals with a consistent occurrence of F1 hybrids every year. Sylvester et al. (2019) also found that there are changes in the relative proportions of hybrid genetic classes but detected a relative decrease in F1s. These temporal changes in population composition suggest a reduced relative fitness of feral and hybrid offspring compared to pure wild. In addition, hybrids that were only 25% admixed (BCW) performed better in the wild environments relative to more admixed hybrids such as F1s (50% admixed), further supporting high degrees of genetic differentiation between wild and farmed strains and reduced local adaptation in domestic genotypes.

The consistent and high proportions of BCW hybrids throughout the entire time series of my study, suggests that, in line with conclusions in Holborn et al. (2022), hybrid individuals can successfully breed as precocial males in the wild, therefore increasing the rate at which farm introgression occurs. Precocial males of farm origin have previously been found to have superior breeding and fertilization success than wild and hybrid individuals (Garant et al. 2003). Thus, the high rates of male precocial maturation observed throughout southern Newfoundland, will likely lead to higher rates of introgression of farm genotypes into wild Atlantic salmon populations. Over the 8 years of my study, I observed a decrease in the proportion of pure farm individuals, and in certain years they were not encountered within our sample. However, F1 hybrid YoY were present every year, indicating that smaller escapes are likely occurring constantly, and pure farm escapees interbreed with wild salmon successfully enough that offspring from these small escapes are still detectable in every sample. Therefore, the potential impact on native populations from genetic introgression may be critical, and further studying this life history trait in domestic individuals in southern Newfoundland is essential.

Studying populations at a regional level is essential as Atlantic salmon are known to be highly adapted to their local environments (Garcia de Leaniz et al. 2007, Fraser et al. 2011, Watson et al. 2022). However, previous research has considered Atlantic salmon a potential model organism for studying genetic interactions between farm and wild individuals as a general concept (Glover et al. 2017). It has been found that other farmed species are also able to escape and survive in the wild for an extended period of time following the escape. For instance Noble et al. (2014) observed that 12 and 18 months after an escape event of barramundi (*Lates calcarifer*) 31% of their samples, collected from a wild channel, were of farm origin. Similar results have been detected in European sea bass (*Dicentrarchus labrax*) which can survive and integrate into natural populations in large numbers, though it is unknown whether these escapees successfully interbreed with wild populations (Toledo Guedes et al. 2009). In Newfoundland specifically, the spatiotemporal distribution of wild and simulated escapee farmed Atlantic cod has been mapped, and it was determined that native and domesticated individuals have similar distributions and dispersal patterns, which could result in substantial interactions between farmed and wild fish (Zimmermann et al. 2013).

Beyond the aquaculture industry, results from my study are also relevant in the salmon stock enhancement scope, as many systems in Canada rely on hatcheries to increase the abundances of salmon (Fisheries and Environment Canada 1978).It has been found that in both, Pacific salmon species and Atlantic salmon, hatchery and wild juveniles have equivalent marine dispersion, yet hatchery fish have significantly lower survival than wild individuals (Jonsson et al. 2003, Beamish et al. 2012). This suggests that the genetic differences in hatchery reared fish may have similar effects to those I observed in farm salmon. Therefore, my results can also help inform decisions regarding salmon enhancement programs and predict how selection pressures and genetic differences between the two rearing groups can affect their subsequent survival at sea and the return to rivers as spawners. Finally, studying wild-farm interactions of aquaculture species may also be relevant to invasive biology, particularly in relation to conspecific invasions. Interactions of domesticated individuals with their wild conspecifics are becoming a pressing problem as human dependence on organisms raised in captivity increases, and previous research has identified various categories of invasive organisms that populate an area where their wild conspecifics naturally occur (Laikre et al. 2010). However, most current invasive biology studies have focused on alien species, rather than those that are the same species as the recipient wild populations. Therefore, research such as the analyses presented here, can be critical in beginning to understand conspecific invasions, which are often the most significant in many areas (Laikre et al. 2010). As human reliance on domesticated organisms continues to increase with a growing population and the expansion of aquaculture and agriculture, it is critical to consider the potential impacts of such species on their wild conspecifics. Research such as this contributes to our understanding of genetic and ecological interactions between farm and wild individuals of the same species and provide data to inform mitigation and management strategies for the negative effects of domestication on wild populations.

References

Beamish RJ, Sweeting RM, Neville CM, Lange KL, Beacham TD, Preikshot D (2012) Wild chinook salmon survive better than hatchery salmon in a period of poor production. Env Biol Fish 94:135-148.

Bolstad GH, Karlsson S, Hagen IJ, Fiske P, Urdal K, Sægrov H, Florø-Larsen B, Sollien VP, Østborg G, Diserud OH, Jensen AJ, Hindar K (2021) Introgression from farmed escapees affects the full life cycle of wild Atlantic salmon. Sci Adv 7:eabj3397.

Bradbury IR, Duffy S, Lehnert SJ, Jóhannsson R, Fridriksson JH, Castellani M, Burgetz I, Sylvester E, Messmer A, Layton K, Kelly N, Dempson J, Fleming IA (2020) Model-based evaluation of the genetic impacts of farm-escaped Atlantic salmon on wild populations. Aquacult Environ Interact 12:45–59.

Clifford SL, McGinnity P, Ferguson A (1998) Genetic changes in an Atlantic salmon population resulting from escaped juvenile farm salmon. Journ Fish Biol 52:118–127.

COSEWIC. 2010. COSEWIC assessment and status report on the Atlantic Salmon *Salmo salar* (Nunavik population, Labrador population, Northeast Newfoundland population, South Newfoundland population, Southwest Newfoundland population, Northwest Newfoundland population, Quebec Eastern North Shore population, Quebec Western North Shore population, Anticosti Island population, Inner St. Lawrence population, Lake Ontario population, Gaspé-Southern Gulf of St. Lawrence population, Eastern Cape Breton population, Nova Scotia Southern Upland population, Inner Bay of Fundy population, Outer Bay of Fundy population) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xlvii + 136 pp.

Crowley SE, Bradbury IR, Messmer AM, Duffy SJ, Islam SS, Fleming IA (2022) Common-garden comparison of relative survival and fitness-related traits of wild, farm, and hybrid Atlantic salmon *Salmo salar* parr in nature. Aquacult Environ Interact 14:35–52.

Dalley EL, Andrews CW, Green JM (1983) Precocious male Atlantic salmon parr (*Salmo salar*) in insular Newfoundland. Can J Fish Aquat Sci 40: 647−652

Einum S, Fleming IA (1997) Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. Journal of Fish Biology 50:634–651.

Ferguson A, Fleming IA, Hindar K, Skaala O, McGinnity P, Cross T, Prodoehl P (2007) Farm Escapes in The Atlantic Salmon–Genetics, Conservation and Management. Verspoor E, Stradmeyer L, Nielsen JL.

Fisheries and Environment Canada (1978) The salmonid enhancement program. A public discussion paper. Information Branch. Fisheries and Marine Science Service, Vancouver

Fleming IA, Einum S (1997) Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. ICES Journ Mar Sci 54:1051–1063.

Fleming IA, Hindar K, Mjølnerød IB, Jonsson B, Balstad T, Lamberg A (2000) Lifetime success and interactions of farm salmon invading a native population. Proc Biol Sci 267:1517–1523.

Forseth T, Barlaup BT, Finstad B, Fiske P, Gjøsæter H, Falkegård M, Hindar A, Mo TA, Rikardsen AH, Thorstad EB, Vøllestad LA, Wennevik V (2017) The major threats to Atlantic salmon in Norway. ICES Journ Mar Sci 74:1496–1513.

Fraser DJ, Weir LK, Bernatchez L, Hansen MM, Taylor EB (2011) Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. Heredity 106:404–420.

Garant, D, Fleming IA, Einum S, Bernatchez,L (2003) Alternative male life-history tactics as potential vehicles for speeding introgression of farm salmon traits into wild populations. Ecol 6(6):541-549.

Garcia de Leaniz C, Fleming IA, Einum S, Verspoor E, Jordan WC, Consuegra S, Aubin-Horth N, Lajus D, Letcher BH, Youngson AF, Webb JH, Vøllestad LA, Villanueva B, Ferguson A, Quinn TP (2007) A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biol Rev 82:173–211.

Glover KA, Otterå H, Olsen RE, Slinde E, Taranger GL, Skaala Ø (2009) A comparison of farmed, wild and hybrid Atlantic salmon (*Salmo salar* L.) reared under farming conditions. Aquaculture 286:203–210.

Glover KA, Quintela M, Wennevik V, Besnier F, Sørvik AGE, Skaala Ø (2012) Three Decades of Farmed Escapees in the Wild: A Spatio-Temporal Analysis of Atlantic Salmon Population Genetic Structure throughout Norway. PLOS ONE 7:e43129.

Glover KA, Solberg MF, McGinnity P, Hindar K, Verspoor E, Coulson MW, Hansen MM, Araki H, Skaala Ø, Svåsand T (2017) Half a century of genetic interaction between farmed and wild Atlantic salmon: Status of knowledge and unanswered questions. Fish Fish 18:890–927.

Holborn M, Crowley S, Duffy S, Messmer A, Kess T, Dempson J, Wringe B, Fleming I, Bentzen P, Bradbury I (2022) Precocial male maturation contributes to the introgression of farmed Atlantic salmon into wild populations. Aquacult Environ Interact 14:205–218.

Jensen Ø, Dempster T, Thorstad EB, Uglem I, Fredheim A (2010) Escapes of fishes from Norwegian sea-cage aquaculture: causes, consequences and prevention. Aquacult Environ Interact 1:71-83.

Jonsson N, Jonsson B, Hansen LP (2003) The marine survival and growth of wild and hatchery-reared Atlantic salmon. Journ Appl Ecol 40(5):900-911.

Laikre L, Schwartz MK, Waples RS, Ryman N. 2010. Compromising genetic diversity in the wild: Unmonitored large-scale release of plants and animals. Trends Ecol Evol 25(9):520–529.

Letcher BH, Gries G (2003) Effects of life history variation on size and growth in stream-dwelling Atlantic salmon. J Fish Biol 62: 97−114

McGinnity P, Stone C, Taggart JB, Cooke D, Cotter D, Hynes R, McCamley C, Cross T, Ferguson A (1997) Genetic impact of escaped farmed Atlantic salmon (Salmo salar L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. ICES Journ Mar Sci 54:998–1008.

McGinnity P, Prodöhl P, Ferguson A, Hynes R, Maoiléidigh NO, Baker N, Cotter D, O'Hea B, Cooke D, Rogan G, Taggart J, Cross T (2003) Fitness reduction and potential extinction of wild populations of Atlantic salmon, Salmo salar, as a result of interactions with escaped farm salmon. Proc Biol Sci 270:2443–2450.

Myers RA (1984) Demographic consequences of precocious maturation of Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 41: 1349−1353

Myers RA, Hutchings JA, Gibson RJ (1986) Variation in male parr maturation within and among populations of Atlantic salmon, *Salmo salar*. Can J Fish Aquat Sci 43:1242-1248.

Noble T, Smith-Keune C, Jerry D (2014) Genetic investigation of the large-scale escape of a tropical fish, barramundi Lates calcarifer, from a sea-cage facility in northern Australia. Aquacult Environ Interact 5:173–183.

Saloniemi I, Jokikokko E, Kallio-Nyberg I, Jutila E, Pasanen P (2004) Survival of reared and wild Atlantic salmon smolts: size matters more in bad years. ICES Journ Mar Sci 61(5):782-787.

Saunders RL, Henderson EB, Glebe BD (1982) Precocious sexual maturation and smoltification in male Atlantic salmon (*Salmo salar*). Aquaculture 28: 211−229.

Skaala Ø, Besnier F, Borgstrøm R, Barlaup B, Sørvik AG, Normann E, Østebø BI, Hansen MM, Glover KA (2019) An extensive common-garden study with domesticated and wild Atlantic salmon in the wild reveals impact on smolt production and shifts in fitness traits. Evol Appl 12:1001–1016.

Skaala Ø, Glover KA, Barlaup BT, Svåsand T, Besnier F, Hansen MM, Borgstrøm R (2012) Performance of farmed, hybrid, and wild Atlantic salmon (*Salmo salar*) families in a natural river environment. Can J Fish Aquat Sci 69:1994–2006.

Skilbrei OT, Heino M, Svåsand T (2015) Using simulated escape events to assess the annual numbers and destinies of escaped farmed Atlantic salmon of different life stages from farm sites in Norway. ICES Journ Mar Sci 72:670–685.

Solberg MF, Skaala Ø, Nilsen F, Glover KA (2013a) Does Domestication Cause Changes in Growth Reaction Norms? A Study of Farmed, Wild and Hybrid Atlantic Salmon Families Exposed to Environmental Stress. PLOS ONE 8:e54469.

Solberg MF, Zhang Z, Nilsen F, Glover KA (2013b) Growth reaction norms of domesticated, wild and hybrid Atlantic salmon families in response to differing social and physical environments. BMC Evol Biol 13:234.

Sylvester EVA, Wringe BF, Duffy SJ, Hamilton LC, Fleming IA, Bradbury IR (2018) Migration effort and wild population size influence the prevalence of hybridization between escaped farmed and wild Atlantic salmon. Aquacult Environ Interact 10:401–411.

Sylvester EVA, Wringe BF, Duffy SJ, Hamilton LC, Fleming IA, Castellani M, Bentzen P, Bradbury IR (2019) Estimating the relative fitness of escaped farmed salmon offspring in the wild and modelling the consequences of invasion for wild populations. Evol Appl 12:705–717.

Toledo Guedes K, Sánchez-Jerez P, González-Lorenzo G, Brito Hernández A (2009) Detecting the degree of establishment of a non-indigenous species in coastal ecosystems: sea bass Dicentrarchus labrax escapes from sea cages in Canary Islands (Northeastern Central Atlantic). Hydrobiologia 623:203–212.

Watson KB, Lehnert SJ, Bentzen P, Kess T, Einfeldt A, Duffy S, Perriman B, Lien S, Kent M, Bradbury IR (2022) Environmentally associated chromosomal structural variation influences fine-scale population structure of Atlantic Salmon (*Salmo salar*). Molec Ecol 31:1057–1075.

Wringe BF, Jeffery NW, Stanley RRE, Hamilton LC, Anderson EC, Fleming IA, Grant C, Dempson JB, Veinott G, Duffy SJ, Bradbury IR (2018) Extensive hybridization following a large escape of domesticated Atlantic salmon in the Northwest Atlantic. Commun Biol 1:1–9.

Zimmermann EW, Purchase CF, Fleming IA, Brattey, J (2013) Dispersal of wild and escapee farmed Atlantic cod (*Gadus morhua*) in Newfoundland. Can J Fish Aquat Sci 70:747-755.

Appendices

Supplementary Table 2.1. Results of analysis of deviance (ANODEV) of binomial generalized linear model for 2018, 2019, and 2020 recapture by site, cross type, year, and all possible interaction terms. df is degrees of freedom, ΔDeviance refers to change in deviance, and LR refers to likelihood ratio.

Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
Null		2984.24	35			
Year	2	575.72	33	2408.52	< 0.001	
Cross type	3	439.11	30	136.61	< 0.001	4.62×10^{29}
Site	2	86.89	28	352.22	< 0.001	3.05×10^{76}
Cross type x Site	6	61.21	22	25.68	0.00026	376999.82
Cross type x Year	6	31.52	16	29.69	4.49×10^{-05}	2.80×10^{6}
Site x Year	4	19.07	12	12.45	0.014	505.22
Cross type x Site x Year	12	0	0	19.07	0.087	13835.60

Supplementary Table 2.2. Results of analysis of deviance (ANODEV) of binomial generalized linear model for 2018 recapture by site, cross type, and their interaction. Abbreviations of terms are defined as in Table S2.1.

Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		384.95	11			
Cross type	3	244.01	8	140.94	< 0.001	4.019×10^{30}
Site	2	11.34	6	232.68	< 0.001	3.35×10^{50}
Cross type x Site	6	0	0	11.34	0.079	289.31

Supplementary Table 2.3. Results of analysis of deviance (ANODEV) of binomial generalized linear model for 2019 recapture by site, cross type, and their interaction. Abbreviations of terms are defined as in Table S2.1.

Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		155.16	11			
Cross type	3	141.35	8	13.81	0.0032	995.26
Site	2	26.54	6	114.82	< 0.001	8.55×10^{24}
Cross type x Site	6	0	0	26.54	0.00018	5.79×10^{5}

Supplementary Table 2.4. Results of analysis of deviance (ANODEV) of binomial generalized linear model for 2020 recapture by site, cross type, and their interaction. Abbreviations of terms are defined as in Table S2.1.

Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		31.01	11			
Cross type	3	20.99	8	10.02	0.018	149.90
Site	2	6.42	6	14.57	0.001	1458.26
Cross type x Site	6	0	0	6.42	0.377	24.82

Supplementary Table 2.5. Results of analysis of deviance (ANODEV) of binomial generalized linear model for 2020 recapture across all sites. Abbreviations of terms are defined as in Table S2.1.

Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		10.02	3			
Cross type	3	0	0	10.02	0.018	149.90

Supplementary Table 2.6. ANOVA table for linear model of 2019 recapture length by site, cross type, and one possible interaction. LR is the likelihood ratio, MS is mean squares, SS is sums of squares, df is degrees of freedom.

Source	Df	SS	MS	F	p	LR
Cross type	3	2828	942.70	9.43	$5.14\text{x}10^{-06}$	$3.53\text{x}10^{6}$
Site	2	34845	17422.40	174.27	< 0.001	$6.41\text{x}10^{58}$
Cross type x Site	6	2169	361.50	3.62	0.0017	$1.16\text{x}10^{5}$
Residuals	365	36491	100.00			

Supplementary Table 2.7. Results of analysis of deviance (ANODEV) of gamma generalized linear model for 2020 recapture weight by site, cross type, and their interaction. Abbreviations of terms are defined as in Table S2.1.

Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		77.00	403			
Cross type	3	73.98	400	3.01	$1.67\text{x}10^{-07}$	4.51
Site	2	35.49	398	38.50	< 0.001	$2.29\text{x}10^{8}$
Cross type x Site	6	32.98	392	2.51	$7.19\text{x}10^{-05}$	3.51

Supplementary Table 2.8. Results of analysis of deviance (ANODEV) of gamma generalized linear model for 2020 recapture length by site, cross type, and their interaction. Abbreviations of terms are defined as in Table S2.1.

Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR

NULL		0.55	38			
Cross type	3	0.48	35	0.07	0.016	1.04
Site	2	0.20	33	0.28	$1.08\text{x}10^{-09}$	1.15
Cross type x Site	4	0.20	29	0.01	0.87	1.00

Supplementary Table 2.9. Results of analysis of deviance (ANODEV) of gamma generalized linear model for 2020 recapture weight by site, cross type, and their interaction. Abbreviations of terms are defined as in Table S2.1.

Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		4.90	38			
Cross type	3	4.33	35	0.57	0.00070	1.33
Site	2	1.08	33	3.24	< 0.001	5.06
Cross type x Site	4	1.00	29	0.08	0.67	1.04

Supplementary Table 2.10. ANOVA table for linear model of 2019 recapture length from each site individually. Abbreviations of terms are defined as in Table S2.6.

Source	Df	SS	MS	F	p	LR
Site 1						
Cross type	3	2363.9	787.98	8.12	0.00020	40312.89
Residuals	45	4364.5	96.99			
Site 2						
Cross type	3	71	23.66	0.57	0.64	2.41
Residuals	124	5137.7	41.43			
Site 3						
Cross type	3	4443.4	1481.1	10.76	$1.42\text{x}10^{-06}$	$4.16\text{x}10^{6}$
Residuals	196	26988.7	137.7			

Supplementary Table 2.11. ANOVA table for linear model of 2020 recapture condition factor by cross type, site, and their interaction. Abbreviations of terms are defined as in Table S2.6.

Source	Df	SS	MS	F	p	LR
Cross type	3	101.34	33.78	3.34	0.033	325.07
Site	2	3.69	1.84	0.18	0.834	1.28
Cross type x Site	4	1.21	0.30	0.030	0.998	1.08
Residuals	29	293.49	10.12			

Supplementary Table 2.12. Results of analysis of deviance (ANODEV) of goodness of fit test used to analyze the effect of cross type on proportions of mature males in 2019. Abbreviations of terms are defined as in Table S2.1.

	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		11.003	3			
Cross type	3	0	0	11.003	0.0117	245.06

Supplementary Table 2.13. Results of analysis of deviance (ANODEV) of goodness of fit test used to analyze family representation through time. Abbreviations of terms are defined as in Table S2.1.

Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		957.42	207			
Year	2	957.41	205	0.02	0.99	1.01
Site	2	943.45	203	13.96	0.00093	1074.92
Family	24	292.09	179	651.35	< 0.001	$2.75\text{x}10^{141}$
Year x Site	4	292.09	175	0	0.99	1

Year x Family	48	227.1	127	65	0.052	1.30x10^{14}
Site x Family	43	62.94	84	164.15	4.72x10^{-16}	4.41x10^{35}
Year x Site x Family	84	957.42	0	62.94	0.96	4.65x10^{13}

Supplementary Table 2.14. Results of analysis of deviance (ANODEV) of gamma generalized linear model for 2018 recapture weight by family, site, and their interaction. Abbreviations of terms are defined as in Table S2.1.

Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		232.72	1241			
Family	24	180.81	1217	51.91	< 0.001	1.87x10^{11}
Site	2	131.3	1215	49.513	< 0.001	5.64x10^{10}
Family x Site	47	114.29	1168	17.006	< 0.001	4929.54

Supplementary Table 2.15. Results of analysis of deviance (ANODEV) of gamma generalized linear model for 2019 recapture weight by family, site, and their interaction. Abbreviations of terms are defined as in Table S2.1.

Source	df	Residual Deviance	Residual df	ΔDeviance	P	LR
NULL		76.995	403			
Family	24	64.171	379	12.824	< 0.001	609.11
Site	2	27.958	377	36.213	< 0.001	7.30 x10^{7}
Family x Site	42	20.578	335	7.38	< 0.001	40.04

Supplementary Table 2.16. Results of analysis of deviance (ANODEV) of gamma generalized linear model for 2020 recapture weight by family, site, and their interaction. Abbreviations of terms are defined as in Table S2.1.

Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		4.9005	38			
Family	13	3.2314	25	1.66902	< 0.001	2.30
Site	2	0.5643	23	2.66711	< 0.001	3.80
Family x Site	5	0.4158	18	0.14851	0.2801	1.08

Supplementary Table 2.17. Results of analysis of deviance (ANODEV) of gamma generalized linear model for 2018 recapture length by family, site, and their interaction. Abbreviations of terms are defined as in Table S2.1.

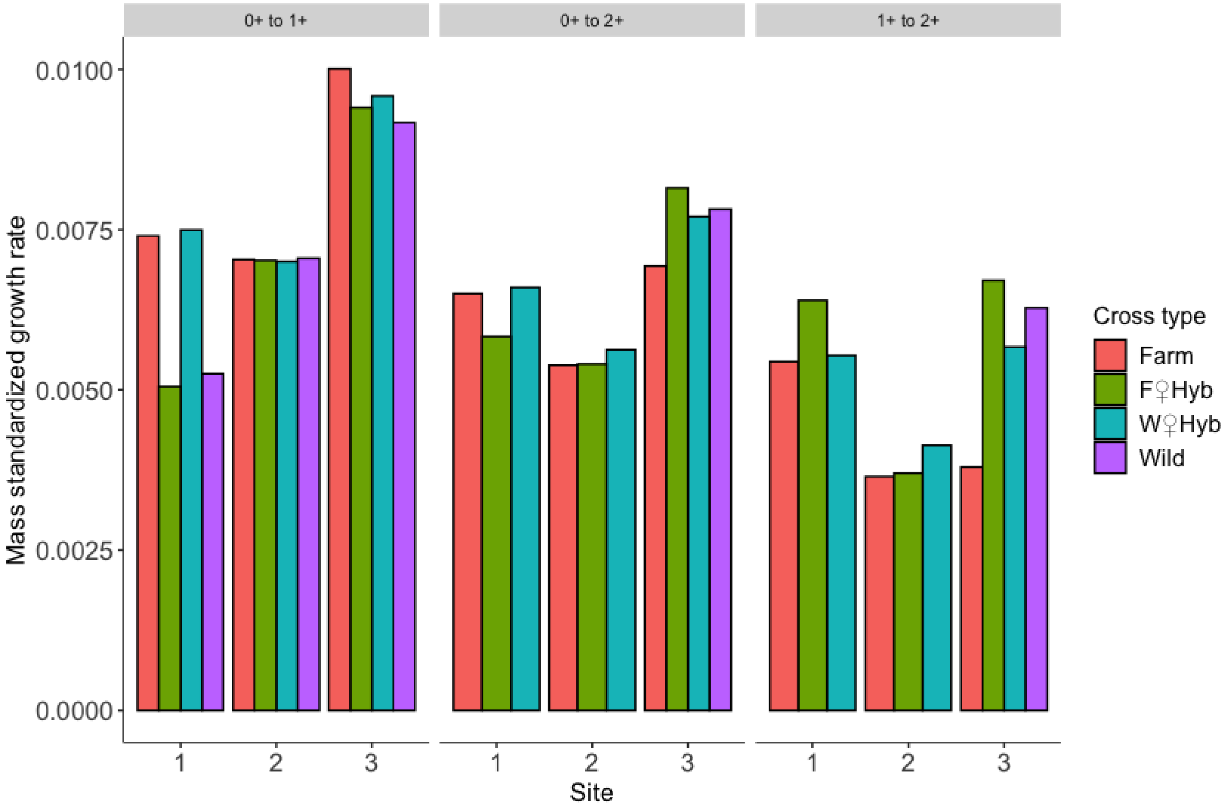
Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		22.849	1239			
Family	24	17.706	1215	5.143	< 0.001	13.09
Site	2	12.512	1213	5.1944	< 0.001	13.43
Family x Site	47	11.226	1166	1.2857	< 0.001	1.90

Supplementary Table 2.18. Results of analysis of deviance (ANODEV) of gamma generalized linear model for 2019 recapture length by family, site, and their interaction. Abbreviations of terms are defined as in Table S2.1.

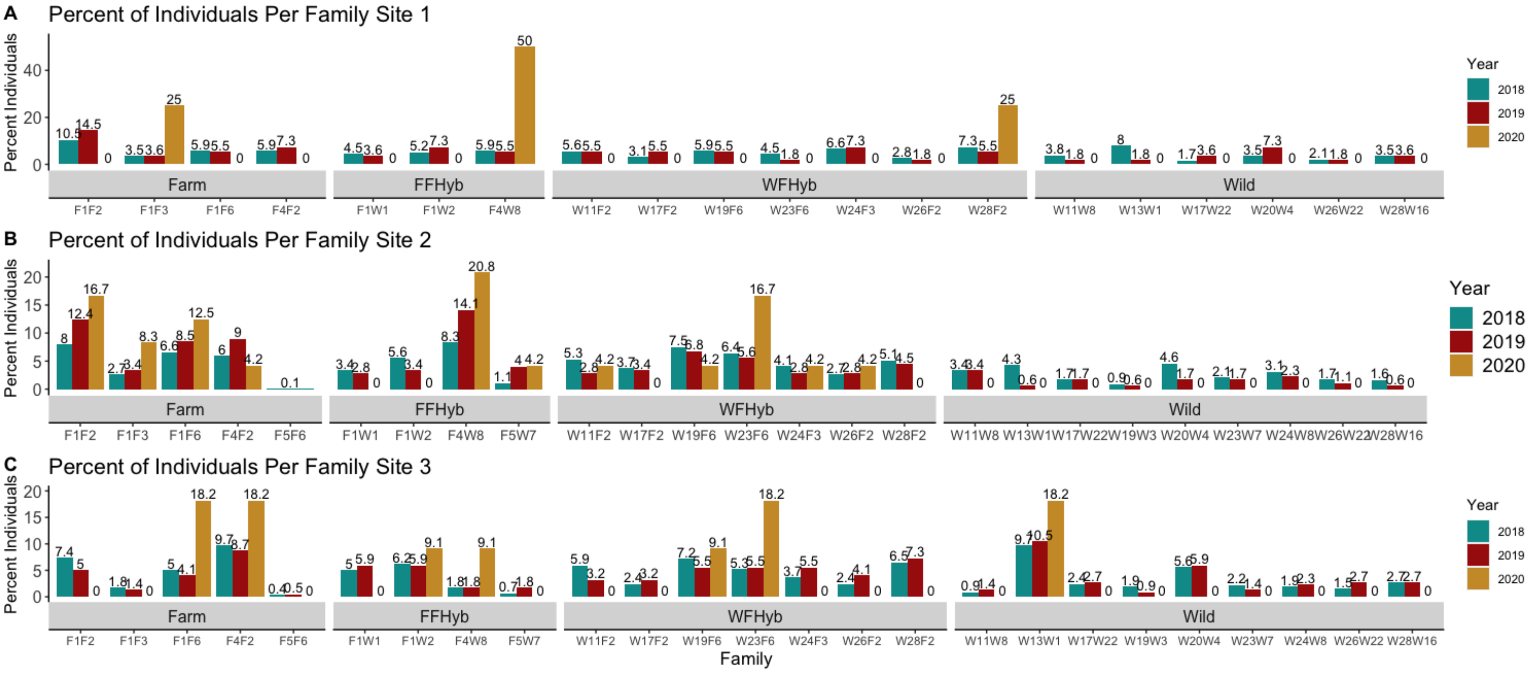
Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		8.4094	376			
Family	24	7.0899	352	1.3195	< 0.001	1.93
Site	2	3.1563	350	3.9336	< 0.001	7.15
Family x Site	41	2.482	309	0.6742	< 0.001	1.40

Supplementary Table 2.19. Results of analysis of deviance (ANODEV) of gamma generalized linear model for 2020 recapture length by family, site, and their interaction. Abbreviations of terms are defined as in Table S2.1.

Source	df	Residual Deviance	Residual df	ΔDeviance	p	LR
NULL		0.55148	38			
Family	13	0.34149	25	0.209989	< 0.001	1.11
Site	2	0.07145	23	0.27004	< 0.001	1.14
Family x Site	5	0.06133	18	0.010122	0.7067	1.01



Supplementary Figure 2.1. Average growth rate for the different cross types for each site between three different recapture periods. 0+ to 1+ refers to the period between the 3-month post-release and 1-year post-release recapture events, 0+ to 2+ refers to the period between the 3-month-post release and 2-year post-release recapture events, and 1+ to 2+ refers to the period between the 1-year post-release and 2-year post-release recapture events.



Supplementary Figure 2.2. Percentages of Atlantic salmon individuals recaptured per family each year in Sites (A)1, (B) 2, and (C) 3.

Supplementary Table 3.1. Linear mixed model of the genetic class temporal analysis, using the logit transformed proportions of each genetic class as a response variable, year and genetic class as regression variables, and river as a random effect. MS is mean squares, SS is sums of squares, and df is degrees of freedom. *log()* refers to the natural logarithm.

Source	SS	MS	NumDf	DenDf	F	p
log(Year)	1.646	1.646	1	684	0.943	0.332
Class	109.393	21.879	5	684	12.533	<0.001
*log(Year)*Class*	109.483	21.897	5	684	12.543	<0.001

Supplementary Table 3.2. Linear mixed model for the temporal analysis of (A) pure wild, (B) pure farm/feral, (C) F1 hybrids, (D) F2 hybrids, (E) backcross wild hybrids, and (F) backcross farm hybrids, using the logit transformed proportions of individuals per genetic class as a response variable, year as a regression variable, and river as a random effect. *log()* refers to the natural logarithm.

A.

	Estimate	Std.Err	t-value	P-value
Intercept	-1977.17	923.6	-2.141	0.0348
log(Year)	259.94	121.38	2.142	0.0347

B.

	Estimate	Std.Err	t-value	P-value
Intercept	1909.32	577.48	3.306	0.00129
log(Year)	-251.34	75.89	-3.312	0.00127

C.

	Estimate	Std.Err	t-value	P-value
Intercept	3889.92	605.1	6.429	<0.001
log(Year)	-511.59	79.52	-6.433	<0.001

D.

	Estimate	Std.Err	t-value	P-value
Intercept	73.87	459.73	0.161	0.873
log(Year)	-10.13	60.42	-0.168	0.867

E.

	Estimate	Std.Err	t-value	P-value
Intercept	-3453.49	737.95	-4.68	<0.001
log(Year)	453.56	96.98	4.677	<0.001

F.

	Estimate	Std.Err	t-value	P-value
Intercept	2118.46	437.15	4.846	<0.001
log(Year)	-278.83	57.45	-4.854	<0.001

Supplementary Table 3.3. Linear mixed model for the temporal analysis of domestic admixture levels per river, using the logit transformed domestic admixture estimates as a response variable, year as a regression variable, and river as a random effect. *log()* refers to the natural logarithm.

	Estimate	Std.Err	t-value	P-value
Intercept	2442	356.9	6.843	< 0.001
log(Year)	-321.1	46.9	-6.848	< 0.001

Supplementary Table 3.4. Linear model for the spatial analysis of average proportion of hybrids in rivers from 2014-2018, using the logit transformed proportions as a response variable and river size, propagule pressure, and estimated wild population size as regression variables. *log()* refers to the natural logarithm.

	Estimate	Std.Err	t-value	P-value
(Intercept)	200.6	57.17	3.51	0.00564

log(Axial Distance)	-81.29	29.35	-2.77	0.0198
log(Estimated Wild Population Size)	-42.08	11.29	-3.73	0.00393
log(Propagule Pressure)	-27.06	7.639	-3.54	0.00534
log(Axial Distance) log(Estimated Wild Population Size)*	15.91	5.194	3.06	0.0120
log(Axial Distance) log(Propagule Pressure)*	11.07	3.936	2.81	0.0184
log(Estimated Wild Population Size) log(Propagule Pressure)*	5.659	1.518	3.73	0.00393
log(Axial Distance) log(Estimated Wild Population Size)* log(Propagule Pressure)*	-2.171	0.700	-3.10	0.0112

Supplementary Table 3.5. Results of analysis of deviance (ANODEV) of the generalized linear model for the spatial analysis of average proportion of hybrids in rivers in relation to (A) estimated wild population size, (B) river size (axial distance), and (C) propagule pressure from 2014-2018.

A.

	df	Deviance	Resid. Df	Resid. Dev	p
NULL			17	4.726	
Wild Population Size	1	1.344	16	3.382	0.0172

B.

	df	Deviance	Resid. Df	Resid. Dev	p
NULL			17	4.726	
Axial Distance	1	1.1292	16	3.597	0.0186

C.

	df	Deviance	Resid. Df	Resid. Dev	p
NULL			17	4.726	
Propagule Pressure	1	0.19752	16	4.529	0.387

Supplementary Table 3.6. Linear model for the spatial analysis of domestic admixture levels in rivers from 2014-2018, using the logit transformed admixture values as a response variable and river size, propagule pressure, and estimated wild population size as regression variables. *log()* refers to the natural logarithm.

	Estimate	Std.Err	t-value	P-value
(Intercept)	257.4	88.53	2.908	0.0156
log(Axial Distance)	-79.41	45.44	-1.747	0.111
log(Estimated Wild Population Size)	-56.39	17.49	-3.225	0.0091
log(Propagule Pressure)	-34.19	11.83	-2.89	0.0161
log(Axial Distance) log(Estimated Wild Population Size)*	16.85	8.044	2.094	0.0627
log(Estimated Wild Population Size) log(Propagule Pressure)*	7.438	2.351	3.164	0.0101
log(Axial Distance) log(Propagule Pressure)*	10.66	6.095	1.749	0.111
log(Axial Distance) log(Estimated Wild Population Size)* log(Propagule Pressure)*	-2.257	1.084	-2.083	0.0638

Supplementary Table 3.7. Results of analysis of deviance (ANODEV) of the generalized linear model for the spatial analysis of domestic admixture levels in rivers in relation to (A) estimated wild population size and (B) propagule pressure from 2014-2018.

A.

	df	Deviance	Resid. Df	Resid. Dev	p
NULL			17	4.069	
Wild Population Size	1	0.756	16	3.313	0.0648

B.

	df	Deviance	Resid. Df	Resid. Dev	p
NULL			17	4.069	
Propagule Pressure	1	0.00183	16	4.067	0.932

Supplementary Table 3.8. Linear model for the spatial analysis of average proportion of hybrids in rivers from 2014-2021, using the logit transformed proportions as a response variable and river size, propagule pressure, and estimated wild population size as regression variables. *log()* refers to the natural logarithm.

	Estimate	Std.Err	t-value	P-value
(Intercept)	5.628	163.4	0.034	0.974
log(Axial Distance)	27.62	81.27	0.34	0.751
log(Estimated Wild Population Size)	-12.003	27.75	-0.433	0.688
log(Propagule Pressure)	-2.373	20.96	-0.113	0.915
log(Axial Distance) log(Estimated Wild Population Size)*	-1.803	13.49	-0.134	0.9
log(Axial Distance) log(Propagule Pressure)*	-2.963	10.55	-0.281	0.793
log(Estimated Wild Population Size) log(Propagule Pressure)*	1.945	3.533	0.55	0.611
log(Axial Distance) log(Estimated Wild Population Size)* log(Propagule Pressure)*	0.087	1.741	0.05	0.963

Supplementary Table 3.9. Results of analysis of deviance (ANODEV) of the generalized linear model for the spatial analysis of domestic admixture levels per river 2014-2021 in relation to estimated wild population size, river size (axial distance), propagule pressure, and all possible interactions.

	df	Deviance	Resid. Df	Resid. Dev	p
NULL			11	1.996	
Axial Distance	1	0.299	10	1.697	0.268
Estimated Wild Population Size	1	0.466	9	1.231	0.167
Propagule Pressure	1	0.083	8	1.147	0.559
*Axial Distance*Estimated Wild Population Size*	1	0.020	7	1.127	0.776
Axial Distance Propagule Pressure*	1	0.008	6	1.120	0.859
*Estimated Wild Population Size * Propagule Pressure*	1	0.027	5	1.093	0.738
*Axial Distance*Estimated Wild Population Size* Propagule Pressure*	1	0.200	4	0.893	0.366