GENETIC INFLUENCE ON SURVIVAL AND FITNESS-RELATED TRAITS OF JUVENILE FARMED, WILD, AND HYBRID ATLANTIC SALMON (*SALMO SALAR*) IN NATURE

By

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Abstract

Farmed Atlantic Salmon (*Salmo salar*) have experienced multiple generations of selection pressures different from those experienced by their wild counterparts. Farmed fish escape from aquaculture facilities regularly, and their interbreeding with wild fish can result in lower wild population productivity and altered life history traits. Therefore, understanding the genetic basis of relative performance of farmed, wild, and hybrid salmon is critical to predicting impacts on wild populations from farmed escapees. In my first data chapter, I compared the relative survival, size, morphology, and parr marks of Atlantic Salmon parr (wild, farmed, and reciprocal F1 hybrids) over the first summer of growth at three replicate sites in southern Newfoundland. There was a consistent pattern of relative survival across all sites (wild-mother hybrids > pure wild > pure farmed > farmed-mother hybrids), with wild fish consistently smallest in size, and wild-mother hybrids and farmed fish largest. In addition, I found small differences in body shape related mainly to body depth, and differences among sites for parr mark size. In my second data chapter, I compared lipid and fatty acid profiles at release and recapture for farmed, wild and hybrid parr. There were lipid profile differences among cross types at both time points and in addition, pure farmed fish displayed a greater decrease in storage lipids and certain fatty acids characteristic of freshwater invertebrate prey over the experimental period when compared with other cross types. Overall, there were measurable differences in survival and fitness-related traits among cross types, even over a relatively short experimental period under favourable conditions. Ultimately, this research provides key data on relative cross type performance for North American populations of Atlantic Salmon that may help inform predictive models, and subsequent aquaculture management and mitigation decisions.

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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
Csize	Centroid Size
ANODEV	Analysis of Deviance
SE	Standard error
TAG	Triacylglycerol
PL	Phospholipid
ST	Sterol
FA	Fatty acid
EFA	Essential fatty acid
ALA	Alpha-linolenic acid
LNA	Linoleic acid
ARA	Arachidonic acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
AMPL	Acetone mobile polar lipid
FFA	Free fatty acid
FAME	Fatty acid methyl ester
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
n-3	Omega-3

Co-Authorship Statement

The work described in this thesis was conducted by Samantha Crowley 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 this study and reared them to release. Steve Duffy chose the field sites and coordinated field work for release and recapture. Amber Messmer coordinated genetic lab work and assisted with trials for parentage analysis of juvenile salmon. Chris Parrish offered input for experimental design in Chapter 3 as well as interpretation of results. Data analyses were conducted, and manuscripts written by Samantha Crowley with assistance from Ian Fleming, Ian Bradbury, and Chris Parrish, with contributions to the editing process from Matt Rise. Anticipated publications arising from this thesis will be co-authored by S. Crowley, I. Bradbury, A. Messmer, S. Duffy, S. Islam, and I. Fleming (Chapter 2); and S. Crowley, I. Bradbury, C. Parrish, A. Messmer, S. Duffy, S. Islam, and I. Fleming (Chapter 3).

Chapter 1: General Introduction

With the world's growing population increasing the demand for protein, there is a push to find alternative sources that do not place additional pressure on strained wild systems (FAO 2018). Though the production of capture fisheries has plateaued since the 1980s, worldwide consumption of fish has increased at twice the rate of the global population since 1961, a feat made possible by the development and expansion of aquaculture industries (FAO 2018). In the past, marine capture fisheries supplied more than 80% of the world's fish (Tidwell and Allan 2001), while in 2016 global aquaculture supplied 53% of this resource, excluding non-food use (FAO 2018). Aquaculture of Atlantic Salmon (*Salmo salar*) has expanded particularly rapidly since beginning in the late 1960s (Glover et al. 2017), growing to produce more than 2.2 million tons as a global industry in 2016 (FAO 2018).

Though the expansion of aquaculture greatly reduces the world's direct reliance on wild salmon populations for food, aquaculture has introduced a new set of threats to wild salmon. Farmed Atlantic Salmon, in the case of the oldest breeding strains, have undergone >12 generations of artificial selection for traits desirable for the aquaculture environment and/or economic profitability, as well as unintentional domestication selection, and random changes such as genetic drift and founder effects (reviewed by Glover et al. 2017). Overall, farmed Atlantic Salmon are considered one of the most domesticated food fish species (Teletchea and Fontaine 2014), and it has even been suggested that they be considered biologically separate (i.e. *Salmo domesticus*) from their wild counterparts (Gross 1998). Regardless of taxonomic designation, farmed Atlantic Salmon are genetically different from wild salmon (e.g. Skaala et al. 2005; Karlsson et al. 2011; Besnier et al. 2015; Wringe et al. 2019), and farmed divergence from a given wild stock may be even further pronounced due to differences in ancestry of the

stocks in question (Glover et al. 2017). Ultimately, these domestication-caused genetic differences can result in expression of traits that are maladaptive to life in the wild (e.g. Fleming and Einum 1997; Fleming et al. 2000; McGinnity et al. 2003; Skaala et al. 2012; reviewed by Glover et al. 2017).

Escapes of farmed fish from net pen aquaculture are at this point inevitable, and wild-maladapted farm fish may interact both ecologically and genetically with wild fish after escaping. Direct genetic interactions occur when escaped farm fish survive to breed with wild or hybrid individuals, which can lead to the introgression of maladapted farmed genes into wild populations and changes in the frequencies of wild genotypes (Verspoor et al. 2015). Since Atlantic Salmon may be highly locally adapted (reviewed by Taylor 1991; Garcia de Leaniz et al. 2007), this introgression can have a negative effect on the wild population through outbreeding depression (Verspoor et al. 2015). Offspring resulting from farm-wild interbreeding can have reduced survival (Fleming and Einum 1997; Fleming et al. 2000; McGinnity et al. 2003; Skaala et al. 2012; Skaala et al. 2019; Sylvester et al. 2019), and ultimately farmed introgression may lead to a reduction in fitness of entire populations (McGinnity et al. 2003).

Since both environment and genetics can influence the traits expressed by salmon, in order to isolate the influence of genetics on differences in farm, wild and hybrid traits, experimental individuals must be subjected to common environmental conditions (Glover et al. 2017). Studies investigating genetic differences among farm, wild, and hybrid Atlantic Salmon have been approached at different scales of biological organization, from looking at differences in gene transcription or genetic polymorphisms, to phenotypic and behavioural differences (reviewed by Glover et al. 2017). At the latter level of organization, a broad range of phenotypic traits have been studied under common-garden conditions, such as those related to growth (e.g.

Einum and Fleming 1997; Solberg et al. 2013; Harvey et al. 2016), external morphology (e.g. Fleming et al. 1994; Solem et al. 2006; Wringe et al. 2016), and body lipid content (Neregård et al. 2008; Glover et al. 2009). With each cross type raised under common conditions, the phenotypes that they express should show the influence of genetics alone under those particular environmental conditions (De Villemereuil et al. 2016),

Farmed fish typically grow faster than wild conspecifics, as this is a desirable economic trait selected for by aquaculture production (Gjøen and Bentsen 1997). A larger size at age can provide a competitive advantage for these larger fish relative to their smaller conspecifics; for example, larger farm parr have been found to displace slower-growing wild parr from suitable habitat in the wild (Fleming et al. 2000; McGinnity et al. 2003). However, farmed fish with larger body size may also have a disadvantage in the wild compared with wild fish. Farmed fish outgrow their wild counterparts by two to three times in culture while only somewhat outgrowing them in the wild (reviewed by Glover et al. 2017). A study by Glover et al. (2018) tested whether this environment-based difference in farm:wild growth rates was due to selection against faster-growing farmed fish in the wild, or a genetic influence on growth plasticity in farmed fish leading to their higher growth in culture. They concluded that neither selection nor plasticity was the sole cause of the difference in growth rates, but rather that it was due to a combination of the two mechanisms. Overall, the results of these studies show that the nature of the link between body size and survival for different cross types is likely to be context-dependent.

Farmed and wild salmon differ morphologically (Fleming et al. 1994; Fleming and Einum 1997; Solem et al. 2006), which may be attributed to the fact that salmon can be adapted to their local environments (reviewed by Taylor 1991; Garcia de Leaniz et al. 2007), and culture

and wild environments differ significantly from each other. For example, smaller heads in farmed fish may be adaptive because farmed fish feed from above, and a smaller head may better facilitate looking up at food (Solem et al. 2006). Similarly, wild fish typically also have larger fins than farmed fish (Fleming and Einum 1997; Solem et al. 2006), with larger fins potentially useful for stabilization in higher water velocities (Riddell and Leggett 1981). In addition, farmed fish have also been found to have a smaller eye diameter and larger mouth than certain wild populations (Solem et al. 2006). Farmed fish can also have deeper bodies than wild fish, thought to be due to a lack of selection pressure for the streamlined body associated with stronger swimming performance (Fleming and Einum 1997). Comparing morphological features such as these among cross types may provide information regarding each cross type's level of adaptation to the wild environment, and therefore their relative abilities to survive therein.

As lipids are the most important macromolecule in the diet of fishes, a diet deficient in lipids and essential fatty acids (EFAs) can cause problems for a fish's growth and pathogen resistance, ultimately affecting survival and reproductive capacity (Tocher 2010). Therefore, investigating the lipid and fatty acid content of wild, farmed, and hybrid juveniles may provide some insight into their relative performance in the wild. Previous work has focused on differences in lipid and fatty acid content in farmed and wild salmon when raised in their respective environments and indicates that farmed salmon typically have higher lipid content than wild salmon (Johnston et al. 2006). While artificial selection for higher growth rate in farmed Atlantic Salmon is correlated with higher fat content (Quinton et al. 2005; Powell et al. 2008), a study comparing farmed, wild, and hybrid Norwegian salmon in common-garden culture conditions (where all cross-types were fed the same commercial diet) found no significant differences in lipid content and most individual fatty acids between cross-types

(Glover et al. 2009). However, there is still question as to whether farmed, wild, and hybrid Atlantic Salmon raised under common conditions in the wild will exhibit differences in lipid and fatty acid (FA) content, and whether these differences are indicative of differential foraging and energy storage abilities that may affect survival.

To date, only a few European studies (Fleming et al. 2000; McGinnity et al. 2003; Skaala et al. 2012; Skaala et al. 2019) have investigated genetic influence on trait differences of juvenile wild, farm, and hybrid Atlantic Salmon in a wild common-garden environment, given the logistical difficulties of maintaining common-garden conditions therein (reviewed by Glover et al. 2017). These types of wild-environment experiments are crucial to understanding the real-world impacts of introgression on wild populations, since the environmental and ecological conditions of a wild system are complex and cannot be fully replicated in a lab experiment. Given the limited number of such wild-environment common-garden studies, work remains to be done in characterizing the impacts of escapees in the wild, particularly for non-European populations in regions with high potential for aquaculture impacts.

In Newfoundland and Labrador, Canada, wild Atlantic Salmon populations are currently at-risk along the south coast, having gained a COSEWIC classification of "threatened" in 2010 (COSEWIC 2010). From 1996 to 2010, south coast populations underwent an overall decline of 45%, with some individual populations near farms experiencing much larger declines (DFO 2013), and this decline has since continued (DFO 2020). One cause of concern for these declines is genetic introgression from farmed fish (DFO 2013), which has been found to be widespread in the region. A study conducted after a large escape event in 2013 of approximately 20,000 sexually mature adult farmed fish found hybrid offspring in 17 of the 18 rivers assessed (with an overall hybridization rate of 27.1%), and feral farm offspring in 13 of 18 rivers (Wringe et al.

2018). A subsequent study by Sylvester et al. (2019) built upon this work and quantified the strength of selection against each cross type, with modeling results predicting wild offspring to have the highest relative fitness, farm the lowest, and F1 hybrids intermediate. Since small within-river sample sizes prohibited predictions at the individual river scale in that study, predictions were made at the southern Newfoundland regional scale; therefore, opportunity exists to further build on these studies and assess relative survival of farm, wild, and hybrid parr at smaller geographic scales. In addition, a wild common-garden experiment with known parents and numbers of offspring initially produced would allow for accounting of maternal effects and parental spawning success when comparing proportions of different cross types in a river, providing a better estimate of relative survival for a given life stage. Finally, while some North American common-garden lab studies have looked at farm-wild differences in early life traits and survival (e.g. Darwish and Hutchings 2009; Fraser et al. 2010; Debes and Hutchings 2014; Hamoutene et al. 2017), there is a gap in the literature regarding how cross type differences in survival-related traits manifest for North American Atlantic Salmon in a wild environment, and how these differences may relate to survival differences in the wild.

This study couples the quantification of relative survival of farm, wild, and hybrid Atlantic Salmon parr in a Newfoundland river system along with comparisons of multiple fitness-related traits, to further the understanding of what drives differences in survival and fitness. In Chapter 2, I examined size (weight and length) and external morphology (including parr mark characteristics) among juveniles of each cross type, in addition to comparing their relative odds of recapture as a proxy of survival. I aimed to isolate the influence of genetics on these traits in the wild by subjecting all cross types to common environmental conditions. I used three separate common-garden experimental sites, which provided an ecological gradient along

which I could compare the expression of these survival-related traits for each cross type. In Chapter 3, I compared the lipid and fatty acid content, as well as size and condition of individuals of each cross type at recapture from this common-garden, wild environment. I also compared these traits among cross types at release (up until which point they were raised under laboratory conditions and a commercial diet), with the patterns of change in lipid profiles during the experimental period providing insight on each cross type's relative ability to capture and store energy resources in the wild. Overall, quantifying these differences in survival and fitness of wild, farm, and hybrid Atlantic Salmon will be crucial for making predictions regarding the state of wild populations in the future.

Chapter 2: A Common-Garden Comparison of Relative Survival, Size, External Morphology, and Parr Marks of Wild, Farmed, and Hybrid Atlantic Salmon (*Salmo salar*) Parr in Nature

Abstract

When farmed Atlantic Salmon escape and breed with wild fish, the resulting introgression of maladaptive genes can lower wild population productivity and alter key life history traits. To date, only a few European studies have compared wild, farmed, and hybrid salmon under common conditions in the wild, which is necessary for isolating the influence of genetics on survival and fitness-related traits. Here, I examine the performance of experimentally derived Atlantic Salmon fry from four cross-types (wild, farmed, and reciprocal F1 hybrids) during the first summer of growth at three replicate locations in southern Newfoundland. Overall survival was high, and the cross type rank order for survival was consistent across all sites (wild-mother hybrids > pure wild > pure farmed > farmed-mother hybrids). Pure wild fish were smaller on average than wild-mother hybrids and pure farmed fish across all sites, but were only slightly different in size compared with farmed-mother hybrids. At two sites, wild-mother hybrids were considerably larger than all cross types except pure farmed fish (over whom they had only a small size advantage), with the opposite pattern for pure farmed and wild-mother hybrid size at the third site. Morphological differences were small and mainly related to body depth, with the largest differences existing between pure wild and farmed fish. Wild-mother hybrids had fewer parr marks on average than other cross types at a single site, and cross type differences for size of marks were minimal. Overall, these results show that genetic differences exist for fitness-related traits among wild, farmed, and hybrid juveniles even over short temporal scales and under favourable environmental conditions, and may contribute to patterns of reduced farmed-mother hybrid and feral farmed survival.

Introduction

Since beginning in the late 1960s, the domestication of Atlantic Salmon (*Salmo salar*) has subjected farmed fish to directional selection, inadvertent domestication selection, and relaxed wild natural selection pressures over multiple generations (reviewed by Glover et al. 2017). As a result, farmed fish differ from wild fish both genetically (e.g. Skaala et al. 2005; Karlsson et al. 2011; Besnier et al. 2015; Wringe et al. 2019) and phenotypically for a variety of traits such as growth, morphology, behaviour, age at maturity, and reproductive success (e.g. Einum and Fleming 1997; Fleming et al. 2000; McGinnity et al. 2003; Skaala et al. 2012; Skaala et al. 2019). In the wild, Atlantic Salmon are characterized by significant adaptive diversity in response to their local environments (Taylor 1991; Garcia de Leaniz et al. 2007; Fraser et al. 2011), and the hybridization of escaped farm fish with wild fish can erode this local adaptation and lead to a reduction in fitness (McGinnity et al. 2003; Baskett et al. 2013; Skaala et al. 2019; Sylvester et al. 2019) and population productivity (Fleming et al. 2000; Bradbury et al. 2020). Therefore, investigating differences in fitness-related traits as well as differences in survival between wild, farm, and hybrid salmon is key to determining and managing the impacts of escaped farmed salmon on wild salmon populations.

Generally, field and laboratory studies have shown that farmed Atlantic Salmon typically grow faster than wild conspecifics (e.g. Glover et al. 2009; Solberg et al. 2013; Harvey et al. 2016; Skaala et al. 2019). This may be advantageous, as faster-growing farmed fish have been found to displace slower-growing wild fish from suitable habitat during the parr stage in the wild (Fleming et al. 2000; McGinnity et al. 2003). However, faster growth may also be a liability for farmed fish in the wild, with evidence for selection against such fastest-growing individuals (Solberg et al. 2020), in addition to the lower risk-aversion of farm fish (e.g. Fleming and Einum

1997). The influence of growth on survival therefore appears to depend on the specific nature of the ecological interactions between cross types, as well as the environment in which they live.

Differences in morphology between wild, farm, and hybrid Atlantic Salmon (Fleming et al. 1994; Fleming and Einum 1997; Solem et al. 2006) may also be indicative of differential adaptation and survival capabilities. For example, differences in head size may be reflective of different feeding/foraging environments (Solem et al. 2006), while fin sizes may be reflective of the water velocities experienced by each cross type in their respective environments (Riddell and Leggett 1981), and body shape may be a result of selection pressures necessitating a certain level of swimming performance (Fleming and Einum 1997). In addition, differences in number and contrast of parr marks (the dark vertical bands on the sides of parr) among wild and hatchery populations may impact the juveniles' abilities to camouflage against the streambed and thus avoid predators (Culling et al. 2013). Given these implications, the degree of morphological differentiation between interacting cross types has the potential to contribute to differences in performance, and ultimately survival.

To date, just a few studies have quantified survival and phenotypic differences among wild, farmed, and hybrid individuals in the wild, and all have been done in Europe (Einum and Fleming 1997; Fleming et al. 2000; McGinnity et al. 2003; Skaala et al. 2012; Skaala et al. 2019). In general, these studies show that farmed juveniles typically have lower survival compared with their wild conspecifics, while hybrid survival is generally intermediate to the two pure crosses (McGinnity et al. 1997; Fleming et al. 2000; Skaala et al. 2012; Skaala et al. 2019). However, since Atlantic Salmon populations tend to be highly differentiated genetically between the eastern and western Atlantic (King et al. 2001; Lehnert et al. 2019; Lehnert et al. 2020), the nature of wild-farm interactions in these European studies may not be directly applicable to

North American farm-wild interactions. It is crucial to characterize differences between farmed, wild, and hybrid salmon at local scales, since the extent to which farmed introgression has an impact on a wild population seems often to be dependent on factors such as the demographics of the wild population in question and its original relatedness to the invading farm stock (e.g. Heino et al. 2015; Wringe et al. 2018).

This study aimed to assess differences in survival, growth, and morphology of North American first-feeding Atlantic Salmon fry from four cross types (wild, farmed, and reciprocal F1 hybrids) during the first summer of growth in multiple tributaries of a natural river on the south coast of Newfoundland. Specifically, the four main objectives were to compare among all cross types within each study site 1) odds of recapture, 2) recapture size (weight and length) and condition, 3) external morphology, and 4) parr marks. The overarching goal was to examine differences among these four cross types while isolating the influence of genetics on these traits by subjecting fish to common environmental conditions throughout the study. The use of three different experimental sites/tributaries allowed for investigation as to whether farm, wild, and hybrid fish differ in their traits by the same degree across an environmental gradient. This study extends existing research on wild, farm, and hybrid Atlantic Salmon performance in the wild in southern Newfoundland (Wringe et al. 2018) to better inform predictions of population responses to escaped farmed salmon in Atlantic Canada (e.g. Keyser et al. 2018; Sylvester et al. 2019; Bradbury et al. 2020).

Methods

Crosses, Release and Recapture

Between 28 November and 21 December 2017, four different crosses of Atlantic Salmon were generated: 9 families of wild offspring from wild parents of the Garnish River, 6 families of farmed offspring from parents of the Saint John River farmed strain, and 6 families of each of the reciprocal F1 hybrids of the above-mentioned farm and wild parents (denoted: farm-mother hybrids [F♀hyb] and wild-mother hybrids [W♀hyb]). Parents of each cross were fin-clipped, with samples stored in 100% ethanol for later use in parentage assignment of offspring. The Garnish River system is located on the Burin Peninsula on the south coast of Newfoundland emptying into Fortune Bay (mouth of river 47.2352808, -55.3442614), and is near an area of intensive Atlantic Salmon aquaculture. The Saint John River (New Brunswick) strain of Atlantic Salmon is, to date, the only farmed strain used in Atlantic Canada commercial aquaculture operations.

Embryos were incubated in Heath trays on ambient water at the Ocean Sciences Centre of Memorial University (St. John's, Newfoundland, Canada), where ambient water temperature was monitored daily (ranged 3-8 ^{o}C) and dead embryos removed every 4-5 days. At emergence (i.e. first feeding), juveniles were pooled (22 May 2018) 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 and fed a combination of *Artemia* and salmonid starter dry feed (crumbles (0.5 g; caloric content: 55% protein and 15% fat), EWOS-Cargill, BC, Canada) for one month, followed by only the dry feed until release. Release occurred on 11 July 2018 at three tributary sites of the Garnish River (Figure 2.1), which were chosen to determine whether the effect of cross type on survival and phenotype was consistent across a gradient of environmental conditions. Prior to release, all fry were adipose fin-clipped (anaesthetized using MS-222 (AQUALIFE TMS, Syndel Laboratories

Ltd., Nanaimo, BC, Canada) at a dose of 50 mg L^{-1} buffered with an equal amount of sodium bicarbonate) to distinguish them from wild fish upon later recapture. In addition, fish to be released at site 2 were photographed. Two thousand juveniles were to be released at each site, consisting of 500 of each of the four cross types. However, since some fry died during transport to the sites, the actual numbers released at each site were 1932 at site 1, 1980 at site 2, and 1972 at site 3. Fry 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.

Recapture occurred from 2– 5 October 2018 using multiple pass electrofishing. For site 3, a single electrofishing unit (LR-24 Backpack Electrofisher, Smith Root, Vancouver, WA, USA) was used, and for sites 1 and 2 (which had wider channels) two electrofishing units were used on opposite sides of the channel. The electrofishing units were set at 550 volts and 60 Hz, with a duty cycle of 25%. Recapture began approximately 150-200 m downstream from the first release points, over which the first ~20 metres no fish were recaptured (sites 1 and 2), or at a culvert downstream from the first release point (site 3). Electrofishing continued upstream until a natural barrier was reached (site 3) or to when no experimental individuals were recaptured over approximately 25 metres (sites 1 and 2). Sites 2 and 3 were each sampled on two different days, while site 1 was sampled only one day due to logistical constraints.

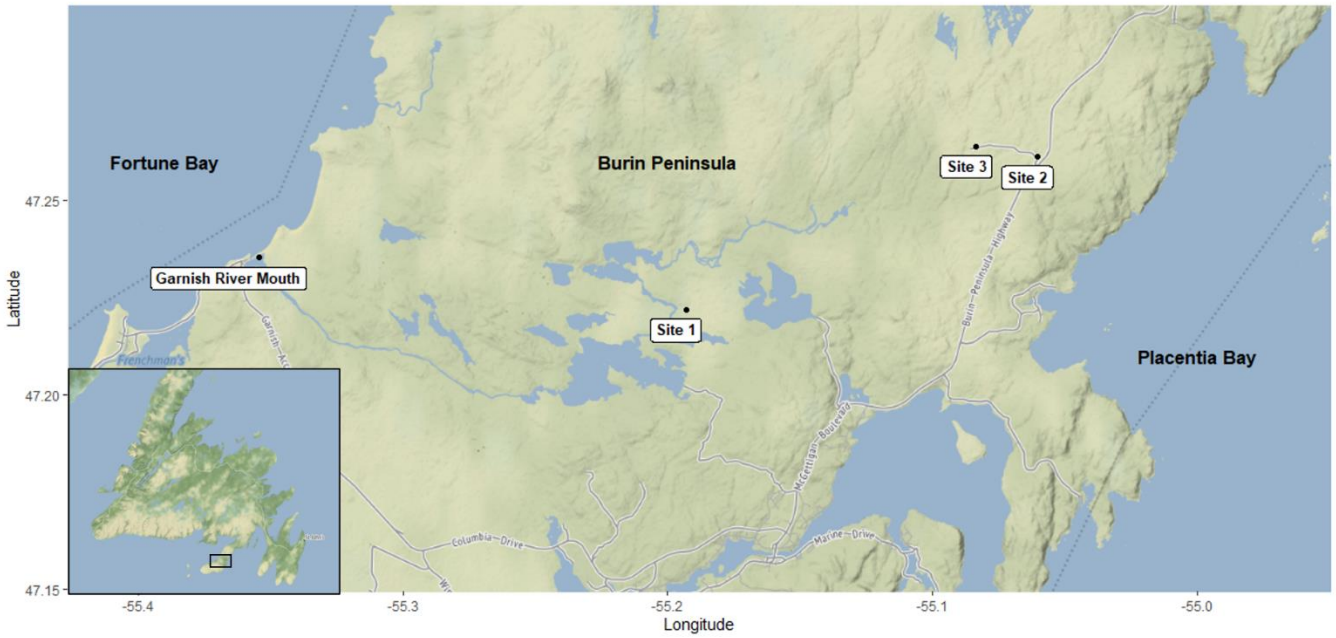

Figure 2.1. Location of three tributary study sites of the Garnish River, Newfoundland, Canada, that were used for the release and recapture experiment. Inset shows the island of Newfoundland with the dark rectangle indicating the general location of the study area on the Burin Peninsula.

Recaptured fish were kept alive until processing, which occurred approximately two h following the end of electrofishing each day. Fish were euthanized using MS-222 (400 mg L^{-1} buffered with an equal amount of sodium bicarbonate), and immediately photographed (Sony Alpha a5000) on the left side using a photo stand set at fixed height. A size and colour standard was photographed for each set of photos (i.e. at the beginning of each sampling session). All fish were then weighed (±0.01 g). The caudal fin was clipped and stored in 100% ethanol for later parentage analysis. Most whole specimens (minus caudal fin) were frozen at -80 ^{o}C, however approximately 100 fish from each site were bulk collected in ethanol.

Genetic Analysis

Parentage analysis was performed using caudal fin tissue samples to assign recaptured individuals to family, and therefore either wild, farm, or one of the two hybrid groups. Parentage

analysis was done using a panel of 31 microsatellite loci with a total of 277 alleles (multiplex panel 1a from Bradbury et al. (2018)), which are a subset of a larger panel of 101 loci for the Atlantic Salmon genome in Atlantic Canada. Of these 31 loci, ultimately 25 were used for parentage assignment, with six original loci excluded due to either a high percentage of missing offspring genotypes or a high estimated allelic dropout rate. The 25 loci used included two with a tetranucleotide repeat sequence, and 23 with 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).

DNA was extracted using the DNeasy 96 Blood and Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol for Purification of Total DNA from Animal Tissues. Microsatellite loci were PCR amplified following the protocol described by Zhan et al. (2017). Sequencing was run on an Illumina MiSeq and scored using MEGASAT software (Zhan et al. 2017). Each individual fish was assigned back to its family (and therefore also its cross type) using the software program COLONY (Jones and Wang 2010). Test trials for COLONY included genotype data for all unique samples, parents, within-plate redundants, and cross-plate controls, to ensure the assignment sensitivity and accuracy of COLONY given the set of input parameters used. The final run included only unique samples (no redundants or controls).

Image Analysis

Fork length measurements were taken from recapture photos of all fish at each site using ImageJ software (version 1.52a). Two hundred photos taken of fish pre-release were also measured for fork length, for a total of 50 per cross type. Care was taken to follow the lateral line

of the fish's body, to account for any body arching when present. Condition factor at recapture was calculated as the residuals taken from the regression of ln(recapture weight) on ln(recapture length) (Bolger and Connolly 1989; Wootton 1998).

For morphometric analysis, landmarks were selected as in Figure 2.2 and digitized using ImageJ software. All photos of fish were visually pre-screened for excess body curvature caused by fish positioning, lopsidedness, or other positioning factors that would cause problems with analysis. Ultimately, 734 fish were landmarked, comprised of 166 from site 1, 290 from site 2, and 278 from site 3, of which 191 were wild, 163 were farmed, 102 were F♀hyb, and 278 were W♀hyb fish. Landmarks were saved as XY coordinates. In addition to measurements using landmarks, 120 fish (10/site/cross type) were selected for pectoral fin length measurements, which were taken manually (due to variation in fin positioning) in ImageJ.

For parr mark measurements, 10 fish from each site by cross type combination were chosen randomly for analysis (total 120 fish). Parr marks were counted manually, and each mark was measured width-wise across its widest point parallel to the lateral line, and lengthwise perpendicular to the lateral line, from the lateral line to the bottom of the mark. Measurements of marks were performed using ImageJ.

Statistical Methods

Models for Recapture, Weight, and Parr Marks

All statistical analyses were performed in R (version 4.0.2). The probability of recapture is the product of two probabilities: the probability of survival to time of recapture, and the probability of being encountered at time of recapture (Skalski et al. 2009). However, since the design of this experiment did not allow for the separation of these two probabilities, probabilities

and odds of recapture were used as estimates of survival here. Since the recapture (survival) data is presence/absence in form, a generalized linear model with binomial family and logit link was used for the analysis, with odds of recapture as the response.

For comparisons of release length, a linear model was used for analysis with cross type included as a fixed factor. Recapture length and condition factor were also analyzed using linear models. All recapture size models included site and cross type as fixed factors, in addition to mean egg weight (for each mother x cross type x site combination) as a covariate, and all possible interaction terms. Model fits were assessed by visual examination of residual-fit plots and normal QQ plots of residuals. As diagnostic plots indicated a general linear model was not a good fit for recapture weight data, a generalized linear model with the Gamma family (identity link) was used, since the Gamma model family is also appropriate for positive continuous data. The analysis of the parr mark data used linear models to test for the effects of site and cross type on a) number of parr marks, b) mean width of marks, and c) mean length of marks below the lateral line. Parr mark length and width were ln transformed prior to model analysis, and ln transformed standard length was included as a covariate. Finally, the linear model for pectoral fin length (ln transformed) also included standard length (ln transformed), site, and cross type.

Though I do report p-values for model parameters, based on the recommendations of Wasserstein et al. (2019) I do not use the terms "statistically significant" or "non-significant" (or indeed, "difference *vs*. no difference") with the p-value of 0.05 serving the delineator of this dichotomy. Instead, the relative evidence for the data given the models and their parameter estimates was assessed using likelihood ratios (LRs), with assessments of relative magnitude of evidence given with LR=8 indicating "strong" evidence, and LR=32 for "quite strong" evidence (Royall 1997). Likelihood ratios give the likelihood of the data given two different/competing

models (Glover and Dixon 2004); that is, the likelihood of the data given a model including a specific variable *vs*. given a model lacking it. For generalized linear models, likelihood ratios were calculated using the change in deviance (Δ Dev) (e.g. Christensen 1990) from an analysis of deviance (ANODEV) on the given generalized linear model. For linear models, likelihood ratios were calculated using the sums of squares and were corrected for number of model parameters as in Glover and Dixon (2004). Finally, the *emmeans* package (Lenth 2020) was used on the results of the respective models to calculate pairwise differences in effect sizes and their confidence intervals, approximated as +/- twice the standard error of the effect size estimate.

Morphometric Analysis

Raw landmark coordinates were corrected for fish body arching using tpsUtil (Rohlf 2015), and the "unbend" function along landmarks 1, 5, and 9 (Figure 2.2). Following correction for arching, coordinates were aligned using a Generalized Procrustes Analysis (GPA, Gower 1975; Rohlf and Slice 1990) to provide Procrustes coordinates using the *geomorph* package in R (Adams et al. 2020). Subsequently, a Procrustes ANOVA was performed on the aligned coordinates using the *procD.lm* function to test for differences in overall shape between sites and cross types. The Procrustes ANOVA was performed using a residual randomization permutation method, with 9999 permutations. Site and cross type were included as fixed factors, and centroid size (Csize) was included as a covariate to test for differences in allometry between groups. All possible interaction terms were also initially included. Pairwise differences in mean shape between crosses were then compared within each site using the pairwise() function in the package RRPP (Collyer and Adams 2018; Collyer and Adams 2020), with the distance statistic being the length of vectors between least-squares mean vectors for shape.

Figure 2.2. Location and numbering of morphometric landmarks used for Generalized Procrustes Analysis. Landmarks are a subset of those described by Winans (1984): (1) point of snout on upper jaw, (2) most posterior point of maxillary, (6) origin of dorsal fin, (8) anterior attachment of dorsal membrane from caudal fin, (9) base of middle caudal rays, (10) anterior attachment of ventral membrane from caudal fin; in addition to a subset of those described by Fleming et al. (1994): (3) most anterior point of eye orbit , and (7) point directly below anterior dorsal fin origin on ventral body surface. Two additional landmarks were also included: (4) most posterior point of eye orbit, and (5) most posterior point of operculum.

Results

Survival (Recapture)

The total number of fish recaptured in 2018 was 1284 (21.8% total fish released), and the total number successfully genotyped and assigned parentage was 1242 (21.1% total fish released) (Figure 2.3A). The likelihood ratio results from the binomial generalized linear model of the recapture data indicate good evidence for the inclusion of site, cross type, and the interaction term in the model (Table 2.1).

Given the strong evidence for the site by cross type interaction term, comparisons of the odds of recapture among the four cross types were subsequently performed within each site

separately. The probabilities of recapture (not shown) for all cross types were higher at sites 2 and 3 than at site 1, which is not surprising given that sites 2 and 3 were sampled twice while site 1 was only sampled once.

W♀hyb had the highest numbers recaptured across all sites, followed by pure wild, pure farm, and then F♀hyb (Figure 2.3A). However, only certain cross type pairs had consistently large differences from one another in their recapture odds across all three sites (Figure 2.3C). W♀hyb had higher odds of recapture than both F♀hyb and farm at all three sites, as did wild *vs*. F♀hyb. On the other hand, differences in recapture odds for wild *vs*. W♀hyb and farm (respectively) were smaller, with error bars overlapping the 1:1 odds ratio line at two out of three sites for each pairing (i.e. indicating a result of no difference in recapture odds for these pairings was also reasonably likely).

Table 2.1. Results of analysis of deviance (ANODEV) of binomial generalized linear model for recapture data.

Source	df	Δ Deviance	Residual df	Residual Deviance	p-value	Likelihood Ratio
Null			11	270.58		
Site	2	126.01	9	144.57	<2.0E-16	2.31E+27
Cross type	3	131.67	6	12.90	<2.0E-16	3.91E+28
Site*Cross type	6	12.90	0	0	0.045	631.75

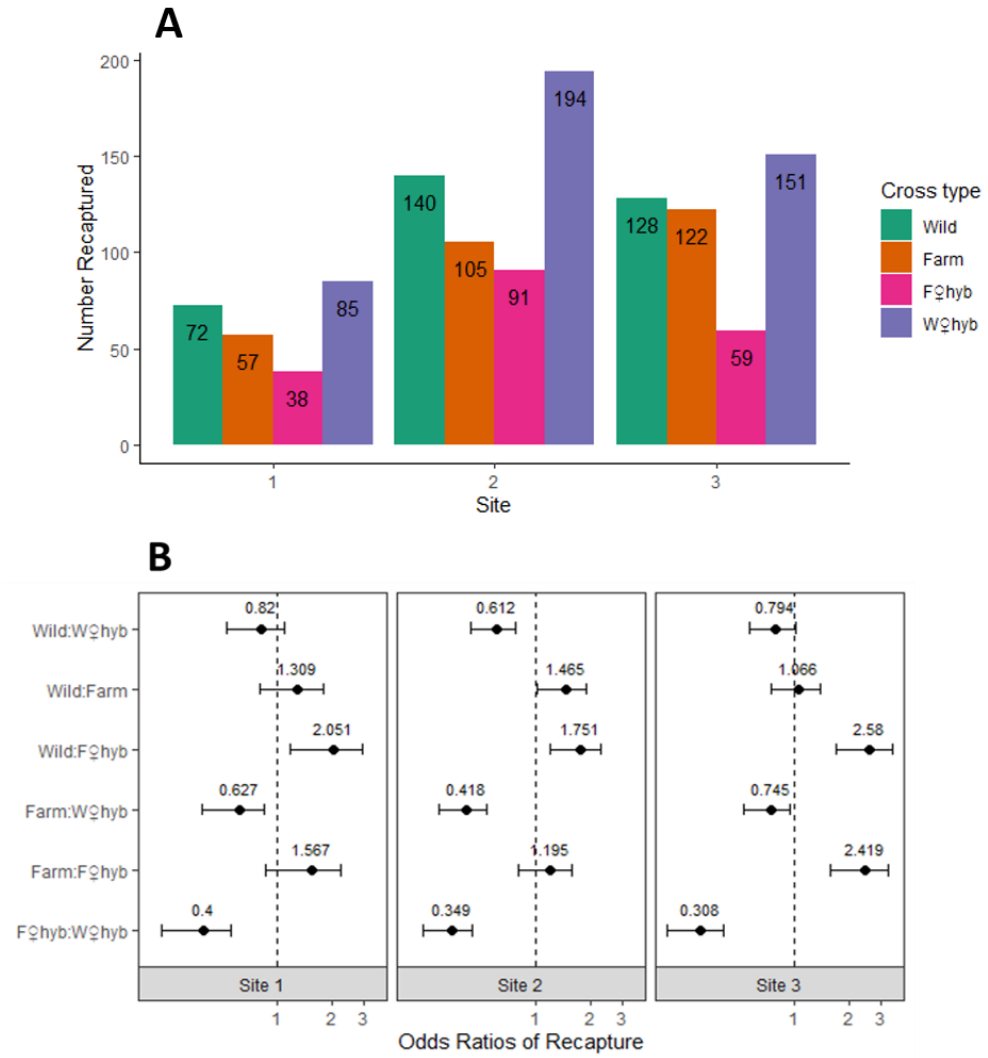

Figure 2.3. Numbers of fish recaptured by cross type and site (A) and odds ratios of recapture for pairwise cross type combinations at each site (B). Error bars in B represent twice the standard error (2*SE) of the odds ratio estimates. Odds ratios and standard errors were back-transformed from the logit scale.

Release Length

There was good evidence for the effect of cross type on length at release ($F_{3,196}$ = 5.53, LR = 147.6, p= 0.0012). However, differences were very small overall, with the largest mean difference in release length between any cross type being 2.03mm (equivalent to only 6.3% of mean release length for all cross types; see Figure 2.4b).

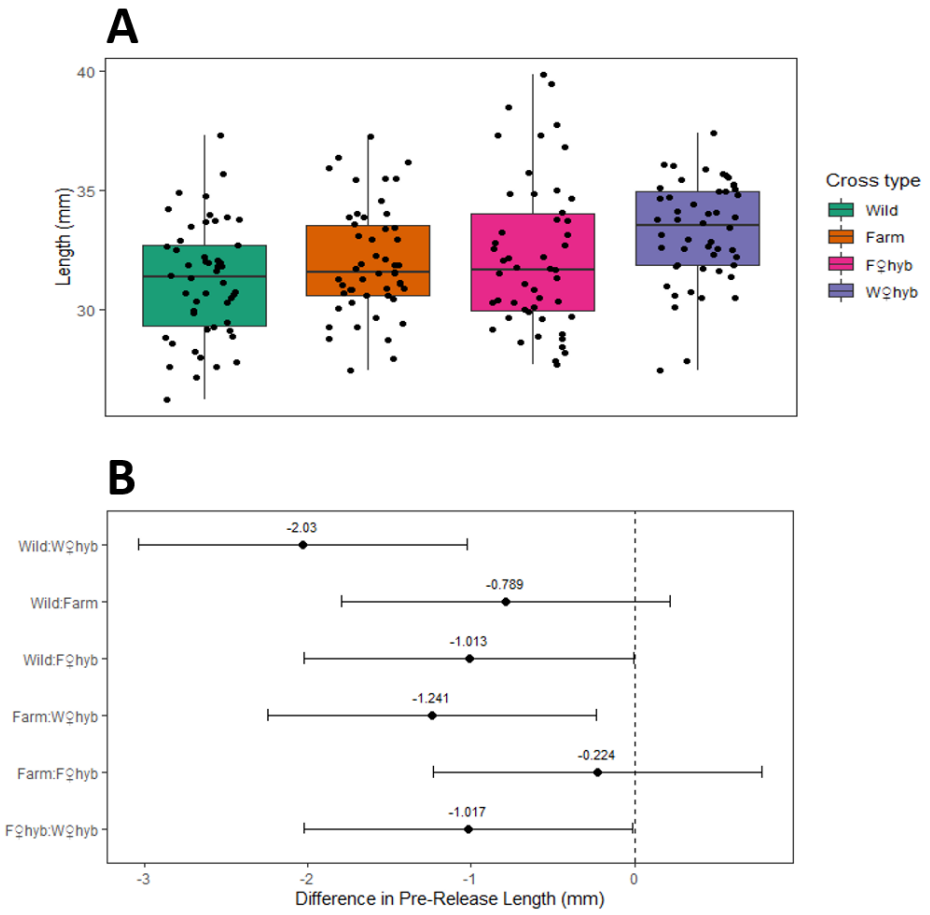

Figure 2.4. Boxplots of pre-release length by cross type (A) and pairwise differences in mean pre-release length for all cross types (B). Error bars on pairwise differences represent 2x the standard error of the difference estimate.

Size at Recapture

There was insufficient evidence for an effect of cross type on egg weight (LR= 0.49, p= 0.081), with the mean egg weight (± standard deviation) from farmed mothers 94.0 ± 19.3 mg, and that of wild mothers 91.6 ± 11.5 mg. However, there was strong evidence for an effect on mother identity (ID) nested within cross type on egg weight (LR= 8.53E+39, p< 2.0E-16). Therefore, mean egg weight for each individual mother was included as a covariate in the models for recapture weight, length, and condition.

There was strong evidence for an effect of mean egg weight, site, and cross type on offspring recapture weight and length, as well as for the interactions of mean egg weight by cross type, and site by cross type (Tables 2.2 & 2.3). Given the evidence for the site by cross type interaction term, pairwise comparisons in mean recapture weights and lengths among cross types were subsequently assessed within each site separately. These comparisons were made at the grand mean value for egg weight (93.2 mg), thereby taking into account the effect of egg weight on recapture size. Predictions of recapture weights and lengths over the entire egg weight range were also calculated for each site by cross type combination (Figures 2.5E & 2.5F and Supplementary Table 2.1).

Table 2.2. Analysis of deviance (ANODEV) table for generalized linear model of recapture weight by mean egg weight, site, cross type, and all possible interactions. Model was specified using the Gamma family and identity link. Null deviance for the model was 232.72 on 1241 degrees of freedom (df), and residual deviance was 142.21 on 1218 df. Δ dev refers to change in deviance, and LR refers to likelihood ratio.

Source	df	Δ dev	F	p-value	LR
NULL					
Egg weight	1	8.46	69.90	<2.2E-16	68.75
Site	2	53.86	222.52	<2.2E-16	4.97E+11
Cross type	3	10.72	29.5	<2.2E-16	212.41
Egg weight*Site	2	1.04	4.31	0.014	1.68
Egg weight*Cross type	3	8.82	24.28	2.92E-15	82.11
Site*Cross type	6	6.34	8.73	2.51E-09	23.78
Egg weight*Site*Cross type	6	1.28	1.76	0.10	1.89

Table 2.3. ANOVA table for linear model of recapture length by mean egg weight, site, cross type, and all possible interactions. 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-value	LR
Egg weight	1	2311	2310.7	76.62	<2.2E-16	1.63E+10
Site	2	13044	6522	216.27	<2.2E-16	4.69E+68
Cross type	3	3098	1032.8	34.25	<2.2E-16	1.64E+18
Egg weight*Site	2	244	121.9	4.044	0.018	5.23
Egg weight*Cross type	3	2972	990.5	32.85	<2.2E-16	9.25E+18
Site*Cross type	6	1086	181	6.00	3.37E-06	1.37E+05
Egg weight*Site*Cross type	6	272	45.3	1.50	0.17	0.19
Residual	1216	36670	30.2			

At the grand mean egg weight value, all cross types were their smallest (weight and length) at site 1, and largest at site 3 (Figures 2.5A & 2.5B, and Supplementary Table 2.1). Wild were smallest on average consistently across all three sites. At sites 2 and 3, W♀hyb were largest, followed by farm and then F♀hyb. However, at site 1, the order of cross types from largest to smallest was farm > F♀hyb > W♀hyb > wild.

The largest pairwise differences in recapture weight and length between cross types occurred at site 3, except for the wild:F♀hyb pairing, for which the largest difference was at site 1 (Figures 2.5C & 2.5D). W♀hyb and farm were consistently larger than wild across all sites. On the other hand, farm and W♀hyb were similar in size at all sites (and error bars overlap zero difference in recapture size), as were wild *vs*. F♀hyb at sites 2 and 3, F♀hyb *vs*. W♀hyb at site 1, and farm *vs*. F♀hyb at sites 1 and 2.

The effect of egg weight on recapture weight and length was small and positive for most site by cross type combinations (Figures 2.5E & 2.5F and Supplementary Table 2.1). However,

for farm at sites 1 and 2, and W♀hyb at site 2, increasing egg weight had a (very small) negative effect on recapture weight and length. Egg weight had the largest absolute effect on F♀hyb out of all cross types consistently across sites, with the largest effect at site 3, though the uncertainty in estimates is largest for this cross type at high egg weights (Figures 2.5E and 2.5F).

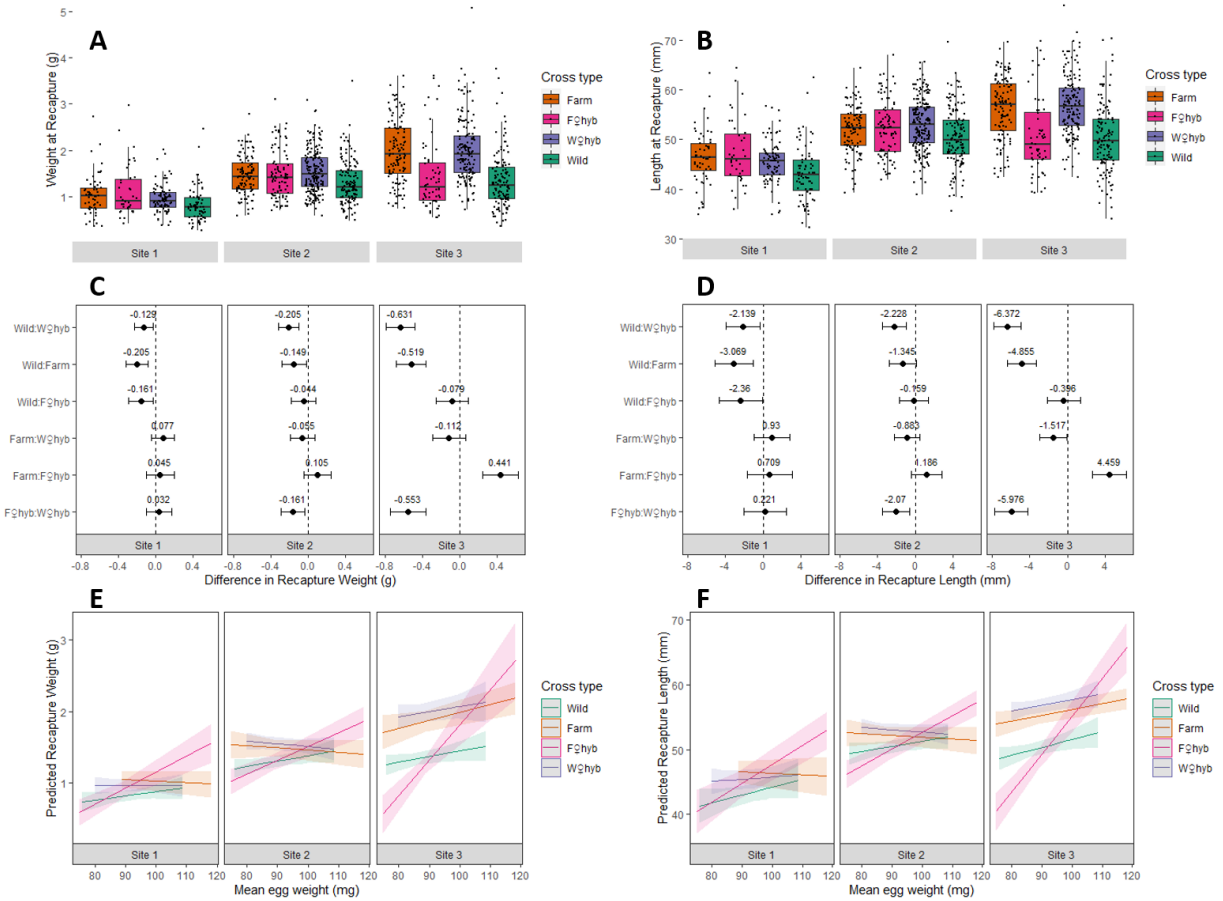

Figure 2.5. Weights (A) and lengths (B) of each fish at recapture for each cross type by site pairing; pairwise differences in mean recapture weight (C) and length (D) for each cross type by site pairing; and predicted recapture weight (E) and length (F) for each cross type at each site across the range of experimental egg weights. Lines in boxplots represent median value, upper and lower hinges represent third and first quartiles (respectively), and upper and lower whiskers extend to furthest value no more than 1.5 times the inter-quartile range above and below the upper and lower hinges (respectively). Error bars on pairwise differences represent 2x the standard error of the difference estimate. Bands around regression lines in (E) and (F) are 2x the standard error of their associated prediction value.

Condition Factor at Recapture

There was strong evidence for an effect of site, cross type, and all interactions except egg weight by site on condition factor (Figure 2.6A and Table 2.4). Pairwise differences were very similar for each cross type pair at sites 2 and 3, with Wild having lower condition than all other cross types and farm higher condition than W♀hyb (Figure 2.6C). By contrast, the 2*SE bars for all pairwise comparisons at site 1 spanned zero difference in condition factor. The effect of egg weight on condition factor varied over cross types and sites in terms of positive or negative effect and magnitude (Figure 2.6C).

Table 2.4. ANOVA table for linear model of recapture condition factor by mean egg weight, site, cross type, and all possible interactions. Abbreviations of terms are defined as in Table 2.3.

Source	df	SS	MS	F	p-value	LR
Egg weight	1	0.003	0.003	0.872	0.35	0.54
Site	2	0.289	0.145	42.195	<2.2E-16	9.83E+15
Cross type	3	0.145	0.048	14.065	5.13E-09	3.40E+07
Egg weight*Site	2	0.003	0.002	0.496	0.61	0.21
Egg weight*Cross type	3	0.045	0.015	4.333	0.0048	28.91
Site*Cross type	6	0.058	0.010	2.803	0.01	9.46
Egg weight*Site*Cross type	6	0.064	0.011	3.086	0.0053	23.68
Residuals	1216	4.168	0.003			

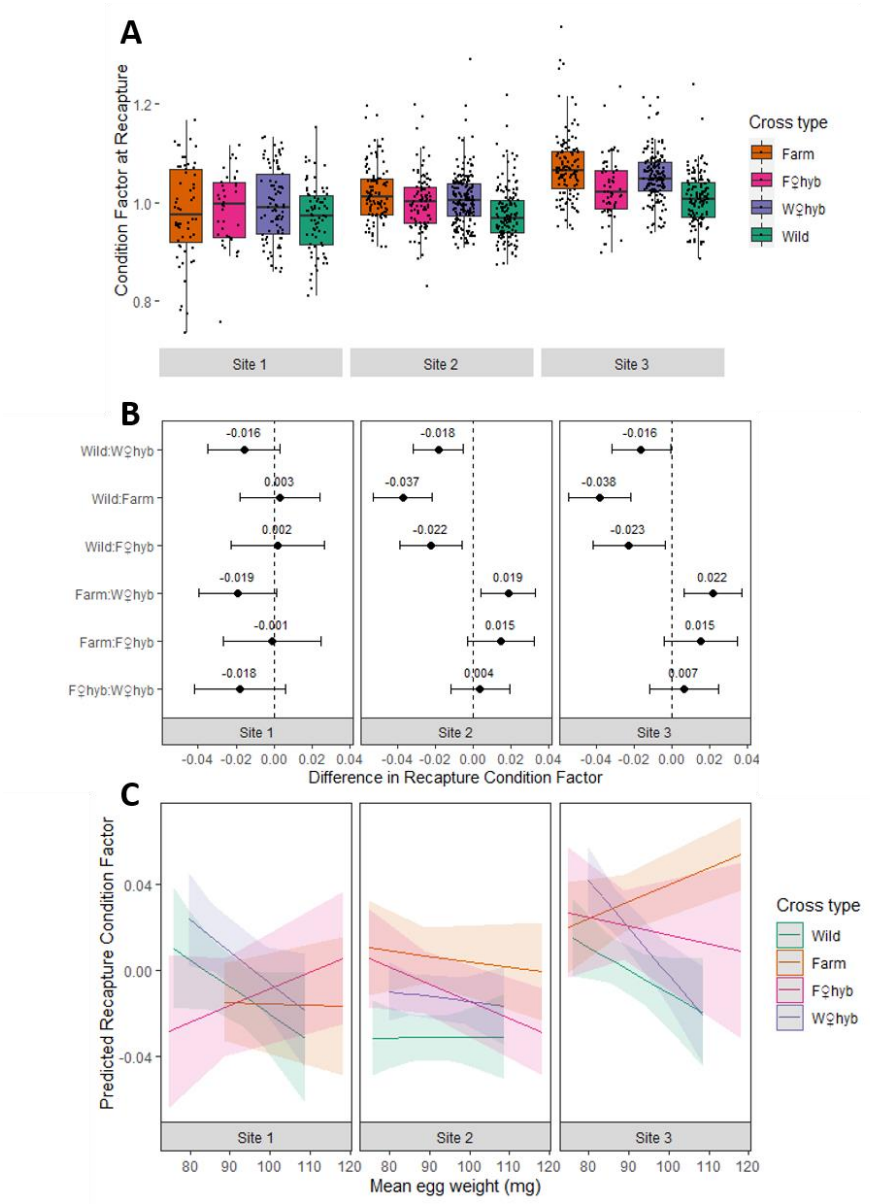

Figure 2.6. Condition (A) of each fish at recapture for each cross type by site pairing; pairwise differences in mean condition (B) for each cross type by site pairing; and predicted recapture condition (C) for each cross type at each site across the range of experimental egg weights. Lines in boxplots represent median value, upper and lower hinges represent third and first quartiles (respectively), and upper and lower whiskers extend to furthest value no more than 1.5 times the inter-quartile range above and below the upper and lower hinges (respectively). Error bars on pairwise differences represent 2x the standard error of the difference estimate. Bands around regression lines in (C) are 2x the standard error of their associated prediction value.

Morphology

Initially, a linear model with centroid size, site, cross type, and all possible interaction terms was run on the Procrustes coordinates. The likelihood ratios for centroid size, site, and cross type were >100, and these terms were thus selected for a reduced model (all other terms had LRs < 1). The lack of evidence for interactions of centroid size with other terms indicates that the effect of fish size on shape does not vary greatly depending on site or cross type.

The results of the Procrustes ANOVA on the reduced linear model are shown in Table 2.5. Likelihood ratios for the effects of centroid size, site, and cross type provided strong evidence for their inclusion in the model; however, the size of their effects on shape looks ultimately to be small. Centroid size explained ~8.9% of the variation in the data, site explained ~6.3%, and cross type explained ~8.6% (Table 2.5). Pairwise comparisons of distances between least-squares means of overall shape were made between cross types and between sites (Table 2.6), after accounting for centroid size. For cross type comparisons, distances between means were largest for farm *vs*. wild and smallest for farm: F♀hyb, and for sites distances were largest between sites 1 and 2 and smallest between sites 2 and 3. The largest cross type differences appeared to be for landmarks associated with body depth, with wild having smaller depths than other cross types, however once again it is important to note that differences overall were small.

Table 2.5. Results of Procrustes ANOVA of overall fish shape by centroid size (Csize), site, and cross type. Sums of squared Procrustes distances are used as a measure of SS (sums of squares) with this approach. SS are calculated sequentially. Z-scores (effect sizes) indicate standard deviation of observed SS for each term relative to the mean of the distribution of resampled SS values (note that the values from the resampled distribution are log-transformed by the function prior to the estimation of effect sizes). Rsq is the amount of variation explained by each term, MS is mean squares, and LR is likelihood ratio.

Source	df	SS	MS	Rsq	F	Z	p-value	LR
Csize	1	0.026	0.026	0.089	85.71	7.16	1.0E-04	2.27E+14
Site	2	0.022	0.011	0.073	35.42	7.98	1.0E-04	3.00E+12
Cross type	3	0.025	0.008	0.086	27.78	9.36	1.0E-04	9.26E+15
Residuals	727	0.222	0.0003	0.75				
Total	733	0.296						

Table 2.6. Distances (d) between least-squares means for pairwise cross type and site comparisons, as well as the upper 95% confidence limit (UCL) of the distribution generated through the resampling process, and the number of standard deviations the distance is away from the mean distance generated through the resampling procedure (Z).

Cross type Comparison	d	UCL	p-value	Z
Farm:F♀hyb	0.00447	0.00354	0.0054	3.125
Farm:W♀hyb	0.0112	0.00280	0.001	16.993
Farm:Wild	0.0149	0.00304	0.001	21.206
F♀hyb:W♀hyb	0.00945	0.00327	0.001	11.485
F♀hyb:Wild	0.0123	0.00349	0.001	14.610
W♀hyb:Wild	0.00529	0.00268	0.001	6.811
Site Comparison				
1:2	0.00968	0.00278	0.0001	14.495
1:3	0.0135	0.00281	0.0001	20.650
2:3	0.00603	0.00237	0.0001	9.723

For pectoral fin length, after accounting for the effect of fish standard length ($F_{1,96}$ = 483.4, LR= 7.04E+35), there was good evidence for an effect of site on this trait ($F_{2,96}$ = 19.5, LR= 2.19E+06), but insufficient evidence for an effect of cross type or any interactions (LR < 1). Mean pectoral fin lengths were back-calculated from ln-transformed emmeans predictions: 11.59 mm (site 2), 10.48 mm (site 3), and 10.38 mm (site 1). All site means were calculated at the

grand mean fish standard length of 43.8 mm (which was also back-calculated from the ln-transformed mean output by the model).

Number of Parr Marks

There was insufficient evidence for the standard length covariate term ($F_{1,97}$ = 3.26, LR = 1.7, p= 0.074), as well as the site term and its interactions with standard length (LR < 1.0) on number of parr marks. However, there was evidence for the cross type term ($F_{3,97}$ = 2.53, LR = 16.3, p=0.062), as well as its interaction with site ($F_{6,97}$ = 1.93, LR = 263.6, p= 0.083) on parr mark number. Therefore, pairwise comparisons of mean numbers of marks between cross types were conducted within each site separately. The largest overall pairwise differences occurred at site 3, with W♀hyb having fewer parr marks than all three of the other crosses (differences all > 1.7 marks; Figure 2.7A and Supplementary Table 2.2). In contrast, differences for all other pairs were < 1.2 marks, with their 2*SE marks spanning zero difference in number of marks.

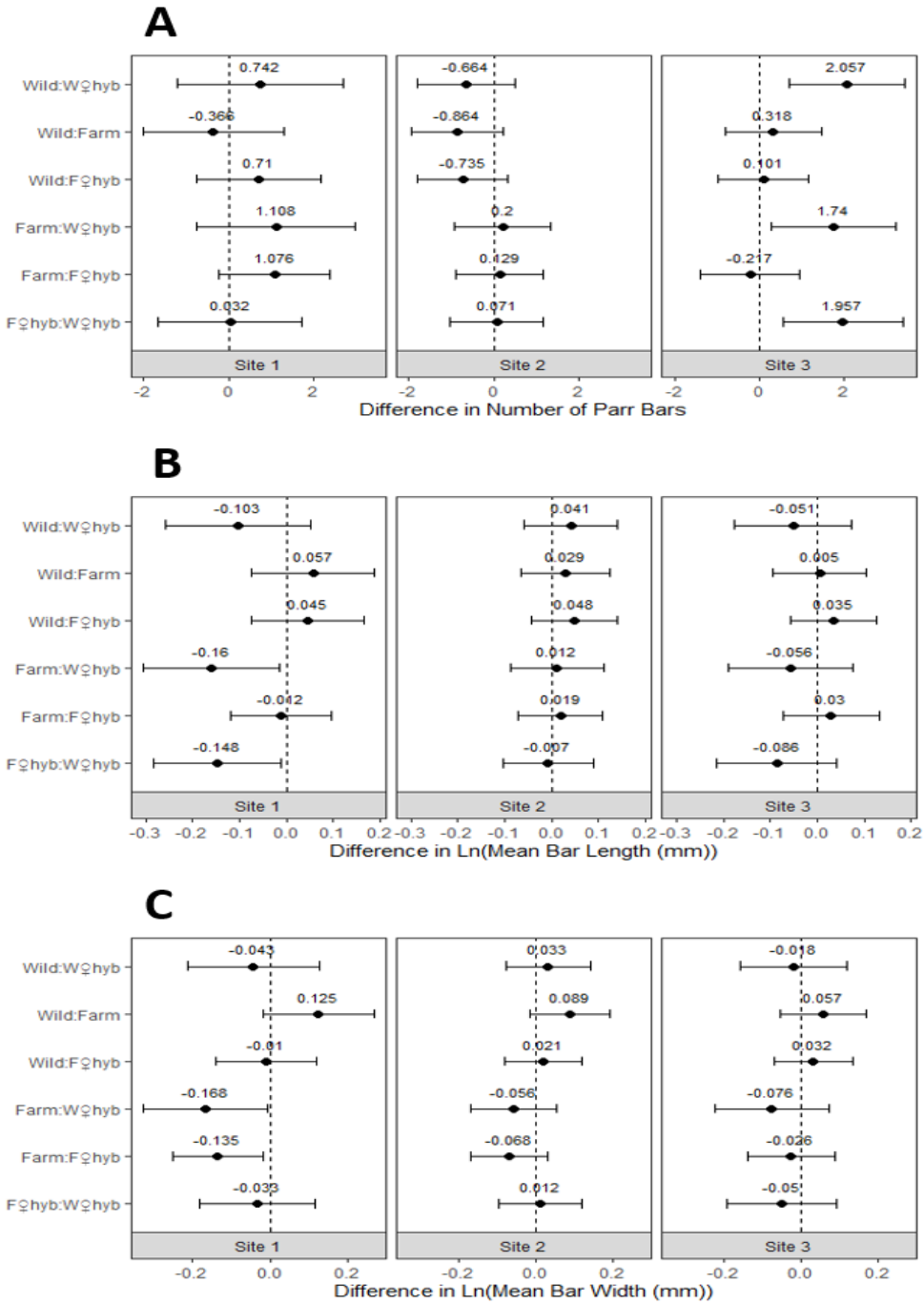

Figure 2.7. Differences in (A) mean number of parr marks; (B) mean ln(parr mark length) below lateral line; and (C) mean ln(parr mark width) for each cross type pair at each site. Error bars represent 2x the standard error of the difference estimate. Differences were calculated at the grand mean of standard length (45.20 mm).

Average width and length of parr marks

There was strong evidence for an effect of standard length and site on mean mark length and width (Table 2.7). The largest differences in mean mark length at the grand mean of standard length (44.7 mm; back-calculated from ln-transformed grand mean length output by emmeans function) were at site 1, for F♀hyb:W♀hyb and farm:W♀hyb (Figure 2.7B). However, difference in size of marks for all pairs was small overall, with error bars for difference estimates spanning zero difference across all sites. One consistent result across all sites though was that farm tended to have narrower marks than the other cross types (Figure 2.7C and Supplementary Table 2.2).

Table 2.7. ANOVA results of the linear model for mean parr mark length below lateral line, and mean mark width by site and cross type. The standard length covariate was ln transformed prior to model analysis.

		Ln(parr mark length)					Ln(parr mark width)				
Source	df	SS	MS	F	p-value	LR	SS	MS	F	p-value	LR
Standard length	1	4.60	4.40	487.24	<2.26E-16	6.74E+39	5.376	5.376	467.870	<2.2E-16	4.1E+37
Site	2	0.159	0.079	8.392	4.35E-04	377.404	0.275	0.137	11.958	2.28E-05	7078.48
Cross type	3	0.004	0.002	0.155	0.927	0.043	0.089	0.030	2.567	0.059	1.902
Standard length*site	2	0.009	0.004	0.463	0.631	0.153	0.020	0.010	0.884	0.416	0.248
Standard length*cross type	3	0.083	0.028	2.932	0.037	2.539	0.083	0.028	2.420	0.071	1.327
Site*Cross type	6	0.008	0.001	0.133	0.992	0.0006	0.015	0.003	0.219	0.970	0.0008
Standard length*Site*Cross type	6	0.088	0.015	1.556	0.168	0.030	0.053	0.009	0.771	0.594	0.002
Residuals	97	0.916	0.009				1.115	0.012			

Discussion

Farmed Atlantic Salmon regularly escape aquaculture sites and interbreed with local wild individuals (e.g. Keyser et al. 2018; Glover et al. 2019). The resulting introgression of farmed genes, many of which can be maladaptive, into wild populations can have a strong impact on the viability and persistence of wild populations (e.g. Bolstad et al. 2017; Sylvester et al. 2019). As

such, an understanding of the relative performance of farmed, wild, and hybrid Atlantic Salmon in the wild is needed to make predictions of the impacts of introgression, as well as to inform management decisions (e.g. Sylvester et al. 2019). In this study, I quantified the relative survival of juveniles of different cross types in the wild over the first summer of life, and I examined differences in the survival-influencing traits of size, body shape, and parr mark morphology. I found a consistent pattern of relative cross type survival across multiple study sites, with survival higher for parr from wild mothers (W♀hyb largest overall) than those from farmed mothers. I also found differences in size at recapture among cross types, with environment also influencing patterns of growth. Morphology differences existed between sites and cross types, though were very small in general. Overall, my results extend previous findings of proportional change in cross type abundances in the southern Newfoundland wild (Wringe et al. 2018; Sylvester et al. 2019), as well as size and shape differences (Perriman et al. in prep) among offspring following a large aquaculture escape event in 2013, thereby contributing additional population-specific data to the body of research on wild-farm interactions.

Survival

I observed significant cross type related differences in recapture odds that were consistent across a range of environmental conditions. In line with previous work, I saw that wild offspring had higher recapture/survival than farm offspring (McGinnity et al. 1997; Fleming et al. 2000; McGinnity et al. 2003; Skaala et al. 2012; Skaala et al. 2019). While my results for hybrid relative survival did not follow the generally-observed cross type survival trend (Wild > Hybrids > Farm), it is important to note that some previous studies have reported results of relative survival at the cohort and/or family level that do not agree with the overall cross type pattern, and my results do correspond to some of these findings. For example, McGinnity et al. (2003)

found a fairly high farm:wild relative survival value (farm ~84% of wild) for one 0+ cohort, which falls within the range of my farm:wild recapture estimates (farm 75-95% of wild). In addition, results which correspond to my finding of high W♀hyb survival include McGinnity et al. (1997), who found W♀hyb had the highest overall survival for one cohort of 0+ parr and spring smolts, and Skaala et al. (2012), who found that for one year cohort W♀hyb fish had essentially the same relative survival as pure wild (farm 96% of wild). Instances of higher hybrid success may be critical given that most escaped farm fish that breed do so with wild rather than other farm fish, meaning hybrid offspring are more likely to occur than pure feral farm offspring (e.g. Fleming et al. 1996, 2000). Therefore, it is the relative performance of hybrid offspring that will be especially important for predicting future impacts of escapees on wild populations.

It should be expected that differences in temporal scale between previous studies and this one would contribute to differences in relative recapture results. Studies that quantified freshwater survival at the smolt stage (Skaala et al. 2012; Skaala et al. 2019) have results that reflect patterns of survival over a longer period of time and a broader range of environmental conditions (perhaps most notably, winter) than those experienced by my fish. In addition, studies that began at earlier life stages would have had early-life (i.e. egg, larval) mortality, and potentially parental spawning success influence their 0+ recapture numbers, while my study looks exclusively at relative survival over the first summer of growth and controls for these factors. For example, compared with my results, the larger proportion of F♀hyb 0+ recaptures relative to W♀hyb found by Fleming et al. (2000) (who began their study at the breeding stage) appeared to be largely due to F♀hyb being the more likely of the two F1 hybrid groups given differential parental spawning success (Fleming et al. 1996). In addition, the fact that I found a

smaller farm:wild survival differential than McGinnity et al. (1997) (farm 51-53% of wild) and Fleming et al. (2000) (farm ~70% of wild), could be because my study does not reflect mortality during the egg and larval stages.

Factors other than cross type, such as egg size, may also be an important influence on the relative survival of juvenile Atlantic Salmon, and influence of egg size may vary among populations and studies. Skaala et al. (2012), whose farmed eggs were slightly larger than those from wild fish, found that when the effect of egg size was controlled for, farmed juveniles had lower survival overall from egg to smolt stage than both wild and hybrids, however when it was not controlled for, the families with the highest and lowest overall survival were both farmed in origin. On the other hand, Skaala et al. (2019) used farm eggs that were smaller than wild, and found that while egg-smolt survival of farm fish was lower than that of wild overall, it was relatively higher after controlling for egg size than before. In my study, it was not possible to include egg weight as a covariate in the survival model since the number of fry from each mother initially released was unknown. However, there was substantial variation in egg weight among mothers, though not between farm and wild mothers overall (farm egg weight mean= 94.0 ± 19.3 mg, wild egg weight mean= 91.6 ± 11.5 mg), so this could very well mean that egg weight played a role in family-specific survival and simply remains undetectable here.

Finally, it is possible that some of the difference in patterns of relative survival in my study *vs*. previous wild-environment studies may reflect differences between North American farmed and wild populations and European ones. A few previous studies have investigated survival and survival-influencing traits of juvenile North American Atlantic Salmon in a lab environment and have also found results that differ from the norm of those from European studies. Hamoutene et al. (2017) found that North American F♀hyb, while the most likely of the

F1 hybrids to occur (as in Fleming et al. 1996), also had the highest mortality rates at the egg and fry stages in a lab environment. While there is considerable uncertainty here, there is the potential for differences in relative performance of cross types in a trans-Atlantic context to play a role in some of the differences seen between my study and European ones. However in general, my results corroborate previous research showing that fish from farmed mothers tend to have lower survival than those from wild mothers, and differences from previously-established general patterns that I see are reflective of the need for more population-specific work in order to account for this variability.

Size

I did not find evidence that larger size consistently confers higher survival for cross types as a group (though it is important to note that I was not able to investigate a link between individual size at recapture and survival within cross type groups). While W♀hyb were the largest cross type at sites 2 and 3 and had the highest relative survival overall, this link between larger size and higher survival does not extend to the other cross types. In particular, wild parr having the second-highest survival but being smallest at all three sites suggests that being small does not automatically confer a relative survival disadvantage over the first summer. Even if wild parr were being displaced from beneficial habitat by larger farmed fish (Fleming et al. 2000; McGinnity et al. 2003), it does not seem to have been overly detrimental during this time period. In addition, the lack of evidence for larger size of farm fish conferring higher survival is also consistent with results of previous studies finding evidence for at least some selection against fast-growing farm fish in the wild (e.g. Glover et al. 2018), as larger farm fish may be bolder and therefore more risk-prone than their wild counterparts (Fleming and Einum 1997; Islam et al. 2020; Solberg et al. 2020). Ultimately, my results suggest that relative size rankings of cross

types cannot be used as a reliable predictor of their relative survival across a range of environmental conditions.

The result of wild parr being smallest on average across all sites is consistent with results from similar European studies (e.g. Einum and Fleming 1997; Fleming and Einum 1997; Fleming et al. 2000; Glover et al. 2009; Solberg et al. 2013; Harvey et al. 2016). In contrast, while a few recent studies on Newfoundland Atlantic Salmon juveniles have found wild fish to be largest at certain early life stages, it is likely that a cross type-based maternal egg size effect contributed to these results, and likely did not play a role in my study. For example, Perriman et al. (in prep) found wild fish to be largest overall at first-feeding and 80 days later in a semi-natural environment and larger than hybrids at age 0+ in the wild, Hamoutene et al. (2017) found wild to be largest at hatch in a hatchery environment, and (Islam et al. submitted) found wild fish were longer than farmed (though not heavier) at first-feeding in a lab environment. Authors of all three of these studies attributed the larger relative size of their wild juveniles to the fact that the eggs they used from wild females were larger than those from farmed females. In contrast, in my study there was little difference in average egg size of wild and farmed mothers, therefore wild juveniles as a group would not be expected to have this particular size advantage initially (and indeed, wild juveniles were also the smallest cross type at release). Overall, my size results for the pure crosses are in line with results of previous research, demonstrating that farmed fish tend to outgrow wild across a range of environmental conditions, though to a lesser extent in more challenging growth conditions (reviewed by Glover et al. 2018).

A potentially notable result I report is the apparent size advantage of W♀hyb observed at two sites, since hybrids have typically been found to be intermediate in size between pure crosses (e.g. Einum and Fleming 1997; Fleming and Einum 1997; Fleming et al. 2000; Glover et al.

2009; Solberg et al. 2013; Harvey et al. 2016). Nevertheless, my observations are supported by results of Skaala et al. (2012), who found that for a cohort using hybrids from wild mothers, the hybrids were indeed the largest cross type as smolts, while in two other cohorts using hybrids from farm mothers, the hybrids were either intermediate to the pure crosses or smallest as smolts. There is often variability for growth among populations of the same cross type, and certain populations under certain environmental conditions may over- or under-perform relative to their overall cross type (e.g. Harvey et al. 2016); therefore it is possible that the particular parental crosses used in my study resulted in a W♀hyb strain that was a particularly good performer under conditions conducive to high growth (i.e. sites 2 and 3). Alternatively, since W♀hyb were also the largest cross type at release, their relative size at recapture could be reflective of their initial size; though since farm and F♀hyb fish outgrew W♀hyb at site 1, initial size is likely to be only a partial influencer of recapture size and/or only an important factor under certain environmental conditions.

Morphology

Though wild Atlantic Salmon populations have been found to be genetically distinct from one another (e.g. Fraser et al. 2011; Bourret et al. 2013), the degree to which variation in morphology is related to genetics *vs*. environment is not yet clear. My results indicate that there is evidence for effects of both environment and cross type on body morphology with the largest differences in shape being for landmarks associated with body depth; however overall shape differences were small. Fleming and Einum (1997) also found that tank-reared Norwegian farmed juveniles had deeper bodies than tank-reared wild juveniles did, as did Perriman et al. (in prep) for tank-reared Newfoundland juveniles at 80 days post-first feeding. Aquaculture

selection for greater weight in salmonids has potentially resulted in a corresponding increase in body depth (Gjerde and Schaeffer 1989), and the aquaculture environment may "release" farmed fish from the natural selection pressures for a more streamlined body that is often associated with a stronger swimming performance (Fleming and Einum 1997). These genetic-based morphological differences between cross types may be less expressed phenotypically in the wild *vs*. culture (e.g. Solem et al. 2006), and instances of morphological convergence among cross types with increasing time spent in a wild environment have also been observed (e.g. Fleming et al. 1994; Von Cramon-Taubadel et al. 2005, Perriman et al. in prep), results which are in line with my observations of small overall differences in the wild. In addition, the lack of evidence for a cross type effect but good evidence for a site effect on pectoral fin length suggests that environment plays a stronger role in shaping this trait than does genetics, perhaps through differences in flow characteristics of the three sites. Overall, my results suggest that any genetic differences for morphological traits for the wild and farmed populations I used are not strongly expressed in a wild environment, at least over the first summer.

Parr Marks

Our results show good evidence for an effect of cross type, as well as a site by cross type interaction on number of parr marks, corroborating the previous studies showing that both genetics and environment play a role in determining parr mark numbers. Juvenile salmon in different environments are likely to experience selection for certain parr mark patterns because this trait influences their ability to camouflage against the riverbed, and thus hide from predators (Donnelly and Dill 1984; Culling et al. 2013). Boulding et al. (2008) found three quantitative trait loci that influenced parr mark numbers, a result which was supported by the results of their common garden experiment finding different numbers of marks on fish from different genetic

backgrounds raised in a common environment. Since I found that W♀hyb had the fewest parr marks and outgrew the other cross types by the largest margin at site 3, it is possible that they had an environment-specific genetic advantage for parr mark number (i.e. enabling better camouflage, allowing more energy to be spent on feeding and less on predator avoidance). At the other two sites cross type differences in parr mark number were minimal, despite W♀hyb remaining the cross type with highest recapture odds. This suggests that the influence of parr mark number on overall survival is not particularly strong, or perhaps this trait interacts with others in varying ways under different conditions to influence survival.

On the other hand, for parr mark size (length and width), there was a lack of evidence for a cross type effect, but good evidence for a site effect, suggesting that plasticity plays a larger role than genetics for this trait. Indeed, Jørgensen et al. (2018) found that the environment played a larger role in determining spot pigmentation patterns on Atlantic Salmon than did genetics (though the latter did still have an effect). In general, my results for parr mark number and size were quite variable, and pairwise differences between cross types were small. I observed the largest pairwise cross type differences for parr mark size at site 1 (where pure farm fish had narrower marks than both hybrids), which was also the site with the lowest growth overall. This perhaps suggests that selection for parr mark size (and thus camouflage ability) operates more strongly under more challenging growth conditions, potentially due to trade-offs in energy allocation for feeding *vs*. predator avoidance.

Conclusion

Overall, my results show that there are differences among farmed, wild, and hybrid Atlantic Salmon parr for fitness-related traits and survival even at small temporal scales under favourable environmental conditions. I show plasticity for these traits exists within cross types, with differing relative performance in terms of size and morphology at replicate study sites. The fact that the pattern of relative survival was consistent across sites but was not obviously linked to patterns in any of the fitness-related traits suggests that other traits (unexamined here) may differ among cross types and influence survival, or perhaps multiple traits combine to have a cumulative effect on survival. Ultimately, as the first North American wild-environment study of its kind, this work could serve as a lead-in for more research on these specific populations in the wild, especially to further investigate potentially critical findings such as the possibility of wild-mother hybrid vigour. Such research provides key data for these populations in areas of high aquaculture activity, and will help to inform predictive modeling of escapee impacts and subsequent management decisions.

Chapter 3: Lipid and Fatty Acid Profiles of Farmed, Wild, and Hybrid Atlantic Salmon (*Salmo salar)* Parr Before and After Release into an Experimental Common-Garden Environment in Nature

Abstract

Atlantic Salmon aquaculture has subjected farm fish to artificial selection for several generations, resulting in fish that are genetically maladapted to life in the wild. When farm escapees breed with their wild counterparts, the consequent farm genetic introgression can impact the fitness and viability of wild populations. As such, an understanding of genetic-based differences in fitness-related traits for farm, wild, and hybrid salmon in the wild is key for making predictions of the impacts of introgression as well as subsequent mitigation strategies. In this study, I used lipid and fatty acid (FA) analyses to investigate differences in storage and foraging ability among Atlantic Salmon fry of four cross types (farm, wild, and reciprocal F1 hybrids), at the beginning and end of a common-garden experimental release in the Newfoundland wild. I found differences in overall lipid class and FA profiles among cross types at both release and recapture, with farm fish being the most differentiated cross type at recapture. In addition, my results point towards the possibility of a feeding disadvantage for farm fish in the wild, with low recapture levels of triacyglycerols, as well as low recapture levels of certain FAs indicative of freshwater prey species. Overall, I show that lipid and FA profiles of juvenile salmon can change over just a short period of time even under favourable (summer) conditions in the wild, and my results point to farm fish having genetic differences affecting energy acquisition and storage that may negatively impact their survival and fitness in the longer term.

Introduction

Aquaculture of Atlantic Salmon (*Salmo salar*) has expanded rapidly since beginning in the late 1960s (Glover et al. 2017) as a response to plateauing capture fisheries and an ongoing increase in the world's demand for protein (FAO 2018). Aquaculture as an alternative to capture fisheries may reduce harvest pressure on wild stocks, however wild populations now face a new set of threats due to interactions with their farm counterparts. For example, intentional and non-intentional artificial selection pressures experienced by farm fish under domestication have resulted in genetic differences and traits that are often maladaptive to life in the wild (Fleming and Einum 1997; Fleming et al. 2000; McGinnity et al. 2003; Skaala et al. 2012; reviewed by Glover et al. 2017). Escapes of farm fish from aquaculture are a common occurrence, and farm traits can enter wild populations when escaped fish survive to breed with wild or hybrid individuals, which can result in reductions in wild population productivity (Fleming et al. 2000; Bradbury et al. 2020).

In order to survive to reproduce, any juvenile organism must balance how they allocate energy to processes such as growth, foraging and predator avoidance, and long-term storage (Post and Parkinson 2001), and therefore diet quality can play a critical role in an organism's success (Orlov et al. 2006). Lipid and FA analyses can be key tools for assessing the impact of diet and storage on fish health and fitness. Lipids (along with proteins) are the primary macronutrients for fish, providing their main source of metabolic energy (Tocher 2003). Total lipid content is often used as a metric of storage energy for fish (e.g. Berg et al. 2000; Finstad et al. 2003), and the assessment of relative percentage of different lipid classes can provide insight into mortality risk (e.g. Finstad et al. 2004). The roles essential fatty acids (EFAs) play in the health of fish are numerous, such as in the structure and function of cell membrane phospholipid

bilayers and in immune response, reproduction, and organ function (reviewed by Tocher 2010). FAs are therefore useful in assessing fish health, and can also provide information about the origin of lipids and FAs a fish contains, since they can be used as diet "signatures" (e.g. Bell et al. 1994; Budge et al. 2002; Heintz et al. 2010; Budge et al. 2012).

Evidence from previous studies suggests that farm and wild salmon have different abilities to store the lipids and fatty acids (FAs) they assimilate. Directional selection employed by the aquaculture industry for increased growth has likely also resulted in selection for increased fat content (e.g. Quinton et al. 2005; Powell et al. 2008). When raised under their respective diets and environments, farm fish have indeed been found to have higher fat content than wild fish (Johnston et al. 2006), however, when raised in common laboratory conditions under a commercial diet, differences in fat content and most FAs were found to be small (Glover et al. 2009). Therefore, the exact nature and magnitude of the influence of genetics on the relative lipid and FA content of different salmon cross types remains to be determined, especially in a wild environment where differential foraging abilities (e.g. Orlov et al. 2006) of cross types may also impact their ultimate nutritional states.

In this study, I aimed to investigate differences in storage and foraging ability among farm, wild, and hybrid Atlantic Salmon fry by comparing lipid and FA content among these cross types before and after an experimental release into a common-garden, wild environment (prior to which fry were raised for a short period under a common commercial diet). Building upon previous findings (Quinton et al. 2005; Johnston et al. 2006; Powell et al. 2008), I hypothesized that farm fish are genetically predisposed to have higher fat content than wild fish, if they have equal and plentiful access to a commercial diet. Should this be the case, farm fish should have a higher total lipid content than wild fish before being released into the wild. I also

hypothesized that farm fish are less capable of obtaining food resources in the wild than wild fish (e.g. Orlov et al. 2006). If this is the case, it would be expected that farm fish would have a greater decrease in their total lipid content, as well as the storage lipid classes, at recapture versus at release when compared to wild fish. In addition, if farm fish are poorer foragers than wild fish, it would be expected that recaptured farm fish would have lower levels of FAs characteristic of freshwater prey items compared with wild fish. Ultimately, comparing these traits among cross types in the wild could improve our understanding of mechanisms associated with differences in survival and growth observed between wild, farm, and hybrid juvenile salmon reported in previous studies, and inform predictions of impact of escaped farm salmon on wild salmon populations.

Methods

Cross Types, Release and Recapture

Between 28 November and 21 December 2017, four experimental cross types of Atlantic Salmon were generated at the Ocean Sciences Centre of Memorial University (St. John's, Newfoundland, Canada). The four cross types were pure wild (9 families), pure farm (6 families), and reciprocal F1 hybrids (6 families for each of wild-mother hybrids (denoted "W♀hyb") and farm-mother hybrids (denoted "F♀hyb")). Wild parents came from the Garnish River, located on the Burin Peninsula on the south coast of Newfoundland emptying into Fortune Bay (Figure 3.1). Farm parents came from the Saint John River strain, which is to date the only farm strain used in Atlantic Canada commercial aquaculture operations. Parent adipose fins were clipped with samples stored in 100% ethanol for later use in parentage assignment of offspring.

Embryos were incubated in Heath trays on ambient water until yolk absorption, at which point they were transferred (22 May 2018) to 470-liter flow-through circular holding tanks (0.9

m diameter x 0.5 m height) and pooled by cross type. Fry were kept in ambient water and initially fed a combination of *Artemia* and salmonid starter feed (crumbles (0.5 g; caloric content: 55% protein and 15% fat), EWOS-Cargill, BC, Canada) for one month, after which they were fed pellets only until release. Release occurred on 11 July 2018 at a tributary site of the Garnish River (Figure 3.1). Prior to release, all fry were anaesthetized using MS-222 (AQUALIFE TMS, Syndel Laboratories Ltd., Nanaimo, BC, Canada; 50 mg L^{-1} dosage buffered with an equal amount of sodium bicarbonate) and subsequently had their adipose fins clipped to distinguish them from wild fish upon later recapture. A sample of approximately 20 fish from three cross types (wild, farm, and F♀hyb) were sacrificed on 10 July 2018 (using an overdose of MS-222 at 400 mg L^{-1} buffered with sodium bicarbonate) and kept for use as a baseline. A total of 1972 fry were released, comprised of approximately 500 per cross type. The fry were released at four locations approximately 50 metres apart. Animal use was approved by the Memorial University of Newfoundland Institutional Animal Care Committee (IACC) following Canadian Council on Animal Care (CCAC) guidelines, under protocol number 18-01-IF.

Recapture occurred on 2 and 5 October 2018 using multiple pass electrofishing. A single electrofishing unit (LR-24 Backpack Electrofisher, Smith Root, Vancouver, WA, USA) was used and was set at 550 volts and 60 Hz, with a duty cycle of 25%. Recapture began at a culvert downstream from the first release point and continued upstream past the final release point until reaching a natural barrier.

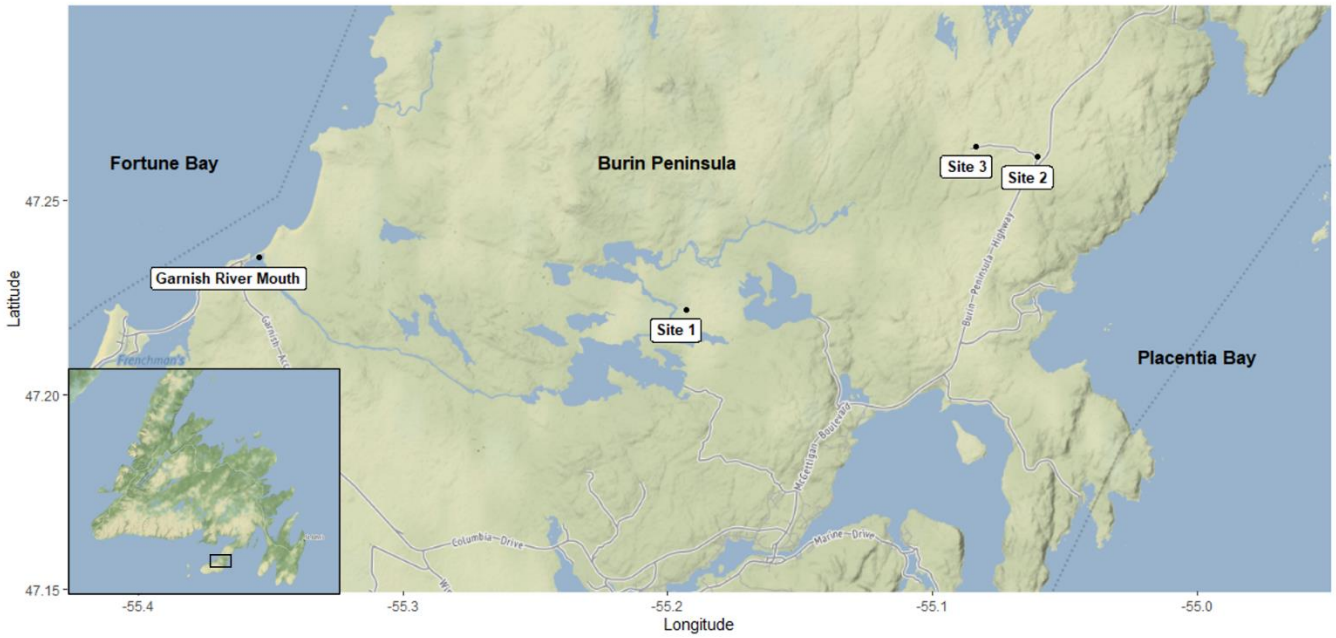

Figure 3.1. Location of three tributary sites of the Garnish River, Newfoundland, that were used for the release and recapture experiment. Site 3 was the study site used for the lipid and FA analyses described in this chapter. Inset shows the island of Newfoundland with the dark rectangle indicating the general location of the study area on the Burin Peninsula.

Recaptured fish were euthanized using an overdose of MS-222 (400 mg L^{-1} buffered with an equal amount of sodium bicarbonate) approximately two hours post-capture, then weighed (±0.01 g), photographed, and caudal fin-clipped. Fin clips were stored in 100% ethanol for later parentage analysis, and whole specimens (minus caudal fin) were frozen at -80 ºC.

Genetic Analysis

Parentage assignment involved a panel of 25 microsatellite loci (277 alleles), which was a subset of a larger panel of 101 loci for Atlantic Canada *S. salar* (Bradbury et al. 2018). DNA was extracted from fin clips of recaptured offspring and their parents using the DNeasy 96 Blood and Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions for the Purification of Total DNA from Animal Tissues protocol. Microsatellite loci were PCR

amplified following the protocol described by Zhan et al. (2017). Sequencing was run on an Illumina MiSeq and scored using MEGASAT software (Zhan et al. 2017). Recaptured offspring were assigned back to their parents and cross type using COLONY (Jones and Wang 2010). See Chapter 2 for further details of genetic analysis and COLONY assignment.

Lipid Sample Preparation

Lipid analyses were performed on the sample of fish sacrificed prior to release in the river, as well as a sample taken at recapture. For recaptured fish, ten fish from each of the four cross types were randomly chosen for analysis, with at least 1 fish from each family (as determined by parentage analysis; see Chapter 2) within a cross type included. For release samples, ten fish from each of the three available cross types were chosen randomly for analysis, as family information for these samples was unknown.

For the recapture samples, the digestive tracts of fish were removed prior to lipid extraction so as not to not bias the samples by the gut contents. The gut was not removed from release samples, due to all these fish having been fed the same diet up until release and fasted for 24 h prior to sacrificing (i.e. expected to have evacuated all/most of their gut contents). In addition, removal of the gut from these samples would have been inaccurate due to their small size. Following removal from longer-term storage at -80^{o}C, all samples were weighed, placed in chloroform, capped under nitrogen, and stored at -20^{o}C until extraction.

Lipid Extraction

Lipids were extracted according to the protocol described by Parrish (1999). Briefly, samples were homogenized in a mixture of 2:1 chloroform:methanol (2 ml and 8 ml chloroform

for release and recapture samples, respectively), followed by a rinse of the homogenizer with 2:1 chloroform:methanol (1 ml release, 4 ml recapture) and chloroform-extracted water (0.5 ml release, 2 ml recapture) into the sample. Samples were then sonicated for 4 minutes and centrifuged for 2 minutes to separate layers. The organic layer was subsequently removed using the double pipetting technique and transferred to another lipid-cleaned vial. The sonication-centrifugation-organic layer removal step was repeated an additional 3 times for each sample, with an addition of chloroform (4 ml release, 12 ml recapture) occurring between each removal step. The organic layer transfers were pooled and then concentrated for transfer to 2 ml vials in two steps (recapture only), the first of which involved evaporation to near-dryness using a flash-evaporator (Buchler Instruments, Fort Lee, NJ, USA), and the second involving evaporation under nitrogen (release samples required only this step due to smaller volumes). Chloroform and methanol (3 additions of the former and 1 of the latter; small volumes just enough to wet sample vials) were added to near-dry samples during each transfer process to ensure transfer of all lipids. Samples were then capped under nitrogen, sealed, and stored at -20^{o}C.

Total Lipids and Lipid Class Analysis

Total lipid and lipid class analyses were done using the Iatroscan Mark V1 TLC-FID (Iatron Laboratories, Tokyo, Japan) (Parrish 1999). Chromatograms were analyzed using PeakSimple software (version 4.88, SRI Instruments, Torrance, CA, USA), with each chromatogram visually inspected and peaks cut manually. For 4 out of the total 40 recapture samples, triacylglycerol (TAG) peak areas were close to the blank value for that region of the chromatogram. For computational purposes, these samples were assigned a TAG value based on

the peak area obtained for the lowest calibrant: 1.26 µg. The value for these 4 samples thus represents their maximum possible TAG value.

Derivatization of Lipid Extracts to Fatty Acid Methyl Esters (FAME)

Sample lipid extracts were derivatized to FAME as described in Katan et al. (2019). Briefly, lipid extracts for each sample were transesterified using Hilditch reagent (sulfuric acid and methanol) for 1 h at 100ºC. After cooling, sodium bicarbonate solution and hexane were added to each sample, which were then shaken vigorously (and centrifuged, in the case of release samples) and left to separate for several minutes. After separation, the top organic layer was removed and transferred to a separate lipid-cleaned vial, which was then blown completely dry under nitrogen. A small amount of hexane was added to each sample, with some release samples requiring further dilution with hexane. Finally, FAME were capped with nitrogen, sealed, and sonicated for 4 minutes. FAME were stored at -20°C until analysis on the gas chromatograph.

The FAME were analyzed on an HP 6890 gas chromatograph flame ionization detector (GC FID) equipped with a 7683 autosampler. The GC column was a ZB wax+ (Phenomenex, Torrance, CA, USA). The column length was 30 m with an internal diameter of 0.32 mm. The column temperature began at 65ºC and held this temperature for 0.5 min. The temperature ramped to 195 ºC at a rate of 40 ºC/min, held for 15 minutes then ramped to a final temperature of 220 ºC at a rate of 2 ºC min^{-1}. This final temperature was held for 0.75 minutes. The carrier gas was hydrogen and flowed at a rate of 2 ml min^{-1}. The injector temperature started at 150 ºC and ramped to a final temperature of 250 ºC at a rate of 120 ºC/minute. The detector temperature stayed constant at 260 ºC. Peaks were identified using retention times from standards purchased from Supelco (Bellafonte, PA, USA): 37 component FAME mix (product number 47885-U),

bacterial acid methyl ester mix (product number 47080-U), PUFA 1 (product number 47033) and PUFA 3 (product number 47085-U). Chromatograms were integrated using the Agilent OpenLAB Data Analysis - Build 2.203.0.573. A quantitative standard purchased from Nu-Chek Prep, Inc (Elysian, MN, USA; product number GLC490) was used to check the GC column about every 300 samples (or once a month) to ensure that the areas returned were as expected.

Size and Condition Measurements

Fork length measurements of recaptured fish were taken from photos using ImageJ software (version 1.52a) and were measured for weight to the nearest 0.01 g at sacrificing. Release samples were measured manually for fork length and weight (to the nearest 0.0001 g) just prior to lipid extraction. Condition factor was calculated as the residuals taken from the regression of ln(weight) on ln(length) (Bolger and Connolly 1989; Wootton 1998), and nutritional condition was taken as the ratio of triacylglycerol to sterols (TAG:ST; e.g. Carreón-Palau et al. (2018)).

Statistical Analyses

Both percentage and concentration data of lipid classes and FAs were analyzed here. The former refers to the percentage each lipid class or FA makes up out of the total lipids or FAs, respectively. Concentration is the amount of lipid/FA in milligrams per gram of wet weight of the sample. Percentage and concentration data point to different aspects of the data overall, with the former speaking more to quality and the latter to quantity (Chris Parrish, pers. communication).

All statistical analyses were performed in R (version 4.0.2). Overall differences in lipid and FA profiles (percentage data) among cross types were assessed using principal components analysis (PCA) on release and recapture data, respectively. PCAs were done using the PCA() function (scaled to unit variance) in the *FactoMineR* package (Le et al. 2008), and visualized using the fviz_pca_biplot() command in the *factoextra* package (Kassambara and Mundt 2020). Scores of individuals for the first two principal components (PCs) were extracted and compared among cross types using general linear models, using fish weight as a covariate in addition to the interaction between fish weight and cross type. The likelihood ratio (LR) of a cross type effect (*vs.* no effect) was calculated using the sums of squares and corrected for number of model parameters as in Glover and Dixon (2004). The strength of the evidence given by likelihood ratios was assessed based on the thresholds of LR>8 as "strong evidence", and LR>32 as "quite strong evidence" (Royall 1997). The *emmeans* package (Lenth 2020) was used on linear model results to calculate pairwise differences in mean PC scores and their confidence intervals (approximated as +/- twice the standard error of the mean). Mean scores were calculated at the grand mean value for fish weight at recapture and release, respectively.

Permutational analysis of variance (PERMANOVA) was also performed on overall percentage lipid and FA profile data using the package *vegan* (Oksanen et al. 2012) and the adonis() function therein, with 999 permutations. The distance matrix used was generated using the vegdist() function and the Bray-Curtis dissimilarity index. The assumption of homogeneity of group dispersions (variances) was met, as tested with the betadisper() function in *vegan* (release data: $F_{(2,27)}$= 0.547, LR= 0.14, p= 0.585; recapture data: $F_{(3,35)}$ =0.43, LR= 0.048, p= 0.734). Finally, similarity percentages analysis (SIMPER) was performed to show which lipid classes and FAs were the highest contributors to dissimilarities in lipid profiles, using the simper()

function in the *vegan* package. However, SIMPER shows the contribution of each lipid/FA to *overall* dissimilarities in lipid profile, and singles out lipids/FAs that are the most variable (i.e. have the most within-cross type variation), rather than those with the greatest between-cross type differences (Oksanen et al. 2012). Therefore, permutations (n=999) with randomization of the cross type factor were used to determine which lipid classes/FAs had between-cross type differences that were an important component of their contribution to overall lipid profile dissimilarities. I thus report only lipids/FAs that, when cross types were randomized, had a low (<5%) probability of having a contribution to overall dissimilarities as or more extreme than their observed contribution, in addition to contributing ≥1% to the overall dissimilarities in the lipid profile. Lipids/FAs with contributions < 1% to overall dissimilarities can be found in Supplementary Tables 3.4 and 3.8.

Percentages of major lipid classes, EFAs and FA groups at release and recapture were compared among cross types using beta regression models, which are used for data with a continuous dependent variable bounded by (0,1) (Cribari-Neto and Zeileis 2010). Percentages were converted to proportions, and the betareg() function in the *betareg* package was used to fit models using a maximum likelihood method, with parameterization using the mean in addition to a precision parameter (Cribari-Neto and Zeileis 2010). The default logit link function was used. To assess if there was evidence for an effect of fish weight, cross type, and/or their interaction on the percentage of a given lipid/FA, nested models were compared (i.e. a model including the term in question was compared with a model lacking that term) using LRs. LRs were calculated by exponentiating the log likelihoods for each nested model (given in the betareg summary output), and dividing the likelihood for the model including the given term by the likelihood for the model lacking it (see Table 3.1). These LRs were then corrected for the number of model

parameters using the method of Glover and Dixon (2004). The *emmeans* package (Lenth 2020) was used on betareg model results to calculate mean proportions and their confidence intervals (approximated as +/- twice the standard error of the mean) for each cross type. Mean proportions were calculated without taking into account the effect of fish weight, and the percentages I report were back-calculated from these proportions. For DHA:EPA ratios, TAG:ST ratios, and lipid and EFA concentrations (including total lipids), linear models were used to assess differences among cross types, and likelihood ratios and means were calculated using the method for PC scores described above.

Table 3.1. (A) Structure of nested beta regression models for percentages of lipid classes and FAs, and (B) the method of calculation of likelihood ratios for the effect of a given term (i.e. model with term *vs*. model lacking it).

A	Model no.	Model
	1	Proportion ~ 1 (i.e. null model, intercept only)
	2	Proportion ~ fish weight
	3	Proportion ~ fish weight + cross type
	4	Proportion ~ fish weight + cross type + fish weight*cross type
B	Term in question	LR for term in question
	fish weight	LR= likelihood(Model 2)/likelihood(Model 1)*parameter correction factor
	cross type	LR= likelihood(Model 3)/likelihood(Model 2)*parameter correction factor
	fish weight*cross type	LR= likelihood(Model 4)/likelihood(Model 3)*parameter correction factor

Results

Growth Parameters

Farm fish were heaviest and longest at release, with results of linear models indicating good evidence for a cross type effect (Table 3.2). Farm also had slightly higher condition on average than the other two cross types at release, though there was insufficient evidence for a

cross type effect on this measure. Farm also had the highest TAG:ST index at release, with strong evidence for a cross type effect. Wild fish were smallest in terms of weight and length, though they did have higher mean condition factor than the hybrids; however they had the lowest TAG:ST index of the three cross types at release.

At recapture, there was insufficient evidence for a cross type effect on weight, length, condition, or TAG:ST (Table 3.2), and most among-cross type differences for these metrics were small in magnitude. W♀hybs were largest in terms of weight and length, followed by farm and F♀hyb (similar to each other), with wild slightly smaller. Farm fish had the highest condition and wild the lowest. However, farm had the lowest TAG:ST index at recapture (hybrids had the highest) and were the only cross type to decrease in this ratio (by greater than 50%) from release to recapture.

Table 3.2. Mean values of growth parameters (weight (g), length (mm), and condition factor) of all cross types at release and recapture, ± twice the standard error of the mean, plus F statistics and likelihood ratios (LRs) for cross type term from general linear models. Condition factor is the residual from the regression of the natural logarithm of weight on the natural logarithm of length.

Release	Growth Parameter	Farm	F♀hyb	Wild	F (cross type)	p-value (cross type)	LR (cross type)
	Condition	0.036 ± 0.068	-0.041 ± 0.068	0.006 ± 0.076	$1.3108_{(2,25)}$	0.2875	0.29
	Length	34 ± 1.174	30.5 ± 1.174	26.2 ± 1.312	$38.803_{(2,25)}$	2.16E-08	2.77E+07
	Weight	0.339 ± 0.033	0.211 ± 0.033	0.134 ± 0.033	$40.368_{(2,27)}$	7.70E-09	7.86E+07
	TAG:ST	1.593 ± 0.24	0.546 ± 0.24	0.476 ± 0.24	$27.294_{(2,27)}$	3.28E-07	1.15E+06

Recap	Growth Parameter	Farm	F♀hyb	W♀hyb	Wild	F (cross type)	p-value (cross type)	LR (cross type)
	Condition	0.019 ± 0.041	0.002 ± 0.044	-0.009 ± 0.041	-0.011 ± 0.041	$0.4462_{(3,35)}$	0.7215	0.05
	Length	52.7 ± 5.3	52.4 ± 5.6	58.2 ± 5.3	48.1 ± 5.3	$2.3994_{(3,35)}$	0.08442	0.91
	Weight	1.6 ± 0.552	1.69 ± 0.582	2.2 ± 0.552	1.23 ± 0.552	$2.1241_{(3,35)}$	0.1148	0.62
	TAG:ST	0.76 ± 0.892	2.33 ± 0.892	2.18 ± 0.892	1.88 ± 0.892	$2.5296_{(3,35)}$	0.07257	0.99

Lipid/FA Profile at Release

Principal components analysis (PCA) on lipid class and FA percentage data for release samples indicated that cross types formed distinct clusters and were different in their lipid and FA profiles (Figure 3.2). The first two components for this PCA explained 60.4% of the total variation in the data (Supplementary Table 3.1). A general linear model on PC1 scores indicates strong evidence for an effect of fish weight (Supplementary Table 3.2; $F_{1,24}$ = 179.67 , LR = 5.89E+10, p= 1.22E-12) and cross type ($F_{2,24}$ = 6.83, LR = 44.37, p= 4.50E-03). A general linear model on PC2 scores indicates strong evidence for a cross type effect (Supplementary Table 3.3;

$F_{2,24}$ = 11.27, LR = 916.91, p= 3.53E-04). Pairwise comparisons of mean PC1 scores indicate that all three cross types are largely different in their PC1 scores at the grand mean release weight (0.23 g) (Figure 3.3a), with wild having the lowest scores, farm having the largest, and F♀hyb intermediate to the two pure crosses. Pairwise comparisons of mean PC2 scores indicate that wild and farm have virtually the same mean PC2 score at the grand mean fish weight, while F♀hybs had smaller PC2 scores on average than the two pure crosses (Figure 3.3b).

The results of PERMANOVA on release lipid profile data do not indicate sufficient evidence for a cross type effect; however the probability of observing such an extreme result given the null hypothesis of no cross type effect is small ($F_{(2,24)}$= 3.32, LR= 1.84, p=0.017). This is after accounting for a strong fish weight effect ($F_{(1,24)}$= 24.71, LR= 1337, p= 0.001). For SIMPER analysis, farm:wild had the greatest numbers of lipids/FAs (n= 18) with important between-cross type contributions to overall dissimilarities, with farm:F♀hyb having 11 and wild:F♀hyb having 7 (Supplementary Table 3.4). These results corroborate those of the linear model on PC1, showing farm as the most differentiated cross type in terms of its overall lipid/FA profile. Only four lipids/FAs for both farm:wild and farm:F♀hyb pairs (and zero for wild:F♀hyb) had important between-cross type contributions that actually contributed to greater than 1% of the overall dissimilarities for their cross type pairs (Table 3.3).

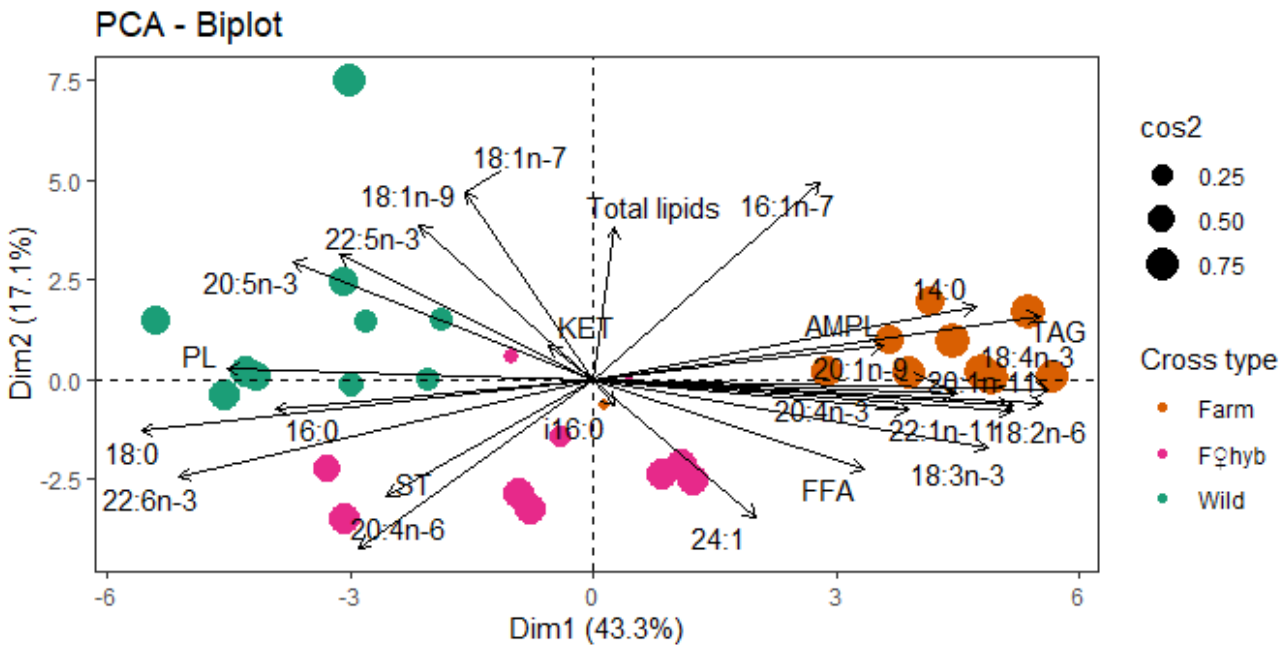

Figure 3.2. PCA biplot of lipid class and FA percentages for fish sampled prior to release (n=30, 10 per cross type). Cos2 refers to the quality of representation on the biplot (i.e. larger circles indicate individuals who are better represented than individuals indicated by smaller circles).

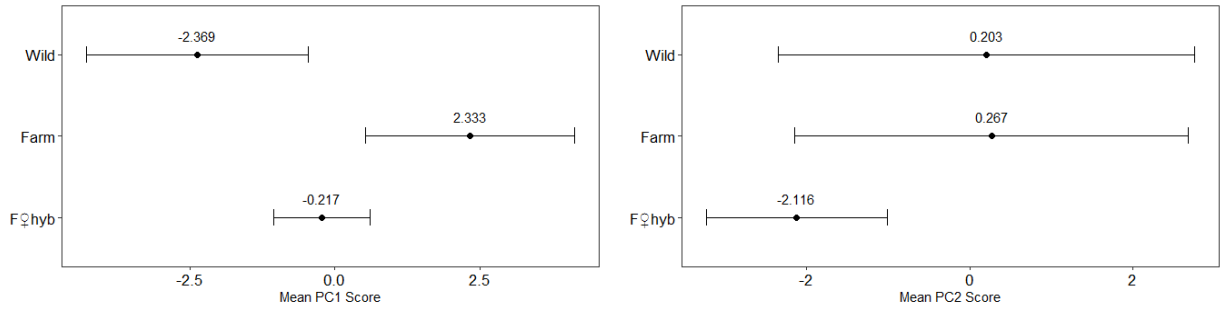

Figure 3.3. Mean PC1 (A) and PC2 (B) scores for each cross type at release. Error bars represent twice the standard error of the mean estimate. Mean score values are calculated at the grand mean release fish weight value (0.23 g).

Table 3.3. Lipid classes and FAs at release and recapture with between-cross type dissimilarities that are an important component of overall dissimilarity in lipid/FA profiles, and with an overall dissimilarity (Avg. Contrib.) of ≥ 1%. Importance of between-cross type dissimilarity to overall dissimilarity was determined by a low (<5%) probability (p) of having a contribution to dissimilarity as or more extreme than their calculated contribution when cross types were randomized over 999 permutations. Abbreviations are as follows: PL= phospholipids, TAG= triacylglycerols, ST= sterols, 22:6n-3 = DHA (docosahexaenoic acid), AMPL= acetone mobile polar lipids, FFA= free fatty acids.

Time	Comparison	Lipid/FA	Avg. Contrib. (%)	SD (%)	p
Release	Wild:Farm	PL	4.0	2.6	0.001
		TAG	3.3	1.4	0.001
		ST	1.5	0.84	0.004
		22:6n-3	1.2	0.53	0.001
	Farm:F♀hyb	PL	3.4	2.1	0.044
		TAG	2.7	1.3	0.002
		22:6n-3	1.1	0.54	0.001
		AMPL	1.0	0.51	0.001
Recapture	W♀hyb:Farm	PL	4.2	2.7	0.049
	Wild:Farm	PL	4.1	2.7	0.046
	Wild:F♀hyb	FFA	1.1	0.73	0.005
	Farm:F♀hyb	TAG	6.8	4.2	0.008
		PL	4.5	2.9	0.013

Lipid/FA Profile at Recapture

The first two components for the PCA on lipid class and FA percentages explained 60.1% of the total variation (Figure 3.4, Supplementary Table 3.5). A linear model on PC1 scores indicated strong evidence for an effect of fish weight (Supplementary Table 3.6; $F_{1,31}$ = 19.66, LR = 255.58, p= 1.08E-04), as well as good evidence for a cross type effect ($F_{3,31}$ = 4.98, LR = 28.12, p= 6.21E-03). Farm fish were the most differentiated cross type in terms of PC1 scores, with F♀hyb and wild having higher PC1 scores (and very similar to one another) at the grand mean recapture fish weight, and W♀hyb intermediate (Figure 3.5a). A linear model on

PC2 scores indicated insufficient evidence for an effect of fish weight (Supplementary Table 3.7; $F_{3,31}$ = 0.175, LR = 0.34, p= 0.678) or cross type (Figure 3.5b; $F_{3,31}$ = 1.51, LR = 0.21, p= 0.232).

Results of PERMANOVA corroborate results of linear models, with good evidence for a fish weight effect on overall lipid profile differences ($F_{(1,31)}$= 18.04, LR= 126.90, p= 0.001), as well as good evidence for a cross type effect ($F_{(3,31)}$= 4.96, LR=18.92, p=0.005). For SIMPER analysis, farm:F♀hyb and farm:W♀hyb had the greatest numbers (ten and eight, respectively) of lipids/FAs with important between-cross type contributions to overall dissimilarities (Supplementary Table 3.8). In contrast, W♀hyb:wild, W♀hyb:F♀hyb, and wild:F♀hyb only had one lipid/FA each with an important between-cross type contribution, and wild:farm only had two. These results corroborate those of the linear model on PC1, showing farm as the most differentiated cross type in terms of its overall lipid/FA profile. However, it is important to note that only four lipids/FAs of all those with important between-cross type contributions actually contributed to greater than 1% of the overall dissimilarities for their cross type pairs (Table 3.3).

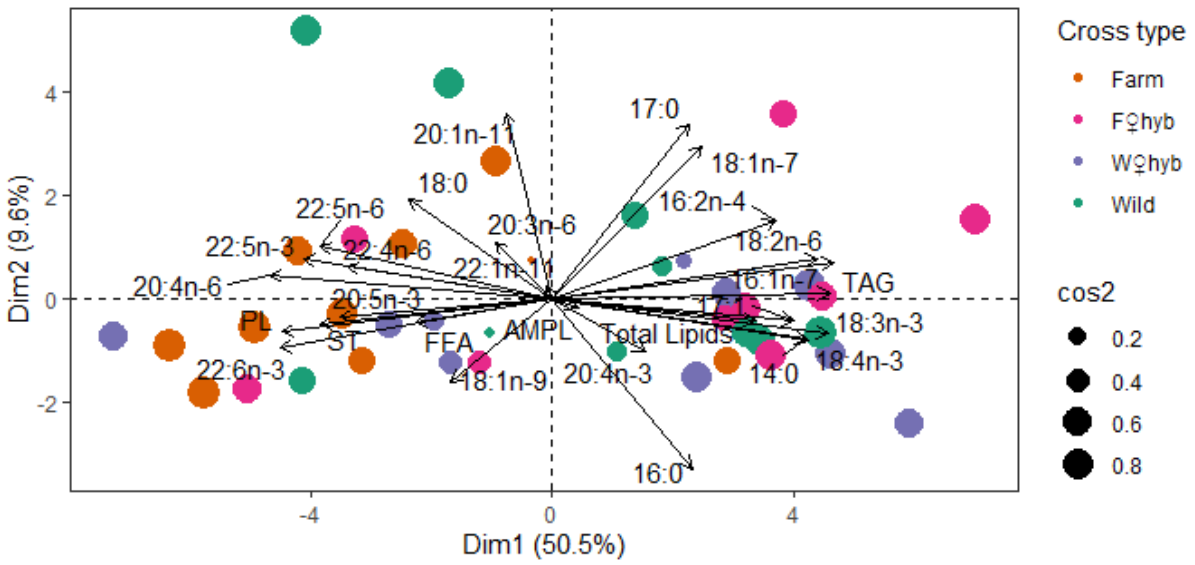

Figure 3.4. PCA biplot of lipid class and FA percentages for fish sampled at recapture (n=40, 10 per cross type). Cos2 refers to the quality of representation on the biplot (i.e. larger circles indicate individuals who are better represented than individuals indicated by smaller circles).

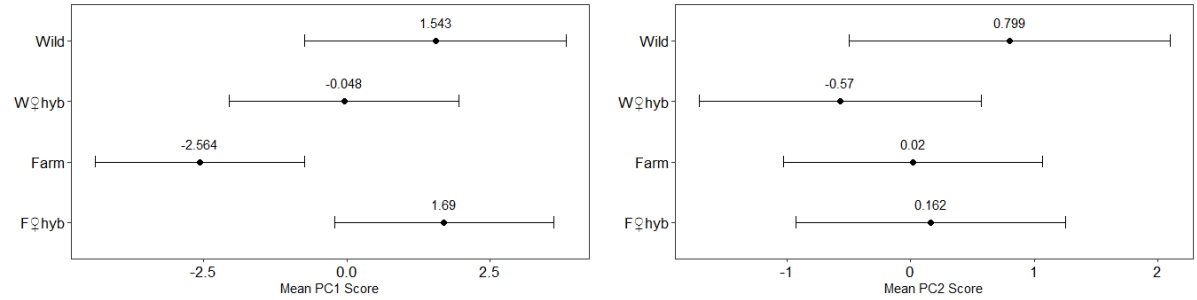

Figure 3.5. Mean PC1 (A) and PC2 (B) scores for each cross type at recapture. Error bars represent twice the standard error of the mean estimate. Mean score values are calculated at the grand mean release fish weight value (1.68 g).

Percentages and Concentrations of Major Lipid Classes

There was insufficient evidence for the effects of cross type, fish weight, or their interaction on total lipid concentration (mg/g wet weight) at both release and recapture (Tables 3.5 & 3.6). The pure crosses were more similar to one another in mean total lipid concentration at release, with hybrids lower, while at recapture all cross types were largely similar in their

concentrations (Table 3.4). All cross types decreased in total lipid concentration while in the river (Figure 3.6).

The largest among-cross type differences for percentages and concentrations of lipid classes at release and recapture occurred for phospholipids (PL), sterols (ST), and triacylglycerols (TAG) (Tables 3.4 & 3.6, Figure 3.6). For percentages of PL and TAG, there was strong evidence for an effect of fish weight at release, while at recapture there was strong evidence for an effect of fish weight and cross type on their percentages, and there was also strong evidence for a fish weight effect on ST percentage at recapture (Table 3.5). Evidence for terms in concentration models varied (discussed below, Table 3.7). At release, farm had the lowest mean percentage of PL and wild had the highest; however farm decreased in its mean percentage of PL by only a slight amount (<3%) while in the river (ending with the highest mean percentage at recapture), while the other cross types decreased substantially (>20%) in their mean PL percentages while in the river. However, while wild also had the highest concentration of PL at release and farm had the highest at recapture (by a slight amount), all crosses decreased in their concentrations of PL while in the river (though Farm by just a slight amount) and there was insufficient evidence for an effect of weight or cross type on concentration of PL at release or recapture (Tables 3.6 and 3.7).

In contrast to patterns for PL, farm had the highest mean TAG percentage and concentration prior to release but was the only cross type to decrease while in the river, ultimately with the lowest mean TAG percentage and concentration at recapture (Figure 3.6, Table 3.6). For TAG concentration, there was good evidence for an effect of weight and cross type at release, but only weight at recapture (Table 3.7), while the opposite was true for TAG percentage. All cross types increased in mean percentage of ST while in the river, however farm

was the only cross type to experience a large change in percent ST, being lowest at release and highest at recapture (Figure 3.6). On the other hand, mean ST concentration was very similar among cross types at both release and recapture, with evidence for an effect of weight only at release and weight and weight by cross type interaction at recapture (Tables 3.6 & 3.7).

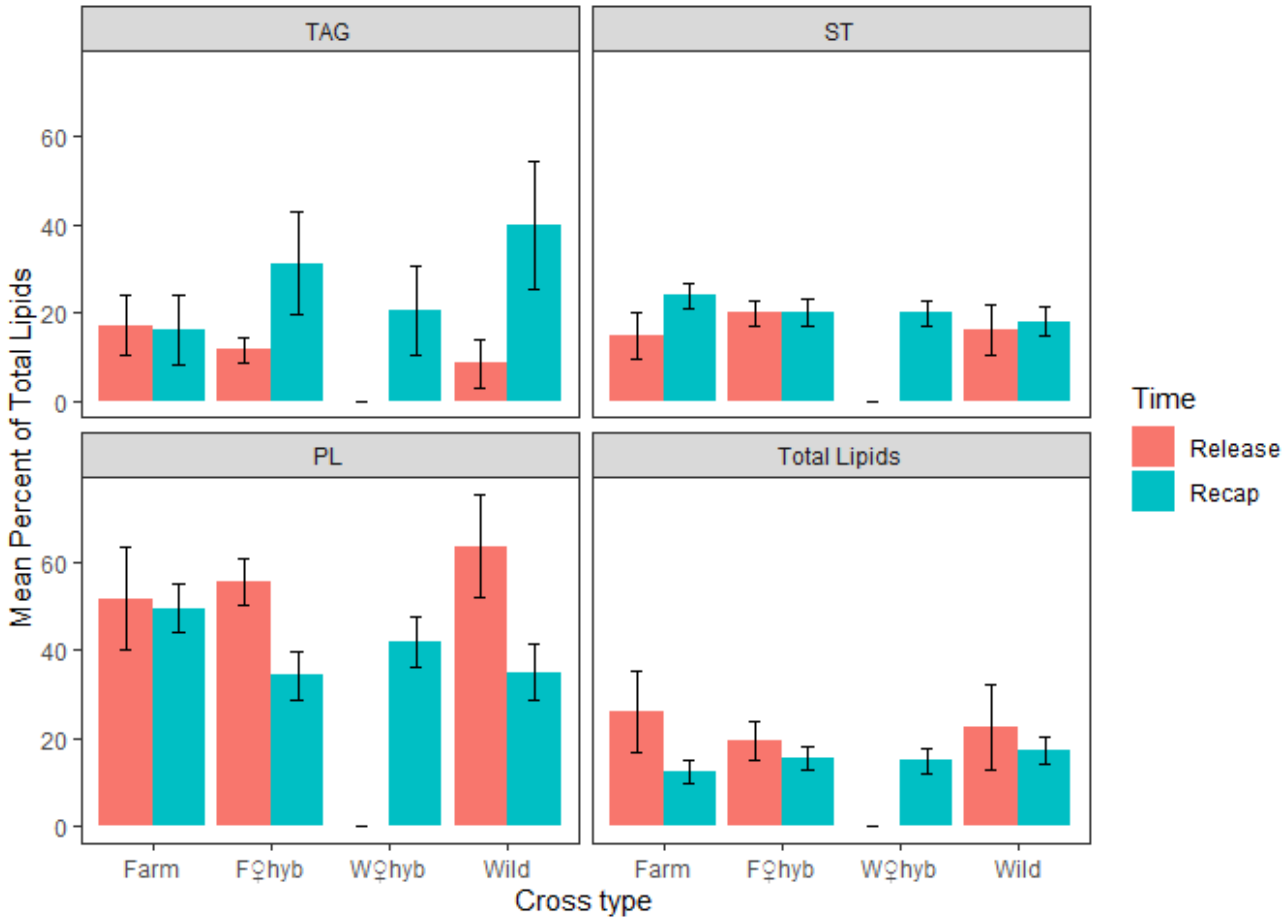

Figure 3.6. Comparison of mean percentages (± 2*SE) of major lipid classes, and total lipid concentration (mg/g wet weight), at release and recapture for the four cross types. Note that there were no release samples for W♀hyb fish (hence lack of data). Abbreviations are as follows: TAG= triacylglycerols, ST= sterols, PL= phospholipids. See Table 3.4 for detailed mean and SE values, and Table 3.5 for likelihood ratio evidence of fish weight and cross type effects on each lipid class.

Percentages and Concentrations of EFAs

At release and recapture, there was strong evidence for an effect of weight, but not cross type, on percent and concentration of LNA (18:2n-6) (Tables 3.5 & 3.7). In addition, percentages and concentrations of LNA did not change much from release to recapture (Tables 3.4 & 3.6; Figure 3.7), though farm went from having the highest mean percentage and concentration to the lowest and was the only cross type to decrease in its mean percentage while in the river. There was strong evidence for an effect of weight on ALA (18:3n-3) concentration at release and recapture, and strong evidence for effects of weight and cross type on percent ALA at release and recapture (Tables 3.5 & 3.7). All crosses increased in their mean percentages and concentrations of ALA while in the river (Figure 3.7, Table 3.6). Farm had the highest percentage and concentration of ALA at release while wild had the lowest, while at recapture farm had the lowest percentage and concentration of this EFA.

There was insufficient evidence for effects of any variable on percent and concentration of ARA at release, however there was strong evidence for the effects of weight and cross type on percent but not concentration at recapture (Tables 3.5 & 3.7). All crosses had similar percent and concentrations of ARA at release, with farm having the highest percentage at recapture. All crosses increased in their mean percentages and concentrations of ARA (20:4n-6) from release to recapture (Figure 3.7, Table 3.6). Wild had the highest percentage of EPA (20:5n-3) at release (strong evidence for effects of weight and cross type), with all crosses decreasing in their percentages while in the river and having very similar percentages at recapture (insufficient evidence for an effect of weight, cross type, or their interaction) (Figure 3.7, Table 3.5). Concentrations of EPA, on the other hand, were similar among all cross types at release and recapture, with insufficient evidence for effects of weight or cross type at both time periods

(Tables 3.6 & 3.7). Percentages of DHA (22:6n-3) were high overall for all cross types at both release and recapture, with all cross types decreasing in their percentages while in the river (Figure 3.7). Farm had the lowest percentage at release, however at recapture farm had the highest percentage. There was strong evidence for effects of weight and cross type on percent DHA at release and recapture (Table 3.5). All crosses were similar in their concentrations of DHA at release and recapture (insufficient evidence for an effect of weight or cross type at both time periods) (Tables 3.6 & 3.7).

Percentages of FA Groups

Overall, there were no large differences in sums of saturated FAs, monounsaturated FAs (MUFAs), or polyunsaturated FAs (PUFAs) among cross types at release or recapture, with insufficient evidence for a cross type effect on percentages at both time points for all groups (Table 3.4, Figure 3.7). There was strong evidence for effect of weight on percentage of saturated FAs at recapture and sum of n-3 (omega-3) FAs at release (Table 3.5). Wild fish were the only cross type to increase slightly in percentage of MUFAs from release to recapture, while farm fish had a slightly higher increase in PUFAs than other cross types from release to recapture. In addition, wild fish had the greatest decrease in mean percentage of n-3 FAs while in the river out of all cross types. For DHA:EPA at release, there was strong evidence for a cross type effect (Tables 3.4 & 3.5). F♀hyb had the highest DHA:EPA ratio at release, while at recapture farm had the highest ratio (and was the only cross type to increase its ratio while in the river); however there was strong evidence for the effects of weight only on DHA:EPA at recapture.

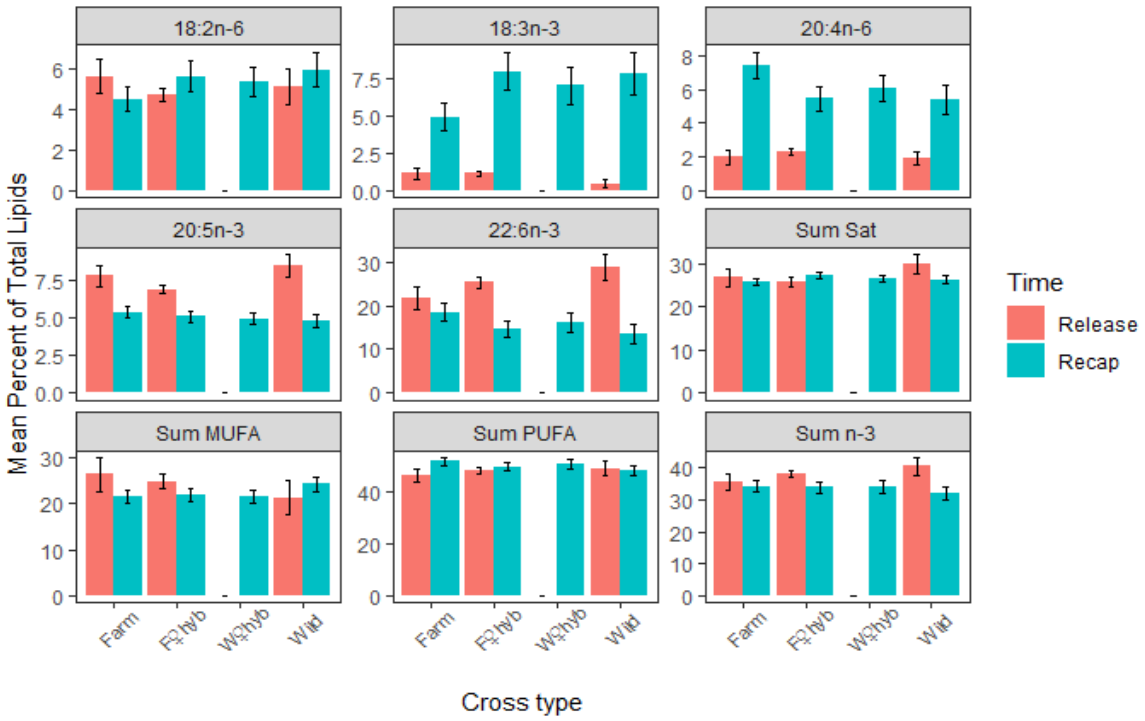

Figure 3.7. Comparison of mean percentages (± 2*SE) of essential FAs and FA groups at release and recapture for the four cross types. Note that there were no release samples for W♀hyb fish (hence lack of data), and that each panel has a different y-axis range. See Table 3.4 for detailed mean and SE values, and Table 3.5 for likelihood ratio evidence of fish weight and cross type effects on each lipid class. Abbreviations are as follows: 18:2n-6 = LNA (linoleic acid), 18:3n-3= ALA (alpha-linolenic acid), 20:4n-6 = ARA (arachidonic acid), 20:5n-3= EPA (eicosapentaenoic acid), 22:6n-3= DHA (docosahexaenoic acid), Sum Sat= sum of saturated fatty acids, Sum MUFA= sum of monounsaturated fatty acids, Sum PUFA= sum of polyunsaturated fatty acids, Sum n-3 = sum of omega-3 fatty acids.

Table 3.4. Release and recapture mean percentages of lipids and FAs, the ratio of DHA to EPA, and total lipids (mg/g wet weight), ± twice the standard error of the mean. Means were calculated for cross types without taking into account potential effect of fish weight. See Table 3.5 for likelihood ratio evidence for fish weight and cross type effects on each lipid class/FA. Abbreviations are as follows: LNA= linoleic acid; ALA= alpha-linolenic acid; ARA= arachidonic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid; ST= sterols, TAG= triacylglycerols; PL= phospholipids, Σ Sat = sum of saturated fatty acids; Σ MUFA= sum of monounsaturated fatty acids, Σ PUFA= sum of polyunsaturated fatty acids, Σ n-3= sum of omega-3 fatty acids.

	Release			Recapture			
Lipid/FA	Farm	F♀hyb	Wild	Farm	F♀hyb	W♀hyb	Wild
LNA (18:2n-6)	5.65 ± 0.82	4.72 ± 0.34	5.14 ± 0.88	4.52 ± 0.63	5.66 ± 0.74	5.38 ± 0.75	5.98 ± 0.87
ALA (18:3n-3)	1.15 ± 0.39	1.13 ± 0.19	0.46 ± 0.28	4.93 ± 0.95	8.02 ± 1.27	7.05 ± 1.23	7.87 ± 1.45
ARA (20:4n-6)	1.98 ± 0.41	2.30 ± 0.20	1.92 ± 0.41	7.41 ± 0.79	5.44 ± 0.70	6.07 ± 0.77	5.37 ± 0.84
EPA (20:5n-3)	7.81 ± 0.68	6.93 ± 0.29	8.50 ± 0.74	5.38 ± 0.38	5.13 ± 0.38	4.97 ± 0.39	4.82 ± 0.45
DHA (22:6n-3)	21.7 ± 2.54	25.32 ± 1.22	28.68 ± 2.95	18.40 ± 2.08	14.60 ± 1.96	16.11 ± 2.11	13.45 ± 2.27
ST	14.84 ± 5.23	19.85 ± 2.69	16.23 ± 5.64	23.91 ± 2.87	20.06 ± 2.87	19.9 ± 2.91	18.06 ± 3.30
TAG	17 ± 6.8	11.54 ± 2.72	8.43 ± 5.55	16.06 ± 7.99	31.14 ± 11.65	20.49 ± 10.15	39.84 ± 14.63
PL	51.7 ± 11.63	55.47 ± 5.3	63.64 ± 11.74	49.51 ± 5.46	34.37 ± 5.43	41.8 ± 5.81	34.89 ± 6.38
Σ Sat	26.86 ± 2.1	25.84 ± 0.95	29.86 ± 2.3	25.83 ± 0.70	27.44 ± 0.74	26.55 ± 0.77	26.34 ± 0.87
Σ MUFA	26.33 ± 3.69	24.76 ± 1.67	21.27 ± 3.58	21.51 ± 1.30	21.92 ± 1.37	21.64 ± 1.43	24.17 ± 1.68
Σ PUFA	46.42 ± 2.72	48.63 ± 1.25	49.12 ± 2.88	51.87 ± 1.62	49.74 ± 1.69	50.88 ± 1.77	48.36 ± 2.02
Σ n-3	35.34 ± 2.61	38.02 ± 1.22	40.37 ± 2.84	34.17 ± 1.70	33.78 ± 1.77	34.05 ± 1.86	31.78 ± 2.08
DHA:EPA	2.78 ± 0.41	3.65 ± 0.19	3.36 ± 0.44	3.44± 0.25	2.86 ± 0.26	3.21 ± 0.27	2.76 ± 0.31
Total Lipids	25.98 ± 9.14	19.49 ± 4.19	22.47 ± 9.67	12.45 ± 2.61	15.43 ± 2.71	14.89 ± 2.85	17.29 ± 3.23

Table 3.5. Likelihood ratios (LRs) for effect (*vs*. no effect) of fish weight, cross type, and fish weight x cross type interaction terms on percentages of lipid classes, FAs, ratio of DHA to EPA, and total lipids (mg/g wet weight) at release and recapture. Abbreviations are as follows: LNA= linoleic acid; ALA= alpha-linolenic acid; ARA= arachidonic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid; ST= sterols, TAG= triacylglycerols; PL= phospholipids, Σ Sat = sum of saturated fatty acids; Σ MUFA= sum of monounsaturated fatty acids, Σ PUFA= sum of polyunsaturated fatty acids, Σ n-3= sum of omega-3 fatty acids.

	Release			Recap		
Lipid/FA	LR weight	LR cross type	LR interaction	LR weight	LR cross type	LR interaction
LNA (18:2n-6)	6.23E+07	0.066	3.17	48.56	0.85	0.94
ALA (18:3n-3)	6.95E+05	508.12	0.047	112.82	69.86	0.048
ARA (20:4n-6)	1.99	3.02	0.107	103.60	106.67	0.016
EPA (20:5n-3)	180.13	3432.58	0.078	1.57	0.10	0.019
DHA (22:6n-3)	1242.31	14.14	0.36	81.16	9.63	0.077
ST	2.40	0.43	0.06	5737	0.21	0.24
TAG	2.14E+06	0.25	0.06	564	16.4	0.12
PL	2571.49	0.10	0.13	20.06	266.21	0.095
Σ Sat	5.54	3.51	0.15	9.55	1.30	0.044
Σ MUFA	0.99	0.44	0.24	0.56	0.51	0.065
Σ n-3	987.48	1.35	0.11	2.15	0.13	0.056
Σ PUFA	0.29	3.83	0.14	2.93	2.05	0.022
DHA:EPA	0.14	3340.31	0.002	55.70	3.89	0.0036
Total lipids	0.084	0.14	0.002	0.21	0.0094	0.00025

Table 3.6. Release and recapture mean concentrations (mg/g wet weight) of lipids and FAs, ± 2x standard error of the means. Means were calculated for cross types without taking into account potential effect of fish weight. See Table 3.7 for likelihood ratio evidence for fish weight and cross type effects on each lipid class/FA. Abbreviations are as follows: LNA= linoleic acid; ALA= alpha-linolenic acid; ARA= arachidonic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid; ST= sterols, TAG= triacylglycerols; PL= phospholipids.

	Release			Recapture			
Lipid/FA	Farm	F♀hyb	Wild	Farm	F♀hyb	W♀hyb	Wild
LNA (18:2n-6)	1.00E+03 ± 326	5.89E+02 ± 149.6	7.65E+02 ± 345	3.71E+02 ± 180.6	6.23E+02 ± 179.8	5.87E+02 ± 200	7.59E+02 ± 221.8
ALA (18:3n-3)	1.98E+02 ± 81.2	1.41E+02 ± 37.2	6.8E+01 ± 86	4.15E+02 ± 278	8.94E+02 ± 278	7.50E+02 ± 308	1.02E+03 ± 342
ARA (20:4n-6)	3.39E+02 ± 139.4	2.86E+02 ± 64	2.63E+02 ± 147.6	5.56E+02 ± 78.4	5.4E+02 ± 78	5.82E+02 ± 86.8	6.39E+02 ± 96.2
EPA (20:5n-3)	1.34E+03 ± 596	8.62E+02 ± 274	1.205E+03 ± 630	4.16E+02 ± 91.2	5.16E+02 ± 90.8	4.85E+02 ± 101	5.79E+02 ± 112
DHA (22:6n-3)	3.784E+03 ± 1722	3.12E+03 ± 790	4.123E+03 ± 1822	1.39E+03 ± 246	1.31E+03 ± 244	1.53E+03 ± 272	1.59E+03 ± 302
ST	3.67E+03 ± 592	3.78E+03 ± 272	3.518E+03 ± 628	2.93E+03 ± 368	3.08E+03 ± 366	2.91E+03 ± 408	3.07E+03 ± 452
TAG	4.357E+03 ± 2040	2.18E+03 ± 936	1.529E+03 ± 2158	2.387E+03 ± 2106	5.978E+03 ± 2096	5.010E+03 ± 2330	7.160E+03 ± 2586
PL	1.386E+04 ± 8762	1.097E+04 ± 4022	1.441E+04 ± 9270	6.08E+03 ± 680	5.11E+03 ± 678	5.93E+03 ± 754	5.818E+03 ± 836

Table 3.7. Likelihood ratios (LRs) for effect (*vs*. no effect) of fish weight, cross type, and fish weight x cross type interaction terms on concentrations of lipid classes and FAs at release and recapture. Abbreviations are as follows: LNA= linoleic acid; ALA= alpha-linolenic acid; ARA= arachidonic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid; ST= sterols, TAG= triacylglycerols; PL= phospholipids.

	Release			Recap		
	LR weight	LR cross type	LR interaction	LR weight	LR cross type	LR interaction
LNA (18:2n-6)	897.97	4.02	0.04	14.75	1.49	0.02
ALA (18:3n-3)	2.53E+06	2.45	0.07	39.79	4.64	0.01
ARA (20:4n-6)	0.38	0.13	0.04	0.35	0.09	0.01
EPA (20:5n-3)	0.37	5.52	0.04	4.19	0.57	0.01
DHA (22:6n-3)	0.51	0.33	0.05	0.34	0.15	0.02
ST	870.03	0.36	1.33	312.16	0.03	12.55
TAG	1.05E+04	12.46	0.11	45.27	6.71	0.01
PL	1.90	0.18	0.04	4.64	0.35	2.06

Discussion

I found evidence that both fish weight and cross type influenced overall parr lipid/FA profiles at release and recapture, as well as a number of major individual lipid classes, EFAs, and FA groups. At release, the effect of cross type on fish weight was strong, and it appears that when looking at the overall lipid/FA profile, the cross type effect is effectively masked by that of weight (see results of PERMANOVA); however when breaking down the overall profile into components, effects of weight and cross type can be better separated (see results of linear models on PCs and betareg models on individual lipids/FAs). Specific compositional data on the pre-release diet is not available; however, given the controlled environment and diet prior to release, any differences that existed up until this point in tissue lipids and FAs should not have been due to differences in diet. Instead, differences at release may have been due to 1) lingering maternal effects, 2) a genetic difference in metabolism among cross types, or a combination of these two factors. Ashton et al. (1993) found that Chinook Salmon eggs and alevin had differences in their FA profiles that mirrored profile differences in the diets of their parents. Though fish in my experiment were sampled after approximately 6 weeks of external feeding, which is later than those in Ashton et al. (1993), the time elapsed since first-feeding was likely not long enough for the maternal FA profile to be completely "washed out" (e.g. Budge et al. 2011), and it is likely a maternal diet signature could still be partially present. In any case, regardless of the cause for the among-cross type variation in lipid and FA profiles at release, these levels exist to provide a baseline with which to compare recapture levels.

Overall lipid and FA profiles at recapture overlap greatly among cross types, as shown by the limited cross type clustering in the PCA biplot (Figure 3.4), however there was evidence for a cross type effect after accounting for fish weight on PC1 scores and in PERMANOVA results.

There was also a lack of evidence for a cross type effect on total lipid concentration. While total lipid is commonly used as a measure of storage energy and a potential predictor of survival for fish, it is important to also consider the influence lipid classes may individually have on fish performance (Næsje et al. 2006). I did find evidence for a cross type effect on recapture percentages of several individual lipid classes, as well as several essential FAs, and this evidence along with patterns of change from release to recapture suggests that there are indeed genetic factors influencing how fish acquire and/or store food resources in the wild.

Farm fish were often the most differentiated in their lipid and FA percentages and concentrations when compared with the other cross types, and relative levels of several lipid classes and essential FAs are suggestive of a possible farm feeding disadvantage. Such a feeding disadvantage has indeed been documented before in the wild, with Orlov et al. (2006) finding that farm parr fed less actively, made more false feeding attempts, and had a higher percentage of poor quality prey items in their stomachs than wild parr. Perhaps most importantly, farm was the only cross type that did not increase in TAG while in the river, which is the main energy storage lipid class for fishes (Tocher 2003), and had the lowest percentage and concentration of TAG of all cross types at recapture. Simply the fact that farm were storing relatively less lipid energy would seem to suggest they were acquiring less while in the river. In addition, while lipid and FA profiles of fish cannot be expected to exactly match those of their prey due to biochemical catabolic and metabolic FA changes that occur in the fish (Budge et al. 2012), the relatively higher TAG levels of wild and hybrid fish at recapture are more similar to TAG levels reported for several species of freshwater invertebrate prey (all greater than 35% of total lipids; Bell et al. (1994)). The fact that farm fish had TAG levels that were less reflective of the lipid profiles of

their would-be prey in the river would suggest that they were feeding relatively less on said prey than other cross types.

Farm also had the lowest percentage and concentration of LNA (18:2n-6) at recapture, despite having the highest levels at release, and was the only cross type to decrease in percentage and concentration of LNA while in the river. Farm also had the lowest concentration of ALA at recapture (but the highest percentage), despite having the highest concentration at release. Given that LNA is one of the most abundant omega-6 (n-6) FAs and ALA one of the most abundant n-3 FAs in freshwater invertebrates (Bell et al. 1994), the farm decrease in LNA and low recapture levels of ALA may again indicate that they were ingesting fewer prey items than other cross types in this environment. Farm also had the highest recapture percentage of DHA (22:6n-3), a FA that was found to be present only in small levels in freshwater invertebrate prey (Bell et al. 1994). Though all cross types experienced a decrease in DHA from release to recapture (which is what could be expected if they were ingesting DHA-lacking prey in the river), the relatively larger percentage of DHA possessed by farm fish may potentially suggest that more DHA was "leftover" from the release profile. Alternatively, since salmonids do have a limited ability to synthesize LC-PUFA from LNA and ALA (e.g. Hixson et al. 2014; Katan et al. 2019), it could be that farm fish have a genetic advantage with regards to DHA synthesis. Indeed, it has been suggested that the high amount of vegetable oils (which are typically lacking in long-chain (LC)-PUFAs) in commercial feed for the past ~20 years has resulted in farm fish being selected for having a higher endogenous LC-PUFA synthesis ability than wild fish when in an EFA-limited environment (Jin et al. 2020).

Farm fish had the highest percentage of ARA (20:4n-6) at recapture, which at first glance does not seem to be consistent with them having a feeding disadvantage in the river, since ARA

is another one of the most abundant n-6 FAs found in freshwater invertebrates (Bell et al. 1994). However, farm having relatively high percentages of ARA may be linked to the fact that they also had the highest recapture percentage of PL (Katan et al. 2019), the lipid class that is a fish's main source of essential FAs such as ARA (Tocher et al. 2008). It does not appear farm fish were acquiring/storing more PL or ARA in absolute terms relative to other cross types (as might be expected if they were feeding relatively more), given that differences in concentrations of PL and ARA among cross types were small (insufficient evidence for a cross type effect, with farm PL concentration greater by only a difference of 2.6% and 4.3% on average compared with W♀hyb and wild, respectively). Farm fish may in fact be able to better synthesize PLs endogenously, as suggested by the results of Jin et al. (2020), who found that wild salmon growth was positively influenced by a diet supplemented with PL but the growth of farm salmon was not. This may help explain the higher relative levels of PL and ARA in farm *vs.* other lipid classes like TAG, even if other results point to lower acquisition of lipids through feeding. A physiological implication of higher percentages of ARA relative to EPA and DHA may mean that farm fish had a higher production of pro-inflammatory eicosanoids (compounds for which these EFAs are precursors). ARA, EPA, and DHA are metabolized by the enzyme 5LOX to produce eicosanoids involved in the fish's inflammatory response (e.g. Rowley et al. 1995), and the one produced from ARA (leukotriene B_4) is the most strongly pro-inflammatory (e.g. Wall et al. 2010). An increased production of transcripts associated with pro-inflammatory eicosanoid synthesis has been previously associated with a higher ARA:EPA ratio (Caballero-Solares et al. 2017), so regardless of the mechanism behind their relatively higher ARA percentage, it may very well be possible that farm fish were at a physiological disadvantage in relation to their inflammatory response compared with other cross types.

In addition, while EPA was found to be one of the most abundant n-3 FAs in freshwater prey invertebrates (Bell et al. 1994), the fact that there was no cross type effect on EPA content at recapture is perhaps not surprising and does not exclude the potential for a difference in feeding efficiency among cross types. EPA has been suggested to be under stricter physiological control than non- EFAs (Budge et al. 2011) and is the more bioactive EFA when compared to DHA (Horn et al. 2019), so it could perhaps be expected that levels of EPA would vary less among cross types, even when feeding and diet are more variable. Farm fish, if they did indeed have a feeding disadvantage, once again may have been able to compensate for a reduced EPA intake by higher endogenous EPA synthesis (as suggested by Jin et al. (2020)).

Ultimately, if this feeding disadvantage did indeed exist, question remains as to why farm fish were still the second-largest cross type on average at recapture. Farm Atlantic Salmon have been found to have higher feed consumption and conversion rates than wild fish (Thodesen et al. 1999), which may mean that even if farm fish were less able to capture food in a natural environment than their wild counterparts, their higher feed conversion rate may have at least partially made up for this (assuming their higher conversion still occurs under wild conditions). Rosenfield et al. (2020) also found that for comparisons of individuals within two species of salmonids, individuals with higher maximum consumption were also the most efficient convertors of food to biomass. Therefore, it could be possible that farmed fish have a higher growth efficiency compared to wild fish. In addition, it is also important to compare the growth of these farm fish in the wild to their own potential growth under culture conditions. Given that the growth differential between farm and wild fish in a culture environment is typically 2-3 times or greater (Glover et al. 2018), the much smaller growth differential I observed here (and the fact that farm were smaller on average than W♀hyb) may indicate that farm fish had a growth

disadvantage while in the river, at least compared to their growth potential in an aquaculture environment. Of course, seeing as there is currently also evidence for selection against the fastest-growing farm fish in the wild (Glover et al. 2018), it is possible that the largest farm fish were removed by selection and were thus not recaptured at all.

In addition to the potential for genetic-based feeding differences among cross types affecting lipid levels, it is also possible that differences in lipid and FA content among cross types may have been at least partially influenced by genetic-based differences in energy allocation. For young fish, when energy is limited there is a trade-off between allocating energy for storage (to avoid starvation) and allocating energy to growth (to escape gape-limited predators) (reviewed by Post and Parkinson 2001). Farm fish have been heavily selected for faster growth, with previous research showing contradictory results regarding if farm fish store more lipid than wild fish do (see Johnston et al. 2006; Glover et al. 2018). However up until now, whether cross types exhibit differences in lipid storage under common garden conditions has remained untested in a wild environment. If, when energy-limited (whether due to the environment, a feeding disadvantage, or both), farm fish still allocate a relatively higher percentage of energy to growth *vs*. storage, they would be likely to have relatively lower lipid stores than wild fish- the result I observed here for TAG. Differences in lipid storage among cross types could also be related to adaptation (or lack thereof) to seasonal changes in prey availability. Given that parr in the wild are vulnerable to energy-related mortality during the winter (e.g. Finstad et al. 2004) whereas farm fish have consistent access to food year-round, it may be that selection for high lipid storage before the winter has operated more strongly on wild parr than farm. Finally, previous results from a study on Norwegian 0+ Atlantic Salmon parr have shown that there are differences in lipid storage at both the latitudinal and local scales (Berg

et al. 2009), so it is possible that lipid storage of farm fish differed from wild fish due to their ancestral geographic backgrounds (St. John River *vs*. Garnish River, respectively).

The implications for differential patterns of lipid and FA levels among cross types would be likely to become most apparent over the harsh winter months. Farm fish with their lower levels of TAG would be at greater risk for energy-related death during the winter period (e.g. Finstad et al. 2004). Also, while parr typically reduce their feeding activity during winter to save energy and avoid predation (Metcalfe and Thorpe 1992), farm fish may be at greater risk of predation during winter if they need to engage in relatively more active feeding periods than wild fish to try and sustain their energy levels. Farm salmon have also been shown to be inherently less risk-averse than wild salmon (e.g. Fleming et al. 1996; Islam et al. 2020; Solberg et al. 2020), potentially compounding this predation risk. Given that farm fish had lower energy storage than wild fish but were not much different in size, allocating more energy to growth would likely still not confer a large predation-avoidance advantage to this cross type, though they would still have the starvation risk due to lower storage. Also, while farm appears to be the cross type showing the starkest differences compared with the others, it is likely the hybrids that merit the most consideration in a conservation context, given that escaped farm fish exhibit lower reproductive success than wild (e.g. Fleming et al. 1996; Fleming et al. 2000), and therefore hybrid offspring are more likely to occur than pure feral farm offspring. My results show that hybrids are often very similar to wild fish in their percentages and concentrations of various lipid classes and FAs, and W♀hyb were largest in size on average at recapture. While results of hybrids do not indicate any obvious disadvantage in terms of feeding, storage, or condition, the previously-discussed results pertaining to farm fish do suggest that there may be a genetic disadvantage affecting levels of energy storage, and certain essential FAs (LNA, ALA)

for fish with farm genes in the wild. It may be, therefore, that these maladaptive genes may be "hidden" and may only be expressed under certain environmental conditions (e.g. Glover et al. 2018). Ultimately, a longer-term common-garden experiment investigating differences over periods of higher risk for these fish may be warranted in the future.

Chapter 4: General Conclusion

Since its beginning approximately fifty years ago, Atlantic Salmon aquaculture has posed the question to scientists and managers as to how to predict and mitigate the effects of maladaptive farmed genetic introgression on wild populations (reviewed by Glover et al. 2017). Predictions of introgression and its impacts are useful for aquaculture management decisions such as siting, as well as for mitigation of escape events (i.e. prioritizing which rivers from which to remove escapees). To make accurate predictions, there is a need for data on a diverse range of populations in order to capture variability in factors such as environment, as well as population demographics and life history (Bradbury et al. 2020). To date, studies that have isolated the influence of genetics on survival and fitness-related traits expressed by wild, farm, and hybrid Atlantic Salmon in the wild are few (Einum and Fleming 1997; McGinnity et al. 1997; Fleming et al. 2000; McGinnity et al. 2003; Skaala et al. 2012; Skaala et al. 2019), with North American populations currently under-represented in this research that provides critical data for predictive models. In this study, I address part of this research gap by providing field data on a North American population of wild Atlantic Salmon and the farmed population with which it would interact after an escape event.

Salmon populations on the south coast of Newfoundland are considered threatened (COSEWIC 2010), with south-coast rivers experiencing substantial farmed introgression since a large escape event in 2013 (Wringe et al. 2018). Given that there is additional aquaculture expansion planned for this area, there is a need to plan for and mitigate the associated risks, and having data from a range of populations will allow for variation in key parameters to be incorporated into predictive models (Bradbury et al. 2020). Indeed, my results of relative farmed versus wild survival indicate a potentially important deviation from results previously reported

for this region. Sylvester et al. (2019) used an individual-based salmon eco-genetic model (IBSEM) to predict impacts to wild populations following farm escape events and found that estimates of high feral farm relative survival (along with increased invasion intensity) resulted in predictions of a more severe wild population decline, longer recovery time, and more severe changes in allele frequency. My results are noteworthy in relation because they are closer to the parameter values Sylvester et al. (2019) specified for this scenario with high farm relative survival, rather than those they used in the scenarios predicting lesser impacts, which they estimated using field data of proportional cross type change following the large 2013 escape event. If the high relative survival of feral farm offspring that I observed at age 0+ was to persist beyond the temporal scope of my experiment, based on the models of Sylvester et al. (2019) I could expect a more negative impact on wild populations than the model scenarios using their field-derived parameter values suggested. In addition, my results of F1 hybrid relative survival as inconsistently intermediate to that of the pure crosses could mean that IBSEM may not predict impacts entirely accurately if the hybrid population is weighted towards one reciprocal F1 cross over the other, since IBSEM does not include direct estimates of relative survival for hybrids and instead infers them based on additive genetic inheritance (Castellani et al. 2015). Ultimately, at a regional scale of management and ecology, my study is valuable in providing data specific to a population implicated in the at-risk area of Southern Newfoundland, and adding to regional data allows for incorporation of multiple cohorts of juveniles in the IBSEM and should make for stronger predictions (Sylvester et al. 2019).

In terms of making predictions of relative cross type performance longer-term (i.e. overwinter), lipid and fatty acid profiles are perhaps some of the most informative, yet least-investigated predictors in the wild. These analyses have been (perhaps predominantly) used to

determine nutritional requirements of farmed fish for the development of optimal aquaculture feeds (e.g. reviewed by Leaver et al. 2008; Taylor et al. 2015; Bou et al. 2017; Katan et al. 2019; Qian et al. 2020). In addition, they have also been used to assess condition and survival of wild salmon (e.g. Næsje et al. 2006; Berg et al. 2009; Finstad et al. 2010), to determine escape history of farmed fish (Skilbrei et al. 2015), and to compare farmed and wild fish raised under their respective environments and diets (Ackman and Takeuchi 1986). Minimal work related to lipid and fatty acid content and genomics of lipid metabolism of wild, farmed, and hybrid Atlantic Salmon raised under common conditions has been done, and only under laboratory conditions (Johnston et al. 2006; Glover et al. 2009; Bicskei et al. 2014; Bicskei et al. 2020; Jin et al. 2020). However, to my knowledge there has been no prior study comparing lipid and fatty acid profiles of farmed, wild, and hybrid Atlantic Salmon in the wild, which is crucial to understanding how differences in energy stores and essential fatty acids may play a role in relative performance of these cross types. Though I only looked at a short temporal scale, the fact that I detect differences in lipid/FA profiles between cross types and interesting patterns of change from release to recapture means that these types of analyses could be very helpful in future studies. Some main takeaways from my study are that farm parr might be expected to do more poorly than wild and hybrids over the winter months, and that the hybrids were generally very similar to wild fish in their percentages and concentrations of various lipid classes and fatty acids, meaning I might speculate that hybrids could perform similarly to pure wild fish over the winter months.

Though the common-garden, wild environment study design used here was novel for investigating juvenile North American Atlantic Salmon performance, and succeeded in providing key population-specific data, the experimental design also had a few potential limitations. Firstly, while fry were released into the river sites early in their development, the life stages prior

to release were likely to have some of the highest mortality rates - for example, McGinnity et al. (1997) found that differential survival between wild and farmed juveniles was greatest from the eyed-egg stage to the first summer. Therefore, my results of relative survival may have been different than if juveniles had experienced wild conditions beginning from hatch or the egg stage. Similarly, the fact that all fry had plentiful access to nutritional food in the laboratory setting for a few weeks prior to release is likely a large contrast to the likely harsher wild foraging conditions, meaning fry of certain cross types may have had a nutritional advantage here that would not have occurred had they spent their full lives in nature. Finally, in this study I could not account for the potential effects of early hatchery rearing on subsequent juvenile performance in the wild. Indeed, recent work has found that hatchery rearing can induce epigenetic changes that may result in reduced survival and fitness in captive-reared fish (e.g. Le Luyer et al. 2017). While the common-garden design of this study means that all cross types would have experienced similar effects due to hatchery rearing, nevertheless it is important to note that epigenetic effects from early hatchery rearing could have impacted the performance of all fish, compared to if they had lived their whole lives in the wild.

While studying the impacts of farmed-wild Atlantic Salmon introgression in the wild has direct implications for predicting and mitigating threats of salmon aquaculture on wild salmon populations, *S. salar* have also come to be considered a model organism for studying genetic aquaculture-wild interactions in general (Glover et al. 2017). Previous research has shown that other cultured species do escape and can survive for a period of time in the wild following escape, such as barramundi (*Lates calcarifer*) (Noble et al. 2014) and European sea bass (*Dicentrarchus labrax*) (Toledo Guedes et al. 2009), though it is currently unknown as to whether these escapees successfully hybridize with wild populations. There is also the potential

for “escape through spawning” for some groups such as gadoids, whereby eggs and sperm produced by adults in sea cages flow through the cage to the surrounding environment (e.g. Uglem et al. 2012). Even species used in aquaculture that are not the target production species, such as corkwing wrasse (*Symphodus melops*) used as cleaner fish in salmon aquaculture, have been found to escape and hybridize with surrounding wild populations (Faust et al. 2018). Overall, Teletchea and Fontaine (2014) reviewed 250 aquaculture species and classified them all as having some level of domestication, with 30% classified as highly domesticated. Though aquaculture species may differ greatly from one another, Atlantic Salmon have been a key reference species from which to compare and make predictions of wild-farm introgression for other species (e.g. Bekkevold et al. 2006). Thus, the more researchers know about the already-existing issue of farm-wild interactions in Atlantic Salmon, the greater the ability to forecast issues related to potential escapes of up-and-coming aquaculture species.

Finally, work such as this on wild-farm interactions of aquaculture species may be applicable to the broader topic of invasive species, which are considered one of five main threats to global biodiversity according to the Millennium Ecosystem Assessment (MA 2005). While the interactions of domesticated individuals with their wild conspecifics are not explicitly addressed in the MA, this type of interaction is becoming a more pressing problem as human dependence on organisms raised in captivity increases (e.g. Laikre et al. 2010). Indeed, in addition to the traditional definition of “invasive” or “alien” (species that are invading an area outside of the range of their wild conspecifics), Laikre et al. (2010) identify three other categories of invaders, all of which involve the invasion of organisms into an area where their wild conspecifics already occur. Much of the existing research on invasive species has focused on those that are non-native, rather than those that are the same species as the recipient wild populations, even when

invasions of the latter categories are often the most significant in many areas (Laikre et al. 2010). Human reliance on captively-raised organisms is likely to increase with the continued expansion of aquaculture and agriculture, as well as augmentation of wild populations with domesticated individuals, and it is likely prudent to consider the potential impacts of these domesticated individuals on wild conspecifics at the level of severity of the "traditionally-classified" invasive species. As such, research such as this contributes to the understanding of the Atlantic Salmon as a type of invasive species upon escape (especially since it has been previously suggested that farmed fish be considered a separate species, *Salmo domesticus* (Gross 1998)), and may help inform subsequent research on other domesticated species and their interactions with wild conspecifics.

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Appendices

Supplementary Table 2.1. Intercept and slope values for regression lines generated using results of generalized linear model (recapture weight) and linear model (recap length), and predicted recapture (Pred) weight and length at mean egg weight value (93.2 mg) with standard error (SE(pred)), for each site by cross type combination. Slope and intercept were calculated using coefficients from model results. Intercept value is theoretical recapture weight or length if mean egg weight was 0, and slope is the change in recapture weight (g) or length (mm) for every 10 mg increase in mean egg weight.

	Recapture Weight				Recapture Length			
Site x Cross	Intercept	Slope* 10	Pred	SE(pred)	Intercept	Slope* 10	Pred	SE(pred)
1 Wild	0.29	0.06	0.836	0.0353	32.17	1.20	43.4	0.659
2 Wild	0.61	0.08	1.33	0.0407	43.31	0.79	50.7	0.477
3 Wild	0.65	0.08	1.391	0.0484	39.12	1.25	50.7	0.544
1 Farm	1.26	-0.02	1.042	0.049	48.641	-0.23	46.5	0.747
2 Farm	1.77	-0.03	1.48	0.0516	54.54	-0.27	52.1	0.548
3 Farm	0.89	0.11	1.911	0.065	47.07	0.91	55.6	0.547
1 F♀hyb	-1.07	0.22	0.997	0.0566	18.77	2.89	45.7	0.952
2 F♀hyb	-0.43	0.19	1.374	0.0509	27.18	2.54	50.9	0.61
3 F♀hyb	-3.14	0.49	1.47	0.0714	-3.46	5.86	51.1	0.716
1 W♀hyb	0.96	0.0001	0.965	0.0367	42.28	0.35	45.5	0.6
2 W♀hyb	1.87	-0.04	1.535	0.039	56.29	-0.36	52.9	0.402
3 W♀hyb	1.35	0.07	2.023	0.0609	48.61	0.91	57.1	0.47

Supplementary Table 2.2. Mean number of parr marks (at grand mean of standard length= 45.2 mm), mean length, and mean width of marks in mm (at grand mean ln(standard length)= 3.08 mm), for each cross type by site combination. SE is the standard error of the mean estimate. Emmeans are estimated marginal means, calculated using the *emmeans* package in R.

		Mean Number of Marks		Mean Length of Marks		Mean Width of Marks	
Cross type	Site	emmean	SE	emmean	SE	emmean	SE
Wild	1	9.56	0.63	0.53	0.05	0.61	0.06
Farm	1	9.92	0.54	0.48	0.04	0.49	0.05
F♀hyb	1	8.85	0.37	0.49	0.03	0.62	0.04
W♀hyb	1	8.81	0.75	0.64	0.06	0.66	0.07
Wild	2	8.18	0.39	0.61	0.03	0.72	0.04
Farm	2	9.04	0.38	0.58	0.03	0.63	0.04
F♀hyb	2	8.92	0.36	0.56	0.03	0.70	0.03
W♀hyb	2	8.84	0.42	0.57	0.04	0.69	0.04
Wild	3	9.48	0.36	0.62	0.03	0.72	0.03
Farm	3	9.16	0.44	0.62	0.04	0.67	0.04
F♀hyb	3	9.38	0.39	0.59	0.03	0.69	0.04
W♀hyb	3	7.42	0.58	0.67	0.05	0.74	0.06

Supplementary Table 3.1. Percent contributions of variables to principal components (PCs) 1 and 2 (respectively) for PCA on prelease samples, arranged descending from largest to smallest. Total lipids are concentrations in mg/g wet weight, while all other lipid classes and fatty acids are percentages. Abbreviations of lipids/FAs mentioned in text are as follows: LNA= linoleic acid; ALA= alpha-linolenic acid; ARA= arachidonic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid; ST= sterols, TAG= triacylglycerols; PL= phospholipids.

PC1		PC2	
Variable	% Contribution	Variable	% Contribution
18:4n-3	7.834558	16:1n-7	15.35748
18:0	7.742916	18:1n-7	13.89563
20:1n-11	7.615917	ARA (20:4n-6)	11.1761
TAG	7.537587	18:1n-9	9.400977
22:1n-11	6.657112	Total Lipids	9.189708
LNA (18:2n-6)	6.641535	24:1	7.536155
DHA (22:6n-3)	6.50567	22:5n-3	6.143107
ALA (18:3n-3)	5.908122	EPA (20:5n-3)	5.524863
14:0	5.539188	ST	5.371723
PL	5.082461	DHA (22:6n-3)	3.753628
20:1n-9	4.994613	Free Fatty Acids	3.20305
16:0	3.822074	14:0	2.106247
20:4n-3	3.756555	ALA (18:3n-3)	1.790698
EPA (20:5n-3)	3.394448	TAG	1.626132
AMPL	3.2023	18:0	1.020828
Free Fatty Acids	2.788156	AMPL	0.464088
22:5n-3	2.454272	Ketones	0.461352
ARA (20:4n-6)	2.08732	LNA (18:2n-6)	0.369733
16:1n-7	1.936484	20:4n-3	0.360206
ST	1.627878	16:0	0.337908
18:1n-9	1.144216	i16:0	0.271679
24:1	1.003136	22:1n-11	0.263572
18:1n-7	0.615653	20:1n-11	0.211972
Ketones	0.074062	20:1n-9	0.07066
Total Lipids	0.017357	PL	0.047413
i16:0	0.016408	18:4n-3	0.045099

Supplementary Table 3.2. ANOVA table results from linear model on release PC1 scores by cross type.

Source	df	SS	MS	F	p-value	LR
Weight	1	277.902	277.902	179.6682	1.224e-12	5.89E+10
Cross type	2	21.116	10.558	6.8258	0.004499	44.37519
Weight*Cross type	2	1.218	0.609	0.3938	0.678759	0.060133
Residuals	24	37.122	1.547			

Supplementary Table 3.3. ANOVA table results from linear model on PC2 scores by cross type.

Source	df	SS	MS	F	p-value	LR
Weight	1	1.833	1.8334	0.6657	0.422576	0.356539
Cross type	2	62.096	31.0482	11.2737	0.000353	916.9074
Weight*Cross type	2	3.077	1.5384	0.5586	0.579267	0.073319
Residuals	24	66.097	2.754			

Supplementary Table 3.4. Lipid classes and fatty acids at release with between-cross type dissimilarities that are an important component of overall dissimilarity in lipid/FA profiles, as determined by their low (<5%) probability (p) of having a contribution to dissimilarity as or more extreme than their calculated contribution when cross types were randomized over 999 permutations (bolded). "Avg. Contrib" indicates the average contribution of each lipid class/FA to overall dissimilarities in profiles, and "sd" indicates the standard deviation of this average. Abbreviations of lipids/FAs mentioned in text are as follows: LNA= linoleic acid; ALA= alpha-linolenic acid; ARA= arachidonic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid; ST= sterols, TAG= triacylglycerols; PL= phospholipids.

Comparison	Lipid/FA	Avg. Contrib	sd	p
Wild:Farm	**PL**	**0.040252**	**0.025848**	**0.001**
	TAG	**0.033293**	**0.013969**	**0.001**
	ST	**0.015088**	**0.008499**	**0.004**
	DHA (22:6n-3)	**0.01234**	**0.005317**	**0.001**
	Free Fatty Acids	0.008334	0.005144	0.006
	AMPL	0.007884	0.004956	0.018
	16:0	0.005808	0.002031	0.001
	LNA (18:2n-6)	0.004687	0.001727	0.001
	18:0	0.004041	0.001646	0.001
	22:1n-11	0.003898	0.001257	0.001
	EPA (20:5n-3)	0.003077	0.001401	0.001
	14:00	0.002539	0.000952	0.001
	ALA (18:3n-3)	0.002491	0.001096	0.001
	20:1n-11	0.002127	0.000729	0.001
	20:1n-9	0.00212	0.001347	0.001
	22:5n-3	0.002075	0.00113	0.001
	18:4n-3	0.001992	0.000639	0.001
	20:4n-3	0.001293	0.000617	0.001
Wild:F♀hyb	16:0	0.005494	0.002236	0.001
	EPA (20:5n-3)	0.0035	0.001589	0.001
	22:5n-3	0.001742	0.001295	0.003
	20:4n-3	0.001366	0.000663	0.001
	24:01	0.001211	0.000538	0.001
	i16:0	0.000949	0.001525	0.023
	18:1n-7	0.000926	0.000608	0.003
Farm:F♀hyb	**PL**	**0.034144**	**0.020867**	**0.044**
	TAG	**0.027307**	**0.013302**	**0.002**
	DHA (22:6n-3)	**0.011143**	**0.005425**	**0.001**
	AMPL	**0.010163**	**0.005119**	**0.001**
	16:1n-7	0.004495	0.001467	0.001
	14:0	0.003388	0.001	0.001
	18:0	0.002785	0.001264	0.027

	22:1n-11	0.002647	0.000804	0.004
	18:4n-3	0.001367	0.000488	0.008
	ARA (20:4n-6)	0.001339	0.000859	0.001

Supplementary Table 3.5. Percent contributions of variables to PCs 1 and 2 (respectively) for PCA on recapture samples, arranged descending from largest to smallest. Abbreviations of lipids/FAs mentioned in text are as follows: LNA= linoleic acid; ALA= alpha-linolenic acid; ARA= arachidonic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid; ST= sterols, TAG= triacylglycerols; PL= phospholipids.

PC1		PC2	
Variable	% Contribution	Variable	% Contribution
TAG	6.704354	20:1n-11	20.73797
ARA (20:4n-6)	6.658696	17:0	18.34883
16:1n-7	6.463627	16:0	17.63173
ALA (18:3n-3)	6.41018	18:1n-7	13.92198
DHA (22:6n-3)	6.18734	18:0	5.973421
PL	6.087601	18:1n-9	4.348898
LNA (18:2n-6)	5.957688	16:2n-4	3.557213
18:4n-3	5.502389	20:3n-6	1.858803
14:0	5.45751	20:4n-3	1.678588
22:5n-3	5.162682	22:5n-6	1.619533
17:1	4.92662	DHA (22:6n-3)	1.481693
ST	4.514212	18:4n-3	1.03751
22:5n-6	4.503575	14:0	1.008875
16:2n-4	4.193102	LNA (18:2n-6)	0.974114
EPA (20:5n-3)	3.770467	22:5n-3	0.919252
22:4n-6	3.528662	TAG	0.785116
Total Lipids	3.509137	ALA (18:3n-3)	0.776142
18:1n-7	1.894868	PL	0.671133
18:0	1.71312	22:4n-6	0.596883
16:0	1.651763	ST	0.418142
17:0	1.574871	Free Fatty Acids	0.368206
Free Fatty Acids	1.533252	ARA (20:4n-6)	0.321151
18:1n-9	0.853227	Total Lipids	0.311519
20:4n-3	0.736411	17:1	0.281754
20:3n-6	0.264828	EPA (20:5n-3)	0.207153
20:1n-11	0.177834	22:1n-11	0.089003
AMPL	0.058949	AMPL	0.058946
22:1n-11	0.003034	16:1n-7	0.016436

Supplementary Table 3.6. ANOVA table results from linear model on PC1 scores by cross type for recapture samples.

Source	df	SS	MS	F	p-value	LR
Weight	1	160.827	160.827	19.6554	0.0001081	255.5776
Cross type	3	122.149	40.716	4.9761	0.0062073	28.11577
Weight*Cross type	3	15.098	5.033	0.6151	0.6104338	0.025643
Residuals	31	253.654	8.182			

Supplementary Table 3.7. ANOVA table results from linear model on PC2 scores by cross type for recapture samples.

Source	df	SS	MS	F	p-value	LR
Weight	1	0.468	0.4683	0.1754	0.6782	0.33662
Cross type	3	12.077	4.0256	1.508	0.2319	0.208599
Weight*Cross type	3	9.36	3.1198	1.1687	0.3375	0.067104
Residuals	31	82.757	2.6696			

Supplementary Table 3.8. Lipid classes and fatty acids at recapture with between-cross type dissimilarities that are an important component of overall dissimilarity in lipid/FA profiles, as determined by their low (<5%) probability (p) of having a contribution to dissimilarity as or more extreme than their calculated contribution when cross types were randomized over 999 permutations. "Avg. Contrib" indicates the average contribution of each lipid class/FA to overall dissimilarities in profiles, and "sd" indicates the standard deviation of this average. Abbreviations of lipids/FAs mentioned in text are as follows: LNA= linoleic acid; ALA= alpha-linolenic acid; ARA= arachidonic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid; ST= sterols, TAG= triacylglycerols; PL= phospholipids.

Comparison	Lipid/FA	Avg. Contrib	sd	p
W♀hyb:Wild	18:00	0.001618	0.001116	0.017
W♀hyb:Farm	**PL**	**0.041626**	**0.027219**	**0.049**
	ALA (18:3n-3)	0.008555	0.005142	0.018
	ARA (20:4n-6)	0.005802	0.003642	0.007
	16:1n-7	0.005026	0.003634	0.022
	14:00	0.001465	0.001017	0.013
	22:4n-6	0.001292	0.000844	0.003
	17:01	0.001272	0.000797	0.018
	AMPL	0.000685	0.001071	0.01
W♀hyb:F♀hyb	18:1n-7	0.005898	0.006142	0.036
Wild:Farm	**PL**	**0.041159**	**0.026608**	**0.046**
	22:4n-6	0.001295	0.00078	0.005
Wild:F♀hyb	**Free Fatty Acids**	**0.010945**	**0.007251**	**0.005**
Farm:F♀hyb	**TAG**	**0.067678**	**0.041965**	**0.008**
	PL	**0.045097**	**0.029544**	**0.013**
	ALA (18:3n-3)	0.009298	0.005727	0.005
	ARA (20:4n-6)	0.005953	0.003646	0.008
	16:1n-7	0.005015	0.003051	0.038
	LNA (18:2n-6)	0.004794	0.003351	0.041
	22:5n-6	0.001721	0.000966	0.038
	18:4n-3	0.001543	0.000903	0.007
	22:4n-6	0.001542	0.000769	0.001
	16:2n-4	0.000994	0.000702	0.034