EVALUATING THE CONSEQUENCES OF HYBRIDIZATION AMONG DIVERGENT FARMED AND WILD ATLANTIC SALMON (*SALMO SALAR*) POPULATIONS

by

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ABSTRACT

Multi-generation domestication selection and distinct geographic and ancestral relationships have raised concerns about potential genetic and ecological interactions between escaped farmed and wild populations. In Newfoundland (NF), Canada, most aquaculture sites use North American (NA) Saint John River strain. However, recently, site-specific permission has been approved to farm a strain of European origin (EO). It has already been documented that if reproductively viable farmed EO salmon escape, it is likely that they will be able to breed successfully and interact genetically and ecologically with local wild populations. In my thesis, using common-garden experiments, I assessed the consequences of interbreeding of divergent EO and NA farmed with NF wild salmon populations. Firstly, in chapter two, I compared a series of early-life fitness-related traits (e.g., development time, size, growth, survival) among them. I then (in chapter three) examined their gene expression profiles at the late yolk sac fry stage, using 44K microarrays and qPCR validation. Subsequently, at the juvenile stage (in chapter four), using two complementary experiments, I investigated their fitness-related traits (e.g., dominance, growth, and survival) differences across the contrasting tank and stream environments. Finally, in chapter five, I compared their behaviour in four different contexts (e.g., exploration, response to a novel object, boldness under predation risk, and aggression). Significant differences were observed in early-life development time, survival, growth, and energy conversion among farmed, F_1 hybrid, and wild populations. All pure farmed strains and wild populations differed among themselves, but I found few differences in fitness-related traits between F_1 hybrids and their maternal wild/farmed strains. The late

yolk sac fry gene expression study indicates that the wild population showed greater transcriptome differences from the EO farmed strain than that of NA farmed strain. I also found the largest differences in global gene expression between the two farmed strains. I detected fewer significantly differentially expressed transcripts between F_1 hybrids and domesticated/wild maternal strains. At the juvenile stage, I found Farm.NA fish were more dominant and less subordinate than NF wild conspecifics, with hybrids being intermediate, not differing from wild fish. Farm.EO fish also tended to dominate NF wild fish. I did not find any differences in the growth of wild fish in sympatry versus allopatry in the tank environment. However, in the stream environment, wild fish in sympatry with Farm.NA and hybrids fish outgrew those in allopatry. Within sympatric treatments, both EO and NA farmed fish similarly outgrew wild fish in the tank environment, but not necessarily always in the stream environment (e.g., Farm.NA). F_1 hybrids tended to display intermediate growth performance relative to farmed and wild fish both in tank and stream environments. No survival differences were detected among cross types both in tank and stream environments. I also found both NA and EO farmed fish were equally more explorative, responsive to a novel object, bold, and aggressive than wild fish and related hybrids. Overall, these findings suggest that early-life fitness-related trait differences among fish of EO and NA farmed, NF wild, and related F_1 hybrid origins are generated by the geographic and ancestral relationship and maternal effects of egg size, but later stage juvenile fitness-related trait differences are mainly generated by domestication selection. Also, the gene transcriptome and fitness-related trait findings suggest that the consequences of hybridization would be greater from escaped EO farmed than NA farmed salmon and may have effects on productivity and viability for local NF populations.

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LIST OF ABBREVIATIONS

°N	Degree-North
°W	Degree-West
µm	Micrometer
actb	Beta-actin
ahcy	Adenosylhomocysteinase
AIC	Akaike Information Criterion
aldoa	Fructose-bisphosphate aldolase A
alg8	Probable dolichyl pyrophosphate alpha-1,3-glucosyltransferase
ANOVA	Analysis of variance
aRNA	Amplified RNA
bglap	Bone Gla protein
BLAST	Basic Local Alignment Search Tool
BLASTn	Nucleotide BLAST
BLASTx	Translated nucleotide sequence searched against protein sequences
BP	Biological process
c1ql2	Complement C1q-like protein 2
c1ql4	Complement C1q-like protein 4
c1qtnf3	Complement C1q tumor necrosis factor-related protein 3
CA	Canada
ca.	Chartered Accountant
cald1	Caldesmon
CC	Cellular component
ccl19	C-C motif chemokine 19
cd8	T-cell surface glycoprotein chain
cDNA	Complementary DNA
cebpd	CCAAT/enhancer-binding protein delta
cGRASP	consortium for Genomic Research on All Salmonids Project
chia	Acidic mammalian chitinase
ckm	Creatine kinase M-type
clec4m	C-type lectin domain family 4 member M
cm	Centimeter
col10a1	Collagen alpha-1(X) chain
col2a1	Collagen alpha-1(II) chain
comt	Catechol O-methyltransferase
contigs	contiguous sequences
csrp1	Cysteine and glycine-rich protein 1
C_T	Cycle Threshold value

ctsa	Cathepsin A
ctsl	Procathepsin L
Cy3	Cyanine3 Fluor
Cy5	Cyanine5 Fluor
cyp27a1	Cytochrome P450 27
d.f	Degrees of freedom
Dam	Paternal identity
dazap1	DAZ-associated protein 1
DD	Degree-Days
Den DF	Denominator Degrees of Freedom
DEPs	Differentially Expressed Probes
DNA	Deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphate
DTT	Dithiothreitol
ef1a1	Elongation factor 1 alpha-1
ef1a2	Elongation factor 1 alpha-2
ehhadh	Peroxisomal bifunctional enzyme
eif3d	Eukaryotic translation initiation factor 3 subunit D
endod1	Endonuclease domain-containing 1 protein
eno1	Alpha-enolase
EO	European origin
ESTs	Expressed Sequence Tags
F	F-value
fads6	Fatty acid desaturase 6
Farm (EO)	European farmed strain
Farm (NA)	North American farmed strain
FDR	False Discovery Rate
fel	Fish-egg lectin
F_{st}	A measure of population differentiation
g	Gram
gapdh	Glyceraldehyde-3-phosphate dehydrogenase
gatb	Glutamyl-tRNA(Gln) amidotransferase subunit B, mitochondrial
GEO	Gene Expression Omnibus
GLMM	Generalized Linear Mixed-effects Model
gmfb	Glia maturation factor beta
gmpr	GMP reductase
GO	Gene Ontology
GOIs	Genes of interest
gpd1	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic

gpx4	Glutathione peroxidase
GTEA	GO term enrichment analyses
hadhb	Trifunctional enzyme subunit beta, mitochondrial
hamp1	Hepcidin-1
hba1	Hemoglobin subunit alpha
hbb	Hemoglobin subunit beta
hbe1	Hemoglobin subunit epsilon
hpdl	4-hydroxyphenylpyruvate dioxygenase-like protein
hpx	Hemopexin
Hyb ($NA_{f♀}$)	F_1 Farm (EO)(♂)-x-Wild (NA_{EO})(♀) hybrid
Hyb 15 ($EO_{f♀}$)	Farm (EO)(♀)-x-Wild (NA_{EO})(♂) hybrid
Hyb 15 ($EO_{w♀}$)	Farm (NA)(♀)-x-Wild (NA_{EO})(♂) hybrid
Hyb16 ($EO_{f♀}$)	Farm.EO (♀)-x-Wild (NA) (♂) hybrid
Hyb16 ($EO_{w♀}$)	Farm.EO (♂)-x-Wild(NA) (♀) hybrid
ighv3-33	Immunoglobulin heavy variable 3-33
K	Fulton's body condition factor
Lat	Latitude
LME	Linear Mixed Effect
Lon	Longitude
Lower. CL	Lower Confidence Limit
LR	Likelihood Ratio
LRTs	Likelihood Ratio Tests
ltbp4	Latent-transforming growth factor beta-binding protein 4
mcoln2	Mucolipin-2
MF	Molecular function
mg	Miligram
mgat2	Mannoside acetylglucosaminyltransferase 2
mgl^{-1}	Miligram per liter
min	Minute
M-MLV	Moloney Murine Leukemia Virus
mRNA	messenger Ribonucleic acid
MS-222	AQUALIFE Tetramethylsilane
mt-co1	Cytochrome c oxidase subunit 1
mt-co2	Cytochrome c oxidase subunit 2
mtmr6	Myotubularin-related protein 6
mybph	Myosin-binding protein H
n	Number
NA	North American origin

NB	New Brunswick
nckap1	Nck-associated protein 1
ND-1000	NanoDrop-1000
NF	Newfoundland
NL	Newfoundland and Labrador
nlk	Nemo-like kinase
nM	nanomole
nr/nt	non-redundant amino acid
NS	Not Significant
NSERC	Natural Sciences and Engineering Research Council of Canada
NTC	No-template control
Num DF	Numerator Degrees of Freedom
ºC	Degree-Celcius
OFI	Ocean Frontier Institute
OSC	Ocean Sciences Centre
p	p-value
pabpc1	Polyadenylate-binding protein 1
pappa2	Pappalysin-2
pbx2	Pre-B-cell leukemia transcription factor 2
PC	Principal Component
pdk3	[Pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 3, mitochondrial
per se	in itself
pers. comm	Personal communication
PFP	Percentage False Positive
pgrmc1a	Membrane-associated progesterone receptor component 1
PMT	Photomultiplier Tube
prcp	Lysosomal Pro-X carboxypeptidase
pygm	Glycogen phosphorylase, muscle form
qPCR	quantitative polymerase chain reaction
Q-Q plot	Quantile-Quantile plot
RNA	Ribonucleic acid
RP	Rank Products
rpl32	60S ribosomal protein 32
rpsa	40S ribosomal protein SA
RQ	Relative Quantity
rras2	Ras-related protein R-Ras2
rsad2	Radical S-adenosyl methionine domain-containing protein 2
s	Second
SAM	Significance Analysis of Microarrays

SE	Standard Errors
senp7	Sentrin-specific protease 7
SGR	Specific Growth Rate
Sire	Maternal identity
slc25a20	Mitochondrial carnitine/acylcarnitine carrier protein
sod1	Superoxide dismutase [Cu-Zn]
spp.	Species
sult6b1	Sulfotransferase 6B1
Sum Sq	Sum of Squares
t	t-value
tcn1	Transcobalamin-1
tcp1	T-complex protein 1 subunit alpha
TIFF	Tag Image File Format
timp2	Metalloproteinase inhibitor 2
Upper. CL	Upper Confidence Limit
USA	United States of America
UV	Ultraviolet
vrk1	Vaccinia-related kinase 1
Wild (NA)	Newfoundland wild population (Garnish River origin)
Wild (NA_{EO})	Newfoundland wild population with a signal of EO ancestry
YCE	Yolk sac Conversion Efficiency
z	Wald z-statistics
χ^2	The value of the chi-square statistics

CO-AUTHORSHIP STATEMENT

As the primary author, I contributed to the conception, planning, data collection, data analyses, and writing for all of my thesis chapters. Similarly, Dr. Ian Fleming contributed to the conception, planning, data analyses, and writing/editing of all manuscripts. Dr. Brendan W. Wringe assisted with the initial establishment of the crosses, and editing of chapters 2, 4, and 5 manuscripts. Dr. Ian R. Bradbury contributed to the conception, and editing of chapters 2, 3, 4, and 5 manuscripts. Drs. Matthew L. Rise, Albert Caballero-Solares, and Xi Xue contributed to experimental design, analysis of the genomics pipeline used, and editing of chapter 3. Dr. Kristin Bøe and Corinne M. Conway assisted with the initial establishment of the crosses of chapter 2 and chapter 4, respectively.

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CHAPTER 1: Introduction

1.1 Introduction

Atlantic salmon (*Salmo salar*) is one of the most economically and ecologically important fish species, and its aquaculture plays a global role in the blue revolution. The commercial production of Atlantic salmon for human consumption first started in the late 1960s (Gjedrem, 2010), and global production is currently estimated at more than 2 million tonnes per annum (April et al., 2021). The phenomenal expansion of the salmon aquaculture industry has not occurred without meeting a diverse array of sustainability-related challenges along the way. Farmed escapees may result in both genetic (Ferguson et al., 2007; Glover et al., 2017) and ecological interactions (Fleming et al., 1996; Thorstad et al., 2008; Bradbury et al., 2020) with wild populations. Direct genetic interaction can arise through introgression, which increases gene flow by genetic mixing (Glover et al., 2017). Also, indirect genetic interaction may alter selective pressures and fitness, and lead to decreased survival, reduction in population size, and increased genetic drift, and can reduce a population's adaptive capacity (Glover et al., 2013; Verspoor et al., 2015; Castellani et al., 2018). The negative ecological interaction of farm progeny, both pure and hybrid, can arise through, for example, direct competition with wild fish (Einum & Fleming, 1997; Fleming & Einum, 1997; Fleming et al., 2000). Many of these factors, individually or collectively, have potentially significant negative consequences for the persistence of wild salmon populations.

Domestication involves adaptation to a captive environment, which is very different from the natural environment experienced by wild conspecifics. In the domestic

environment, intentional selection for production-related traits (e.g., faster growth, delayed maturation, disease resistance), combined with various inadvertent selection on non-target traits (e.g., aggression, risk aversion, feeding behaviour) can lead farmed salmon to show a wide range of phenotypic and behavioural differences relative to wild counterparts (Huntingford, 2004; Glover et al., 2018; Solberg et al., 2020). In contrast to farmed salmon, wild salmon populations are genetically structured, with significant differentiation at the scale of geographic regions, among river systems and within large river systems (Verspoor et al., 2005, Bourret et al., 2013). High homing allows populations to differentiate and potentially adapt to their environment by natural selection (Hindar et al., 2006, Garcia de Leaniz et al., 2007). The resultant gene flow from escaped farmed to wild populations could rapidly reduce the genetic differences and disrupt local adaptations (Taylor, 1991; Fraser et al., 2011). As a result, introgressive hybridization between escaped farmed and wild salmon can lead to reduced fitness and survival in the wild (McGinnity et al., 1997, 2003; Fleming et al., 2000; Skaala et al., 2012, 2019; Sylvester et al., 2019). Additionally, escaped farmed and resultant hybrid fish may compete with wild salmon for food and territories, thereby potentially depressing numbers and production of wild salmon (Fleming et al., 2000, McGinnity et al., 2003, Sundt-Hansen et al., 2015, Robertsen et al., 2019).

Understanding the fitness impact of hybridization between escaped farmed and wild salmon at early-life stages is crucial because they can have knock-on effects for various phenoytpes (e.g., growth and survival) and behavioural traits (e.g., dominance status and aggressiveness) at the latter stages. For example, various early-life traits (e.g., development time, size, growth) can have important fitness consequences (i.e., behaviour, growth, survival) for juvenile salmon (Metcalfe & Thorpe, 1992, Einum & Fleming, 2000a). At the

onset of exogenous feeding, when alevins emerge from the gravel, both large and early emerging offspring can have a competitive advantage in the wild, which in turn increases growth potential and survival probability (Einum & Fleming, 2000a). At emergence, competition for residency and nutritional resources is high, and phenology is crucial (Einum & Fleming, 2000b). However, spawning time in the wild is likely to be dependant on temporal resource availability to secure optimal time of hatch and emergence (Brannon, 1987). In contrast, the reduction in natural selective pressure in the domestic environment may allow for both early and late onset of spawning, and/or prolonged spawning time which may increase development time variance (Solberg et al., 2014). How the patterns of domestication selection in the aquaculture environment may impact early-life development time in salmon originating from differing strains and geographic regions remains unknown. Moreover, empirical evidence suggests that various fitness traits (e.g., body size, growth, survival) at the developmental stages are strongly influenced by both genetic and non-genetic maternal effects (Smith & Fretwell, 1974; Sargent et al., 1987, Einum & Fleming, 2000b, 2004). Empirical studies also suggest that there is a positive correlation between egg size and female size (Hendry et al., 2001; Hendry & Day, 2003; Rollinson & Rowe, 2016). Therefore, the relationships among female size, egg size, and offspring fitness (e.g., alevin and fry development, growth, and survival, and energy utilization patterns) at the population level and in the context of the impacts of escaped farmed salmon are still unclear.

Due to multi-generation domestication selection, farmed and wild salmon differ genetically, which raise concerns about potential genetic interactions and disruption of

local adaptation through introgression (Glover et al., 2017). However, when farm strains from different continents are used, we do not know if the genetic risks posed to local wild populations by escaped farmed fish will be greater than that when more geographically close farmed strains are used. Examining population-specific gene expression effects of interbreeding among divergent farmed, wild and F_1 hybrids under a common-garden environment is one of the best ways to explore the consequences of hybridization. Although a handful of genomic studies have investigated global gene expression profiles of farmed and wild salmon, and revealed a large number of differentially expressed genes (Roberge et al., 2006; Bicskei et al., 2014, 2016), none of them have studied gene transcription effects of hybridization, while comparing population-specific differences among divergent farmed, wild and F_1 hybrid salmon. Studies of gene expression in Atlantic salmon have identified processes that may be linked with domesticated-associated evolutionary changes. For example, processes that involve environmental information processing and signalling pathways and immune-relevant pathways have been reported to be more highly expressed in wild than farmed salmon (Bicskei et al., 2014, 2016). On the other hand, processes linked to metabolism and protein synthesis have been demonstrated to be upregulated in farmed strains compared to wild populations (Roberge et al., 2006; Bicskei et al., 2014). Overall, the degree to which the changes in these processes reflect genomic differences due to domestication selection among multiple divergent salmon strains remains unquantified. Furthermore, the transition stage from endogenous to exogenous feeding is a critical stage for Atlantic salmon's fitness (e.g., metabolism, development, growth, immune system, and survival). Gene expression studies at early life stages comparing divergent farmed, wild,

F_1 hybrid salmon can, therefore, provide insight into the fitness consequences of hybridization between escaped farmed and their wild conspecifics.

Behaviour represents one of the major areas where the trait differences between farmed and wild salmon have been observed. Behavioural traits are highly important for juvenile salmon in the wild environment, enabling individuals to be able to compete for resources such as territories and food while avoiding predation (Solberg et al., 2020). Behavioural changes have been linked directly or indirectly with the process of domestication selection in salmon (Huntingford, 2004). However, it has been suggested that the direction of behavioural response is likely to be specific to the conditions in which the domestication selection was imposed, and therefore, which behaviour favours access to and use of resources under the context-specific conditions (Ruzzante, 1994). For example, domesticated fish species, such as farmed Atlantic salmon, may exhibit a behavioural advantage over their wild conspecifics in the domestic environment (Einum & Fleming, 1997). Moreover, social interaction and hierarchies are well documented in salmonids (Ruzzante 1994; Huntingford, 2004), where body size can affect the outcome of the competition and can provide faster-growing farmed salmon with a further competitive advantage in an environment with little or no predation risk (Abrahams & Sutterlin, 1999; Biro et al., 2004, 2006). As a whole, bigger, bolder, and dominant salmonid fish may get better access to food and territories than smaller, shy, and submissive fish (Sundstróm et al., 2004). This may, in turn, result in a competitive advantage in a wild environment (Fleming et al., 2000; McGinnity et al., 2003; Skoglund et al., 2011). Limited research, to date, has been conducted to evaluate dominance status among farmed, wild, and related

hybrid fish, particularly in the context of distinct geographical and ancestral relationships and domestication selection.

Moreover, the fitness trait exhibiting the largest and most consistent difference between farmed and wild salmon is growth. Selection for increased growth rate has been the backbone of aquaculture breeding programmes (Gjedrem, 2000, 2010), and it is thus expected that this trait displays the greatest divergence. Recent studies typically reveal between 2 to 3-fold differences in size at age between farmed and wild Atlantic salmon when reared together in fish tanks (Solberg et al., 2013a,b; Harvey et al., 2016a; Glover et al., 2018). However, growth is highly plastic in salmon, suggesting the existence of differences in reaction norms between domesticated and wild salmon (Solberg et al., 2013a,b; Harvey et al., 2016b; Solberg et al., 2016), and in the natural environment, growth of farm salmon, is only marginally higher than that of wild salmon (Fleming et al., 2000; Skaala et al., 2012; Glover et al., 2018). This may be driven by a difference in energy budgets between the two environments. For example, the natural diets can vary considerably in terms of type and form of prey, the density of calories, and nutrient composition (Jonsson et al., 1998a, b) and may be limited in the natural environment, thus limiting the energy available to farmed salmon to utilize their high genetic growth-potential and outgrow wild salmon. Moreover, domesticated salmon displaying the highest growth potential may be more susceptible to predation in the wild than those displaying lower growth potential (Glover et al., 2018). Such a selection mechanism could result in more similar growth among surviving farm and wild salmon in the natural environment. Although some of the selection during the freshwater phase is density-dependent (Jonsson et al., 1998a), studies have suggested that deviating growth-potential mortality may

influence the growth relationship between farm and wild salmon (Solberg et al., 2013b), and that growth potential may be negatively correlated with survival in the wild (Biro et al., 2004, 2006; Tymchuck et al., 2007).

Farmed and wild salmon often display consistent differences in behaviour across situations and contexts. Such behavioural differences are likely to arise due to directed and inadvertent selection in farmed salmon. In particular, fish from farmed stocks tend to be bolder and to take greater risks when foraging (Huntingford & Adams, 2005). They may also be aggressive, depending both on conditions during selection (Lahti et al., 2001). However, the extent of risk posed by escaped farmed and resultant hybrids to wild populations mediated by behavioural interaction will be dependent on the relationship between farm strains and wild populations resulting from domestication selection and the ancestral relationship of farm strains to wild populations. Comparative studies of behavioural trait differences across different contexts (e.g., exploration in an unfamiliar environment, novel object response, boldness under predation risk, and aggressiveness) and association among divergent farmed strains and wild populations can provide valuable insights into how this might operate.

Hybridization between escaped farmed and wild salmon has been extensively documented throughout Atlantic Canada (Keyser et al., 2018; Wringe et al., 2018; Sylvester et al., 2019). Ultimately, the impact of introgression of domestic escapes in wild populations depends on the degree of differentiation between domestic and wild populations which arises due to both the process of domestication and the sources of strains chosen from domestication (Baskett et al., 2013). In the Northwest Atlantic, to date all aquaculture companies have been using North American (hereafter “NA”) farmed strains

primarily originating from the Saint John River, New Brunswick (NB), Canada, although European sourced farmed salmon have been used historically in Maine, and will be used in Placentia Bay, Newfoundland (NF). Significant continental divergence between European (hereafter "EO") and NA wild salmon populations have been reported in both single loci and karyotype (i.e., Ssa01/Ssa23 translocation and Ssa08/Ssa29 fusion; Lehnert et al., 2019, 2020) due to trans-Atlantic isolation during Pleistocene glaciations (King et al., 2007; Nilsson et al., 2001; Rougemont & Bernatchez, 2018). Despite considerable evidence for trans-Atlantic divergence, genetic data has provided evidence of trans-Atlantic secondary contact in EO and NA wild populations both in Europe and North America (Bradbury et al., 2015). In southern Newfoundland, genetic and genomic data suggests postglacial secondary contact has occurred in the southeast and is primarily evident in populations on the southern Avalon Penisnula including Northeast Placentia River). Recently, in NF, permission has been approved to import a strain of EO farmed salmon (StofnFiskur), a Norwegian origin strain, to be farmed as triploids. It is already evident that the efficacy of the triploidization process is not 100% (Benfey, 2015), and if a proportion of non-triploid farmed EO salmon escape, it is likely that they will be able to breed successfully and interact genetically and ecologically with the local NF wild populations (O'Reilly et al., 2006). We do not know yet whether the resultant genetic and ecological impacts would be greater from escaped EO farmed than NA farmed (population differentiation, F_{st}> 0.44, between EO farmed and NA farmed; S.J. Lehnert, pers. comm.) to the NF local wild populations. It will depend on the specific nature of trait differences between the respective farmed strains and native wild populations.

1.2 Overall objectives of the thesis

In my thesis, I designed common-garden experiments (i.e., testing groups under common environmental conditions) to assess the consequences of ecological and genetic interactions among divergent EO and NA farmed, NF wild, and related F_1 hybrid salmon. The main objective of the second chapter of this thesis was to compare early-life fitness-related traits (e.g., development time, size, growth, and survival) of EO and NA farm strains with NF wild fish and conspecific hybrids. I hypothesized that: (i) both EO and NA farmed fish will exhibit similar patterns of fitness trait differences (development time, size, growth, and survival) relative to wild fish; (ii) the early-life fitness trait differences will be reflected by their geographic and ancestral relationships; (iii) hybrids will display altered fitness traits relative to wild and farmed fish; and (iv) the association between maternal effects of egg size and early-life fitness traits will differ among EO and NA farmed, NF wild salmon, and F_1 hybrids.

The overall objective in the third chapter of this thesis was to quantify the gene expression differences among EO and NA farmed, NF wild, and F_1 hybrid salmon at the late sac fry stage using 44K microarrays, combined with real-time quantitative polymerase chain reaction (qPCR) validation approaches. I hypothesized that: (i) due to a common multi-generation domestication effect, EO and NA farmed fish will exhibit some similar gene expression patterns; (ii) due to geographic and ancestral relationships, global gene expression patterns of NF wild fish will be more similar to that of NA farm than that of EO farm fish; and (iii) F_1 hybrids will display altered gene expression relative to wild and farmed fish. My main goal was to understand the potential genetic consequences of the

hybridization of EO and NA farmed escapees on NF wild populations to better guide sustainable aquaculture practices and the maintenance of wild populations.

For the fourth chapter of this thesis, using two complementary experiments, I investigated (a) differences in dominance status; and (b) growth and survival differences among divergent EO and NA farmed, NF wild, and related hybrids across contrasting tank and semi-natural stream environments. I tested the hypotheses that: (i) EO and NA farmed will be more dominant than NF wild salmon, and F_1 hybrids will be intermediate; (ii) the growth and survival of wild fish in allopatry will be higher than that of those in sympatry (i.e., competing with farmed and related hybrids); (iii) both EO and NA farmed and F_1 hybrids will display higher growth and survival than wild fish within sympatry; and (iv) multi-generation domestication selection and the geographic and ancestral relationships will be reflected in the growth, survival, and dominance status among divergent EO and NA farmed, NF wild and related hybrids.

The overall objective of the fifth chapter of this thesis was to measure how individual fish from NA and EO farmed strains behaved relative to NF wild fish and conspecific hybrids in four different contexts: exploration in an unfamiliar environment, response to a novel object, boldness under predation risk and levels of aggression. This experiment aimed specifically to understand both (i) variation in behavioural traits across individuals among populations and (ii) behavioural correlations (syndromes) within populations. I tested the hypotheses that: (i) multi-generation domestication selection in both EO and NA farm fish has resulted in similar directions of behavioural trait differences relative to wild fish; (ii) the geographic and ancestral relationships of the fish will be reflected in behavioural trait differences; (iii) interbreeding will cause hybrids to display

altered behaviours relative to wild and farmed fish; and (iv) behavioural syndromes within populations will differ reflective of their different selective histories (wild vs. domesticated).

1.3 References

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CHAPTER 2

Early-life fitness trait variation among divergent European and North American farmed and Newfoundland wild Atlantic salmon populations

Preface

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2.1 Abstract

It has long been clear that interbreeding between domesticated and wild Atlantic salmon can lead to negative fitness consequences for native populations. Few studies, however, have examined these consequences at critical early life stages, particularly in the context of distinct geographical and ancestral relationships among populations as well domestication selection. In Newfoundland (NF), Canada, while the majority of aquaculture sites use North American (NA) Saint John River strain, site-specific permission has been granted to farm a strain of European origin (EO). I designed a common-garden experiment to compare fitness-related traits (e.g., development time, survival, size, and growth) at different early-life stages (eye development, hatch, and yolk absorption) among EO and NA farmed, two NF wild and F_1 hybrid groups. Significant differences ($P < 0.001$) were observed in development time, survival, growth, and energy conversion among farmed, F_1 hybrid, and wild populations. While pure populations (farmed and wild) differed amongst one another, I found few differences in fitness-related traits between F_1 hybrids and their maternal wild/farmed strains. This suggests that the early-life fitness consequences of F_1 hybridization will be largely manifested through the action of maternal effects. Additionally, significant associations between the maternal effects of egg size and alevin development time, size, survival, growth, condition, and energy conversion efficiency were found. These findings suggest that early-life fitness-related trait differences among farmed, wild, and their related F_1 hybrids are generated by the geographic and ancestral relationship and maternal effects of egg size, and less so by domestication selection.

2.2 Introduction

It is increasingly clear that interactions between domesticated and wild organisms have the potential to lead to negative effects on wild populations, and as such, are of concern (Rhymer & Simberloff, 1996; Fleming & Petersson, 2001; Ellstrand, 2003; Laikre et al., 2010; Frankham et al., 2011; Glover et al., 2017). Successful interbreeding between domestic and wild conspecifics may result in the loss of adaptive genetic variation with maladaptive fitness consequences for wild populations. These fitness effects may become lasting within the wild population should the hybrids themselves successfully reproduce, leading to introgression (Edmands, 2002, 2007; Garcia de Leaniz et al., 2007; Reed et al., 2015). The Atlantic salmon (*Salmo salar*) is one of the species for which there are concerns that successful breeding of escaped domesticated individuals and the resultant hybridization with native individuals will cause ecological and genetic impacts on wild populations and threaten local adaptation (McGinnity et al., 1997, 2003; Fleming et al., 2000; Fraser et al., 2011; Skaala et al., 2012, 2019; Bradbury et al., 2020).

Farmed salmon are often genetically distinct from wild counterparts because of geographical origin (Gjedrem, 2010), founder effects (Skaala et al., 2006), genetic drift (Glover et al., 2012, 2013), and especially domestication selection in captivity (Solberg et al., 2013; Harvey et al., 2016). Directed selection for economically important traits (e.g., faster growth, delayed maturation, disease resistance), in combination with unintentional and relaxed selection on non-target traits (e.g., aggression, risk aversion, feeding behaviour) can lead to rapid genetic changes in farm strains (Einum & Fleming, 1997; Fleming & Einum, 1997; Huntingford & Adams, 2005; Houde et al., 2010a,b; Debes &

Hutchings, 2014; Perry et al., 2019; Solberg et al., 2020). This has, for example, resulted in farmed salmon displaying a growth rate that is over two- to three-fold higher than that of wild conspecifics reared under identical culture environments (Glover et al., 2018). In contrast to farm strains, wild salmon populations are genetically structured, with substantial genetic differences among populations at multiple spatial scales (Garcia de Leaniz et al., 2007; Verspoor et al., 2005; Bourret et al., 2013). Gene flow from escaped farmed to wild salmon could rapidly reduce the genetic diversity and local adaptation inherent in wild populations (Taylor, 1991; Hinder et al., 2006; Fraser et al., 2011). Introgressive hybridization between escaped farmed and wild salmon may then lead to reduced fitness in the wild (Sylvester et al., 2019). Cultured salmon have lower fitness in the wild, and empirical evidence has mounted that farmed-wild hybrids may also have reduced survival (McGinnity et al., 2003; Tymchuck et al., 2007; Skaala et al., 2012, 2019). Likewise, offspring of escaped farmed and hybrid fish may compete with wild salmon for food, habitat, and other resources, thereby potentially altering the genetics and depressing numbers and productivity of wild salmon (Fleming et al., 2000; McGinnity et al., 2003; Sundt-Hansen et al., 2015; Robertsen et al., 2019; Bradbury et al., 2020).

Understanding the ultimate impact of interbreeding between wild and escaped farm salmon requires measures of reproductive and post-reproductive success following interaction. For instance, the timing of (i.e., phenology) and size at early life stages can have important fitness consequences for juvenile salmonids. Juveniles that emerge relatively sooner or are larger at emergence can have a competitive advantage over smaller conspecifics in the establishment of a feeding territory, which in turn increases their growth opportunities and survival probability (Metcalfe & Thorpe, 1992; Einum & Fleming,

2000a). Hence, spawning and development time in wild populations is believed to be adapted to the seasonal resource availability in order to secure optimal time of hatch and alevin emergence (Brannon, 1987; Webb & McLay, 1996; Einum & Fleming, 2000a). In the domestic environment, on the other hand, the reduction in natural selective pressure may allow for both early and late onset of spawning and/or prolonged spawning time, which may increase development time variance (Solberg et al., 2014). How the patterns of domestication selection may impact early-life development time in salmon originating from differing strains and geographic regions remains unknown.

Empirical evidences suggest that traits expressed during early life tend to be influenced strongly by maternal effects (Sargent et al., 1987, Einum & Fleming, 1999, 2004; Houde et al., 2011, 2015; Thorn & Morbey, 2018). However, maternal influences on early-life history traits are driven by trade-offs faced by mothers and shaped by environmental conditions (Smith & Fretwell, 1974; Einum & Fleming, 2000b). Furthermore, the trade-off between egg size and fecundity can vary by female size (Hendry et al., 2001; Hendry & Day, 2003). These relationships among female size, egg size, and offspring fitness can be scaled up to generate hypotheses describing influences of selection on females and egg size at the population level. Taken together, a potentially complex set of interacting selective pressures act to maximize female reproductive fitness in terms of optimum number and size of eggs produced, that in turn influences offspring fitness through alevin and fry development, growth, and survival, and energy utilization patterns.

Hybridization is expected to lead to a reduction in fitness during early life stages with the increasing genetic divergence between parental populations, their origin, and

patterns of domestication (Edmands, 1999; Barton, 2001; Frankham et al., 2002; Neff, 2004; Baskett & Waples, 2013; Huisman & Tufto, 2013). At present, salmon aquaculture practices in Atlantic Canada are using farmed strains that originate from the Saint John River, NB. A recent approval has also been granted to allow culture, as triploids, of a farmed strain from Europe (hereafter "EO"), which was domesticated from wild Norwegian populations, to be farmed in Newfoundland (NF), Canada. Evidence indicates that the North American farmed strain (Saint John River strain; hereafter "NA") and EO farmed strain (Norwegian) are highly divergent genetically ($F_{st} > 0.40$; S.J. Lehnert, pers. comm.; Jeffery et al., 2018). Although phenotypic and genetic differences exist among wild salmon populations within NF (e.g., $F_{st} = 0.12$, between Northeast Placentia and Garnish River populations; S.J. Lehnert, pers. comm.), the divegence between NF wild and NA farmed salmon populations is larger ($F_{st} = 0.14 - 0.20$; see Bradbury et al., 2018). It has already been documented that if a portion of non-triploid EO farmed salmon escape and breed successfully, they will interact genetically and ecologically with wild populations (O'Reilly et al., 2006). The outcome of genetic and ecological interactions of EO farmed strain, compared to NA farmed strain, with local NF wild populations is still unknown and may depend on the genetic differences between these farm strains and local wild populations.

Here, I designed a common-garden experiment (i.e., examining cross types under common environmental conditions) to compare early-life development time, size, growth, and survival of EO and NA farm strains with NF wild fish and conspecific hybrids. I hypothesized that: (i) both EO and NA farm fish will exhibit similar patterns of fitness trait differences (development time, size, growth, and survival) relative to wild fish; (ii) the early-life fitness trait differences will be reflected by their geographic and ancestral

relationships; (iii) hybrids will display altered fitness traits relative to wild and farm fish; and (iv) the association between maternal effects of egg size and early-life fitness traits will differ among EO and NA farmed, NF wild salmon, and F_1 hybrids.

2.3 Materials & Methods

2.3.1 Parental populations and crosses

The experiment was conducted across two years with two cohorts of fish. Crosses were created using salmon gametes collected from four base populations (see Fig. 2.1 for the geographical origin of study populations; modified from Islam et al., 2020). Farm (EO) was a Norwegian farm strain produced in an Icelandic facility (StofnFiskur), that had been recently approved for aquaculture as triploids in southern NF. Diploid gametes were transported by air from Iceland to St John's, NF, and crosses were generated within 24 hours of stripping under the authority of an experimental permit. Farm (NA), a major aquaculture strain in Atlantic Canada, originated from the Saint John River, NB. Farm (NA) gametes were provided by Northern Harvest, a local aquaculture company with operations on the south coast of NF. The Wild population for the 2015 cohort, which derived from the Northeast Placentia River, NF (Lat: 47.2408 °N, Lon: 53.9566 °W) has a signal of EO ancestry (hereafter "Wild (NA_{EO})"), were captured at a fishway facility and transported on July 27, 2015, to the Ocean Science Centre (OSC; at Memorial University of Newfoundland) where they were held in broodstock tanks until performing the crosses. This population shows evidence of EO introgression because of historical trans-Atlantic straying and colonization in southeastern NF, Canada (~10,000 years before present; see Bradbury et al., 2015). The Wild population for the 2016 cohort (hereafter "Wild (NA)"),

which derived from the Garnish River, NF (Lat: 47.2348 °N; Lon: 55.3615 °W), were collected at the salmon fence facility and transported to the OSC on August 9, 2016, where they were held in broodstock tanks until the crossing. The 2015 cohort of crosses was generated between 20 November and 5 December 2015. Six cross types were generated to produce a total of 76 families: (i) 20 Farm (NA); (ii) 11 Wild (NA_{EO}); (iii) 13 F_1 Farm (NA)(♀)-x-Wild (NA_{EO})(♂) hybrid (referred to as "Hyb ($NA_{f♀}$)"); (iv) 10 Farm15 (EO); (v) 12 F_1 Farm (EO)(♂)-x-Wild (NA_{EO})(♀) hybrid (referred to as "Hyb15 ($EO_{w♀}$)"); and (vi) 10 F_1 Farm (EO)(♀)-x-Wild (NA_{EO})(♂) hybrid (referred to as "Hyb15 ($EO_{f♀}$)"). The 2016 cohort was generated on 27 November 2016. Four cross types were generated to produce in a total of 40 families: (i) 10 Farm16 (EO) (again gametes were collected from the Icelandic facility; the same strain which was used for the 2015 cohort); (ii) 10 Wild (NA); (iii) 10 F_1 Farm.EO (♂)-x-Wild(NA) (♀) hybrid (referred to as "Hyb16 ($EO_{w♀}$)"): and (iv) 10 F_1 Farm.EO (♀)-x-Wild (NA) (♂) hybrid (referred to as "Hyb16 ($EO_{f♀}$)") (see Fig. 2.2 for schematic crossing design). Crosses for the 2015 and 2016 cohorts were conducted and reared in Heath-tray incubation facilities at the OSC. Biological information about the parental salmon used in the crosses is provided in supplementary Table S2.1.

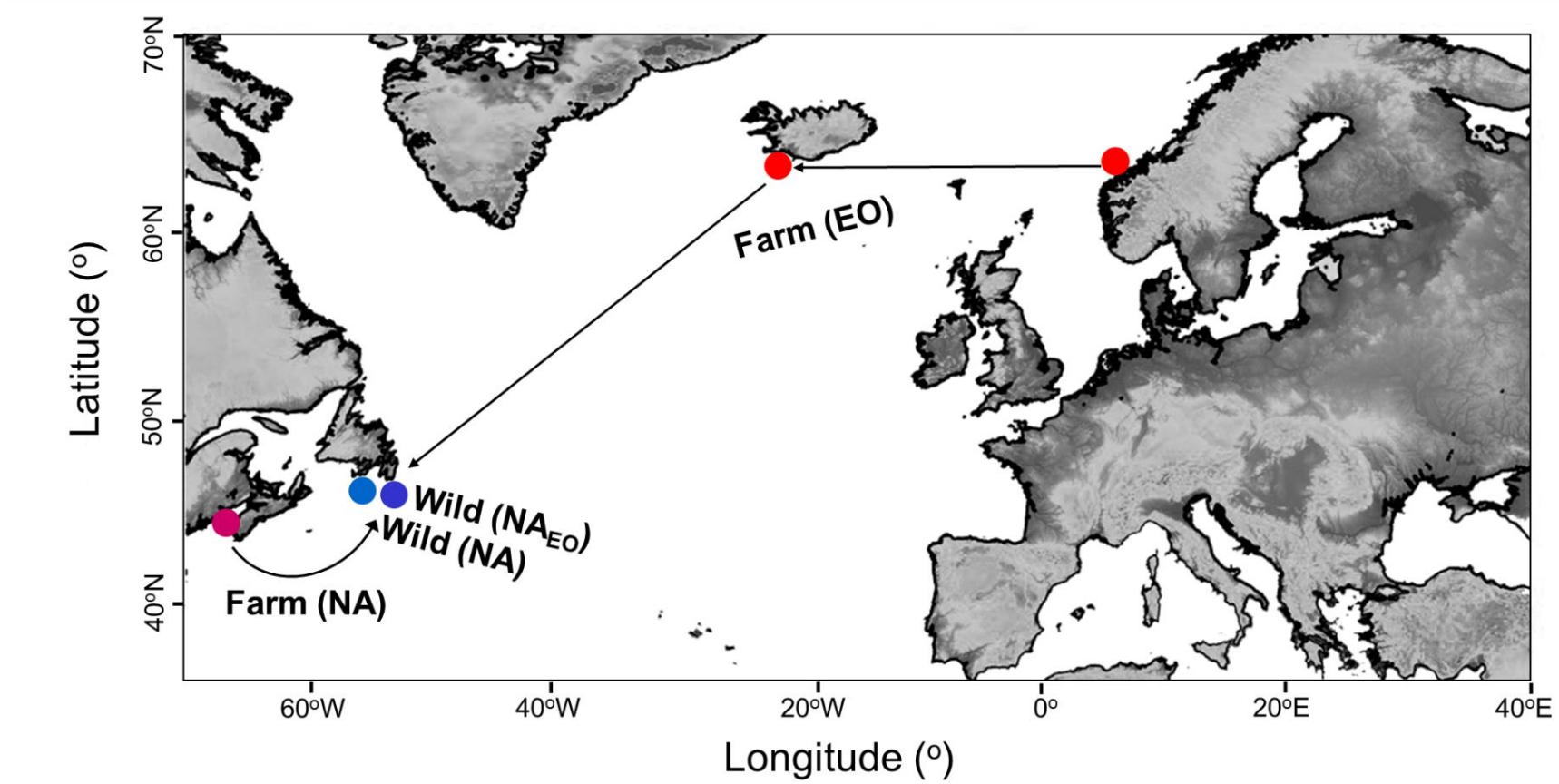

Figure 2.1: Approximate geographical origin of wild (Wild (NA) and Wild (NA_{EO})) populations and farmed (Farm (NA) and Farm (EO)) strains. The Farm (EO) strain (StofnFiskur) derives from Norwegian strains that is produced in an Icelandic facility (adapted from Islam et al., 2020).

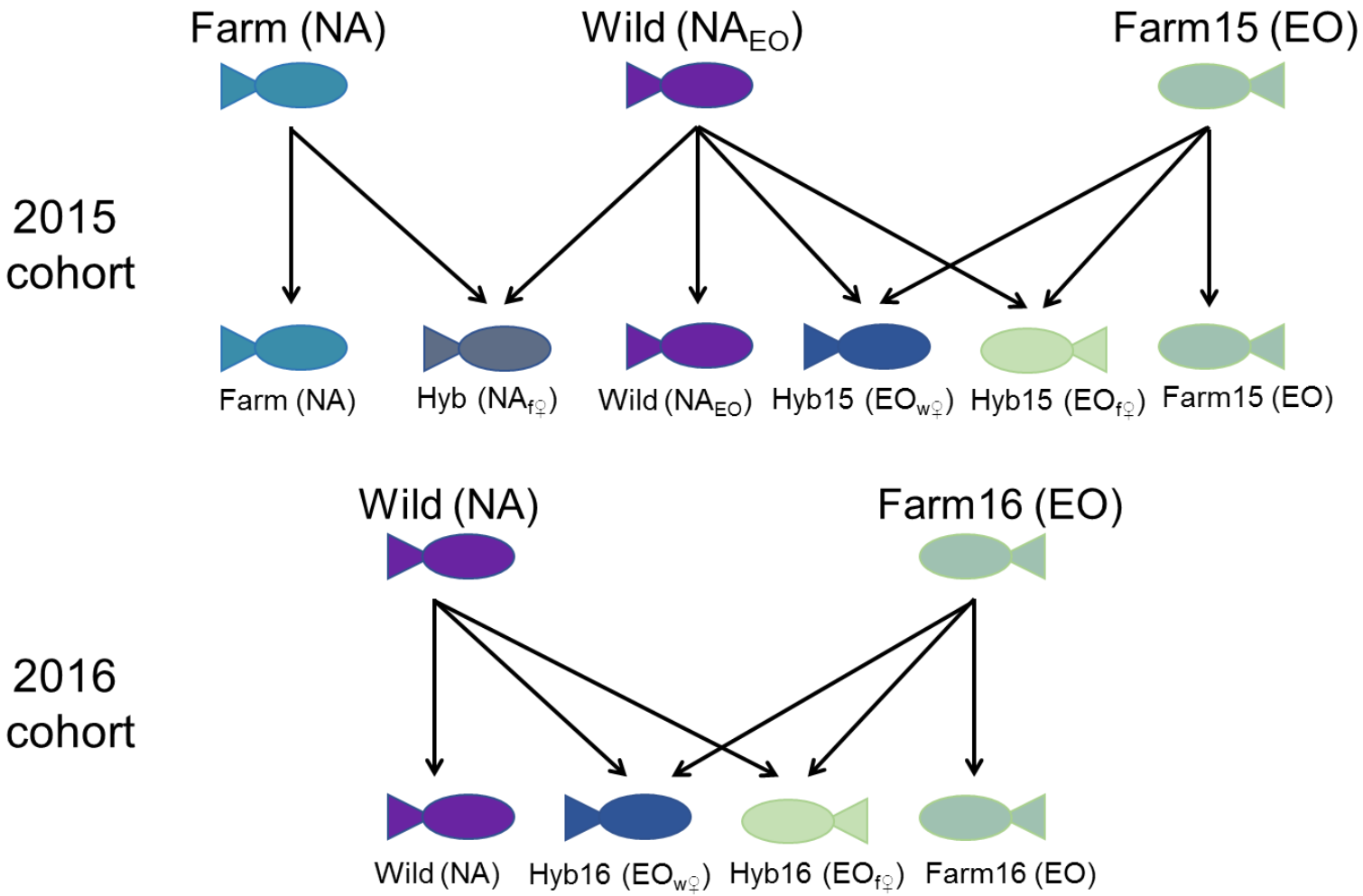

Figure 2.2: Schematic of crossing design among wild (Wild (NA) and Wild (NA_{EO})) populations and farmed (Farm (NA) and Farm (EO)) strains. For the 2015 cohort, the number of generated families was: 20 Farm (NA); 13 Hyb ($NA_{f♀}$); 11 Wild (NA_{EO}); 12 Hyb15 ($EO_{w♀}$); 10 Hyb15 ($EO_{f♀}$); and 10 Farm15 (EO). For the 2016 cohort, 10 families of each cross type were generated.

2.3.2 Experimental protocol

Experimental conditions and protocols were the same between years. Following fertilization, the eggs were water-hardened and disinfected with 0.5% Ovadine (Syndel, Nanaimo, BC, Canada) for 30 minutes, which is not expected to affect egg survival (Fowler & Banks, 1991). Fertilized eggs were then incubated in Heath trays and raised under common environmental conditions (ambient water temperature = 3.1-7.9 ºC; pH = 5.7-6.2, dissolved oxygen = 8.0-8.5 $mg \cdot L^{-1}$). To minimize density effects, each family consisted of ca. 500 eggs. Dead and unfertilized eggs were counted and removed every two to three days. As the eggs hatched, the number of alevins were counted daily, and at 50% hatch, 10 alevins per family were weighed and photographed digitally to determine yolk sac dimensions and total length (using ImageJ, Rasband, 2014).

Similarly, at yolk sac absorption, 10 randomly chosen fry from each family were photographed for total length and weighed. All animals were treated following the guidelines provided by the Canadian Council on Animal Care during holding and experimentation, and approval was granted by the Memorial University Animal Care Committee (15-21-IF).

2.3.3 Fitness-related trait differentiation

I first compared maternal body length and egg size and then quantified a series of traits that are known to be linked to early-life fitness in salmonids: development time, survival, size, specific growth rate (SGR), condition factor, yolk sac volume, and yolk sac conversion efficiency (YCE) (Metcalfe & Thorpe, 1992; Koskinen et al., 2002; Fraser et al., 2010a; Houde et al., 2013). Development time was measured in cumulative degree-days (DD = $\sum^{\circ}C$ per day) from fertilization to the eyed stage (i.e., when black dots

representing the eyes first appear), eyed to hatch, and hatch to yolk absorption. Specific growth rate (SGR) of length and mass were calculated as 100 × [ln (body length or mass at yolk sac absorption) – ln (body length or mass at hatch)]/ ∑ ˚C per day (Koskinen et al., 2002; Houde et al., 2013). Fulton's body condition factors ($K = 100(mass/length^3)$) were calculated separately at hatch and at yolk absorption (Robinson et al., 2008). Yolk sac volume was calculated as yolk sac length × yolk sac $width^2 \times \pi/6$ (Koskinen et al., 2002, Houde et al., 2013), and yolk sac conversion efficiency (YCE) was calculated as (body length at yolk sac absorption - body length at hatch) / yolk sac volume (Fraser et al., 2010a; Houde et al., 2013).

2.3.4 Statistical Analyses

All statistical analyses were performed in R version 4.0.5 (R Core Team, 2021). Statistical significance was inferred if $P < 0.05$ after sequential Bonferroni adjustment (Rice, 1989). All data were checked visually (using Q-Q plots, and histograms), and a Shapiro-Wilk's test was also applied to examine normality (Crawley, 2005). A Bartlett test was performed to check the constancy of variance, and homoscedasticity was checked visually (using residuals vs. fitted values) (Crawley, 2005).

Overall development time, length and mass at yolk absorption, length and mass specific growth rate (SGR), condition factor at yolk absorption, yolk sac volume, and yolk sac conversion efficiency (YCE) were analysed using linear mixed effects (LME) models with the *lme4* package (Bates et al., 2014). Cross type was included as a categorical fixed effect and egg size as a continuous fixed covariate. Maternal identity (dam), paternal identity (sire), and Heath tray unit (for position effect) were included as random intercepts. Mass data were log_{10}-transformed. The final fitted model was selected from the full model

with the *lmerTest* package, using the *step* function, which allowed for automatic model selection (Kuznetsova et al., 2017). This function performed backwards selection; non-significant random covariates were eliminated first, followed by the removal of non-significant fixed covariates. Non-significant interaction terms were removed before the fixed covariates, and if significant interaction terms were found, all fixed covariates were included in the final model, regardless of their significance level. While significance values for the fixed effects were obtained using a F - test based on Satterthwaite's approximation, the P - values for random effects were estimated using likelihood ratio tests (LRTs). The final fitted model was confirmed by using plots of the model residuals, and the normality of the fitted model residuals was confirmed visually using histograms.

Overall survival was analysed using a generalized linear mixed-effects model (GLMM) with a binomial distribution (logit-link function) again with the *lme4* package (Bates et al., 2014) using Laplace approximation. Cross type was included as a categorical fixed effect and egg size as a continuous fixed covariate. Again, dam, sire, and Heath tray unit were included as random intercepts. Non-significant interaction terms and covariates were removed backwards stepwise using LRTs. The model residual plots and normality check were confirmed for the final model, as for the development time, length, and mass data.

Estimated marginal means (see Supplementary Table S2.2) and Tukey adjusted post hoc multiple comparisons (using Kenward-Roger's degrees-of-freedom method) were carried out using the *emmeans* package (Lenth et al., 2018). This test estimated all pairwise cross type contrasts (see Supplementary Tables S2.3 and S2.4) and reported parameter

estimates, *t* - values (for normally distributed data), *z* - values (for binary data), and *P* - values.

2.4 Results

2.4.1 Maternal body length and egg size

Maternal body length varied significantly among populations ($P < 0.001$; Table 2.1; Fig. 2.3). Farm (EO) females were longer than Farm (NA) females in both years while both farm types were longer than both wild (Wild (NA_{EO}) and Wild (NA)) types. However, there was no difference between wild types (Table 2.1, Figure 2.3). Generally, larger females produced larger eggs than smaller females ($P < 0.001$; Table 2.1, Fig. 2.3). However, despite Farm (EO) females of both cohorts being of similar sizes, egg size of Farm15 (EO) was smaller than that of Farm16 (EO) and did not differ from Farm (NA). The relationship between maternal body length and egg size was positive within all populations (Farm (NA): $r = 0.87$, $P < 0.001$; Wild (NA): $r = 0.78$, $P < 0.05$; Farm16 (EO): $r = 0.89$, $P < 0.001$); Wild (NA_{EO}): $r = 0.91$, $P > 0.05$; Farm15 (EO): $r = 0.39$, $P > 0.05$).

2.4.2 Early life fitness-related traits

Significant differences in development time from fertilization to the eyed stage and from eyed to hatch were detected among cross types of EO and NA farmed, NA wild, and related F_1 hybrids in both cohorts ($P < 0.001$; Table 2.1), but there was no difference from hatch to yolk absorption ($P > 0.05$; Table 2.1). From fertilization to the eyed stage, Wild (NA_{EO}) eggs had faster embryonic development than Wild (NA), both Farm (EO) cohorts and Farm (NA) eggs. Farm16 (EO) eggs took more cumulative degree-days (DD) to reach the eyed stage than Farm15 (EO), with Farm (NA) displaying an intermediate development

time that did not differ from that of Wild (NA). In general, the difference in embryonic development time among most F_1 hybrids and their maternal strains were not significant at the eyed stage.

Similarly, Wild (NA_{EO}) eggs hatched earlier than Wild (NA) and Farm16 (EO), but did not differ from Farm15 (EO) and Farm (NA). Moreover, Farm16 (EO) took longer (DD) to hatch than Farm15 (EO), whereas Farm (NA) hatched earlier than both Farm (EO) cohorts. Yet, again, most of the hybrids had similar hatching times as their maternal strains. Overall development time from fertilization to yolk absorption was best described by a model that included cross type (Sum of Squares [SS] = 6094.3, DF = 9,81.98, F = 2.59, $P < 0.05$), egg size (SS = 16907.4, DF = 1,81.84, F = 64.74, $P < 0.0001$), and their two-way interaction (cross-x-egg) (SS = 6148.8, DF = 9,81.89, F = 2.62, $P < 0.05$; Table 2.2). In general, Farm16 (EO) alevins had a longer development time than Farm15 (EO), Farm (NA), and Wild (NA_{EO}) alevins, which did not differ among themselves (Fig. 2.4A). All hybrids exhibited similar overall development time as their maternal strains.

Table 2.1: Multiple comparisons of family-level mean differences in fitness-related traits among different cross types from the 2015 and 2016 cohorts (Tukey-adjusted pairwise comparisons). The *P* values are represented by significance level whereby * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: Not Significant. For development time, DD denotes degree days. Different letters denote significant family-level mean trait differences among cross types (for visual representation, see Figs. 2.4-2.5 for cross type effects using mixed-effects models).

Trait	Farm (NA)	Hyb ($NA_{f♀}$)	Wild (NA_{EO})	Hyb15 ($EO_{w♀}$)	Hyb15 ($EO_{f♀}$)	Farm15 (EO)	Wild (NA)	Hyb16 ($EO_{w♀}$)	Hyb16 ($EO_{f♀}$)	Farm16 (EO)	Significance level
Maternal traits											
Maternal body fork length (cm)	77.6^{a}		54.3^{b}			107.0^{c}	55.5^{b}			105.8^{c}	***
Maternal egg size (mm)	5.7^{a}		5.25^{b}			5.77^{a}	5.37^{b}			6.14^{c}	***
Development time (DD)											
Fertilization to eyed	310.3^{a}	305.2^{b}	277.3^{cd}	279.3^{ce}	282^{de}	284.2^{e}	313.8^{af}	318.1^{f}	326.4^{g}	337.9^{h}	***
Eyed to hatch	196.4^{a}	200.7^{a}	233.3^{b}	233.5^{b}	229^{bd}	232.3^{bd}	248.1^{c}	222.2^{d}	254.9^{ce}	265^{e}	***
Hatch to yolk absorption	369.9	382.4	360.9	383	376.6	379.4	359.2	365.5	361.9	353.6	NS
Overall development time	879^{a}	888^{ac}	871^{a}	896^{acd}	887^{ac}	896^{acd}	927^{bc}	906^{ab}	943^{bd}	951^{b}	***
Survival (%)											
Fertilization to eyed	80.5^{a}	82.2^{a}	99.7^{b}	99.4^{b}	82^{a}	83.5^{a}	90.5^{a}	72.9^{a}	42.3^{c}	46.8^{c}	***
Eyed to hatch	69.9^{ac}	85.7^{bcd}	98.5^{b}	97.6^{b}	53.5^{a}	62.7^{ad}	96.3^{b}	92.7^{bc}	71.4^{ac}	75.8^{ab}	***
Hatch to yolk absorption	93.7^{ab}	90.4^{ab}	82.2^{ab}	96.1^{ab}	85.3^{ab}	89.2^{ab}	97.5^{b}	95.8^{ab}	82.7^{ab}	81.1^{a}	***
Overall survival	55.6^{ac}	67.6^{bc}	80.6^{bc}	93.2^{b}	35.0^{ad}	46.1^{ac}	85.2^{bc}	65.6^{bcd}	29.3^{a}	30.9^{a}	***
Size traits											
Length at hatch (cm)	1.83^{ad}	1.82^{ad}	1.79^{ab}	1.76^{b}	1.69^{ce}	1.63^{c}	1.67^{c}	1.84^{d}	1.77^{ab}	1.74^{be}	***
Mass at hatch (mg)	107.7^{a}	106.1^{ac}	95.1^{bd}	98.5^{bce}	88.8^{de}	87.0^{d}	102^{ac}	105^{ac}	118.4^{f}	110^{a}	***
Length at yolk absorption (cm)	2.61^{ab}	2.54^{a}	2.66^{ab}	2.76^{b}	2.57^{ac}	2.70^{bc}	2.59^{ac}	2.7^{bc}	2.76^{b}	2.68^{ab}	***
Mass at yolk absorption (mg)	133.7^{a}	126.8^{a}	120.2^{ab}	136.3^{ac}	96.2^{b}	120^{ab}	134.2^{ad}	141.3^{ac}	162.4^{c}	155.3^{cd}	***
Energy conversion											
SGR_{length}	0.095^{ac}	0.087^{a}	0.104^{ab}	0.115^{bc}	0.105^{ab}	0.13^{b}	0.123^{b}	0.107^{ab}	0.123^{b}	0.121^{b}	***
SGR_{mass}	0.06	0.05	0.07	0.08	0.03	0.09	0.08	0.09	0.08	0.09	NS
Condition factor ($g.cm^{-3}$ x 100) at hatch	1.77^{ad}	1.75^{ad}	1.69^{a}	1.81^{ad}	1.83^{acd}	2.0^{bd}	2.24^{b}	1.69^{a}	2.26^{bc}	2.14^{b}	***

Condition factor ($g.cm^{-3}$ x 100)at yolk absorption	0.76^{a}	0.77^{a}	0.63^{b}	0.65^{b}	0.57^{b}	0.59^{b}	0.77^{a}	0.72^{ab}	0.77^{a}	0.81^{a}	***
Yolk sac volume at hatch (cm^{3})	0.077^{ab}	0.061^{a}	0.07^{a}	0.074^{ab}	0.053^{a}	0.056^{a}	0.069^{a}	0.081^{ab}	0.111^{b}	0.147^{c}	***
Yolk sac conversion efficiency ($cm.cm^{-3}$)	10.9^{ac}	12.3^{ac}	15.2^{bc}	14.2^{bc}	17.1^{b}	18.2^{b}	13.8^{bc}	12.2^{ac}	9.4^{ac}	7.5^{a}	***

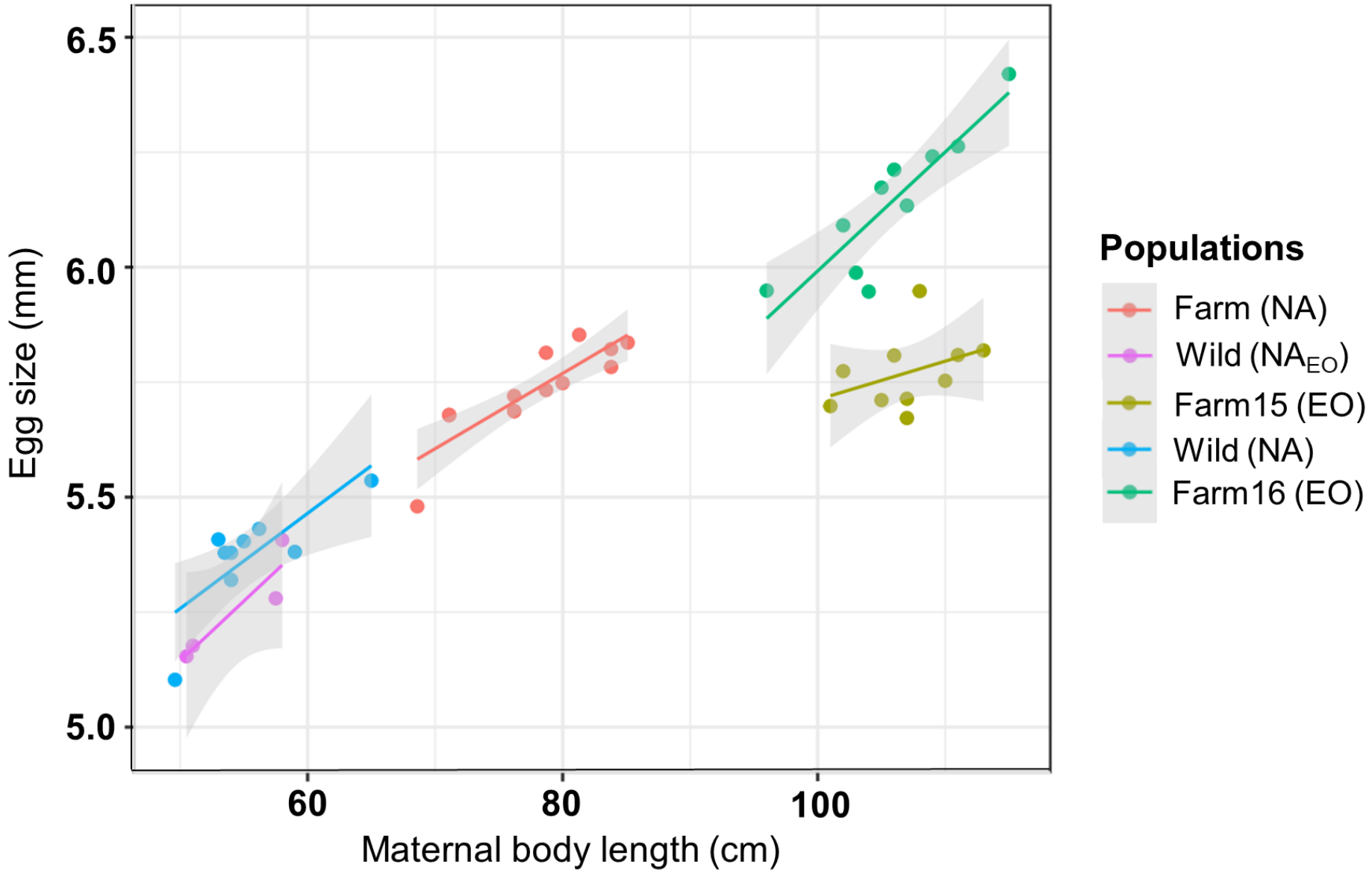

Figure 2.3: Regression plot of egg size in relation to maternal body fork length for the different farmed strains and wild populations [Farm (NA); Farm15 (EO); Farm16 (EO); Wild (NA); and Wild (NAEO)]. The shaded areas represent 95% CI.

Significant differences in survival at the three different early life stages examined (eyed, hatch, and yolk absorption) were detected among cross types ($P < 0.001$; Table 2.1). From fertilization to the eyed stage, Wild (NA_{EO}) embryos had higher survival than Wild (NA), both Farm (EO) cohorts, and Farm (NA) embryos. No detectable differences in embryo survival were observed among Wild (NA), Farm (NA), and Farm15 (EO), but Farm16 (EO) had significantly lower survival than other cross types. The difference in embryonic survival between F_1 hybrids and their maternal strains were not significant at the eyed stage. From eyed to hatch, both Wild populations had higher survival than Farm (NA) and Farm15 (EO) but did not differ from Farm16 (EO) embryos. Again, all F_1 hybrids had similar survival as their maternal strains at this stage. From hatch to yolk absorption, no significant differences in alevin survival were detected among cross types, except that Farm16 (EO) had lower survival than Wild (NA).

Table 2.2: Summary of Linear Mixed Effects model selection for investigating differences in overall development time, body length and mass at yolk absorption, specific growth rate (SGR) length and mass, condition factor at yolk absorption, yolk sac volume at hatch, and yolk sac conversion efficiency for different cross types of the 2015 and 2016 cohorts. Maternal identity (dam), paternal identity (sire), and Heath tray unit (for position effect) were included as random intercepts.

Variable	**Sum Sq**	**Num DF**	**Den DF**	**F**	***P***
Overall development time					
Cross	6094.3	9	81.98	2.59	**<0.05**
Egg	16907.4	1	81.84	64.74	**<0.0001**
Cross x egg	6148.8	9	81.89	2.62	**<0.05**
Length at yolk absorption					
Cross	0.05	9	55.61	1.97	0.06†
Egg	0.36	1	41.35	132.51	**<0.0001**
Cross x egg	0.06	9	55.40	2.27	**<0.05**
Mass at yolk absorption					
Cross	491.4	9	33.34	6.0	**<0.0001**
Egg	1150.8	1	26.86	126.5	**<0.0001**
Cross x egg	491.7	9	31.65	6.0	**<0.0001**
SGR_{length}					
Cross	0.45	9	42.32	15.11	**<0.0001**
Egg	0.001	1	25.87	12.28	**<0.01**
Cross x egg	0.001	9	35.46	1.25	0.30
SGRmass					
Cross	0.01	9	55.86	0.86	0.57
Egg	0.02	1	63.03	13.91	**<0.0001**
Cross x egg	0.01	9	53.42	0.92	0.52
Condition factor at yolk absorption					
Cross	0.05	9	61.86	3.17	**<0.01**
Egg	0.002	1	66.68	1.02	0.32†
Cross x egg	0.05	9	58.89	3.10	**<0.01**
Yolk sac volume at hatch					
Cross	0.004	9	30.57	4.40	**<0.001**
Egg	0.005	1	22.35	48.9	**<0.0001**
Cross x egg	0.004	9	30.50	4.36	**<0.01**
Yolk sac conversion efficiency					
Cross	668.77	9	34.45	25.97	**<0.0001**
Egg	173.65	1	28.99	107.45	**<0.0001**

Cross x egg	23.24	9	32.99	1.60	0.16

Significant fixed effects in bold were retained in the final model. †Cross (length at yolk absorption) and egg (condition factor at yolk absorption) were also retained in the final model as the interaction terms were significant. Significant random effects (dam, sire, Heath tray unit) were also retained in the final model. Sum Sq, sum of squares. Num DF, numerator degrees of freedom. Den DF, denominator degrees of freedom based on Sattherwaithe's approximations. F, F-value.

Table 2.3: Summary of Generalized Linear Mixed-effects Model selection using likelihood ratio tests (LRTs) for investigating difference in overall survival for different cross types of the 2015 and 2016 cohorts.

Model No.	**Terms included in GLMM model**	**Term tested**	**Versus model No.**	**Log likelihood**	**AIC**	**df**	χ^2	***P***
Overall survival								
0†	Cross + egg + cross x egg			-799.09	1644.2	23		
1	Cross + egg	Cross x egg	0	-816.09	1660.2	14	34.0	**<0.0001**
2	Cross	Egg	1	-817.86	1661.7	13	3.54	**<0.05**
3	Egg	Cross	1	-865.70	1741.1	5	99.2	**<0.0001**

†Retained final model. AIC, Akaike Information Criterion.Df, degrees of freedom. χ^2, the value of the chi-square statistics.

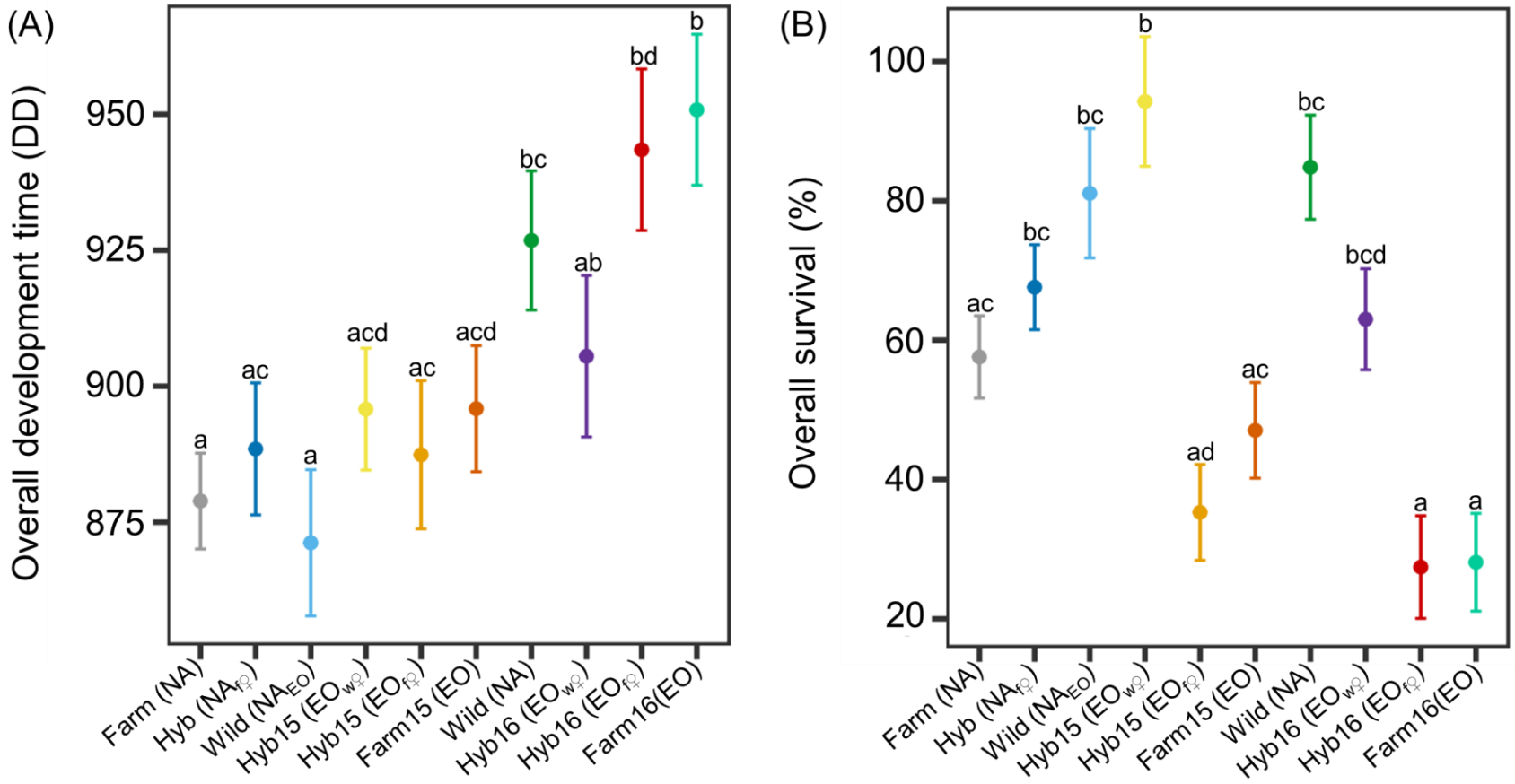

Figure 2.4 (A-B): Cross type effects on (A) overall development time (DD) and (B) overall survival (%) using mixed-effects models. Displayed are marginal means and standard errors. Different letters denote significant differences in estimated marginal mean (family-level) traits among cross types. See Supplementary Table S2.2 for estimated marginal means and Supplementary Tables S2.3 and S2.4 for Tukey-adjusted pairwise contrasts among cross types fitted in the final models. Cross types: Farm (NA); Hyb ($NA_{f♀}$); Wild (NA_{EO}); Hyb15 ($EO_{w♀}$); Hyb15 ($EO_{f♀}$); and Farm15 (EO) from the 2015 cohort. Cross types: Wild (NA); Hyb16 ($EO_{w♀}$); Hyb16 ($EO_{f♀}$); and Farm16 (EO) from the 2016 cohort.

In terms of overall survival, the significant terms retained after model selection were cross type (χ^2 = 99.2, P < 0.0001), egg size (χ^2 = 3.54, P < 0.05), and the cross-x-egg interaction term (χ^2 = 34.0, P < 0.0001) (Table 2.3). In general, both wild populations had higher survival than Farm16 (EO), but did not differ from Farm (NA) and Farm15 (EO) (Fig. 2.4B). There was no difference in overall survival between F_1 hybrids and their related maternal strains.

Significant differences in size (body length and mass) were detected among EO, and NA farmed strains, NA wild populations, and related hybrids at hatch and yolk absorption (P < 0.001; Table 2.1). At hatch, Wild (NA) and Farm15 (EO) alevins were shorter than Wild (NA_{EO}), Farm (NA), and Farm16 (EO) alevins. Farm (NA) alevins were longer than Farm16 (EO) but did not differ from Wild (NA_{EO}), which in turn did not differ from Farm16 (EO). There was no difference in alevin length between F_1 hybrids and their related maternal strains, except Hyb16 ($EO_{w♀}$) > Wild (NA). In terms of body mass at the same stage, Farm15 (EO) alevins weighed less than Wild (NA), Farm (NA), and Farm16 (EO), but no different than Wild (NA_{EO}). There was no difference in alevin mass between F_1 hybrids and their respective maternal strains, except Hyb16 ($EO_{f♀}$) > Farm16 (EO). For fry length at yolk absorption, egg size (SS = 0.36, DF = 1,41.35, F = 132.51, P < 0.0001) and the cross-x-egg interaction term (SS = 0.06, DF = 9,55.40, F = 2.27, P < 0.05) (Table 2.2) were significant, and therefore retained in the final model. As the higher-order interaction term was significant, the lower-order non-significant fixed term cross type was also retained in the final model. There was no difference in fry length at yolk absorption among farmed strains and wild populations (Fig. 2.5A). However, Hyb16 ($EO_{f♀}$) and Hyb15 ($EO_{w♀}$) were larger than Hyb ($NA_{f♀}$), Hyb15 ($EO_{f♀}$), and Wild (NA). For mass at

yolk absorption, the model terms cross type (SS = 491.4, DF = 9,33.34, F = 6.0, P < 0.0001), egg size (SS = 1150.8, DF = 1,26.86, F = 126.5, P< 0.0001), and their interaction (SS = 491.7, DF = 9,31.65, F = 6.0, P < 0.0001) were significant, and therefore retained in the final model (Table 2.2). The random effect term dam was also significant (LR = 18.58, P < 0.0001) and retained in the final model. Farm16 (EO) fry weighed more than Farm15 (EO), Farm (NA), and Wild (NA_{EO}) but did not differ from Wild (NA) (Fig. 5B). No difference in fry mass was observed between F_1 hybrids and their respective maternal strains.

Significant differences in growth, body condition, yolk sac volume, and yolk sac conversion efficiency were observed among cross types (P < 0.001; Table 2.1). In terms of length specific growth rate (SGR), cross type (SS = 0.45, DF = 9,42.32 F = 15.11; P < 0.0001) and egg size (SS = 0.001, DF = 1,25.87 F = 12.28; P < 0.01) were significant (Table 2.2), and therefore, retained in the final model. The random covariate dam was also significant (LR = 6.39, P < 0.05) and retained. Of the pure crosses, Farm15 (EO), Farm16 (EO), and Wild (NA) had the highest SGR in terms of length from hatch to yolk absorption, which differed significantly from Farm (NA), with Wild (NA_{EO}) being intermediate between the former pure crosses and Farm (NA) (Fig. 5C). There was no difference in length SGR between F_1 hybrids and their respective maternal strains. For mass SGR, the only significant term retained in the final model was egg size (SS = 0.02, DF = 1,63.03 F = 13.91, P < 0.0001) (Table 2.2). I did not find any significant differences in mass SGR among cross types (Tables 2.1, 2.2).

In terms of Fulton's condition factor (K) at hatch, Wild (NA) and Farm16 (EO) had the highest K, followed by Farm15 (EO), which did differ from Farm (NA), while Wild

(NA_{EO}) had the lowest K, but did not differ from Farm (NA). There was no difference in K at hatch between F_1 hybrids and their respective maternal strains, except Hyb16 ($EO_{w♀}$) < Wild (NA). With regards to K at yolk absorption, cross type (SS = 0.05, DF = 9,61.86, F = 3.17, $P < 0.01$) and the cross-x-egg interaction term (SS = 0.05, DF = 9,58.89, F = 3.10, $P < 0.01$) were significant and retained in the final model (Table 2.2). As the two-way interaction term was significant, the fixed term egg size was also retained. Farm16 (EO) fry had the highest K at yolk absorption, which did not differ from that of Farm (NA) and Wild (NA) (Fig. 2.5D). Whereas, Farm15 (EO) had the lowest K of the pure crosses, and did not differ from Wild (NA_{EO}). There were no differences in fry K between F_1 hybrids and their respective maternal strains. With respect to yolk sac volume at hatch, both cross type (SS = 0.004, DF = 9,30.57, F = 4.40, $P < 0.001$), egg size (SS = 0.005, DF = 1,22.35, F = 48.9, $P < 0.0001$), and their interaction (SS = 0.004, DF = 9,30.50, F = 4.36, $P < 0.01$) were significant, and therefore retained after model selection (Table 2.2).The random covariate dam was also significant ($LR = 4.87$, $P < 0.05$) and also retained. Farm16 (EO) alevins had the largest yolk sac volume at hatch (Fig. 2.5E). There was no difference in yolk sac volume among Farm (NA), Farm15 (EO), and both Wild populations.

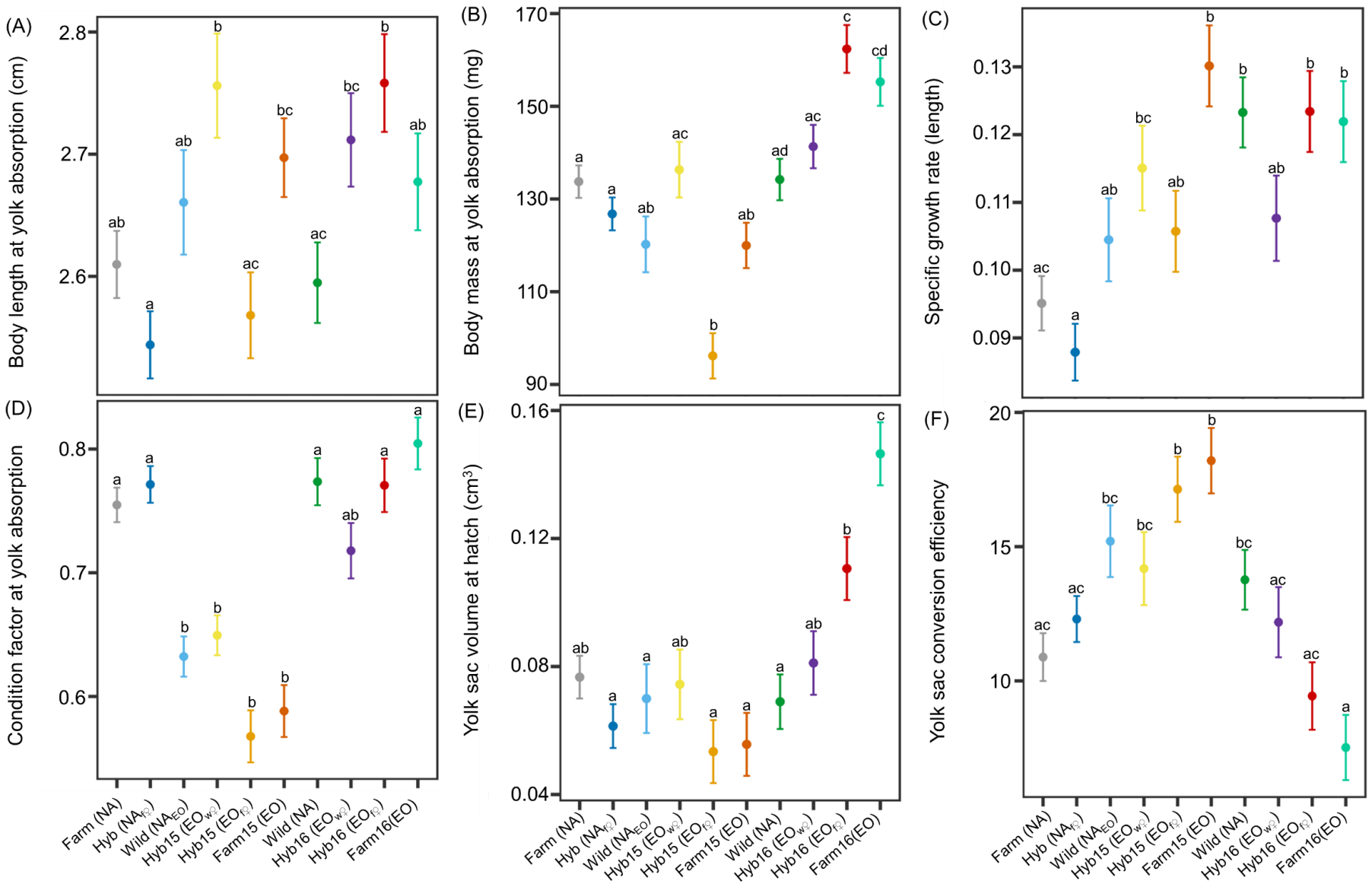
(A)
Body length at yolk absorption (cm)
2.8
2.7
2.6
ab
a
ab
b
ac
bc
ac
bc
b
ab
(B)
Body mass at yolk absorption (mg)
170
150
130
110
90
a
a
ab
ac
b
ab
ad
ac
c
cd
(C)
Specific growth rate (length)
0.13
0.12
0.11
0.10
0.09
ac
a
ab
bc
ab
b
ab
b
b
(D)
Condition factor at yolk absorption
0.8
0.7
0.6
a
a
b
b
b
b
a
ab
a
a
(E)
Yolk sac volume at hatch (cm³)
0.16
0.12
0.08
0.04
ab
a
a
ab
a
a
a
ab
b
c
(F)
Yolk sac conversion efficiency
20
15
10
ac
ac
bc
bc
b
b
bc
ac
ac
a
Farm (NA)
Hyb (NA$_{F2}$)
Wild (NA$_{EO}$)
Hyb15 (EO$_{W2}$)
Hyb15 (EO$_{F2}$)
Farm15 (EO)
Wild (NA)
Hyb16 (EO$_{W2}$)
Hyb16 (EO$_{F2}$)
Farm16(EO)

Figure 2.5 (A-F): Cross type effects on (A) length at yolk absorption; (B) mass at yolk absorption; (C) specific growth rate (length); (D) condition factor at yolk absorption; (E) yolk sac volume at hatch; and (F) yolk sac conversion efficiency using mixed-effects models. Displayed are marginal means and standard errors. Different letters denote significant differences in estimated marginal mean (family-level) traits among cross types. See Table S2 for estimated marginal means and Table S3 for Tukey-adjusted pairwise contrasts for different cross types fitted in the final models. Cross types: Farm (NA); Hyb ($NA_{f♀}$); Wild (NA_{EO}); Hyb15 ($EO_{w♀}$); Hyb15 ($EO_{f♀}$); and Farm15 (EO) from the 2015 cohort. Cross types: Wild (NA); Hyb16 ($EO_{w♀}$); Hyb16 ($EO_{f♀}$); and Farm16 (EO) from the 2016 cohort.

No differences in alevin yolk sac volume were observed between F_1 hybrids and their respective maternal strains, except Hyb16 ($EO_{f♀}$) < Farm16 (EO). In terms of yolk sac conversion efficiency (YCE), cross type (SS = 668.77, DF = 9,34.45, F = 25.97, P < 0.0001) and egg size (SS = 173.65, DF = 1,28.99, F = 107.45, P < 0.0001) were significant and retained in the final model (Tables 2.2). Of the pure crosses, Farm15 (EO) had the largest YCE, followed by both Wild populations, which did not differ from Farm15 (EO) and Farm (NA) (Fig. 5F). Farm16 (EO) had the lowest YCE and differed from all other pure crosses except Farm (NA). Again, no difference was observed in alevin YCE between F_1 hybrids and their related maternal strains.

2.5 Discussion

The present study has demonstrated early-life fitness-related trait differences among divergent EO and NA farmed strains, NA wild populations, and related F_1 hybrids, and these differences can provide insight into the impact of hybridization in the wild. The main findings can be summarized in four key points: (i) significant differences were detected in development time (except hatch to yolk absorption), survival, size, and energy conversion among EO and NA farm, wild and F_1 hybrid offspring during early life; (ii)

fitness-related traits differed between Farm (EO) and Farm (NA), and also between Wild (NA) and Wild (NA_{EO}); (iii) few differences in fitness-related traits between F_1 hybrids and their respective maternal strains were detected; and (iv) significant associations were found between the maternal effects of egg size and many of the traits measured. These findings suggest geographical and ancestral relationships and maternal effects were more important in generating early-life trait differences among farmed, wild, and their related hybrids than effects of domestication selection.

I found significant differences in development time to the eyed and hatch stages in salmon of farmed, wild, and hybrid origin which was not unexpected based on previous observations of other domesticated salmonid populations (Beacham & Murray, 1987; Donaghy & Verspoor, 1997; Fraser et al., 2010b; Solberg et al., 2014). However, I did not find a significant difference in development time from hatch to yolk absorption among cross types. It is possible that the increases in temperature at this latter incubation stage might decrease among-population differences in development time, given that temperature variation does not necessarily affect all life stages equally (Thorn & Morbey, 2018). Moreover, developmental trait differences were not always the same at different developmental stages (eyed, hatch, and yolk absorption) in each farmed, wild and F_1 hybrid cross comparison. Overall, however, Farm16 (EO) had a longer developmental time than the other farm strains, which appears likely to be related to the larger egg size of Farm16 (EO) females. Wild (NA) displayed intermediate development time in each of the three early-life developmental phases. Whereas Wild (NA_{EO}) always had the shortest development time and smallest egg size, however, despite this, its overall development time did not differ from that of Farm (NA).

All hybrid fry exhibited similar development times to yolk absorption as their respective maternal strains, which implies that maternal effects, likely associated with egg size (Hendry et al., 1998; Einum & Fleming, 2004; Green 2008), were important determinants of development time (Thorpe et al., 1984; Beacham & Murray, 1985, 1987; Einum & Fleming, 2000b). It is also possible that maternal transcript factors contributed to this pattern (Bougas et al., 2013; Bicskei et al., 2016; Bizuayehu et al., 2019). Emergence timing, which will be affected both by adult spawning time and embryo development time, is likely to affect competitive ability due to prior residency (Cutts et al., 1999; Kvingedal & Einum, 2011). Thus, the extended developmental time of Farm16 (EO) and the related maternal hybrid (Hyb16 ($EO_{f♀}$)) may be maladaptive, compromising survival and growth in the wild (Einum & Fleming, 2000a). Put simply, the delayed emergence of Farm16 (EO) offspring may inhibit introgressive hybridization. However, this effect seems to be cohort specific as a delayed emergence was not observed in Farm15 (EO).

The results demonstrated significant differences in early-life survival among farmed, wild, and F_1 hybrid conspecifics, which is consistent with the observations of other salmonid populations (Granath et al., 2004; Fraser et al., 2010a., Houde et al., 2013, 2015; Falica et al., 2017). The survival differences were quite consistent at different stages in each farmed, wild, and F_1 hybrid cross comparison. In general, both Wild (NA) and Wild (NA_{EO}) had the highest survival, Farm (NA) had intermediate survival, whereas both Farm (EO) cohorts had the lowest survival. I cannot rule out the possibility of an egg quality effect on early life survival, as gametes of both wild parental populations derived from adults stripped immediately prior to fertilization on-site, while gametes of the farm strains were stripped at their respective facilities (Iceland and south coast NF) and shipped

immediately to St. John's. Such egg quality effects would be expected to be most evident during the early developmental stage when Farm16 (EO) had the lowest survival from fertilization to the eyed stage, followed by the Farm (NA) and Farm15 (EO) strains. As with development time, survival of F_1 hybrids was most similar to that of their respective maternal strains indicative of maternal effects, though the effect here appears to be more likely related to egg quality than size.

The farmed, wild and F_1 hybrid offspring also differed significantly in early-life size (in terms of both length and mass) at hatch and yolk absorption. Moreover, with regards to growth rate, both cohorts of the Farm (EO) strain had higher length and mass SGR than Farm (NA), while Wild (NA) had higher length SGR than Wild (NA_{EO}). This study was designed for discerning the degree to which farmed-wild early life fitness traits differences may be attributable to the domestication selection and the ancestral relationship among the populations. In this study, the two farmed strains used are historically genetically divergent but have undergone multiple generations of domestication selection, though for differing lengths of time (Farm (EO): 10-12 generations; Farm (NA): 5-7 generations), thus an outstanding question remained was whether these two farmed strains would display similar early-life fitness trait (e.g. growth). However, while these two farmed strains have likely experienced similar domestication selection, there was no strong evidence of similarities in early-life traits, which contrasts with observations of their behaviour as young-of-the-year juveniles where both Farm (EO) and Farm (NA) fish showed similar patterns (Islam et al., 2020). Moreover, it does not appear that the differences can be explained by the maternal effects of egg size, as Farm (NA) and Farm15 (EO) had similar egg sizes, though smaller than Farm16 (EO). While I cannot entirely rule out the possibility that the different numbers

of generations of domestication selection have influenced the differences between these two farmed strains, inconsistencies in their differences relative to the wild populations (i.e., not always differing in the same direction), suggests that their distinct geographical and ancestral origins are more important in explaining the patterns observed.

F_1 hybrids had similar overall growth to their maternal strains, and I also found a significant dam effect (for mass at yolk absorption and SGR length) which again suggests that maternal effects, likely due to egg size, largely determine this pattern, as also seen in earlier studies (Houde et al., 2011; Debes et al., 2013; Solberg et al., 2014). However, there was still cross type effect for length SGR (although not for mass SGR), even after controlling for egg size and the dam effect in the model. Energy conversion (i.e., utilization of endogenous yolk resources; YCE) also differed significantly among cross types, even after controlling for the effects of egg size, in contrast with some earlier studies that did not find differences between farmed strains and wild populations (Fraser et al., 2010a; Debes et al., 2013). There were also differences in yolk sac volume at hatch, with Farm16 (EO) having the greatest volume (and largest initial egg size) compared to other cross types, while no differences were observed among Farm (NA) and the two wild populations, despite differences in initial egg size. Taken together, although, it appeared that there were significant differences in growth and energy conversion, these findings provide little indication that domestication selection has resulted in changes in early alevin size, growth and endogenous resource utilization, but rather suggest that distinct geographic and ancestral relationships of the farmed strains and maternal effects have mainly contributed in early life growth differences.

In conclusion, the differences in the early-life fitness traits observed among divergent EO and NA farmed, wild and F_1 hybrid populations appear to largely reflect the influence of geographic and ancestral relationships of the farmed strains and maternal effects and less so domestication selection. Briefly, although the traits nearly always differed significantly among cross types, the differences were not necessarily consistent among the different early-life stages. Moreover, I did not see many consistent trait differences among farmed strains relative to the wild populations, suggesting that domestication selection had relatively less effect on fitness at this relatively early life stage than maternal effects likely due to differences in egg size. Along the same line, one of my main hypotheses was that interbreeding would cause F_1 hybrids to display altered fitness, reflected in differences in fitness-related traits relative to farm and wild populations. However, I observed few differences in fitness-related traits between F_1 hybrids and their respective maternal farmed/wild strains. As the principal route of hybridization/introgression is likely to occur through farm females rather than males because of sex differences in their reproductive capabilities (Fleming et al., 1996, 2000), the maternal contributions of farm females will be important in understanding the fitness consequences of interbreeding. Escaped farmed salmon have been detected in rivers in Southern NF, Canada (Keyser et al., 2018; Wringe et al., 2018), and successful breeding between farmed and wild salmon was detected in 17 out 18 rivers studied. Wringe et al. (2018) also detected successful reproduction of pure farm (i.e., production of feral offspring) in a number of rivers, which is of particular note given the results presented in this study. It has long been clear that successful breeding of escaped farmed salmon within wild populations can have fitness impacts (e.g., on growth and survival) at subsequent life

stages, influencing lifetime success and threatening the native wild populations (McGinnity et al., 1997, 2003; Fleming et al., 2000; Skaala et al., 2019). Thus understanding the effect of hybridization and, consequently, early-life fitness trait differences among divergent farmed, wild and F_1 hybrid populations can provide valuable insight for the conservation and management of Atlantic salmon.

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2.7 Appendices

Supplementary Table S2.1: Body size measurements of parental salmon of the cohort 2015 and 2016

Cohort	Population	Sex	n	Fork length (cm)	
				Mean	Range
2015	Farm (NA)	Female	13	77.75	68.6-85.1
		Male	13	81.6	76.2-88.9
	Wild (NA_{EO})	Female	4	54.25	50.5-58
		Male[†]	11	14.5	11-20.5
	Farm15 (EO)	Female	10	107	101-113
		Male	10	103.1	95-110
2016	Wild (NA)	Female	9	55.48	49.6-65
		Male	6	55.62	50-60
	Farm16 (EO)	Female	10	105.8	96-115
		Male	8	116.25	106-124

[†]Wild(NA_{EO}) male parr (2015 cohort) were used to generate the cross.

Supplementary Table S2.2: Estimated marginal means of different cross types from the 2015 and 2016 cohorts derived from the fitted final models for overall development time, overall survival, length at yolk absorption, mass at yolk absorption, specific growth rate (SGR) length and mass, condition factor at yolk absorption, yolk sac volume at hatch, and yolk sac conversion efficiency. SE, standard errors. Lower. CL, lower confidence limit.Upper.CL, upper confidence limit.

Cross types	**Estimated marginal mean**	**±SE**	**Lower. CL**	**Upper. CL**
Overall development time (DD)				
Farm (NA)	879	8.81	774	984
Hyb ($NA_{f♀}$)	888	12.13	780	997
Wild (NA_{EO})	871	13.44	764	978
Hyb15 ($EO_{w♀}$)	896	11.21	696	1096
Hyb15 ($EO_{f♀}$)	887	13.62	792	982
Farm15 (EO)	896	11.59	749	1042
Wild (NA)	927	12.79	875	978
Hyb16 ($EO_{w♀}$)	906	14.84	847	964
Hyb16 ($EO_{f♀}$)	943	14.84	885	1002
Farm16 (EO)	951	13.88	909	992
Overall survival (%)				
Farm (NA)	57.6	5.91	45.7	69.4
Hyb ($NA_{f♀}$)	67.6	6.09	55.4	79.7
Wild (NA_{EO})	81.1	9.28	62.3	99.8
Hyb15 ($EO_{w♀}$)	94.3	9.31	75.4	113.1
Hyb15 ($EO_{f♀}$)	35.3	6.87	21.6	49
Farm15 (EO)	47	6.86	33.4	60.7
Wild (NA)	84.8	7.47	69.9	99.8
Hyb16 ($EO_{w♀}$)	63	7.25	48.5	77.5
Hyb16 ($EO_{f♀}$)	27.4	7.37	12.7	42.1
Farm16 (EO)	28.1	7.02	14.1	42.1
Length at yolk absorption (cm)				
Farm (NA)	2.61	0.028	2.55	2.67
Hyb ($NA_{f♀}$)	2.54	0.028	2.49	2.6
Wild (NA_{EO})	2.66	0.043	2.57	2.75
Hyb15 ($EO_{w♀}$)	2.76	0.043	2.67	2.84
Hyb15 ($EO_{f♀}$)	2.57	0.035	2.5	2.64
Farm15 (EO)	2.7	0.032	2.63	2.76
Wild (NA)	2.59	0.033	2.53	2.66

Hyb16 ($EO_{w♀}$)	2.71	0.038	2.64	2.79
Hyb16 ($EO_{f♀}$)	2.76	0.040	2.68	2.84
Farm16 (EO)	2.68	0.040	2.6	2.76
Mass at yolk absorption (mg)				
Farm (NA)	133.7	3.510	126.6	141
Hyb ($NA_{f♀}$)	126.8	3.540	119.6	134
Wild (NA_{EO})	120.2	6.020	108	132
Hyb15 ($EO_{w♀}$)	136.3	5.990	124.1	149
Hyb15 ($EO_{f♀}$)	96.2	4.890	86.3	106
Farm15 (EO)	120	4.890	110.1	130
Wild (NA)	134.2	4.460	125.2	143
Hyb16 ($EO_{w♀}$)	141.3	4.680	131.9	151
Hyb16 ($EO_{f♀}$)	162.4	5.150	151.9	173
Farm16 (EO)	155.3	5.150	144.8	166
SGR_{length}				
Farm (NA)	0.095	0.004	0.087	0.103
Hyb ($NA_{f♀}$)	0.088	0.004	0.080	0.096
Wild (NA_{EO})	0.105	0.006	0.092	0.117
Hyb15 ($EO_{w♀}$)	0.115	0.006	0.102	0.128
Hyb15 ($EO_{f♀}$)	0.106	0.006	0.094	0.118
Farm15 (EO)	0.130	0.006	0.118	0.142
Wild (NA)	0.123	0.005	0.113	0.134
Hyb16 ($EO_{w♀}$)	0.108	0.006	0.095	0.120
Hyb16 ($EO_{f♀}$)	0.123	0.006	0.111	0.135
Farm16 (EO)	0.122	0.006	0.110	0.134
SGR_{mass}				
Farm (NA)	0.060	0.013	0.034	0.087
Hyb ($NA_{f♀}$)	0.050	0.013	0.023	0.076
Wild (NA_{EO})	0.072	0.014	0.044	0.101
Hyb15 ($EO_{w♀}$)	0.079	0.016	0.048	0.110
Hyb15 ($EO_{f♀}$)	0.025	0.018	-0.011	0.061
Farm15 (EO)	0.087	0.018	0.051	0.123
Wild (NA)	0.082	0.018	0.046	0.117
Hyb16 ($EO_{w♀}$)	0.085	0.022	0.041	0.129
Hyb16 ($EO_{f♀}$)	0.084	0.019	0.046	0.122
Farm16 (EO)	0.090	0.018	0.055	0.126
Condition factor at yolk absorption ($g.cm^{-3}$)				
Farm (NA)	0.755	0.014	0.727	0.783
Hyb ($NA_{f♀}$)	0.771	0.015	0.742	0.801

Wild (NA_{EO})	0.632	0.016	0.600	0.665
Hyb15 ($EO_{w♀}$)	0.649	0.016	0.617	0.682
Hyb15 ($EO_{f♀}$)	0.568	0.021	0.526	0.61
Farm15 (EO)	0.588	0.021	0.546	0.63
Wild (NA)	0.774	0.019	0.735	0.812
Hyb16 ($EO_{w♀}$)	0.718	0.022	0.673	0.763
Hyb16 ($EO_{f♀}$)	0.771	0.022	0.728	0.814
Farm16 (EO)	0.805	0.021	0.763	0.846
Yolk sac volume at hatch (cm^3)				
Farm (NA)	0.077	0.007	0.063	0.090
Hyb ($NA_{f♀}$)	0.061	0.007	0.048	0.075
Wild (NA_{EO})	0.070	0.011	0.048	0.092
Hyb15 ($EO_{w♀}$)	0.074	0.011	0.052	0.097
Hyb15 ($EO_{f♀}$)	0.053	0.010	0.034	0.073
Farm15 (EO)	0.056	0.010	0.036	0.076
Wild (NA)	0.069	0.009	0.052	0.086
Hyb16 ($EO_{w♀}$)	0.081	0.010	0.061	0.101
Hyb16 ($EO_{f♀}$)	0.111	0.010	0.091	0.130
Farm16 (EO)	0.147	0.010	0.127	0.166
Yolk sac conversion efficiency ($cm.cm^{-3}$)				
Farm (NA)	10.890	0.889	9.100	12.68
Hyb ($NA_{f♀}$)	12.300	0.856	10.580	14.02
Wild (NA_{EO})	15.200	1.334	12.480	17.92
Hyb15 ($EO_{w♀}$)	14.180	1.360	11.420	16.95
Hyb15 ($EO_{f♀}$)	17.140	1.214	14.690	19.58
Farm15 (EO)	18.200	1.217	15.750	20.65
Wild (NA)	13.770	1.113	11.530	16
Hyb16 ($EO_{w♀}$)	12.190	1.307	9.580	14.79
Hyb16 ($EO_{f♀}$)	9.440	1.256	6.910	11.96
Farm16 (EO)	7.520	1.214	5.080	9.96

Supplementary Table S2.3: Tukey-adjusted pairwise contrasts of different cross types from the 2015 and 2016 cohorts derived from the fitted final models for overall development time, length at yolk absorption, mass at yolk absorption, specific growth rate (SGR) length and mass, condition factor at yolk absorption, yolk sac volume at hatch, and yolk sac conversion efficiency. Estimate, parameter estimate.SE, standard errors. t, t-value.

Pairwise contrasts	**Estimate**	**±SE**	t	***P***
Overall development time (DD)				
Farm (NA) - Hyb ($NA_{f♀}$)	-9.579	14.99	-0.639	0.999
Farm (NA) - Wild (NA_{EO})	7.678	16.07	0.478	1.000
Farm (NA) - Hyb15 ($EO_{w♀}$)	-16.886	14.26	-1.184	0.965
Farm (NA) - Hyb15 ($EO_{f♀}$)	-8.508	16.22	-0.525	1.000
Farm (NA) - Farm15 (EO)	-16.983	14.56	-1.166	0.968
Farm (NA) - Wild (NA)	-47.899	10.36	-4.626	<0.01
Farm (NA) - Hyb16 ($EO_{w♀}$)	-26.618	17.26	-1.543	0.734
Farm (NA) - Hyb16 ($EO_{f♀}$)	-64.571	17.26	-3.742	< 0.001
Farm (NA) - Farm16 (EO)	-71.918	15.48	-4.646	<0.01
Hyb ($NA_{f♀}$) - Wild (NA_{EO})	17.257	20.04	0.861	0.982
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{w♀}$)	-7.307	16.52	-0.442	1.000
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{f♀}$)	1.071	20.16	0.053	1.000
Hyb ($NA_{f♀}$) - Farm15 (EO)	-7.404	16.78	-0.441	1.000
Hyb ($NA_{f♀}$) - Wild (NA)	-38.320	17.63	-2.173	0.205
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{w♀}$)	-17.039	21.00	-0.811	0.991
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{f♀}$)	-54.993	11.36	-4.840	<0.001
Hyb ($NA_{f♀}$) - Farm16 (EO)	-62.339	18.44	-4.981	<0.001
Wild (NA_{EO}) - Hyb15 ($EO_{w♀}$)	-24.564	17.51	-1.403	0.843
Wild (NA_{EO}) - Hyb15 ($EO_{f♀}$)	-16.185	9.97	-1.623	0.834
Wild (NA_{EO}) - Farm15 (EO)	-24.661	17.75	-1.389	0.854
Wild (NA_{EO}) - Wild (NA)	-55.577	18.56	-2.995	<0.05
Wild (NA_{EO}) - Hyb16 ($EO_{w♀}$)	-34.296	11.59	-2.960	0.104
Wild (NA_{EO}) - Hyb16 ($EO_{f♀}$)	-72.249	22.43	-3.222	<0.05
Wild (NA_{EO}) - Farm16 (EO)	-79.596	19.32	-4.119	<0.001
Hyb15 ($EO_{w♀}$) - Hyb15 ($EO_{f♀}$)	8.379	17.64	0.475	1.000
Hyb15 ($EO_{w♀}$) - Farm15 (EO)	-0.097	9.77	-0.010	1.000
Hyb15 ($EO_{w♀}$) - Wild (NA)	-31.013	17.01	-1.823	0.570
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{w♀}$)	-9.732	18.60	-0.523	1.000
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-47.685	18.60	-2.564	0.116
Hyb15 ($EO_{w♀}$) - Farm16 (EO)	-55.032	17.84	-3.084	<0.05
Hyb15 ($EO_{f♀}$) - Farm15 (EO)	-8.475	17.88	-0.474	1.000

Hyb15 ($EO_{f♀}$) - Wild (NA)	-39.391	18.69	-2.108	0.227
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{w♀}$)	-18.110	11.79	-1.536	0.874
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{f♀}$)	-56.064	22.53	-2.488	0.488
Hyb15 ($EO_{f♀}$) - Farm16 (EO)	-63.410	19.44	-3.261	0.352
Farm15 (EO) - Wild (NA)	-30.916	17.27	-1.791	0.600
Farm15 (EO) - Hyb16 ($EO_{w♀}$)	-9.635	18.83	-0.512	1.000
Farm15 (EO) - Hyb16 ($EO_{f♀}$)	-47.589	18.83	-2.527	0.131
Farm15 (EO) - Farm16 (EO)	-54.935	18.08	-3.038	<0.05
Wild (NA) - Hyb16 ($EO_{w♀}$)	21.281	19.59	1.086	0.948
Wild (NA) - Hyb16 ($EO_{f♀}$)	-16.673	19.59	-0.851	0.984
Wild (NA) - Farm16 (EO)	-24.019	20.08	-1.196	0.963
Hyb16 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-37.954	23.29	-1.630	0.792
Hyb16 ($EO_{w♀}$) - Farm16 (EO)	-45.300	20.32	-2.230	0.571
Hyb16 ($EO_{f♀}$) - Farm16 (EO)	-7.347	20.32	-0.362	1.000
Length at yolk absorption (cm)				
Farm (NA) - Hyb ($NA_{f♀}$)	0.066	0.02	2.670	0.219
Farm (NA) - Wild (NA_{EO})	-0.051	0.05	-0.998	0.9906
Farm (NA) - Hyb15 ($EO_{w♀}$)	-0.146	0.05	-2.884	0.1477
Farm (NA) - Hyb15 ($EO_{f♀}$)	0.042	0.04	0.933	0.9946
Farm (NA) - Farm15 (EO)	-0.087	0.04	-2.062	0.5615
Farm (NA) - Wild (NA)	0.015	0.04	0.349	1
Farm (NA) - Hyb16 ($EO_{w♀}$)	-0.102	0.05	-2.165	0.4915
Farm (NA) - Hyb16 ($EO_{f♀}$)	-0.148	0.05	-3.060	0.0926
Farm (NA) - Farm16 (EO)	-0.068	0.05	-1.403	0.9207
Hyb ($NA_{f♀}$) - Wild (NA_{EO})	-0.117	0.05	-2.298	0.4137
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{w♀}$)	-0.212	0.05	-4.182	<0.01
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{f♀}$)	-0.024	0.04	-0.545	0.9999
Hyb ($NA_{f♀}$) - Farm15 (EO)	-0.153	0.04	-3.615	<0.05
Hyb ($NA_{f♀}$) - Wild (NA)	-0.051	0.04	-1.185	0.9715
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{w♀}$)	-0.168	0.05	-3.563	<0.05
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{f♀}$)	-0.214	0.05	-4.416	<0.01
Hyb ($NA_{f♀}$) - Farm16 (EO)	-0.133	0.05	-2.768	0.1748
Wild (NA_{EO}) - Hyb15 ($EO_{w♀}$)	-0.096	0.03	-2.590	0.093
Wild (NA_{EO}) - Hyb15 ($EO_{f♀}$)	0.092	0.05	1.703	0.7868
Wild (NA_{EO}) - Farm15 (EO)	-0.037	0.05	-0.683	0.9995
Wild (NA_{EO}) - Wild (NA)	0.066	0.05	1.217	0.965
Wild (NA_{EO}) - Hyb16 ($EO_{w♀}$)	-0.051	0.06	-0.891	0.996
Wild (NA_{EO}) - Hyb16 ($EO_{f♀}$)	-0.098	0.06	-1.668	0.8064
Wild (NA_{EO}) - Farm16 (EO)	-0.017	0.06	-0.289	1

Hyb15 ($EO_{w♀}$) - Hyb15 ($EO_{f♀}$)	0.188	0.06	3.395	<0.05
Hyb15 ($EO_{w♀}$) - Farm15 (EO)	0.059	0.05	1.130	0.9778
Hyb15 ($EO_{w♀}$) - Wild (NA)	0.161	0.05	3.382	<0.05
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{w♀}$)	0.044	0.06	0.782	0.9985
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-0.002	0.06	-0.037	1
Hyb15 ($EO_{w♀}$) - Farm16 (EO)	0.079	0.06	1.376	0.9269
Hyb15 ($EO_{f♀}$) - Farm15 (EO)	-0.129	0.03	-2.137	0.1134
Hyb15 ($EO_{f♀}$) - Wild (NA)	-0.027	0.05	-0.553	0.9999
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{w♀}$)	-0.144	0.05	-2.774	0.1676
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{f♀}$)	-0.190	0.05	-3.571	<0.05
Hyb15 ($EO_{f♀}$) - Farm16 (EO)	-0.109	0.05	-2.055	0.5654
Farm15 (EO) - Wild (NA)	0.102	0.05	2.220	0.4574
Farm15 (EO) - Hyb16 ($EO_{w♀}$)	-0.015	0.05	-0.293	1
Farm15 (EO) - Hyb16 ($EO_{f♀}$)	-0.061	0.05	-1.189	0.9709
Farm15 (EO) - Farm16 (EO)	0.020	0.05	0.394	1
Wild (NA) - Hyb16 ($EO_{w♀}$)	-0.117	0.04	-3.307	0.0546
Wild (NA) - Hyb16 ($EO_{f♀}$)	-0.163	0.05	-3.289	0.0555
Wild (NA) - Farm16 (EO)	-0.083	0.05	-1.602	0.841
Hyb16 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-0.047	0.06	-0.837	0.9976
Hyb16 ($EO_{w♀}$) - Farm16 (EO)	0.034	0.05	0.645	0.9997
Hyb16 ($EO_{f♀}$) - Farm16 (EO)	0.081	0.03	2.319	0.3971
Mass at yolk absorption (mg)				
Farm (NA) - Hyb ($NA_{f♀}$)	6.964	2.05	3.101	0.107
Farm (NA) - Wild (NA_{EO})	13.527	6.97	1.941	0.6426
Farm (NA) - Hyb15 ($EO_{w♀}$)	-2.575	6.94	-0.371	1
Farm (NA) - Hyb15 ($EO_{f♀}$)	37.582	6.02	6.245	<.0001
Farm (NA) - Farm15 (EO)	13.765	6.02	2.287	0.4188
Farm (NA) - Wild (NA)	-0.453	5.68	-0.08	1
Farm (NA) - Hyb16 ($EO_{w♀}$)	-7.569	5.85	-1.295	0.9494
Farm (NA) - Hyb16 ($EO_{f♀}$)	-28.617	6.24	-4.589	<0.01
Farm (NA) - Farm16 (EO)	-21.517	6.24	-3.451	<0.05
Hyb ($NA_{f♀}$) - Wild (NA_{EO})	6.563	6.99	0.94	0.9937
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{w♀}$)	-9.538	6.96	-1.37	0.9279
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{f♀}$)	30.618	6.04	5.07	<0.001
Hyb ($NA_{f♀}$) - Farm15 (EO)	6.801	6.04	1.126	0.9789
Hyb ($NA_{f♀}$) - Wild (NA)	-7.417	5.70	-1.301	0.9474
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{w♀}$)	-14.532	5.87	-2.477	0.3096
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{f♀}$)	-35.580	6.26	-5.688	<0.001
Hyb ($NA_{f♀}$) - Farm16 (EO)	-28.480	6.26	-4.553	<0.01

Wild (NA_{EO}) - Hyb15 ($EO_{w♀}$)	-16.101	2.21	-2.295	0.4375
Wild (NA_{EO}) - Hyb15 ($EO_{f♀}$)	24.055	7.75	3.102	0.0935
Wild (NA_{EO}) - Farm15 (EO)	0.238	7.75	0.031	1
Wild (NA_{EO}) - Wild (NA)	-13.980	7.49	-1.866	0.6906
Wild (NA_{EO}) - Hyb16 ($EO_{w♀}$)	-21.095	7.62	-2.768	0.1863
Wild (NA_{EO}) - Hyb16 ($EO_{f♀}$)	-42.143	7.92	-5.318	<0.001
Wild (NA_{EO}) - Farm16 (EO)	-35.043	7.92	-4.422	<0.01
Hyb15 ($EO_{w♀}$) - Hyb15 ($EO_{f♀}$)	40.157	7.73	5.192	<0.001
Hyb15 ($EO_{w♀}$) - Farm15 (EO)	16.339	7.73	2.112	0.5315
Hyb15 ($EO_{w♀}$) - Wild (NA)	2.122	7.47	0.284	1
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{w♀}$)	-4.994	7.60	-0.657	0.9996
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-26.042	7.90	-3.294	0.0614
Hyb15 ($EO_{w♀}$) - Farm16 (EO)	-18.942	7.90	-2.396	0.3592
Hyb15 ($EO_{f♀}$) - Farm15 (EO)	-23.817	3.12	-2.639	0.0936
Hyb15 ($EO_{f♀}$) - Wild (NA)	-38.035	6.62	-5.745	<.0001
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{w♀}$)	-45.151	6.77	-6.673	<.0001
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{f♀}$)	-66.199	7.10	-9.318	<.0001
Hyb15 ($EO_{f♀}$) - Farm16 (EO)	-59.099	7.10	-8.319	<.0001
Farm15 (EO) - Wild (NA)	-14.218	6.62	-2.147	0.5072
Farm15 (EO) - Hyb16 ($EO_{w♀}$)	-21.333	6.77	-3.153	0.0778
Farm15 (EO) - Hyb16 ($EO_{f♀}$)	-42.381	7.10	-5.965	<.0001
Farm15 (EO) - Farm16 (EO)	-35.281	7.10	-4.966	<0.001
Wild (NA) - Hyb16 ($EO_{w♀}$)	-7.116	2.86	-2.488	0.3016
Wild (NA) - Hyb16 ($EO_{f♀}$)	-28.164	6.82	-4.13	<0.01
Wild (NA) - Farm16 (EO)	-21.064	6.82	-3.089	0.0936
Hyb16 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-21.048	6.96	-3.024	0.1059
Hyb16 ($EO_{w♀}$) - Farm16 (EO)	-13.948	6.96	-2.004	0.6011
Hyb16 ($EO_{f♀}$) - Farm16 (EO)	7.100	2.88	2.463	0.3159
SGR_{length}				
Farm (NA) - Hyb ($NA_{f♀}$)	0.007	0.00	1.717	0.7804
Farm (NA) - Wild (NA_{EO})	-0.009	0.01	-1.279	0.9516
Farm (NA) - Hyb15 ($EO_{w♀}$)	-0.020	0.01	-2.684	0.2197
Farm (NA) - Hyb15 ($EO_{f♀}$)	-0.011	0.01	-1.475	0.8957
Farm (NA) - Farm15 (EO)	-0.035	0.01	-4.865	<0.001
Farm (NA) - Wild (NA)	-0.028	0.01	-4.299	<0.01
Farm (NA) - Hyb16 ($EO_{w♀}$)	-0.013	0.01	-1.682	0.8012
Farm (NA) - Hyb16 ($EO_{f♀}$)	-0.028	0.01	-3.931	<0.01
Farm (NA) - Farm16 (EO)	-0.027	0.01	-3.723	<0.01
Hyb ($NA_{f♀}$) - Wild (NA_{EO})	-0.017	0.01	-2.233	0.4546

Hyb ($NA_{f♀}$) - Hyb15 ($EO_{w♀}$)	-0.027	0.01	-3.608	<0.05
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{f♀}$)	-0.018	0.01	-2.443	0.3223
Hyb ($NA_{f♀}$) - Farm15 (EO)	-0.042	0.01	-5.786	<.0001
Hyb ($NA_{f♀}$) - Wild (NA)	-0.035	0.01	-5.31	<0.001
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{w♀}$)	-0.020	0.01	-2.614	0.2326
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{f♀}$)	-0.036	0.01	-4.865	<0.001
Hyb ($NA_{f♀}$) - Farm16 (EO)	-0.034	0.01	-4.66	<0.001
Wild (NA_{EO}) - Hyb15 ($EO_{w♀}$)	-0.011	0.00	-2.162	0.4968
Wild (NA_{EO}) - Hyb15 ($EO_{f♀}$)	-0.001	0.01	-0.146	1
Wild (NA_{EO}) - Farm15 (EO)	-0.026	0.01	-2.998	0.1141
Wild (NA_{EO}) - Wild (NA)	-0.019	0.01	-2.343	0.3874
Wild (NA_{EO}) - Hyb16 ($EO_{w♀}$)	-0.003	0.01	-0.363	1
Wild (NA_{EO}) - Hyb16 ($EO_{f♀}$)	-0.019	0.01	-2.212	0.4663
Wild (NA_{EO}) - Farm16 (EO)	-0.017	0.01	-2.037	0.5796
Hyb15 ($EO_{w♀}$) - Hyb15 ($EO_{f♀}$)	0.009	0.01	1.078	0.9842
Hyb15 ($EO_{w♀}$) - Farm15 (EO)	-0.015	0.01	-1.743	0.7649
Hyb15 ($EO_{w♀}$) - Wild (NA)	-0.008	0.01	-1.011	0.9897
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{w♀}$)	0.007	0.01	0.834	0.9976
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-0.008	0.01	-0.965	0.9927
Hyb15 ($EO_{w♀}$) - Farm16 (EO)	-0.007	0.01	-0.793	0.9983
Hyb15 ($EO_{f♀}$) - Farm15 (EO)	-0.024	0.01	-2.025	0.1653
Hyb15 ($EO_{f♀}$) - Wild (NA)	-0.018	0.01	-2.218	0.4576
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{w♀}$)	-0.002	0.01	-0.223	1
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{f♀}$)	-0.018	0.01	-2.092	0.5412
Hyb15 ($EO_{f♀}$) - Farm16 (EO)	-0.016	0.01	-1.915	0.6592
Farm15 (EO) - Wild (NA)	0.007	0.01	0.868	0.9968
Farm15 (EO) - Hyb16 ($EO_{w♀}$)	0.022	0.01	2.59	0.245
Farm15 (EO) - Hyb16 ($EO_{f♀}$)	0.007	0.01	0.796	0.9984
Farm15 (EO) - Farm16 (EO)	0.008	0.01	0.972	0.9927
Wild (NA) - Hyb16 ($EO_{w♀}$)	0.016	0.01	2.457	0.3165
Wild (NA) - Hyb16 ($EO_{f♀}$)	0.000	0.01	-0.018	1
Wild (NA) - Farm16 (EO)	0.001	0.01	0.171	1
Hyb16 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-0.016	0.01	-1.815	0.7234
Hyb16 ($EO_{w♀}$) - Farm16 (EO)	-0.014	0.01	-1.643	0.8218
Hyb16 ($EO_{f♀}$) - Farm16 (EO)	0.001	0.01	0.247	1
SGR_{mass}				
Farm (NA) - Hyb ($NA_{f♀}$)	0.011	0.02	0.571	0.9999
Farm (NA) - Wild (NA_{EO})	-0.012	0.02	-0.622	0.9998
Farm (NA) - Hyb15 ($EO_{w♀}$)	-0.019	0.02	-0.939	0.9943

Farm (NA) - Hyb15 ($EO_{f♀}$)	0.035	0.02	1.58	0.8529
Farm (NA) - Farm15 (EO)	-0.027	0.02	-1.189	0.9715
Farm (NA) - Wild (NA)	-0.022	0.02	-0.981	0.992
Farm (NA) - Hyb16 ($EO_{w♀}$)	-0.025	0.03	-0.972	0.9929
Farm (NA) - Hyb16 ($EO_{f♀}$)	-0.024	0.02	-1.022	0.9896
Farm (NA) - Farm16 (EO)	-0.030	0.02	-1.355	0.9362
Hyb ($NA_{f♀}$) - Wild (NA_{EO})	-0.023	0.02	-1.196	0.9706
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{w♀}$)	-0.030	0.02	-1.466	0.9004
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{f♀}$)	0.025	0.02	1.132	0.9796
Hyb ($NA_{f♀}$) - Farm15 (EO)	-0.037	0.02	-1.669	0.8088
Hyb ($NA_{f♀}$) - Wild (NA)	-0.032	0.02	-1.468	0.8988
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{w♀}$)	-0.036	0.03	-1.389	0.9266
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{f♀}$)	-0.034	0.02	-1.481	0.8947
Hyb ($NA_{f♀}$) - Farm16 (EO)	-0.041	0.02	-1.838	0.709
Wild (NA_{EO}) - Hyb15 ($EO_{w♀}$)	-0.007	0.02	-0.332	1
Wild (NA_{EO}) - Hyb15 ($EO_{f♀}$)	0.047	0.02	2.435	0.3301
Wild (NA_{EO}) - Farm15 (EO)	-0.014	0.02	-0.628	0.9998
Wild (NA_{EO}) - Wild (NA)	-0.009	0.02	-0.417	1
Wild (NA_{EO}) - Hyb16 ($EO_{w♀}$)	-0.013	0.03	-0.488	1
Wild (NA_{EO}) - Hyb16 ($EO_{f♀}$)	-0.012	0.02	-0.489	1
Wild (NA_{EO}) - Farm16 (EO)	-0.018	0.02	-0.785	0.9986
Hyb15 ($EO_{w♀}$) - Hyb15 ($EO_{f♀}$)	0.054	0.02	2.293	0.4062
Hyb15 ($EO_{w♀}$) - Farm15 (EO)	-0.007	0.02	-0.403	1
Hyb15 ($EO_{w♀}$) - Wild (NA)	-0.002	0.02	-0.106	1
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{w♀}$)	-0.006	0.02	-0.232	1
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-0.005	0.02	-0.192	1
Hyb15 ($EO_{w♀}$) - Farm16 (EO)	-0.011	0.02	-0.56	0.9999
Hyb15 ($EO_{f♀}$) - Farm15 (EO)	-0.062	0.03	-2.429	0.3242
Hyb15 ($EO_{f♀}$) - Wild (NA)	-0.057	0.03	-2.26	0.4278
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{w♀}$)	-0.060	0.03	-2.12	0.52
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{f♀}$)	-0.059	0.03	-2.245	0.4371
Hyb15 ($EO_{f♀}$) - Farm16 (EO)	-0.065	0.03	-2.581	0.2455
Farm15 (EO) - Wild (NA)	0.005	0.03	0.199	1
Farm15 (EO) - Hyb16 ($EO_{w♀}$)	0.002	0.03	0.062	1
Farm15 (EO) - Hyb16 ($EO_{f♀}$)	0.003	0.03	0.104	1
Farm15 (EO) - Farm16 (EO)	-0.004	0.02	-0.165	1
Wild (NA) - Hyb16 ($EO_{w♀}$)	-0.003	0.03	-0.118	1
Wild (NA) - Hyb16 ($EO_{f♀}$)	-0.002	0.02	-0.117	1
Wild (NA) - Farm16 (EO)	-0.009	0.03	-0.34	1

Hyb16 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	0.001	0.03	0.036	1
Hyb16 ($EO_{w♀}$) - Farm16 (EO)	-0.005	0.02	-0.218	1
Hyb16 ($EO_{f♀}$) - Farm16 (EO)	-0.006	0.03	-0.238	1
Condition factor at yolk absorption ($g.cm^{-3}$)				
Farm (NA) - Hyb ($NA_{f♀}$)	-0.017	0.02	-0.812	0.9981
Farm (NA) - Wild (NA_{EO})	0.123	0.02	5.722	<.0001
Farm (NA) - Hyb15 ($EO_{w♀}$)	0.105	0.02	4.947	<0.001
Farm (NA) - Hyb15 ($EO_{f♀}$)	0.187	0.03	7.415	<.0001
Farm (NA) - Farm15 (EO)	0.167	0.03	6.616	<.0001
Farm (NA) - Wild (NA)	-0.019	0.02	-0.796	0.9983
Farm (NA) - Hyb16 ($EO_{w♀}$)	0.037	0.03	1.404	0.9212
Farm (NA) - Hyb16 ($EO_{f♀}$)	-0.016	0.03	-0.616	0.9998
Farm (NA) - Farm16 (EO)	-0.050	0.03	-1.973	0.6201
Hyb ($NA_{f♀}$) - Wild (NA_{EO})	0.139	0.02	6.368	<.0001
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{w♀}$)	0.122	0.02	5.566	<.0001
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{f♀}$)	0.204	0.03	7.993	<.0001
Hyb ($NA_{f♀}$) - Farm15 (EO)	0.183	0.03	7.129	<.0001
Hyb ($NA_{f♀}$) - Wild (NA)	-0.002	0.02	-0.094	1
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{w♀}$)	0.054	0.03	1.994	0.6061
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{f♀}$)	0.001	0.03	0.026	1
Hyb ($NA_{f♀}$) - Farm16 (EO)	-0.033	0.03	-1.29	0.9529
Wild (NA_{EO}) - Hyb15 ($EO_{w♀}$)	-0.017	0.02	-0.75	0.999
Wild (NA_{EO}) - Hyb15 ($EO_{f♀}$)	0.065	0.02	2.598	0.2437
Wild (NA_{EO}) - Farm15 (EO)	0.044	0.03	1.66	0.8139
Wild (NA_{EO}) - Wild (NA)	-0.141	0.03	-5.645	<.0001
Wild (NA_{EO}) - Hyb16 ($EO_{w♀}$)	-0.086	0.03	-3.09	0.0792
Wild (NA_{EO}) - Hyb16 ($EO_{f♀}$)	-0.138	0.03	-5.119	<0.001
Wild (NA_{EO}) - Farm16 (EO)	-0.172	0.03	-6.493	<.0001
Hyb15 ($EO_{w♀}$) - Hyb15 ($EO_{f♀}$)	0.082	0.03	3.084	0.0793
Hyb15 ($EO_{w♀}$) - Farm15 (EO)	0.061	0.02	2.558	0.2674
Hyb15 ($EO_{w♀}$) - Wild (NA)	-0.124	0.02	-4.979	<0.001
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{w♀}$)	-0.068	0.03	-2.579	0.2462
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-0.121	0.03	-4.499	<0.01
Hyb15 ($EO_{w♀}$) - Farm16 (EO)	-0.155	0.02	-6.302	<.0001
Hyb15 ($EO_{f♀}$) - Farm15 (EO)	-0.020	0.03	-0.689	0.9995
Hyb15 ($EO_{f♀}$) - Wild (NA)	-0.206	0.03	-7.257	<.0001
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{w♀}$)	-0.150	0.03	-4.884	<0.001
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{f♀}$)	-0.203	0.03	-6.732	<.0001
Hyb15 ($EO_{f♀}$) - Farm16 (EO)	-0.237	0.03	-7.975	<.0001

Farm15 (EO) - Wild (NA)	-0.185	0.03	-6.545	<.0001
Farm15 (EO) - Hyb16 ($EO_{w♀}$)	-0.130	0.03	-4.285	<0.01
Farm15 (EO) - Hyb16 ($EO_{f♀}$)	-0.182	0.03	-6.061	<.0001
Farm15 (EO) - Farm16 (EO)	-0.216	0.03	-7.841	<.0001
Wild (NA) - Hyb16 ($EO_{w♀}$)	0.056	0.03	1.899	0.6695
Wild (NA) - Hyb16 ($EO_{f♀}$)	0.003	0.03	0.116	1
Wild (NA) - Farm16 (EO)	-0.031	0.03	-1.09	0.9842
Hyb16 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-0.053	0.03	-1.7	0.7918
Hyb16 ($EO_{w♀}$) - Farm16 (EO)	-0.087	0.03	-3.089	0.085
Hyb16 ($EO_{f♀}$) - Farm16 (EO)	-0.034	0.03	-1.124	0.9806
Yolk sac volume at hatch (cm^3)				
Farm (NA) - Hyb ($NA_{f♀}$)	0.015	0.01	2.647	0.2259
Farm (NA) - Wild (NA_{EO})	0.007	0.01	0.529	0.9999
Farm (NA) - Hyb15 ($EO_{w♀}$)	0.002	0.01	0.175	1
Farm (NA) - Hyb15 ($EO_{f♀}$)	0.023	0.01	1.956	0.6325
Farm (NA) - Farm15 (EO)	0.021	0.01	1.766	0.7519
Farm (NA) - Wild (NA)	0.008	0.01	0.709	0.9993
Farm (NA) - Hyb16 ($EO_{w♀}$)	-0.004	0.01	-0.37	1
Farm (NA) - Hyb16 ($EO_{f♀}$)	-0.034	0.01	-2.853	0.1505
Farm (NA) - Farm16 (EO)	-0.070	0.01	-5.869	<.0001
Hyb ($NA_{f♀}$) - Wild (NA_{EO})	-0.009	0.01	-0.674	0.9995
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{w♀}$)	-0.013	0.01	-1.014	0.9894
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{f♀}$)	0.008	0.01	0.665	0.9996
Hyb ($NA_{f♀}$) - Farm15 (EO)	0.006	0.01	0.477	1
Hyb ($NA_{f♀}$) - Wild (NA)	-0.008	0.01	-0.696	0.9994
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{w♀}$)	-0.020	0.01	-1.634	0.8259
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{f♀}$)	-0.049	0.01	-4.104	<0.01
Hyb ($NA_{f♀}$) - Farm16 (EO)	-0.085	0.01	-7.096	<.0001
Wild (NA_{EO}) - Hyb15 ($EO_{w♀}$)	-0.004	0.01	-0.669	0.9996
Wild (NA_{EO}) - Hyb15 ($EO_{f♀}$)	0.017	0.01	1.136	0.9772
Wild (NA_{EO}) - Farm15 (EO)	0.014	0.01	0.981	0.9916
Wild (NA_{EO}) - Wild (NA)	0.001	0.01	0.071	1
Wild (NA_{EO}) - Hyb16 ($EO_{w♀}$)	-0.011	0.01	-0.76	0.9988
Wild (NA_{EO}) - Hyb16 ($EO_{f♀}$)	-0.041	0.01	-3.786	<0.05
Wild (NA_{EO}) - Farm16 (EO)	-0.076	0.01	-5.246	<0.001
Hyb15 ($EO_{w♀}$) - Hyb15 ($EO_{f♀}$)	0.021	0.01	1.432	0.9089
Hyb15 ($EO_{w♀}$) - Farm15 (EO)	0.019	0.01	1.278	0.9525
Hyb15 ($EO_{w♀}$) - Wild (NA)	0.005	0.01	0.393	1
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{w♀}$)	-0.007	0.01	-0.452	1

Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-0.036	0.01	-2.463	0.3207
Hyb15 ($EO_{w♀}$) - Farm16 (EO)	-0.072	0.01	-4.906	<0.001
Hyb15 ($EO_{f♀}$) - Farm15 (EO)	-0.002	0.01	-0.275	1
Hyb15 ($EO_{f♀}$) - Wild (NA)	-0.016	0.01	-1.197	0.969
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{w♀}$)	-0.028	0.01	-1.98	0.616
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{f♀}$)	-0.057	0.01	-4.109	<0.01
Hyb15 ($EO_{f♀}$) - Farm16 (EO)	-0.093	0.01	-6.686	<.0001
Farm15 (EO) - Wild (NA)	-0.013	0.01	-1.024	0.9891
Farm15 (EO) - Hyb16 ($EO_{w♀}$)	-0.025	0.01	-1.819	0.7207
Farm15 (EO) - Hyb16 ($EO_{f♀}$)	-0.055	0.01	-3.947	<0.01
Farm15 (EO) - Farm16 (EO)	-0.091	0.01	-6.524	<.0001
Wild (NA) - Hyb16 ($EO_{w♀}$)	-0.012	0.01	-1.38	0.9274
Wild (NA) - Hyb16 ($EO_{f♀}$)	-0.042	0.01	-3.395	<0.05
Wild (NA) - Farm16 (EO)	-0.077	0.01	-5.95	<.0001
Hyb16 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	-0.030	0.01	-2.11	0.529
Hyb16 ($EO_{w♀}$) - Farm16 (EO)	-0.065	0.01	-4.675	<0.001
Hyb16 ($EO_{f♀}$) - Farm16 (EO)	-0.036	0.01	-4.355	<0.01
Yolk sac conversion efficiency ($cm.cm^{-3}$)				
Farm (NA) - Hyb ($NA_{f♀}$)	-1.417	0.80	-1.781	0.7425
Farm (NA) - Wild (NA_{EO})	-4.315	1.61	-2.686	0.2171
Farm (NA) - Hyb15 ($EO_{w♀}$)	-3.295	1.63	-2.027	0.5867
Farm (NA) - Hyb15 ($EO_{f♀}$)	-6.251	1.51	-4.14	<0.01
Farm (NA) - Farm15 (EO)	-7.315	1.51	-4.852	<0.001
Farm (NA) - Wild (NA)	-2.879	1.42	-2.021	0.589
Farm (NA) - Hyb16 ($EO_{w♀}$)	-1.300	1.58	-0.822	0.998
Farm (NA) - Hyb16 ($EO_{f♀}$)	1.450	1.54	0.942	0.9941
Farm (NA) - Farm16 (EO)	3.367	1.51	2.237	0.4473
Hyb ($NA_{f♀}$) - Wild (NA_{EO})	-2.898	1.58	-1.84	0.7065
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{w♀}$)	-1.878	1.60	-1.171	0.9724
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{f♀}$)	-4.834	1.47	-3.289	<0.05
Hyb ($NA_{f♀}$) - Farm15 (EO)	-5.898	1.49	-3.964	<0.01
Hyb ($NA_{f♀}$) - Wild (NA)	-1.462	1.40	-1.041	0.988
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{w♀}$)	0.118	1.56	0.075	1
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{f♀}$)	2.867	1.52	1.886	0.6781
Hyb ($NA_{f♀}$) - Farm16 (EO)	4.785	1.48	3.223	0.0634
Wild (NA_{EO}) - Hyb15 ($EO_{w♀}$)	1.019	0.86	1.184	0.9707
Wild (NA_{EO}) - Hyb15 ($EO_{f♀}$)	-1.937	1.72	-1.128	0.9775
Wild (NA_{EO}) - Farm15 (EO)	-3.000	1.81	-1.663	0.8085
Wild (NA_{EO}) - Wild (NA)	1.436	1.74	0.827	0.9977

Wild (NA_{EO}) - Hyb16 ($EO_{w♀}$)	3.015	1.87	1.611	0.8362
Wild (NA_{EO}) - Hyb16 ($EO_{f♀}$)	5.764	1.83	3.145	0.082
Wild (NA_{EO}) - Farm16 (EO)	7.682	1.80	4.26	<0.01
Hyb15 ($EO_{w♀}$) - Hyb15 ($EO_{f♀}$)	-2.956	1.82	-1.628	0.8263
Hyb15 ($EO_{w♀}$) - Farm15 (EO)	-4.020	1.70	-2.365	0.3806
Hyb15 ($EO_{w♀}$) - Wild (NA)	0.417	1.76	0.237	1
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{w♀}$)	1.996	1.85	1.08	0.9842
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	4.745	1.85	2.563	0.2681
Hyb15 ($EO_{w♀}$) - Farm16 (EO)	6.663	1.73	3.851	<0.05
Hyb15 ($EO_{f♀}$) - Farm15 (EO)	-1.064	1.02	-1.043	0.9875
Hyb15 ($EO_{f♀}$) - Wild (NA)	3.373	1.65	2.048	0.5713
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{w♀}$)	4.952	1.79	2.773	0.1704
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{f♀}$)	7.701	1.75	4.408	<0.01
Hyb15 ($EO_{f♀}$) - Farm16 (EO)	9.619	1.72	5.608	<.0001
Farm15 (EO) - Wild (NA)	4.436	1.65	2.689	0.2072
Farm15 (EO) - Hyb16 ($EO_{w♀}$)	6.016	1.76	3.424	<0.05
Farm15 (EO) - Hyb16 ($EO_{f♀}$)	8.765	1.75	5.008	<0.001
Farm15 (EO) - Farm16 (EO)	10.683	1.61	6.65	<.0001
Wild (NA) - Hyb16 ($EO_{w♀}$)	1.579	1.24	1.279	0.9534
Wild (NA) - Hyb16 ($EO_{f♀}$)	4.328	1.49	2.898	0.1453
Wild (NA) - Farm16 (EO)	6.246	1.65	3.791	<0.05
Hyb16 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	2.749	1.83	1.505	0.8848
Hyb16 ($EO_{w♀}$) - Farm16 (EO)	4.667	1.64	2.841	0.1558
Hyb16 ($EO_{f♀}$) - Farm16 (EO)	1.918	1.07	1.789	0.7378

Supplementary Table S2.4: Tukey-adjusted pairwise contrasts of different cross types from the 2015 and 2016 cohorts derived from the fitted final models for overall survival (logits). Estimate, parameter estimate.SE, standard errors. z, z-value.

Pairwise contrasts	**Estimate**	**±SE**	z	***P***
Overall survival (logits)				
Farm (NA) - Hyb ($NA_{f♀}$)	-0.722	0.369	-1.956	0.630
Farm (NA) - Wild (NA_{EO})	-1.326	0.737	-1.799	0.736
Farm (NA) - Hyb15 ($EO_{w♀}$)	-2.756	0.74	-3.725	<0.01
Farm (NA) - Hyb15 ($EO_{f♀}$)	1.055	0.599	1.76	0.761
Farm (NA) - Farm15 (EO)	0.417	0.6	0.696	1.000
Farm (NA) - Wild (NA)	-1.637	0.673	-2.433	0.306
Farm (NA) - Hyb16 ($EO_{w♀}$)	0.028	0.612	0.046	1.000
Farm (NA) - Hyb16 ($EO_{f♀}$)	1.716	0.662	2.59	0.222
Farm (NA) - Farm16 (EO)	1.653	0.601	2.749	0.154
Hyb ($NA_{f♀}$) - Wild (NA_{EO})	-0.604	0.703	-0.86	0.998
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{w♀}$)	-2.034	0.731	-2.783	0.142
Hyb ($NA_{f♀}$) - Hyb15 ($EO_{f♀}$)	1.777	0.556	3.195	<0.05
Hyb ($NA_{f♀}$) - Farm15 (EO)	1.139	0.589	1.934	0.646
Hyb ($NA_{f♀}$) - Wild (NA)	-0.915	0.663	-1.38	0.933
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{w♀}$)	0.750	0.601	1.249	0.964
Hyb ($NA_{f♀}$) - Hyb16 ($EO_{f♀}$)	2.438	0.653	3.735	<0.01
Hyb ($NA_{f♀}$) - Farm16 (EO)	2.375	0.59	4.027	<0.01
Wild (NA_{EO}) - Hyb15 ($EO_{w♀}$)	-1.430	0.375	-2.809	0.219
Wild (NA_{EO}) - Hyb15 ($EO_{f♀}$)	2.381	0.657	3.621	<0.05
Wild (NA_{EO}) - Farm15 (EO)	1.743	0.752	2.319	0.376
Wild (NA_{EO}) - Wild (NA)	-0.311	0.811	-0.383	1.000
Wild (NA_{EO}) - Hyb16 ($EO_{w♀}$)	1.354	0.762	1.778	0.750
Wild (NA_{EO}) - Hyb16 ($EO_{f♀}$)	3.042	0.803	3.79	<0.01
Wild (NA_{EO}) - Farm16 (EO)	2.978	0.753	3.955	<0.01
Hyb15 ($EO_{w♀}$) - Hyb15 ($EO_{f♀}$)	3.811	0.754	5.054	<.0001
Hyb15 ($EO_{w♀}$) - Farm15 (EO)	3.173	0.66	4.811	<0.0001
Hyb15 ($EO_{w♀}$) - Wild (NA)	1.119	0.815	1.374	0.935
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{w♀}$)	2.784	0.672	2.145	0.217
Hyb15 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	4.472	0.806	5.549	<.0001
Hyb15 ($EO_{w♀}$) - Farm16 (EO)	4.408	0.662	6.659	<.0001
Hyb15 ($EO_{f♀}$) - Farm15 (EO)	-0.638	0.369	-1.729	0.779
Hyb15 ($EO_{f♀}$) - Wild (NA)	-2.691	0.688	-3.911	<0.01
Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{w♀}$)	-1.027	0.629	-1.632	0.833

Hyb15 ($EO_{f♀}$) - Hyb16 ($EO_{f♀}$)	0.661	0.678	0.975	0.994
Hyb15 ($EO_{f♀}$) - Farm16 (EO)	0.598	0.619	0.966	0.994
Farm15 (EO) - Wild (NA)	-2.054	0.69	-2.977	0.086
Farm15 (EO) - Hyb16 ($EO_{w♀}$)	-0.389	0.514	-0.757	0.999
Farm15 (EO) - Hyb16 ($EO_{f♀}$)	1.299	0.679	1.912	0.661
Farm15 (EO) - Farm16 (EO)	1.235	0.501	2.463	0.289
Wild (NA) - Hyb16 ($EO_{w♀}$)	1.665	0.467	2.567	0.142
Wild (NA) - Hyb16 ($EO_{f♀}$)	3.353	0.513	6.533	<.0001
Wild (NA) - Farm16 (EO)	3.289	0.69	4.764	<0.0001
Hyb16 ($EO_{w♀}$) - Hyb16 ($EO_{f♀}$)	1.688	0.69	3.445	<0.01
Hyb16 ($EO_{w♀}$) - Farm16 (EO)	1.624	0.511	3.181	<0.05
Hyb16 ($EO_{f♀}$) - Farm16 (EO)	-0.063	0.467	-0.136	1.000

CHAPTER 3

Distinct early-life stage gene expression effects of hybridization among European and North American farmed and wild Atlantic Salmon populations

Preface

The research described in Chapter 3 is now under review to the journal Molecular Ecology as: Islam, S. S., X. Xue, A. Caballero-Solares, I. R. Bradbury, M. L. Rise, and I. A. Fleming. Distinct early-life stage gene expression effects of hybridization among European and North American farmed and wild Atlantic salmon populations; see Co-authorship statement on page XXIV-XXV.

3.1 Abstract

Due to multi-generation domestication-associated selection, farmed and wild Atlantic salmon have differed genetically. This raises concerns about potential genetic interactions should farmed fish escape and interbreed with local wild populations and thereby disrupt local adaptation through introgression. When farmed strains of distant geographic origin are used, it is unknown whether the genetic risks posed by escaped farmed fish will be greater than if more locally derived strains are used. Quantifying gene expression differences among divergent farmed, wild and F_1 hybrids under controlled conditions is one of the ways to explore the consequences of hybridization. To this end, I compared the transcriptomes of late sac fry of a European (EO) farmed ("StofnFiskur", Norwegian strain), a North American (NA) farmed (Saint John River, NB strain), a Newfoundland (NF) wild population with EO ancestry, and related F_1 hybrids using 44K oligonucleotide microarrays. My findings indicate that the wild population showed greater transcriptome differences from the EO farmed strain than that of the NA farmed strain. I also found the largest differences in global gene expression between the two farmed strains. I detected fewer significantly differentially expressed transcripts between F_1 hybrids and domesticated/wild maternal strains. I also found that the differentially expressed genes between cross types over-represented gene ontology (GO) terms associated with metabolism, development, growth, immune response, and redox homeostasis processes. These findings suggest that the interbreeding of escaped EO/NA farmed and a NF wild population would alter gene transcription, and the consequences of hybridization would be

greater from escaped EO farmed than NA farmed salmon, resulting in potential effects on the fitness of local wild populations.

3.2 Introduction

It has long been recognized that hybridization between domesticated species and their wild conspecifics constitutes a potential threat to the genetic integrity of natural populations, and as such, is now of growing concern (Allendorf et al., 2001; Hamilton & Miller, 2016; Todesco et al., 2016). Successful hybridization between domesticated and wild populations may result in loss of genetic variation, reduced adaptive fitness, and breakdown of local adaptations (Ellstrand et al., 1999; Garcia de Leaniz et al., 2007; McFarlane & Pemberton, 2019). This likely occurs when hybridization breaks up locally co-adapted gene complexes through introgression or brings together allele combinations with negative effects by segregation and recombination (Turelli et al., 2001; Edmands, 2007; Chan et al., 2019). Consequently, such outbreeding depression, a mechanism of fitness reduction, is expected when outbreeding involves more genetically distant populations (Edmands & Timmerman, 2003). Thus, understanding the fitness implications of genetic interaction between divergent domesticated and wild conspecifics is important for the conservation and management of intraspecific biodiversity.

Broadly speaking, domesticated animals comprise a rapidly increasing proportion of life on Earth (Bar-On et al., 2018). Given human-induced environmental change, wild living resources are becoming increasingly unsustainable (Hutchings, 2000; Myers & Worm, 2003; Thiault et al., 2019), and domestication of species represents a necessary component for food security and capture fisheries (Gering et al., 2019; Houston et al.,

2020). Compared with other livestock species (e.g., pigs, cattle, sheep, goats, and poultry), which have been domesticated as a source of food for 5,000 generations (Craig, 1981), domestication has only been implemented in most fishes, aside from carp, for 5 to 15 generations (Gjedrem, 2000). Atlantic salmon (*Salmo salar* L.), an ecologically and culturally significant fish species, have been intensively domesticated since 1970 and undergone intentional selection for a wide range of economically important traits such as increased growth rates, delayed maturation, and enhanced disease resistance (Gjederm, 2010); in combination with unintentional and relaxed selection on non-target traits (e.g., increased aggression, reduced risk-aversion, altered feeding behaviours) (Fleming & Einum, 1997; Huntingford, 2004). Consequently, Atlantic salmon is considered as one of the most domesticated cultured fish species worldwide for food (Teletchea & Fontaine, 2014).

Due to the rapid expansion of salmon farming in the past three to four decades, tens of millions of domesticated salmon have escaped aquaculture facilities, thus raising a persistent concern about their potential direct and indirect genetic interaction with wild salmon (Fleming et al., 2000; McGinnity et al., 2003; Bolstad et al., 2017; Skaala et al., 2019). Direct genetic interaction can arise through introgression, which increases gene flow by genetic mixing (Glover et al., 2017). On the other hand, indirect genetic interaction can occur through altered selective pressures, which can lead to decreased survival, reduction in population size, and increased genetic drift (Bradbury et al., 2020). A pressing question is to what extent interbreeding between farmed and wild individuals will change the genetics of wild populations. The extent of subsequent genetic risk of hybridization will be

dependent on the relationship between wild and farm strains resulting from both the degree of domestication selection and the ancestral relationship of farmed strains to wild populations (Neff, 2004; Baskett & Waples, 2013).

A plethora of genetic studies revealed that the Pleistocene glaciations resulted in a continental divergence between European (hereafter "EO") and North American (hereafter "NA") salmon populations that likely started > 600,000 years before present (Nilsson et al., 2001; King et al., 2007; Rougemont & Bernatchez, 2018). This prolonged reproductive isolation between EO and NA salmon accrued many genetic differences, including differences in chromosome number and structure (Lehnert et al., 2020). However, despite this lengthy isolation, genetic data provide evidence of trans-Atlantic secondary contact in EO and NA populations when glaciers retreated, and salmon recolonized (~10,000 years before present; Bradbury et al., 2015). This secondary contact between divergent EO and NA populations can have significant genomic consequences, including chromosomal inversions, translocations, and fusions (i.e., Ssa01/Ssa23 translocation and Ssa08/Ssa29 fusion; Lehnert et al., 2019). Existing knowledge indicates that our studied Newfoundland (NF) wild population has a natural signal of EO. Presently, the major commercial salmon aquaculture strain in use in Atlantic Canada derives from the Saint John River, New Brunswick (NB). However, site-specific permission has been recently granted to Grieg NL (a Norwegian-based seafood company) to import an EO strain (StofnFiskur strain, domesticated from wild Norwegian salmon), to be farmed as triploids in NF. Should a proportion of non-triploid (Benfey, 2015) EO farmed salmon escape, it is likely that they will be able to breed successfully and interact genetically with local wild populations

(O'Reilly et al., 2006). Whether the resultant genetic impacts would be greater than that resulting from the use of NA farmed strains is unknown.

It is increasingly appreciated that evolutionary changes may strongly depend on alterations of gene transcription regulation (Stern, 2000; Fay & Wittkopp, 2008). Even small gene expression changes can have consequences for development and phenotypic expression (Cho et al., 1998). Transcription profiles have the potential to reveal evolutionary novelty at both the phenotypic and genomic levels (Whitehead & Crawford, 2006; Bernatchez et al., 2010). For example, DNA microarrays, measuring the expression levels of thousands of transcripts simultaneously, represent a powerful tool for identifying evolutionarily important gene expression differences (Bumgarner, 2013). In Atlantic salmon, even 7-12 generations of domestication selection was enough to generate significant changes in gene expression patterns using DNA microarrays between farmed and wild salmon (Roberge et al., 2006; Bicskei et al., 2014). Moreover, the transition stage from endogenous feeding to the onset of exogenous feeding (i.e., late sac fry stage) is a key developmental stage and is critical for the fish's metabolic, development, growth, immune system function, and survival; thus, it can play a major role in shaping evolutionary trajectories at the population level (Einum & Fleming, 2000). The study of the differences in gene transcription levels at an early life stage among farmed, wild, and F_1 hybrid salmon can, therefore, provide insight into the fitness consequences of hybridization between escaped farm and their wild conspecifics.

Here, using a common garden experiment, 44K microarrays (Jantzen et al., 2011) combined with real-time quantitative polymerase chain reaction (qPCR) validation were applied to provide insight into gene expression differences among farmed, wild, and F_1

hybrid salmon at the late sac fry stage. Although some previous studies have compared the transcriptional/genomic signature of domestication between EO and NA (Canadian) multi-generational farmed and wild salmon (Roberge et al., 2006; Vasemägi et al., 2012; Mäkinen et al., 2014; López et al., 2018), to my knowledge, this is the first experiment that characterizes early life (i.e., whole-body transcriptome of late sac fry) gene expression effects of hybridization, while comparing population-specific differences among NA and EO farmed, NF wild with EO ancestry and F_1 hybrid salmon. I hypothesized that: (i) due to a common multi-generation domestication effect, EO and NA farmed fish will exhibit some similar gene expression patterns; (ii) due to geographic and ancestral relationships, global gene expression patterns of NF wild fish will be more similar to that of NA farmed than that of EO farmed fish; and (iii) F_1 hybrids will display altered gene expression relative to wild and farmed fish. My main goal was to understand the potential consequences of hybridization of EO and NA farmed escapees on NF wild populations to better guide sustainable aquaculture practices and the maintenance of wild populations.

3.3 Materials and Methods

3.3.1 Study populations, crosses, and sampling

Gametes used in experimental crosses were derived from parental salmon of three main populations: Farm.NA, Farm.EO, and NF Wild with a signal of EO ancestry. The origin of Farm.NA is the Saint John River, NB, and represents Atlantic Canada's dominant aquaculture strain of Atlantic salmon. Farm.NA gametes were collected from the aquaculture company Northern Harvest, based in Southern NF. Farm.EO, a Norwegian origin farm strain, is now produced at an Icelandic facility (StofnFiskur), from where the

gametes were shipped by air for the experimental crosses. The wild population was derived from the Northeast Placentia River, NF (Lat: 47.2408 °N, Lon: 53.9566 °W). Wild broodstock were captured at a fishway facility and transported to the Ocean Sciences Centre (OSC, Memorial University of Newfoundland) on July 27, 2015, and kept in broodstock tanks (2000 L) until the crosses were made. This wild population was included in the current study as it showed evidence of EO introgression when salmon recolonized after glaciers retreated (~10,000 years before present; see Bradbury et al., 2015). Crosses were generated between 20 November and 5 December 2015 to produce 76 families of six cross types (for biological information about the parental populations and cross details, see chapter two). Crosses were generated and reared in an incubation facility at the OSC. Following crossing, the eggs were water-hardened and disinfected with 0.5% Ovadine (Syndel, Nanaimo, BC, Canada) for 30 min. Fertilized eggs were then incubated in Heath trays and raised under common environmental conditions (ambient water temperature: 3.1 - 7.9 ºC, pH: 5.7 - 6.2, dissolved oxygen: 8.0 – 8.5 mg l^{-1}).

Following yolk sac absorption (i.e., swim-up fry at the onset of exogenous feeding stage), sampling took place; fry were euthanized with AQUALIFE TMS (MS-222; 400 mgl^{-1}, saline buffered with 0.1 M sodium bicarbonate), immediately flash-frozen in liquid nitrogen and stored at -80 ºC until homogenized.

3.3.2 Microarray experimental design

The microarray study was performed using a consortium for Genomic Research on All Salmonids Project (cGRASP)-designed Agilent (Mississauga, ON, CA) 44K salmonid oligonucleotide microarray (GEO accession number: GPL11299; Jantzen et al., 2011). The

microarray experiment comprised 36 arrays: 6 cross types (Farm.NA, Hyb.NA$_{f♀}$, Wild, Hyb.EO$_{w♀}$, Hyb.EO$_{f♀}$, and Farm.EO) x 3 families per cross type x 2 biological replicates per family. Families from each cross type were selected based on their average cumulative survival (%) to yolk sac absorption during incubation (see chapter two for details on survival data). .

3.3.3 RNA extraction, DNase treatment, and column purification

Frozen whole fry were homogenized individually in TRIzol Reagent (Invitrogen/Life Technologies, Burlington, CA) with stainless steel beads (5 mm; QIAGEN, Mississauga, ON, CA) using a TissueLyser (QIAGEN), following the manufacturer's instructions, then further disrupted using QIAshredder spin columns (QIAGEN), and then subjected to RNA extraction (detailed in Umasuthan et al., 2020). The resulting 30 μg of each total RNA samples were treated with DNase I (6.8 Kunitz units; RNase-free DNase Set, QIAGEN) with the manufacturer's buffer (1 x final concentration) to degrade residual genomic DNA, and then purified using the RNeasy Mini Kit (QIAGEN) following the manufacturer's instructions. RNA integrity was confirmed by 1% agarose gel electrophoresis, and RNA purity was assessed using A260/280 and A260/230 via NanoDrop UV spectrophotometry (NanoDrop, Wilmington, DE, USA) at both the pre-cleaned and the cleaned RNA stages. Only high-purity (A260/280 > 2.0, A260/230 > 1.8) column-cleaned RNA samples were used in RNA amplification and cDNA synthesis reactions.

3.3.4 Microarray hybridization and data acquisition

Differences among the six cross types in the whole-body transcriptome were assessed by contrasting individual RNA samples against a common reference pool of equal quantities of RNA from all 36 fry. This microarray experiment was performed as described in Caballero-Solares et al. (2018) and Umasuthan et al. (2020). In short, anti-sense amplified RNA (aRNA) was prepared using Ambion's Amino Allyl MessageAmp II aRNA Amplification kit (Life Technologies). The aRNA samples were column-purified and subjected to the quality and quantity assessment by agarose gel electrophoresis and Nanodrop UV spectrophotometry. A common reference was prepared by pooling 10 µg of each of the 36 experimental aRNA samples. Twenty micrograms of each experimental and common reference aRNA sample was then precipitated overnight following standard molecular biology procedures and re-suspended in coupling buffer. Subsequently, individual and common reference aRNA were labeled with Cy5 and Cy3 (GE HealthCare, Mississauga, ON, CA), respectively. The labeling efficiency was quantified using the "microarray" function in the ND-1000 Nanodrop UV spectrophotometer. Equal quantities (825 ng) of labeled aRNA from each experimental sample and the common reference were fragmented and co-hybridized to a microarray, as per manufacturer protocols (Agilent, Mississauga, ON, CA). Hybridizations were conducted at 65^{o}C for 17 h at 10 rpm rotation in an Agilent hybridization oven. Array slides were then washed with Gene Expression Wash Buffer 1 and 2 (Agilent) immediately after hybridization according to the manufacturer's guidelines, and residual wash buffer was removed by centrifuging at 200xg for 5 min at room temperature.

Each microarray slide was scanned (at 5 µm resolution and 90% of laser power) using a ScanArray Gx Plus scanner and ScanArray Express software (v.4.0; Perkin Elmer, Waltham, MA, USA). The Cy5 and Cy3 photomultiplier tube (PMT) sensitivities were adjusted to balance the fluorescence signal. The resulting TIFF images were then extracted using Imagene software (v.9.0; Biodiscovery Inc., El Segundo, CA, USA). The removal of low-quality/flagged spots, background signal correction, LOWESS normalization, and data transformation ($\log_2$) were performed using R version 3.6.1 (R Core Team 2020) and the Bioconductor package mArray (Booman et al., 2011).

3.3.5 Microarray data analyses

Prior to statistical analyses, missing data points were imputed using the EM_array method from LSimpute (Bø et al., 2004). Based on their ecological relevance, pairwise cross type comparisons of the differentially expressed probes (DEPs) among EO and NA farmed, NF wild, and F_1 hybrid salmon were determined using both Rank Products (RP) (Breitling et al., 2004; Hong et al., 2006) and Significance Analysis of Microarrays (SAM) (Tusher et al., 2001), with the percentage false positive (PFP) and false discovery rate (FDR) cut-off of 10%. The pairwise comparisons between NA hybrid and EO farmed and EO hybrid and NA farmed were not considered in the text in light of their lack of ecological relevance (i.e., the likelihood that such comparisons would occur in nature should escape of a particular strain occur). The resulting DEPs were annotated using the contiguous sequences (contigs) or expressed sequence tags (ESTs) employed to design the 60mer oligonucleotide probes of the array (Jantzen et al., 2011). BLASTn/BLASTx alignment of these sequences against the NCBI non-redundant (nr) amino acid and nucleotide sequence databases (E-value threshold $< 1 \times 10^{-5}$). The best BLASTn/BLASTx hits relating to

putative *Homo sapiens* orthologues were used to obtain gene ontology (GO) terms from the UniProt Knowledgebase (http://www.uniprot.org/) (see details in Appendix A for microarray fold-changes; functional annotation). GO term enrichment analyses (GTEA) were conducted to further inform the functional implications of the lists of DEPs among cross types using the ClueGO (Bindea et al., 2009) plugin in Cytoscape v.3.7.2 version (Shannon et al., 2003). The enrichment analyses were performed using the entire 44K microarray as the reference gene set corresponding to putative *Homo Sapiens* orthologues for biological process (BP), cellular component (CC), and molecular function (MF), and a right-sided hypergeometric test with Benjamini-Hochberg correction (Benjamini & Hochberg, 1995) (FDR < 0.05). For visualization of GO term networks, the GO term fusion strategy was employed to create functionally organized GO cluster networks. The leading terms of each network were selected based on their significance with different p-values for different gene sets with the Kappa-statistics (Cohen, 1968) score threshold set to 0.4. The complete linkage clustering functions in Genesis programs (v.1.8.1) were used in generating the hierarchical clustering and heatmaps of DEPs (Sturn et al., 2002).

3.3.6 Real-time qPCR validation

A total of 108 fry were used for qPCR analysis: 3 fry per family, 6 families per cross type, and 6 cross types (3 fry x 6 families x 6 cross types). The 20 genes of interest (GOIs), including paralogs for 4 GOIs, used in this qPCR study were selected from the DEP lists (for details of qPCR assays, see Supplementary Table S3.1). The genes selected from both RP and SAM gene lists were based on the GTEA, as representative of metabolic, developmental, growth, immune, cellular, and redox homeostasis processes. For each of the selected GOIs, BLASTn searches of NCBI's non-redundant nucleotide (nt) and

expressed sequence tags (EST) databases (*Salmo salar* [taxid: 8030] sequences) were conducted to identify paralogs. The alignment of multiple cDNA sequences corresponding to putative paralogs using Vector NTI (Vector NTI Advance 11, Life Technologies) revealed regions suitable for designing paralog-specific primers (i.e., with at least 3 bp difference). All primers were designed using Primer3 (v.0.4.0) software (available at [http://bioinfo.ut.ee/primer3-0.4.0/]). Each primer pair was quality tested using the 7500 Fast Real Time PCR system (Applied Biosystems/Life Technologies) to ensure that a single product was amplified (dissociation curve analysis) and there was no primer-dimer present in the no-template control (NTC). Amplicons were electrophoretically separated on 2% agarose gels and compared with a 1 Kb Plus DNA Ladder (Invitrogen/Life Technologies) to verify that the correct size fragment was being amplified. Amplification efficiencies were determined from the standard curves generated using 5-point 1:3 dilution series, starting with cDNA representing 10 ng of input total RNA (Pfaffl, 2001). For primer quality testing, three reference RNA pools (Farm.EO, Farm.NA, and Wild) were prepared with an equal contribution of all samples from each cross type included in the qPCR study.

qPCR primer pairs of six candidate normalizer genes (60S ribosomal protein 32 [*rpl32*]; elongation factor 1 alpha-1 [*ef1a1*]; elongation factor 1 alpha-2 [*ef1a2*]; beta-actin [*actb*]; polyadenylate-binding protein 1 [*pabpc1*]; and eukaryotic translation initiation factor 3 subunit D [*eif3d*]), designed for previous studies (Caballero-Solares et al., 2018; Eslamloo et al., 2017; Umasuthan et al., 2020), were quality tested as described above. Template cDNA of each sample (5 ng input total RNA) was used to measure the fluorescence threshold cycle (C_T) for each candidate normalizer. Among the six, three

candidate normalizers genes were selected based on their gene expression stability, which was assessed using geNorm M values (*rpl32,* geNorm M = 0.195*; eif3d,* geNorm M = 0.200*; pabpc1*, geNorm M = 0.206) (Hellemans et al., 2007).

First-strand cDNA templates for qPCR were synthesized in 20 µL reactions from 1 µg of DNaseI-treated, column-purified total RNA using random primers (250 ng; Invitrogen/Life Technologies) and M-MLV reverse transcriptase (200 U; Invitrogen/Life Technologies) with the manufacturer's first strand buffer (1 x final concentration), dNTPs (0.5 mM final concentration), and DTT (10 mM final concentration) at 37 ºC for 50 min. After primer quality testing and normalizer gene selection, qPCR analyses of the transcript levels of the selected GOIs were performed in technical triplicates using Power SYBR Green I dye chemistry in 384-well format on a ViiA 7 Real-Time PCR system (Applied Biosystems/Life Technologies, Foster City, USA). A NTC (in triplicate) was included in each qPCR plate. Assays were performed in 13 µL reactions using 1 x Power SYBR Green PCR Master Mix (Applied Biosystems/Life Technologies), 50 nM of both forward and reverse primers, and 4 µL of diluted cDNA (5 ng input total RNA). The qPCR program involved 1 cycle at 50 ºC for 2 min, 1 cycle at 95 ºC for 10 min, and 40 cycles of 95 ºC for 15 s and 60 ºC for 1 min, with fluorescence detection at the end of each 60 ºC step.

The relative quantity (RQ) of each transcript was determined by a qBase relative quantification framework (Hellemans et al., 2007) by using the C_T values quantified for GOIs and reference genes using the ViiA 7 software (v.1.2.3; Applied Biosystems/Life Technologies), with normalization to the transcript levels of *rpl32*, *eif3d*, and *pabpc1*, and amplification efficiencies incorporated (Umasuthan et al., 2020). For each GOI, the sample

with the lowest normalized expression (mRNA) level was set as the calibrator sample (i.e., assigned as RQ value = 1.0). Fold-change values were calculated from microarray $\log_2$ ratios, and qPCR RQs. Genes were considered to be validated if qPCR showed significant differential expression among cross types, with microarray and qPCR fold-changes in the same direction.

3.3.7 Statistical analyses

All statistical analyses were performed in R version 4.0.5 (R Core Team, 2021). Statistical significance was inferred if $P < 0.05$ after sequential Bonferroni adjustment (Rice, 1989). All data were checked visually (Q-Q plot) and statistically (Shapiro-Wilk's test) for normality, and homoscedasticity was assessed visually (using residuals vs. fitted values) (Crawley, 2005). The validated qPCR datasets of selected metabolic, developmental, growth, immune, cellular, and redox homeostasis relevant genes (n = 12) were analyzed via principal component analysis (PCA) using the Factoextra R packages. Cross type-driven PCA scores (i.e., PC1 and PC2) and differences in qPCR RQs, were analyzed using either ANOVA (parametric) or the Kruskal-Wallis test (non-parametric alternative to one-way ANOVA). Tukey-adjusted multiple comparisons posthoc test was performed for pairwise comparisons between cross types.

3.4 Results

3.4.1 Differentially expressed transcripts between cross types

Pairwise cross type comparisons detected DEPs by RP and SAM analysis among fry of EO farmed, NA farmed, wild, and F_1 hybrid cross types (Table 3.1, Supplementary Table S3.2, Supplementary Fig. S3.1). The largest number of DEPs was observed between

fry of the two domesticated strains, Farm.NA vs. Farm.EO (RP: 223 DEPs; SAM: 168 DEPs). The greatest transcriptomic differences between domesticated farmed and wild populations were observed between Farm.EO vs. Wild (RP: 200 DEPs), as compared to Farm.NA vs. Wild (RP: 46 DEPs; SAM: 21 DEPs). SAM did not find any DEPs between Farm.EO vs. Wild, possibly since SAM is more sensitive to high biological variability (Tusher et al., 2001; Breitling et al., 2004; Jeffery et al., 2006; Brown et al., 2016); therefore, subsequent data analyses were performed using the RP DEP lists. Interestingly, there were fewer DEPs between fry of both EO and NA F_1 hybrid and wild cross types (Hyb.$NA_{f♀}$ vs. Wild [RP: 23 DEPs; SAM: 4 DEPs]; Hyb.$EO_{w♀}$ vs. Wild [RP: 17 DEPs; SAM: 0 DEP]; and Hyb.$EO_{f♀}$ vs. Wild [RP: 9 DEPs; SAM: 13 DEPs]) and EO and NA F_1 hybrid and farm cross types (Hyb.$NA_{f♀}$ vs. Farm.NA [RP: 6 DEPs; SAM: 2 DEPs]; Hyb.$EO_{w♀}$ vs. Farm.EO [RP: 3 DEPs; SAM: 1 DEP]; and Hyb.$EO_{f♀}$ vs. Farm.EO [RP: 5 DEPs; SAM: 3 DEPs]). There were no DEPs detected between Hyb.$EO_{w♀}$ and Hyb.$EO_{f♀}$.

Table 3.1: Number of differentially expressed probes (DEPs) between different cross types identified by RP (Percentage of False Prediction < 0.1) analysis. See Supplementary Table S3.2 for the complete list of DEPs.

Comparisons[†]	**Up-regulated DEPs**	**Down-regulated DEPs**	**Total DEPs**[‡]
Farm.NA vs. Wild	41	5	46
Farm.EO vs. Wild	177	23	200
Farm.NA vs. Farm.EO	139	84	223
Hyb.$NA_{f♀}$ vs. Wild	21	2	23
Hyb.$EO_{w♀}$ vs. Wild	8	9	17
Hyb.$EO_{f♀}$ vs. Wild	6	3	9
Hyb.$NA_{f♀}$ vs. Farm.NA	3	3	6
Hyb.$EO_{w♀}$ vs. Farm.EO	0	3	3

Hyb.EO$_{f♀}$ vs. Farm.EO	3	2	5
Hyb.EO$_{f♀}$ vs. Hyb.EO$_{w♀}$	0	0	0

†Farm.NA: North American farmed strain, derived from Saint John River, NB; Farm.EO: European origin Norwegian farm strain, unfertilized gametes collected from the Icelandic facility; Wild: North American wild derived from the Northeast Placentia River, NF; Hyb.NA$_{f♀}$: F_1 Farm.NA(♀)-x-Wild(♂) hybrid; Hyb.EO$_{w♀}$: F_1 Farm.EO(♂)-x-Wild(♀) hybrid; and Hyb.EO$_{f♀}$: F_1 Farm.EO(♀)-x-Wild(♂) hybrid

‡Total numbers of non-redundant DEPs were 396 (see Fig. 1)

Hierarchical cluster analyses and heatmaps of all RP DEPs provided further insights into mean expression values by cross type (Fig. 3.1) and individual expression patterns among cross types (see Supplementary Fig. S3.2). Heatmap and hierarchical clustering analyses of mean expression values by cross type showed that cross types were grouped into two distinct clusters (Fig. 3.1): Farm.NA, related F_1 hybrid (Hyb.NA$_{f♀}$) and Wild into one cluster, and Farm.EO and their related hybrids (Hyb.EO$_{w♀}$ and Hyb.EO$_{f♀}$) into another cluster. Additionally, three separate clusters of DEPs were evident (left side of Fig. 3.1). In the first cluster (Fig. 3.1, gene cluster I), DEPs were up-regulated in Farm.NA, Hyb.NA$_{f♀}$ and Wild cross types, but down-regulated in Farm.EO and their related hybrids (Hyb.EO$_{w♀}$ and Hyb.EO$_{f♀}$). By contrast, in the second cluster (Fig. 3.1, gene cluster II), DEPs were down-regulated in Farm.NA, Hyb.NA$_{f♀}$ and Wild cross types, but up-regulated in Farm.EO, Hyb.EO$_{w♀}$ and Hyb.EO$_{f♀}$. In the third cluster (Fig. 3.1, gene cluster III), DEPs were generally up-regulated in both Farm.NA and Farm.EO, but down-regulated in Wild. Despite the fact that low numbers of DEPs were detected between Wild and F_1 hybrids, the expression profiles of all microarray-identified genes still show different patterns between Wild and hybrids based on the cluster and heatmap analyses.

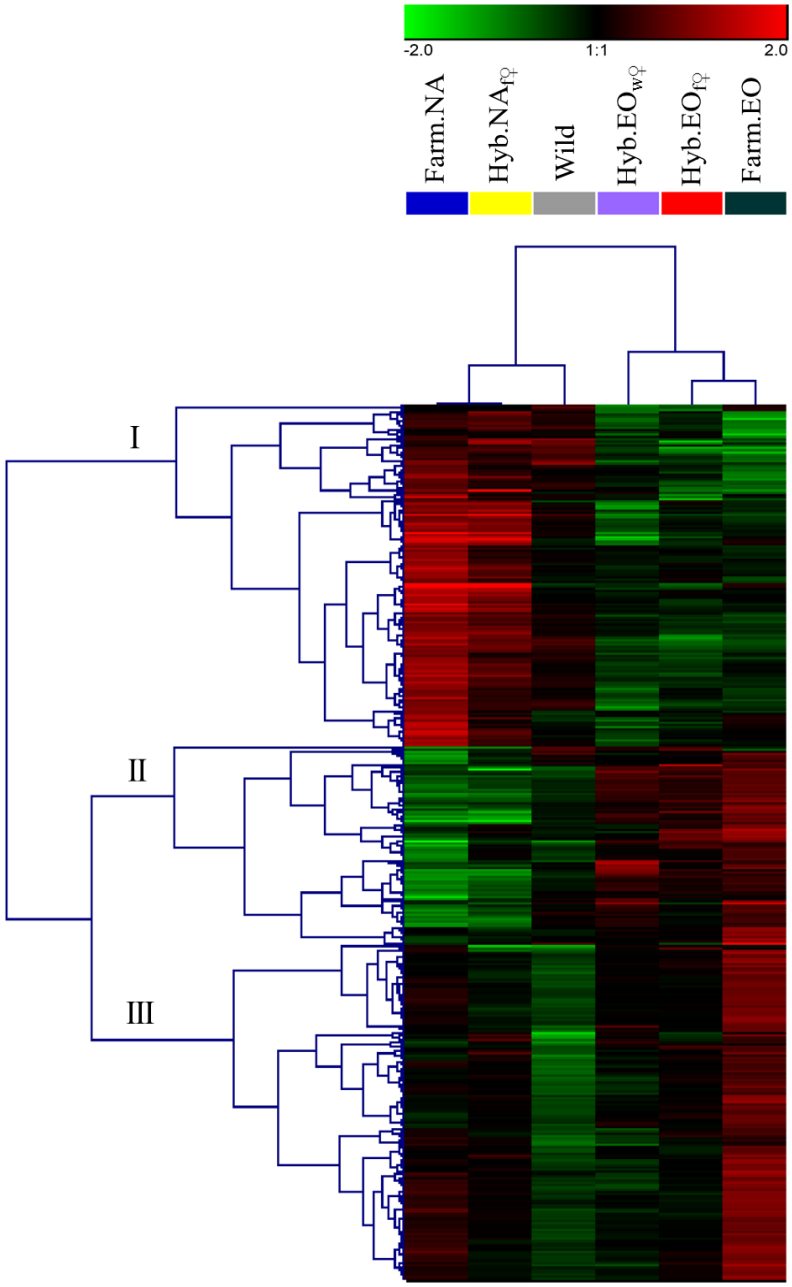


Figure 3.1: Hierarchical clustering analyses of mean expression values of all samples (in a given cross type) using 396 DEPs from RP analysis (after removing redundancy from 532 DEPs). For the individual expression patterns among cross types, see Supplementary Figure S3.2. Coloured panel indicates mean expression values (6 biological replicates) of each cross type: blue-Farm.NA, yellow-Hyb.NA$_{f♀}$, grey-Wild, light blue-Hyb.EO$_{w♀}$, red-Hyb.EO$_{f♀}$, and black-Farm.EO

3.4.2 Functional enrichment analyses of differentially expressed transcripts

To visualize functionally organized enriched Gene Ontology (GO) terms under the domains of biological process (BP), cellular component (CC), and molecular function (MF), GO term network analysis was conducted to show interconnections among different BP (Fig. 3.2), CC (Fig. 3.3), and MF (Supplementary Fig. S3.3) of enriched GO terms. Among all pair-wise cross type comparisons, enriched BP GO term networks were detected between Farm.NA vs. Farm.EO (Fig. 3.2A); Farm.NA vs. Wild (Fig. 3.2B); Farm.EO vs. Wild (Fig. 3.2C), and Hyb.EO♀ vs. Wild (Fig. 3.2D).

The enriched BP GO term networks found between Farm.NA vs. Farm.EO formed four main clusters aligned with 'immune response', 'cellular process', 'metabolic process' and 'redox homeostasis process' (Fig. 3.2A). In particular, "immune response" was composed of two groups connected by GO term "leukocyte mediated immunity"; the first group (top of "Immune Response" section of Fig. 3.2A) was composed of interconnected GO terms including "innate immune response", "defense response", "adaptive immune response", "T cell-mediated immunity", and "lymphocyte-mediated immunity", and the second group (bottom of "Immune Response" section of Fig. 3.2A) was composed of interconnected GO terms including "leukocyte activation", "myeloid leukocyte mediated immunity", "neutrophil degranulation", and "secretion". Cellular process comprised only one main cluster including GO terms such as "cellular response to nutrient levels", "cellular response to external stimulus", "cellular response to starvation", and "regulation of mRNA splicing, via spliceosome" (Fig. 3.2A). "Metabolic process" relevant enriched GO terms among the Farm.NA vs. Farm.EO DEPs formed one cluster of interconnected GO terms,

including “canonical glycolysis”, “glucose metabolic process”, “carbohydrate catabolic process”, “pyruvate metabolic process”, and “carboxylic acid metabolic process” (Fig. 3.2A). “Redox homeostasis process” relevant enriched GO terms in this comparison formed a cluster of interconnected terms including “hydrogen peroxide catabolic process”, hydrogen peroxide metabolic process”, “oxygen carrier activity”, “peroxidase activity”, and “antioxidant activity” (Fig. 3.2A).

Significantly enriched BP GO terms represented by genes differentially expressed between Farm.NA and Wild were associated with biological processes including metabolism (e.g., “glycolytic process”; “regulation of ATP metabolic process”; “purine nucleotide metabolic process”), redox homeostasis (e.g., “antioxidant activity”; “hydrogen peroxide metabolic process”), apoptosis (e.g., “apoptotic signaling pathway”), RNA localization (e.g., “localization”; “processing”; “transport”), and immune response (e.g. “viral entry into host cell”; “interleukin-6 production”) (Fig. 3.2B). I did not find any clusters formed in Farm.EO vs. Wild, but the enriched GO terms included “complement activation, classical pathway”, “complement activation, alternative pathway”, and “regulation of complement activation” (Fig. 3.2C). Lastly, enriched BP GO terms in Hyb.EO$_{f♀}$ vs. Wild comprised of one main cluster associated with immune response, with interconnected terms including “defense response”, “innate immune response”, “vesicle-mediated transport”, and “establishment of localization in cell” (Fig. 3.2D).

Among all pair-wise cross type comparisons, enriched CC GO term networks were detected between Farm.NA vs. Farm.EO (Fig. 3A); Farm.NA vs. Wild (Fig. 3B); Hyb.EO$_{f♀}$ vs. Wild (Fig. 3C); and Hyb.EO$_{f♀}$ vs. Farm.EO (Fig. 3D). The gene list corresponding to Farm.NA

vs. Farm.EO was enriched with CC GO terms related to tertiary/secretory granules (e,g., “lysosomes”; “hemoglobin complex”; “blood microparticle”; “vacuole”), extracellular matrix (e.g., “extracellular exome”; “vesicle”), and sacromere and myofibril (e.g., “supramolecular fiber”) (Fig. 3A). The Farm.NA vs. Wild DEPs were enriched with CC GO terms related to “extracellular space/exosome and sarcomere” (Fig. 3B). The Hyb.EO♀ vs. Wild gene list only presented one enriched CC GO term, i.e., “focal adhesion” (Fig. 3C). Two CC GO terms related to “cytoplasmic vesicle” and “bounding membrane of organelle” were enriched in the Hyb.EO♀ vs. Farm.EO DEPs (Fig. 3D). Only a few MF GO terms networks have been observed in this study (see supplementary Fig. S4). For example, enriched MF GO terms for Farm.NA vs. Farm.EO were related to “tetrapyrrole binding and peroxidase activity” (Supplementary Fig. S4A); and “Rho GTPase binding and pre-mRNA binding” functions in Farm.NA vs. Wild (Supplementary Fig. S4B).

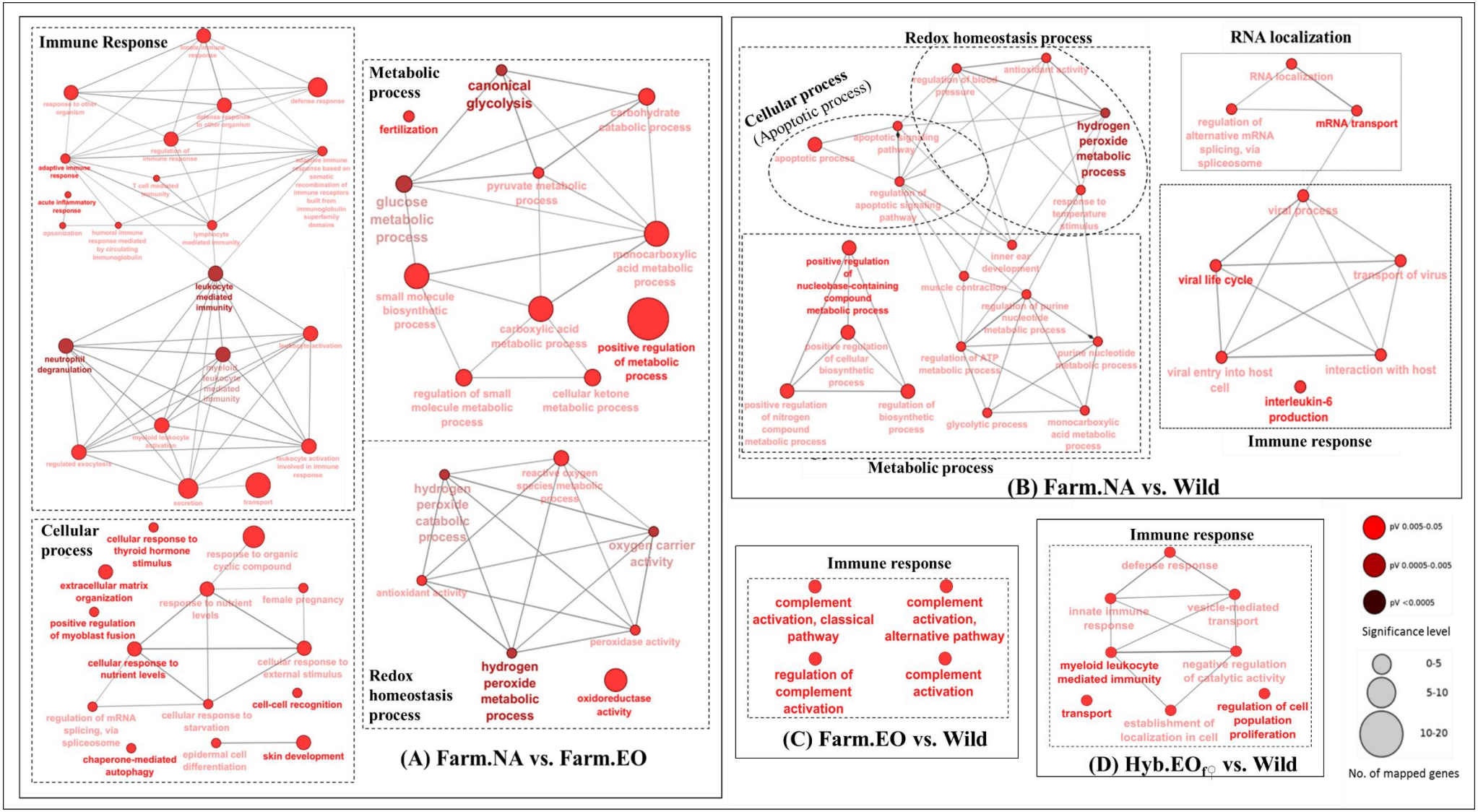


Figure 3.2 (A-D): Enriched biological process (BP) gene ontology (GO) term networks in different cross types comparisons [(A) Farm.NA vs. Farm.EO; (B) Farm.NA vs. Wild; (C) Farm.EO vs. Wild, and (D) Hyb.EO♀ vs. Wild]. Enrichment analysis and visualization of GO term networks were done by ClueGO plugin in Cytoscape. The nodes (round shape) represent GO terms node color represents the level of significance as indicated in the legend, while node size reflects the number of genes in each enriched GO term.

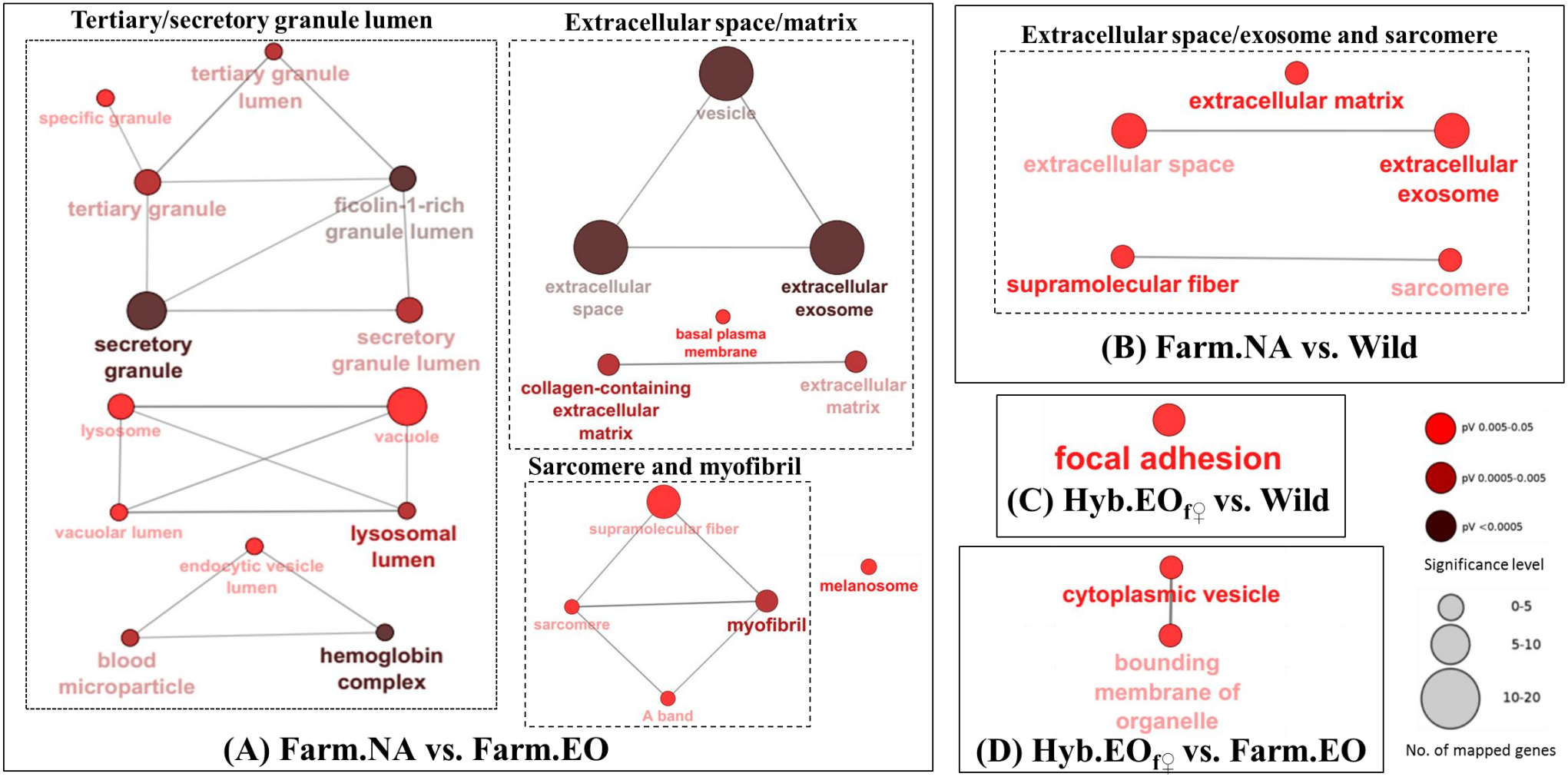


Figure 3.3 (A-C): Enriched cellular component (CC) gene ontology (GO) term networks in different cross types comparisons [(A) Farm.NA vs. Farm.EO; (B) Farm.NA vs. Wild; (C) Hyb.EO$_{f♀}$ vs. Wild; and (D) Hyb.EO$_{f♀}$ vs. Farm.EO]. Enrichment analysis and visualization of GO term networks were done by ClueGO plugin in Cytoscape. The nodes (round shape) represent GO terms node color represents the level of significance as indicated in the legend, while node size reflects the number of genes in each enriched GO term.

To visualize expression patterns of differentially expressed transcripts contributing to the identified metabolism, development and growth, immune, and redox-related processes, hierarchical clustering and heatmaps were performed (Fig. 3.4 and 3.5). Heatmaps and cluster analyses comparisons showed that metabolic, development, and growth relevant transcripts were grouped into three distinct clusters (Fig. 3.4A). In the first cluster (Fig. 3.4A, gene cluster I), many metabolic, development, and growth relevant transcripts (e.g., *col10a1*, *timp2*, *ckm*, *aldoa*, *pdk3*, *gapdh*, *pygm*, *gpd1*, *eno1*, and *mybph*) had higher expression levels in Farm.NA, their related F_1 hybrid (Hyb.$NA_{f♀}$) and Wild cross types, but lower mRNA expression levels in Farm.EO and their related hybrids (Hyb.$EO_{w♀}$ and Hyb.$EO_{f♀}$). In the second cluster (Fig. 3.4A, gene cluster II), some other metabolic, development, and growth relevant transcripts (e.g., *alg8*, *dazap1*, *gatb*, *ltbp4*, *mtmr6*, *mgat2*, *fads6*, and *sult6b1*) had higher expression levels in Farm.NA and Farm.EO, but lower mRNA expression levels in Wild and related hybrids (Hyb.$NA_{f♀}$, Hyb.$EO_{w♀}$, and Hyb.$EO_{f♀}$). In the third cluster (Fig. 3.4A, gene cluster III), many other metabolic, development, and growth relevant transcripts (e.g., *tcn1*, *pappa2*, *timp2*, *cald1*, *csrp1*, *col2a1*, *bglap*, and *comt*) had lower mRNA expression levels in Farm.NA, Hyb.$NA_{f♀}$, and Wild cross types but had higher expression levels in Hyb.$EO_{w♀}$, Hyb.$EO_{f♀}$, and Farm.EO. Immune relevant transcripts were also grouped into three separate clusters (Fig. 3.4B). In the first cluster (Fig. 3.4B, gene cluster I), some immune relevant transcripts (e.g., *nckap1*, *cd8*, *fel*, *ccl19*, *endod1*, *chia*, and *clec4m*) had lower expression levels in Farm.NA, their related F_1 hybrid (Hyb.$NA_{f♀}$) and Wild cross types, but higher mRNA expression levels in Farm.EO and their related hybrids (Hyb.$EO_{w♀}$ and Hyb.$EO_{f♀}$). By contrast, in the second cluster (Fig. 3.4B, gene cluster II), many immune relevant transcripts (e.g., *pbx2*, *rsad2*,

hamp1, *cebpd*, *tcp1*, *ctsa*, *ctsl*, *c1ql2*, *c1ql4*, *c1qtnf3*, *ahcy*, *mcoln2*, and *hpx*) had higher expression levels in Farm.NA, Hyb.NA$_{f♀}$ and Wild cross types, but lower expression levels in Farm.EO, Hyb.EO$_{w♀}$ and Hyb.EO$_{f♀}$. However, in the third cluster (Fig. 3.4B, gene cluster III), some immune relevant transcripts (e.g., *gmfb*, *c3*, *serpinh1*, *ighv3-33*, *senp7*, *vrk1*, *nlk*, and *rras2*) had higher expression levels only in Farm.EO, but lower mRNA expression levels in Farm.NA, Wild, and related F_1 hybrids. Redox homeostasis relevant transcripts were grouped into two separate clusters (Fig. 3.5). In the first cluster (Fig. 3.5, gene cluster I), some redox homeostasis relevant transcripts (e.g., *hba*, *cyp27a1*, *ehhadh*, *gpx4*, and *prcp*) had lower mRNA expression levels in Farm.NA, their related F_1 hybrid (Hyb.NA$_{f♀}$) and Wild cross types, but higher expression levels in Farm.EO and their related hybrids (Hyb.EO$_{w♀}$ and Hyb.EO$_{f♀}$). By contrast, in the second cluster (Fig. 3.5, gene cluster II), many redox homeostasis and oxygen transport relevant transcripts (e.g., *hba2*, *hbe1*, *gmpr*, *hpdl*, *mt-co1*, *mt-co2*, *sod1*, *hbb* and *hadhb*) had higher mRNA expression levels in Farm.NA, Hyb.NA$_{f♀}$ and Wild cross types, but lower expression levels in Farm.EO, Hyb.EO$_{w♀}$ and Hyb.EO$_{f♀}$.

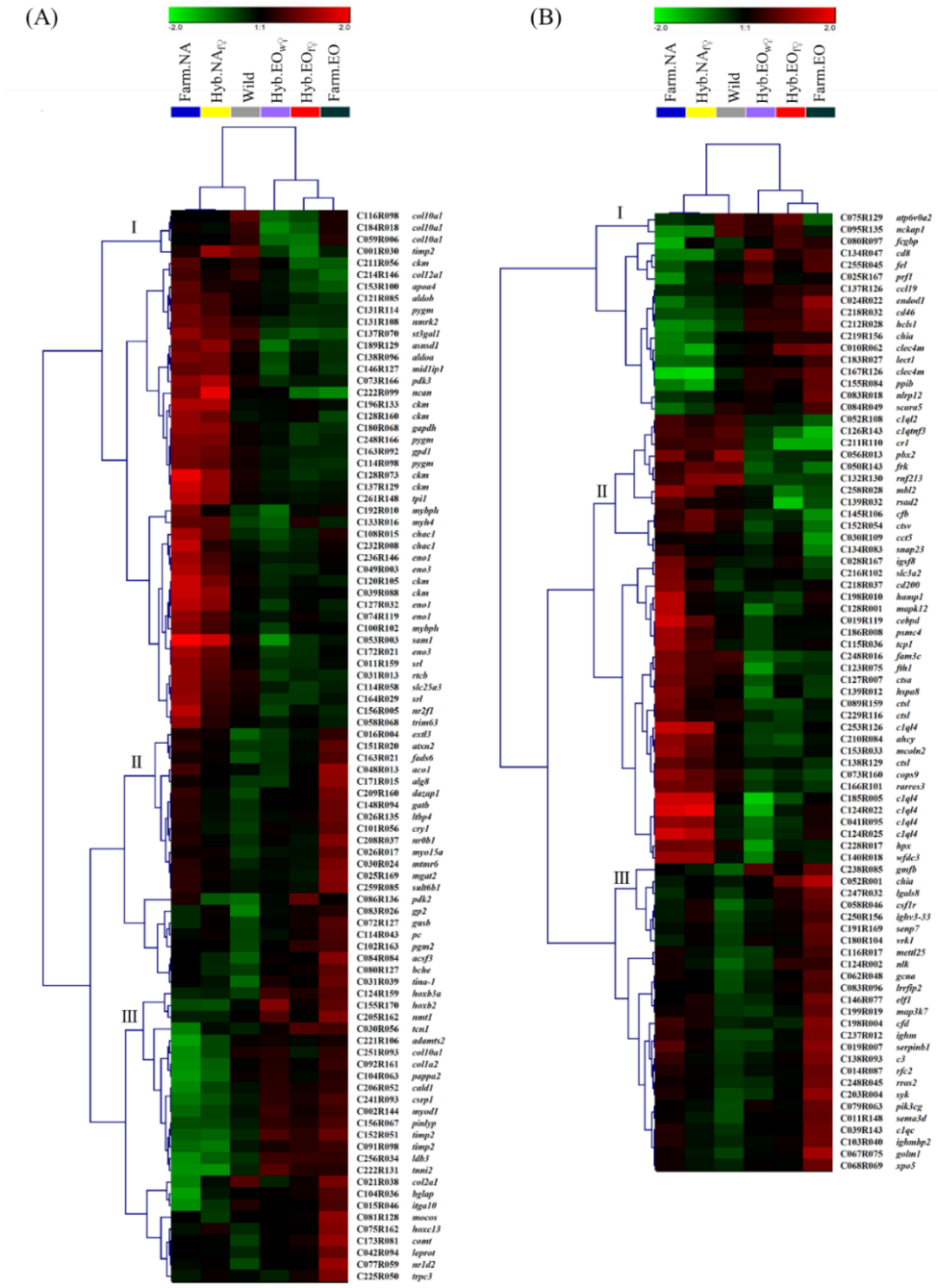


Figure 3.4 (A-B): Hierarchical clustering analyses of (A) metabolic, development and growth relevant genes; and (B) immune relevant genes from RP DEPs list that were differentially expressed in different cross types. Coloured panel indicates mean expression values (6 biological replicates) of each cross type: blue-Farm.NA, yellow-

Hyb.NA$_{f♀}$, grey-Wild, light blue-Hyb.EO$_{w♀}$, red-Hyb.EO$_{f♀}$, and black-Farm.EO.

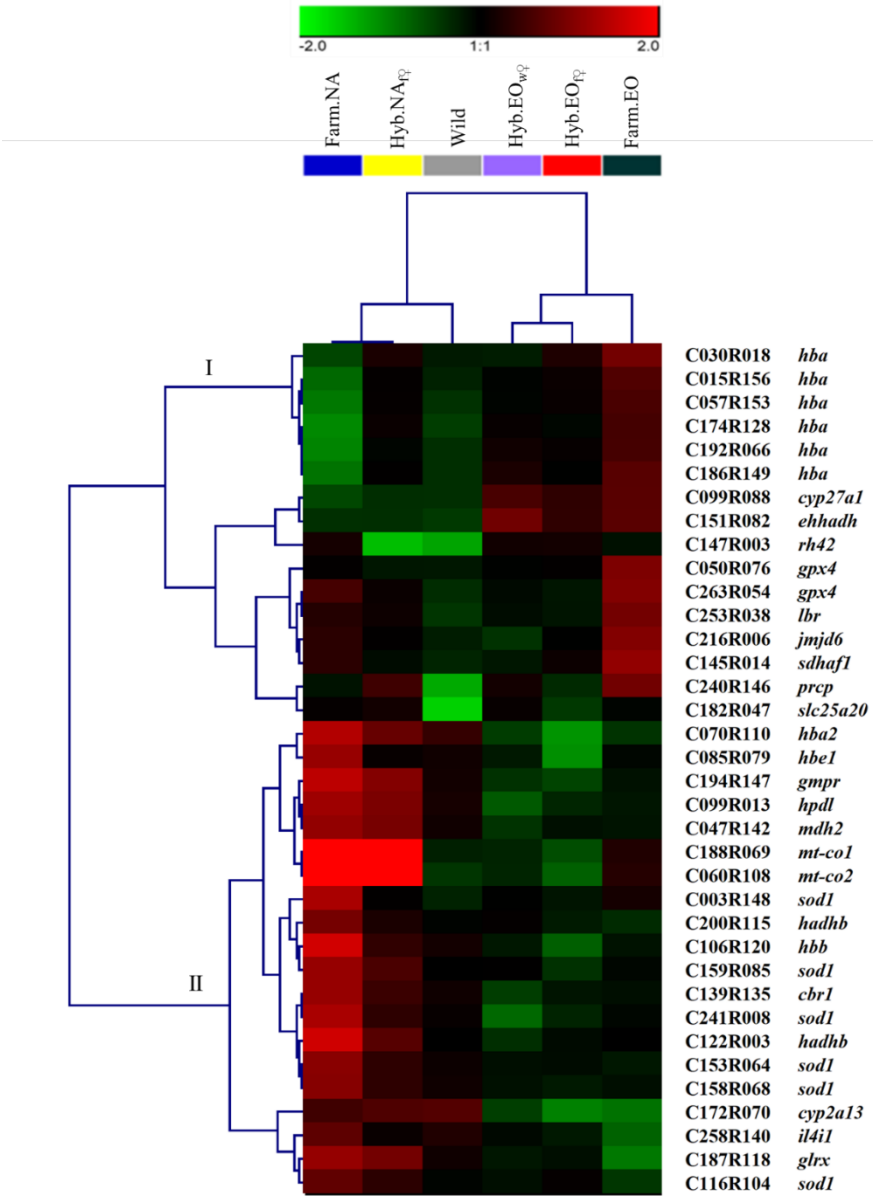


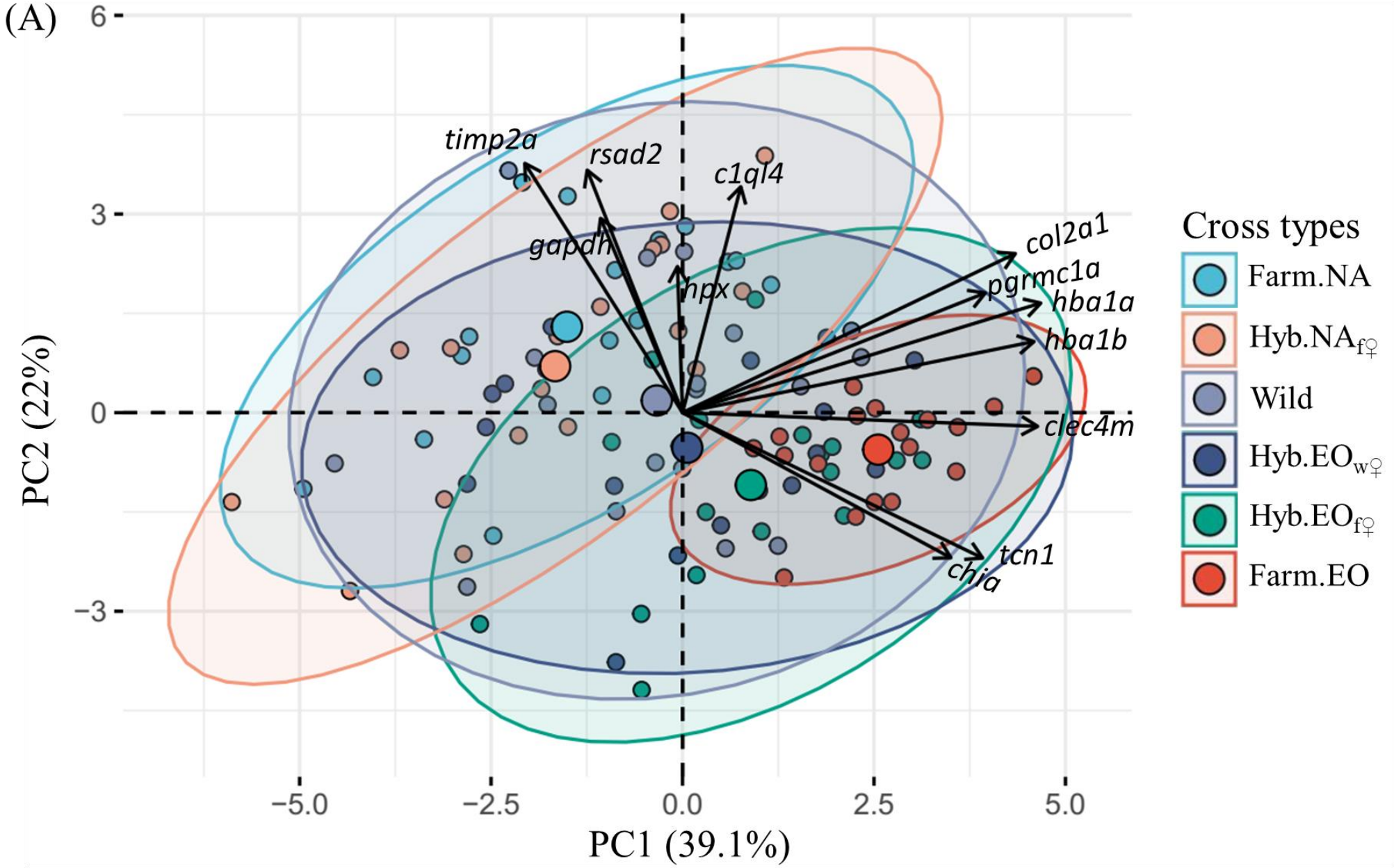
Figure 3.5: Hierarchical clustering analyses of redox homeostasis relevant genes from RP DEPs list that were differentially expressed in different cross types. Coloured panel indicates mean expression values (6 biological replicates) of each cross type: blue-

Farm.NA, yellow-Hyb.NA$_{f♀}$, grey-Wild, light blue-Hyb.EO$_{w♀}$, red-Hyb.EO$_{f♀}$, and black-Farm.EO.

3.4.3 qPCR confirmation

Twenty microarray-identified genes were selected for qPCR validation based on their functional annotations related to metabolic, development and growth, immune, cellular, and redox-related processes. Twelve out of 20 qPCR-analyzed genes (*col2a1, tcn1, gapdh, timp2a, hpx, c1ql4, rsad2, chia, clec4m, pgrmc1a, hba1a,* and *hba1b*) were validated, showing significant differential expression among cross types and the same direction of change as in the microarray analyses (Supplementary Table S3.3). Four qPCR genes (*rpsa, pgrmc1b, eno1,* and *slc25a20*) did not show significant differential expression, but the direction of change was similar between the microarray and qPCR analyses. Four other qPCR genes (*timp2b, sod1, mt-co2a,* and *mt-co2b*) differed in the direction of change from that of the microarray, so they were considered as non-validated genes.

A PCA for the validated qPCR genes (n = 12) revealed among cross type differences along PC1 and PC2 (Fig. 6A-C). PC1 and PC2 explained 39.1% and 22.0% of the variance in validated qPCR genes, respectively (Fig. 3.6A), and showed significant cross type differences along PC1 ($P < 0.001$) and PC2 ($P < 0.001$) (Fig. 3.6B-C). Farm.NA differed significantly in PC1 and PC2 scores from Farm.EO, but did not differ from Wild. Farm.EO differed from Wild in PC1, but not PC2 scores. Hyb.NA$_{f♀}$ differed significantly in PC1 and PC2 scores from Hyb.EO$_{w♀}$ and in PC1 scores from Hyb.EO$_{f♀}$.


(A)
6
3
0
−3
PC2 (22%)
−5.0
−2.5
0.0
2.5
5.0
PC1 (39.1%)
timp2a
rsad2
c1ql4
gapdh
hpx
col2a1
pgrmc1a
hba1a
hba1b
clec4m
tcn1
chia
Cross types
Farm.NA
Hyb.NA♀f
Wild
Hyb.EO♀w
Hyb.EO♀f
Farm.EO

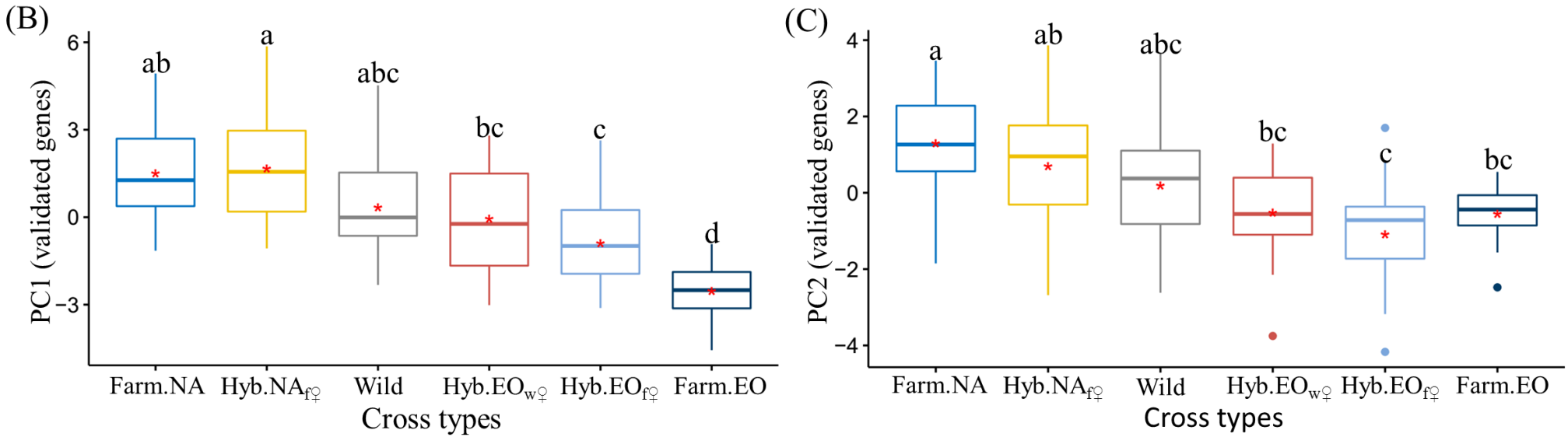


Figure 3.6 (A-C): (A) Principal component analysis (PCA-Biplot) of selected significantly differentially expressed genes validated by qPCR (n= 12); metabolic, development and growth responses (*col2a1*; *tcn1*; *gapdh*; and *timp2a*); immune response (*hpx*; *c1ql4*; *rsad2*; *chia*; and *clec4m*); cellular process (*pgrmc1a*); and redox homeostasis process (*hba1a* and *hba1b*). Four non-significant qPCR genes (*rpsa, pgrmc1b, eno1,* and *slc25a20*) agreed in direction with the microarray, and four non-validated (either significant qPCR genes disagreed in direction with the microarray or non-significant qPCR genes disagreed in direction) genes (*timp2b, mt-co2a, mt-co2b,* and *sod1*) were not included in the PCA. The log_2-transformed relative quantity (RQ) values from qPCR for all cross types were combined to generate PC1 and PC2. Log_2-transformed relative quantity (RQ) values from all validated genes for each cross type were combined to generate boxplots of (B) PC1 and (C) PC2 scores. Bold line represents median, boxes 25 and 75 % quartiles, whiskers 95% confidence interval, dots outliers, and red asterisk (*) mean. Different letters denote significant differences in mean RQ values among cross types.

More specifically, among four qPCR validated metabolic, development and growth relevant genes (*col2a1*, *tcn1*, *gapdh*, and *timp2a*; Fig. 7A-D), Farm.EO salmon showed significantly higher *col2a1* and *tcn1* mRNA expression levels than Farm.NA, Hyb.NA$_{f♀}$, Wild, and Hyb.EO$_{w♀}$, but did not differ from Hyb.EO$_{f♀}$ (Fig. 7A and 7B). On the contrary, Farm.NA and Hyb.NA$_{f♀}$ exhibited significantly higher *gapdh* and *timp2a* mRNA expression levels than Farm.EO (Fig. 7C and 7D). Wild showed similar *gapdh* mRNA levels to other cross types, but showed higher *timp2a* mRNA expression levels than Hyb.EO$_{f♀}$ and Farm.EO.

Among five qPCR validated immune relevant genes (*hpx*, *c1ql4*, *rsad2*, *chia*, and *clec4m*,; Fig. 7E-7I), Farm.NA and Hyb.NA$_{f♀}$ salmon showed significantly higher *hpx*, *c1ql4*, and *rsad2* mRNA expression levels than Hyb.EO$_{f♀}$, and Farm.EO (except, no *c1ql4* mRNA expression levels differences among Hyb.NA$_{f♀}$, Hyb.EO$_{f♀}$, and Farm.EO) (Fig. 7E-7G). The Wild population showed similar *hpx* mRNA expression levels to Farm.NA, but higher than Farm.EO. In contrast, the Wild population showed similar *c1ql4* mRNA expression levels to Farm.EO, but lower than Farm.NA. However, Wild fish exhibited similar *rsad2* mRNA expression levels to Farm.NA and Farm.EO. Farm.NA and Hyb.NA$_{f♀}$ cross types displayed significantly lower *chia* and *clec4m* mRNA expression levels compared to Hyb.EO$_{f♀}$, and Farm.EO (Fig. 7H-7I). The Wild population showed similar *chia* mRNA expression levels to Farm.NA, but lower than Farm.EO. On the contrary, the Wild population showed similar *clec4m* mRNA expression levels to Farm.EO, but higher than Farm.NA.

Lastly, one cellular process (*pgrmc1a*; Fig. 3.7J) and two redox homeostasis and oxygen transport (*hba1a* and *hba1b*; Fig. 3.7K-L) relevant genes were validated by qPCR. Farm.NA, Hyb.NA$_{f♀}$, and Wild cross types displayed lower *pgrmc1a*, *hba1a*, and *hba1b* mRNA expression levels than Farm.EO. There were no differences in *pgrmc1a*, *hba1a*, and *hba1b* mRNA expression levels between Hyb.NA$_{f♀}$, Hyb.EO$_{w♀}$, and Hyb.EO$_{f♀}$.

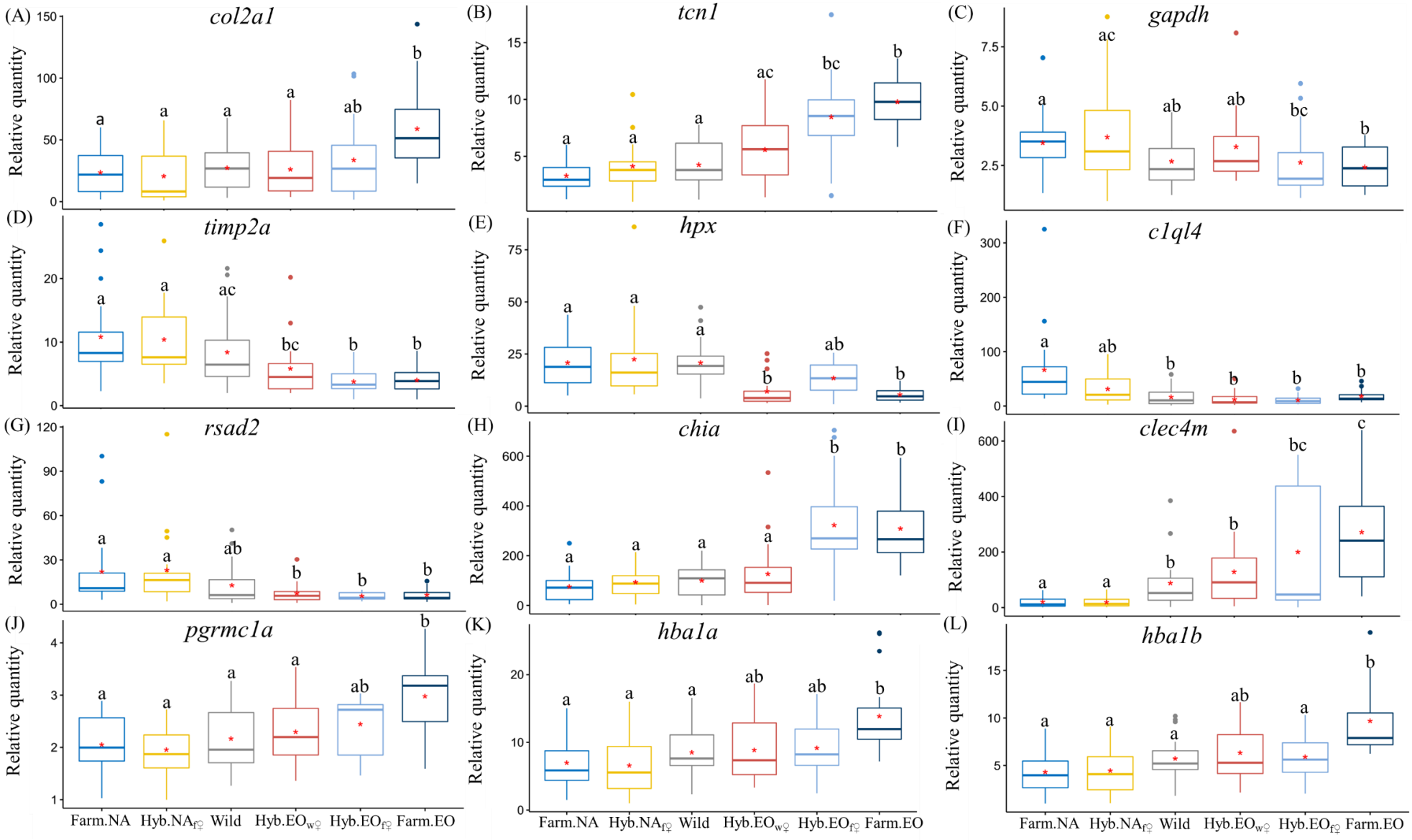


Figure 3.7 (A-L): Boxplots from the qPCR analysis of selected significantly differentially expressed validated qPCR genes (n= 12) involved in metabolic, development and growth responses (A-D) [A: *col2a1*; B: *tcn1*; C: *gapdh*; and D: *timp2a*]; immune response (E-I) [E: *hpx*; F: *c1ql4*; G: *rsad2*; H: *chia*; and I: *clec4m*]; cellular process (J) [*pgrmc1a*]; and redox homeostasis process (K-L) [K: *hba1a* and L: *hba1b*]. Bold line represents median, boxes 25 and 75 % quartiles, whiskers 95% confidence interval, dots outliers and red asterisk (*) mean. Different letters denote significant differences in mean RQ values among cross types.

3.5 Discussion

The present study has demonstrated early-life stage global gene expression differences among divergent EO and NA farmed, NF wild, and related F_1 hybrid salmon to provide insight into the potential consequences of hybridization. The main findings can be summarized as: (i) the Wild population showed greater transcriptome differences from the Farm.EO strain than that of Farm.NA strain; (ii) among the ecologically relevant comparisons, the largest differences in gene expression were observed between the two farm strains (Farm.NA and Farm.EO); (iii) fewer significantly differentially expressed transcripts were detected between F_1 hybrids and domesticated/wild maternal strains; and (iv) significantly enriched GO terms represented by genes differentially expressed between cross types were associated with biological processes including metabolism, development, and growth processes; immune response; cellular process; and redox homeostasis process. These findings suggest that the interbreeding of escaped EO/NA farmed with the NF local wild population would alter gene transcription, and the consequences of hybridization would be greater from escaped EO farmed than NA farmed, resulting in potential effects on the fitness of NF local wild populations.

3.5.1 Differences between EO and NA farmed, NF wild, and F_1 hybrids

The NF wild population showed greater transcriptome differences relative to the Farm.EO (RP: 200 DEPs) than to the Farm.NA strain (RP: 46 DEPs). Consequently, from a gene expression perspective, the NF wild population was more distinct genetically from the Farm.EO than the Farm.NA strain as hypothesized, which was not unexpected based on the population genetic data (F_{st} = 0.14 - 0.20, between NF wild and NA farmed; whereas

F_{st} > 0.40, between NF wild and EO farmed salmon; S.J. Lehnert, pers. comm. based on data from Jeffery et al., 2018). Moreover, recent studies have demonstrated large genomic differences (Lehnert et al., 2020), and little overlap on allele frequencies/haplotype differentiation (López et al., 2018), between EO (Norwegian) and NA Atlantic salmon, raising additional concerns about interactions of introduced Norwegian salmon with wild populations in NF. Additionally, among the ecologically relevant comparisons, I found the largest gene transcript level differences between domesticated EO and NA farmed strains (RP: 223 DEPs), which is concordant with an earlier observation by Roberge et al. (2006) on country-specific (Norway-Canada) changes in transcription profiles by the domestication of farmed strains. This finding, however, contrasts with our hypothesis that commonalities due to domestication selection between Farm.NA and Farm.EO would lead to reduced gene expression differences, as was observed previously in regards to behavioural differences (Islam et al., 2020). Given that the Farm.EO strain has already undergone 12-15 generations of domestication selection and the Farm.NA strain 7-8 generations (Glover et al., 2017), I cannot simply rule out the possibility that differences in the form and number of generations of domestication selection also contributed to the global gene expression differences between Farm.EO and Farm.NA strains, especially when only a single generation of domestication selection may change hundreds of gene expression patterns (Christie et al., 2016).

There were relatively fewer gene transcript differences observed between F_1 hybrids and domesticated/wild maternal strains than among the pure strains themselves. This finding supports my hypothesis that interbreeding would affect hybrid global gene expression patterns relative to that of wild fish, but the effect was not large. Moreover,

hierarchical clustering from this study revealed that the gene expression patterns of F_1 hybrids were more a reflection of their maternal than parental origins, suggesting that maternal effects might have contributed to this. It is noteworthy that maternal effects from the yolk sac (e.g., highly abundant maternal ribosome and maternally deposited RNAs or other yolk sac components such as hormones, proteins, or nutrients) can influence the F_1 hybrid's gene expression (e.g., Atlantic cod, Lanes et al., 2013; Atlantic salmon, Bicskei et al., 2016, Bizuayehu et al. 2019). Also, maternal effects related to egg size (Einum & Fleming, 2000) can contribute to the F_1 hybrids' gene expression patterns. Moreover, gene expression differences at the population level can also be due to environmental effects (Amaral et al., 2008; Toews et al., 2019), but this common garden experimental approach was designed to minimize such effects on transcriptional variation. Although maternal effects are known to be strongly influential during the embryonic stage of fish, paternal effects have also been observed to influence F_1 hybrid gene expression patterns (e.g., brook charr, Bougas, Audet, & Bernatchez, 2013; zebrafish, Jiang et al., 2013).

3.5.2 Functional significance of gene expression differences among farmed, wild, and F_1 hybrids

I observed differentially expressed genes among Farm.EO, Farm.NA, Wild, and F_1 hybrid cross types that encode functionally relevant (i.e., likely to influence fitness in early life stage salmon) proteins related to metabolism, development and growth, immune responses, cellular processes, and redox homeostasis. One of the key findings of this study is that metabolic, development, and growth relevant genes involved in glycolysis and gluconeogenesis (e.g., *gapdh*), and embryogenesis (e.g., *timp2*), showed higher levels of expressions in Farm.NA, Hyb.$NA_{f♀}$, and Wild than in Farm.EO, Hyb.$EO_{w♀}$ and Hyb.$EO_{f♀}$.

For example, one of the qPCR validated genes, *gapdh*, involved in energy metabolism, is particularly compelling as it may shape the most important traits (e.g., development and growth) targeted in directed domestication selection of salmonid (Xu et al., 2011). Decreased mRNA levels of this gene could lower the functional capacity in the pathway or can involve reduced yield of their components. Another examined development-relevant gene, *timp2*, has been reported to have growth factor activities during embryonic development (Zhang, Bai, Tanase, Nagase, & Sarras, 2003), and limb (fin) development and regeneration in zebrafish (Bai et al., 2005). Thus, different expression levels of this gene among cross types could potentially alter salmon embryogenesis and potentially influence fitness. Other metabolic, development and growth-relevent genes explored in this study presented lower mRNA levels in Farm.NA, Hyb.$NA_{f♀}$, and Wild than in Hyb.$EO_{w♀}$, Hyb.$EO_{f♀}$, and Farm.EO. For example, we examined *col2a1*, which encodes a key regulatory protein involved in cartilage development, endochondral ossification in medaka and zebrafish (Matsumoto et al., 2012), and is involved in notochord development and growth during embryonic stages in Atlantic salmon (Wang et al., 2014); therefore, higher expression levels of this structural gene may influence salmon growth and aquaculture production. Another qPCR validated gene, *tcn1*, encodes a protein that is involved in protecting cobalamin (i.e., vitamin B12), a basis for the synthesis of nucleotides, amino acids, and fatty acids (Banerjee & Ragsdale, 2003) found in teleosts such as zebrafish, trout, and salmon (Greibe et al., 2012a, b). The different expression levels among Farm.EO, Farm. NA, NF Wild, and F_1 hybrids, thus, can alter its' roles in Atlantic salmon metabolism and tissue function.

Notwithstanding farmed salmon strains have been intensely selected for production-related traits (e.g., growth) on both sides of the Atlantic Ocean, I found different expression levels of genes encoding functionally relevant proteins between the two farmed strains in the present study, suggesting that population-specific selection may have acted upon different genes (e.g., see Mäkinen et al., 2014; Gutierrez et al., 2015; López et al., 2018 for Atlantic salmon; also see Elmer et al., 2014 for cichlids; Pujolar et al., 2017 for three-spined sticklebacks; Amaral et al., 2011 for pig domestication). Also, the GO term networks evidenced interconnections between many development and growth-relevant genes' putative functions and metabolism, which was expected based on other earlier studies of domesticated salmonid populations (Fleming et al., 2002; Overturf et al., 2010), and can be relevant when considering hybridization between farm and wild fish.

Moreover, farmed salmon are often subjected to high levels of pathogens, such as parasitic sea lice (Bicskei et al., 2016), which are known to be among the strongest selective forces driving the evolution of wild populations (Zueva et al., 2014). In this study, I examined immune relevant genes that are involved in inflammatory response (e.g., *chia*), and adaptive immune response, antigen processing, and presentation (e.g., *clec4m*), which had lower mRNA expression levels in Farm.NA, NA hybrid, and Wild cross types than Farm.EO and EO hybrids. Higher transcript expression levels of *clec4m* and *chia* are associated with a lice-resistant salmon species (Sutherland et al., 2014); the higher expression of these genes in Farm.EO and EO hybrids suggest that these cross types would be potentially more immune robust (e.g., resistant to parasites) than Farm.NA, NA hybrid, and Wild. In contrast, my examination of *viperin* (alias *rsad2*), a key antiviral effector in

immune responses of Atlantic salmon (Ignatz et al., 2020; Zanuzzo et al., 2020), sockeye salmon (Long et al., 2019), and Atlantic cod (Eslamloo et al., 2019), showed higher expression levels in Farm.NA, NA hybrid, and Wild cross types than in Farm.EO and EO hybrids. Another qPCR validated gene, *c1ql4*, which plays a pivotal role in the inflammatory network, angiogenesis, and tumor necrosis in animals (Liu et al., 2017), similarly presented higher mRNA levels in Farm.NA, NA hybrid, and Wild fish than Farm.EO and related hybrids. Lastly, I examined another immune relevant gene, *hpx*, suggested to be involved in fish acute-phase response (Atlantic cod, Solbakken et al., 2019; sturgeon, Castellano et al., 2020), which also showed higher expression levels in Farm.NA and Wild than Farm.EO, suggesting that it may impact the immune response of these cross types differently.

Additionally, in the present study, I examined a cellular process relevant gene, *pgrmc1a*, which plays an important role in cell survival, morphology, differentiation, and apoptotic process in animals (Aizen & Thomas, 2015; Thejer et al., 2020a, b). It showed lower expression levels in NA farm and Wild than EO farm, suggesting that various cellular processes might be differentially regulated among these cross types during salmon embryogenesis. I also explored a redox homeostasis and oxygen transport relevant gene, *hba*, which showed lower expression levels in Farm.NA, NA hybrid, and Wild than Farm.EO and EO hybrids. In fishes, hemoglobin proteins have shown to be the primary molecules responsible for transporting oxygen from gills to tissues for use in cellular respiration (Weber 1990; Nikinmaa 1997), and many fish species bear diverse hemoglobin protein types adapted to oxygen loading and delivery under different environmental

conditions (Andersen et al., 2009; Star et al., 2011; Verde et al., 2012). It has been documented that the hemoglobin transcript and protein divergences between populations from different origins/environments are likely being driven by genetic divergence (Evans et al., 2014). As the farmed and natural habitats are variable in oxygen tension, a number of physiological, morphological, and behavioural processes could be altered by hemoglobin protein divergence among Farm.EO, Farm.NA, Wild, and related hybrids.

3.5.3 Implications for EO/NA farm-wild hybridization

A persistent concern of successful breeding of escaped farmed salmon and the resultant hybridization with local wild populations is the genetic impact and consequent threat to local adaptation. Farm-wild hybridization can lead to homogenization among introgressed populations, thus eroding population structure (Bourret et al., 2013; Skaala et al., 2019). It is unknown, however, whether the genetic effects posed by escaped EO farm fish will be greater than that of escaped NA farm fish for local NF wild populations. The whole-body transcriptomic differences observed in the current study substantiate these concerns as differences were greater between Farm.EO and Wild than between Farm.NA and Wild. Existing knowledge indicates that farmed salmon are known to successfully mate in the rivers of southern NF. For example, escaped farmed salmon have been detected in 17 out of 18 wild salmon rivers in southern NF (Wringe et al., 2018). However, my results suggest few gene transcriptome differences between F1 hybrids and their respective maternal farmed/wild strains. Given that the major route of introgressive hybridization is likely to occur through farmed females (Fleming et al., 1996, 2000), the maternal contributions of farmed females will be important in understanding the fitness consequences for local NF wild populations. For example, there has been a decline in the

abundance of wild salmon in southern NL of ~ 45% between 1996-2010, particularly near the main farming area (e.g., ~70% decline in the Conne River), and these wild populations have been designated as threatened (Bradbury et al., 2018). As such, concern exists that the escape of fertile fish of the genetically distinct EO farmed strain may present further issues.

3.6 Concluding remarks

Overall, my study revealed transcriptome differences observed among divergent EO and NA farmed, NF wild, and F_1 hybrid Atlantic salmon at the late sac fry stage, which may reflect the influence of ancestral relationship and geographic origin. The continental difference and the ancestral relationship in their origin appear responsible for Farm.EO expressing greater global gene expression differences relative to the Wild salmon than did Farm.NA. Among the ecologically relevant comparisons, I have also observed the largest number of DEPs between the two domesticated farmed populations (Farm.EO and Farm.NA), which suggests that there was no indication of any convergence between the two domesticated strains despite both being subjected to domestication selection. Likewise, I found fewer gene transcript differences between F_1 hybrids and domesticated/wild maternal strains, inferring maternal effects. Moreover, I found significantly enriched GO terms associated with differentially expressed genes among cross types (particularly between those of NA and EO origin), with these genes encoding for functionally relevant proteins related to metabolic, development and growth processes, immune response, cellular process, and redox homeostasis and oxygen transport processes. To my knowledge, this is the first study to characterize early-life stage gene expression differences resulting from hybridization by comparing divergent EO and NA farm strains and a NF wild

population. My findings suggest that the interbreeding of escaped EO and NA farmed with wild populations could alter the whole sac fry transcriptome resulting in potential effects on the fitness of local populations. Understanding the impact of hybridization on gene expression differences of salmon at early-life stages is therefore important to wild salmon conservation and management programs.

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3.8 Appendices

Supplementary Table S3.1: Primers used for qPCR validation

Accession number	Genes of interest	Sequence 5'-3'	Efficiency (%)	R^2
†XM_014131770[2]	Acidic mammalian chitinase precursor (*chia*)	F: CAGGGCAGATACCCACTGAT R: TTAAGTGGGTGCTGGGTAGG	109.1	0.995
§EG783083[2]	C-type Lectin domain family 4 member M (*clec4m*)	F: TCAAGGAAGACACGCATCAG R: GTGAGGAGGAGACAGGCAAG	107.3	0.992
§BT057670[1]	Complement C1q-like protein 4 precursor (*c1ql4*)	F: GAGGAGAAACTGCGAACCAC R: AGCCGAAAAGATCACCTTCA	105.4	0.996
§EG878765[2]	Collagen alpha-1 (II) chain precursor (*col2a1*)	F: TGAACAACCCCTTGTGATGT R: AGCTGATTGCTTCAGCAGGT	91.7	0.990
§BT043817[2]	Alpha-enolase (*eno1*)	F: GGTGTGATGGTGTCTCATCG R: GGCCAGACGCTCTGATCTAC	95.8	0.999
§BT043826[2]	Glyceraldehyde-3 phosphate dehydrogenase (*gapdh*)	F: TGAGGCATCTCACAAACGAG R: TCCCTTCTTGGAGTGGAATG	104.7	0.998
§BT058672[2]	Hemoglobin subunit alpha (*hba1a*)	F: TGAGTGCTCTCAGCGATCTG R: GTGCACTTCGGGAGTGAAAT	97.5	0.996
§NM_001123662[2]	Hemoglobin subunit alpha (*hba1b*)	F: GCAAGGGACAAATCTGTGGT R: CAGCCCAGTGGGAGAAGTAG	102.5	0.996
§BG935408[2]	Hemopexin (*hpx*)	F: AAACCCTTGAAGGAGGTGCT R: AACTGGAACACGTGGAGAGG	98.7	0.997
§BT044012[3]	Cytochrome c oxidase subunit 2 (*mt-co2a*)	F: CGAAATTAATGACCCACACCTTA R: GACGCGGATTGGAGATTCTA	97.0	0.996
§DW556807[2]	Cytochrome c oxidase subunit 2 (*mt-co2b*)	F: CGAGAGGCAATAAAGGCTGT R: AGACCATCGAATGGTTGTCC	99.2	0.997
§XM_014197218[2]	Membrane-associated progesterone receptor component 1 (*pgrmc1a*)	F: TTTGAGTCTGCTTGCCCTCT R: AGGCCATCGTACGGTTGTAG	104.3	0.988
§NM_001146359[2]	Membrane-associated progesterone receptor component 1 (*pgrmc1b*)	F: TCGAGGAACCTCTGCCTAAA R: GGCCCGTAGAATTTCTTTCC	107.6	0.993
§BT058861[2]	40S ribosomal protein SA (*rpsa*)	F: CCTGTCGGGAGAAACACAAT R: GGGCACTCCAATCCTCTGTA	101.9	0.961

§BT047340[1]	Radical S-adenosyl methionine domain containing protein 2 (*rsad2*)	F: GTACCGCAGATGCACAACAC R: GCACTGTCGGGTAAAATGGT	117.4	0.971
†BT044930[2]	Mitochondrial carnitine/ acylcarnitine carrier protein (*slc25a20*)	F: ACAACAGAAAACCCCTGACG R: CGCCTGGATCTGTAGGAGAC	108.6	0.997
§DW576971[1]	Superoxide dismutase [CU-ZN] (*sod1*)	F: GATGGTGGTGAAGGCTGTTT R: TGGGCAAGACCTGAAATCTC	101.1	0.961
§XM_014213691[2]	Transcobalamin-1 (*tcn1*)	F: CAAAGCAGCCAATGAGACCT R: ACTCTCCAGGAAGGGACCAT	100.5	0.992
§GE790771[2]	Metalloproteinase inhibitor 2 precursor (*timp2a*)	F: GCCAGAACCTTGCCTGTATCAAG R: GAGTCTCCAATATCCAGTAACTAC	105.4	0.994
§BT058709[2]	Metalloproteinase inhibitor 2 precursor (*timp2b*)	F: TGATGGAAAAGAGCCACAGCG R: GGTCTTCAATATCCAGGAACTCT	97.1	0.995
Accession number	**Selected normalizer genes**	**Sequence 5'-3'**	**Efficiency (%)**	**R^2**
GE777139[4]	Eukaryotic translation initiation factor 3 subunit D (*eif3d*)	F: CTCCTCCTCCTCGTCCTCTT R: GACCCCAACAAGCAAGTGAT	105.0	0.998
EG908498[4]	Polyadenylate-binding protein 1 (*pabpc1*)	F: TGACCGTCTCGGGTTTTTAG R: CCAAGGTGGATGAAGCTGTT	105.9	0.998
BT043656[4]	60S ribosomal protein 32 (*rpl32*)	F: AGGCGGTTTAAGGGTCAGAT R: TCGAGCTCCTTGATGTTGTG	104.9	0.993

†qPCR genes were selected from RP DEPs list only
‡qPCR genes were selected from SAM DEPs list only
§qPCR genes were selected from both RP and SAM DEPs lists
[1]Primers established within the Genomic Applications Partnership Program (GAPP #6604) and quality-tested again using the reference cDNA template of the present study.
[2]Primers were designed for this present study.
[3]Primers previously published in Caballero-Solares et al. 2018, and quality-tested again using the reference cDNA template of the present study.
[4]Primers for normalization previously published in Caballero-Solares et al., 2018; Eslamloo et al., 2017; and Umasuthan et al., 2020, and quality-tested again using the reference cDNA template of the present study.

Supplementary Table S3.2: Number of differentially expressed probes (DEPs) identified by RP (PFP[†] < 0.1) and SAM (FDR[‡] < 0.1) analyses presented as pair-wise cross types

Comparisons[§]	**RP**	**Overlapped DEPs**	**SAM**
Farm.NA vs. Wild	46	4	21
Farm.EO vs. Wild	200	0	0
Farm.NA vs. Farm.EO	223	42	168
Hyb.NA$_{f♀}$ vs. Wild	23	4	4
Hyb.EO$_{w♀}$ vs. Wild	17	0	0
Hyb.EO$_{f♀}$ vs. Wild	9	1	13
Hyb.NA$_{f♀}$ vs. Farm.NA	6	1	2
Hyb.EO$_{w♀}$ vs. Farm.EO	3	1	1
Hyb.EO$_{f♀}$ vs. Farm.EO	5	1	3
Hyb.NA$_{f♀}$ vs. Farm.EO[¶]	167	9	18
Hyb.EO$_{w♀}$ vs. Farm.NA[¶]	374	349	2527
Hyb.EO$_{f♀}$ vs. Farm.NA[¶]	153	122	706
Hyb.EO$_{f♀}$ vs. Hyb.EO$_{w♀}$	0	0	3

[†]PFP: Percentage of False Prediction

[‡]FDR: False Discovery Rate

[§]Farm.NA: North American farmed strain, derived from Saint John River, NB; Farm.EO: European origin Norwegian farm strain, unfertilized gametes collected from the Icelandic facility; Wild: North American wild derived from the Northeast Placentia River, NF; Hyb.NA$_{f♀}$: F_1 Farm.NA(♀)-x-Wild(♂) hybrid; Hyb.EO$_{w♀}$: F_1 Farm.EO(♂)-x-Wild(♀) hybrid; and Hyb.EO$_{f♀}$: F_1 Farm.EO(♀)-x-Wild(♂) hybrid.

[¶]The pairwise comparisons (number of DEPs) between Hyb.NA$_{f♀}$ vs. Farm.EO, Hyb.EO$_{w♀}$ vs. Farm.NA andHyb.EO$_{f♀}$ vs. Farm.NAwere not considered in the text in light of their lack of ecological relevance.

Supplementary Table S3.3: Comparison between the microarray and qPCR fold-change values among different pair-wise cross types identified by both RP and SAM. P-values have been Bonferroni corrected.

Probe ID	Gene Name	Farm.NA vs. Wild		Farm.EO vs. Wild		Farm.NA vs. Farm.EO		Hyb.NA$_{f♀}$ vs. Wild		Hyb.EO$_{f♀}$ vs. Wild		Hyb.EO$_{w♀}$ vs. Wild		qPCR p-value
		Micro-array	qPCR	Micro-array	qPCR	Micro-array	qPCR	Micro-array	qPCR	Micro-array	qPCR	Micro-array	qPCR	
†C052R001	Acidic mammalian chitinase precursor (*chia*)	-	-	2.96	3.08[a]	-2.98	-4.08[b]	-	-	-	-	-	-	<0.001[ab]
†C167R126	C-type Lectin domain family 4 member M (*clec4m*)	-2.57	-4.3[a]	2.09	3.09[b]	-5.4	-13.4[c]	-2.76	-4.6[d]	-	-	-	-	0.008[a] 0.003[b] <0.001[c] 0.003[d]
†C124R022	Complement C1q-like protein 4 precursor (*c1ql4*)	3.91	4.01[a]	-	-	2.94	3.70[b]	4.28	1.89[c]	-	-	-	-	0.001[a] <0.001[b] 0.05[c]
†C021R038	Collagen alpha-1 (II) chain precursor (*col2a1*)	-3.21	-1.2[a]	-	-	-3.87	-2.5[b]	-	-	-	-	-	-	1[a] 0.005[b]
‡C236R146	Alpha-enolase (*eno1*)	2.47	1.14[a]	-	-	2.26	1.16[b]	-	-	-	-	-	-	1[ab]
†C180R068	Glyceraldehyde-3 phosphate dehydrogenase (*gapdh*)	-	-	-	-	2.85	1.42	-	-	-	-	-	-	0.02
†C174R128	Hemoglobin subunit alpha (*hba1a*)	-	-	-	-	-3.09	-1.99	-	-	-	-	-	-	0.001
†C174R128	Hemoglobin subunit alpha (*hba1b*)	-	-	-	-	-3.09	-2.25	-	-	-	-	-	-	<0.001
†C228R017	Hemopexin (*hpx*)	-	-	-	-	2.11	3.68[a]	-	-	-	-	-2.7	-2.93[b]	<0.001[ab]

§C060R108	Cytochrome c oxidase subunit 2 (*mt-co2a*)	10.5	-1.6[a]	-	-	6.32	-1.75[b]	11.1	-1.30[c]	-	-	-	-	<0.001[ab] 0.02[c]
§C060R108	Cytochrome c oxidase subunit 2 (*mt-co2b*)	10.5	-1.1[a]	-	-	6.32	-1.26[b]	11.1	1.06[c]	-	-	-	-	0.26[a] 0.03[b] 1[c]
†C065R158	Membrane-associated progesterone receptor component 1 (*pgrmc1a*)	-	-	3.19	1.37[a]	-2.87	-1.45[b]	-	-	-	-	-	-	0.02[a] 0.001[b]
‡C065R158	Membrane-associated progesterone receptor component 1 (*pgrmc1b*)	-	-	3.19	1.11[a]	-2.87	-1.19[b]	-	-	-	-	-	-	1[a] 0.79[b]
‡C083R035	40S ribosomal protein SA (*rpsa*)	2.49	1.14[a]	-	-	2.86	1.15[b]	-	-	-	-	-	-	1[ab]
†C139R032	Radical S-adenosyl methionine domain containing protein 2 (*rsad2*)	-	-	-	-	2.24	3.45	-	-	-	-	-	-	0.013
‡C182R047	Mitochondrial carnitine/ acylcarnitine carrier protein (*slc25a20*)	3.22	1.09[a]	3.05	1.17[b]	-	-	3.47	-1.14[c]	2.29	1.02[d]	3.26	-1.01[e]	0.76[a] 1[bde] 0.87[c]
§C158R068	Superoxide dismutase [CU-ZN] (*sod1*)	-	-	-	-	2.27	-1.74	-	-	-	-	-	-	<0.001

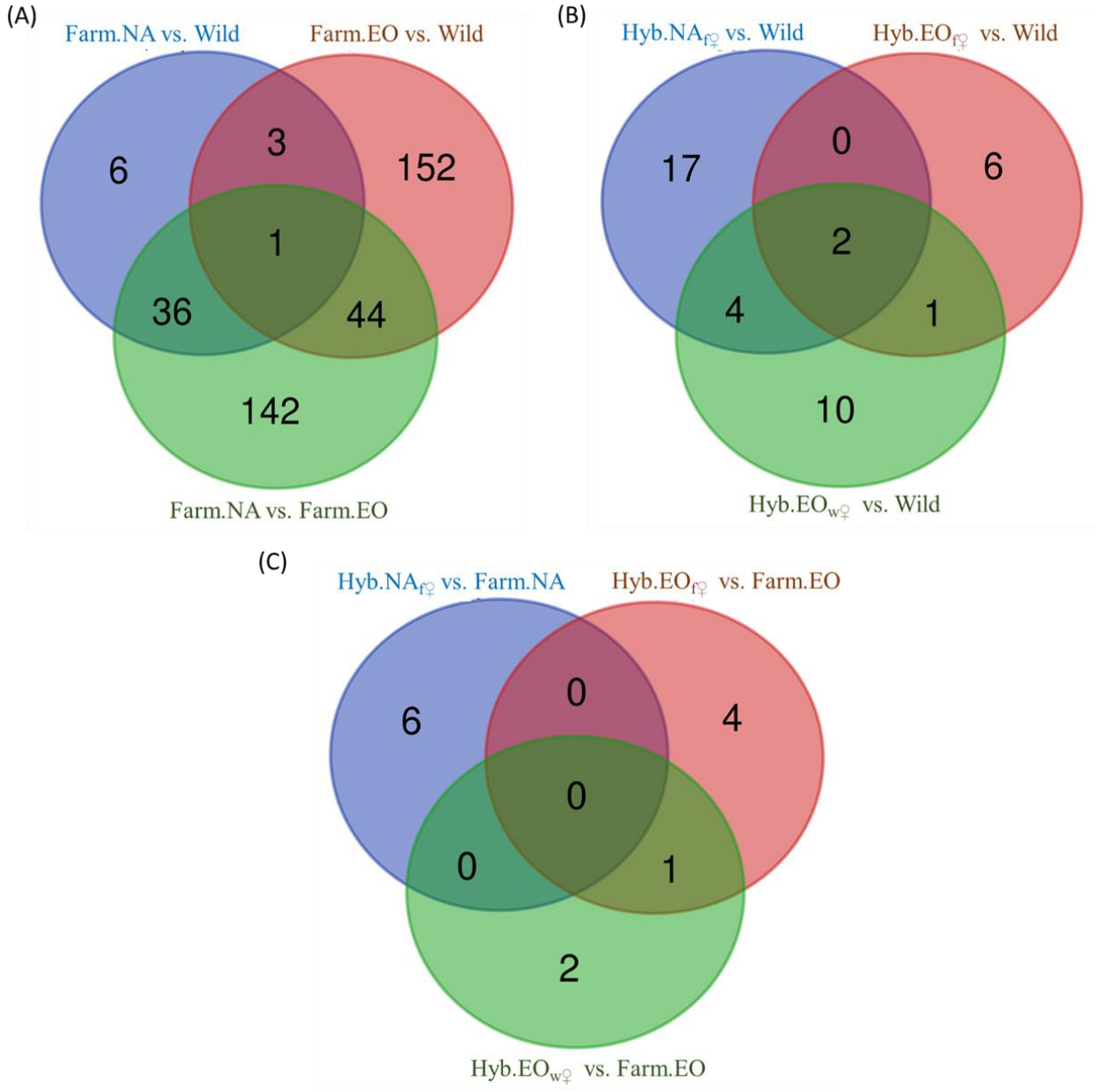
†C030R056	Transcobalamin-1 (*tcn1*)	-	-	-	-	-2.85	-2.98	-	-	-	-	-	-	<0.001
†C001R030	Metalloproteinase inhibitor 2 precursor (*timp2a*)	-	-	-	-	-	-	-	-	-3.63	-2.21	-	-	0.03
§C001R030	Metalloproteinase inhibitor 2 precursor (*timp2b*)	-	-	-	-	-	-	-	-	-3.63	1.22	-	-	0.89

†Validated genes: qPCR shows significant differential expression, with microarray and qPCR fold-changes in the same direction

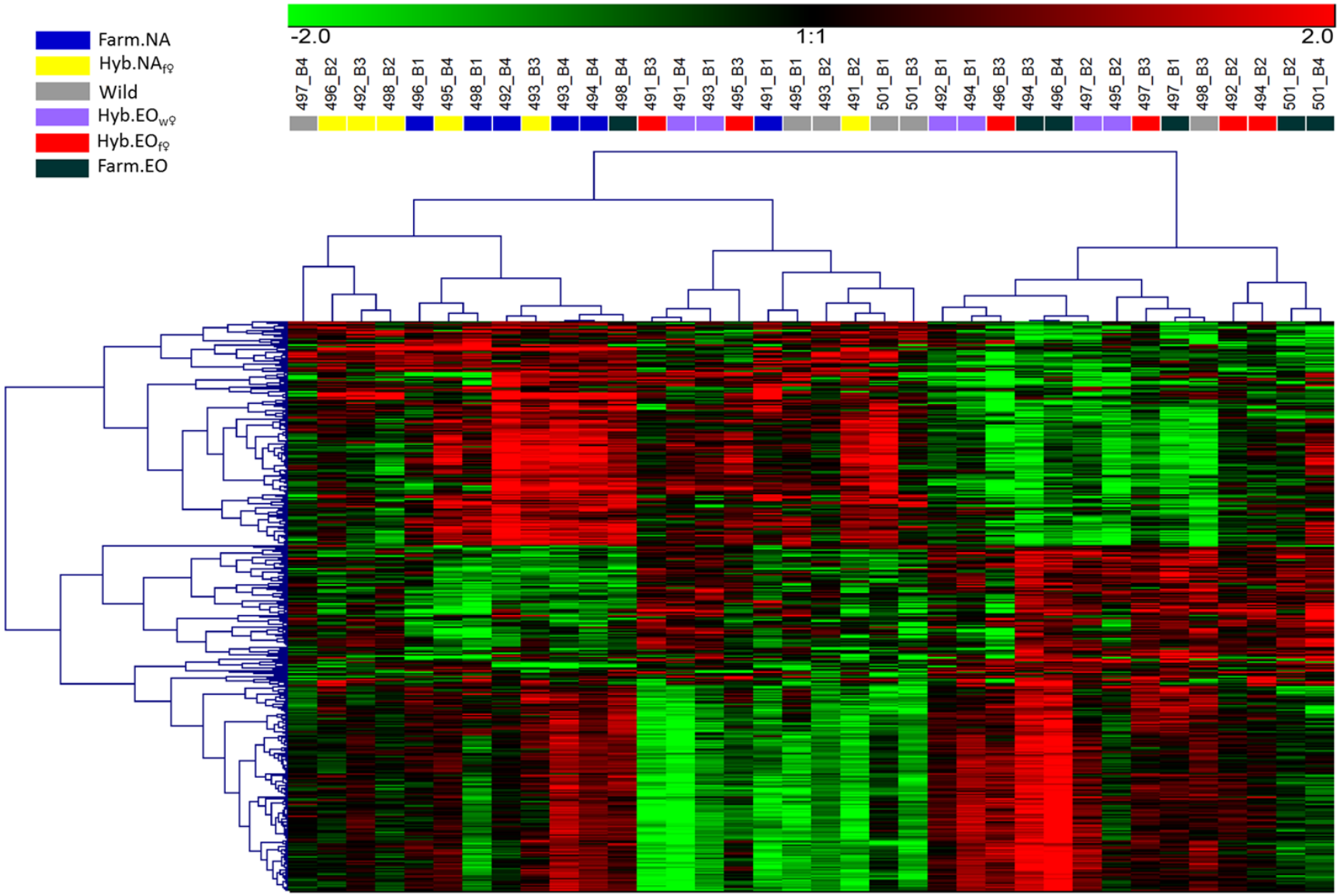
‡Agreement in direction of fold-change: qPCR does not show significant differential expression, but microarray and qPCR fold-changes in the same direction.

§Non-validated genes: qPCR either shows significant or insignificant differential expression, with microarray and qPCR fold-changes in different directions.

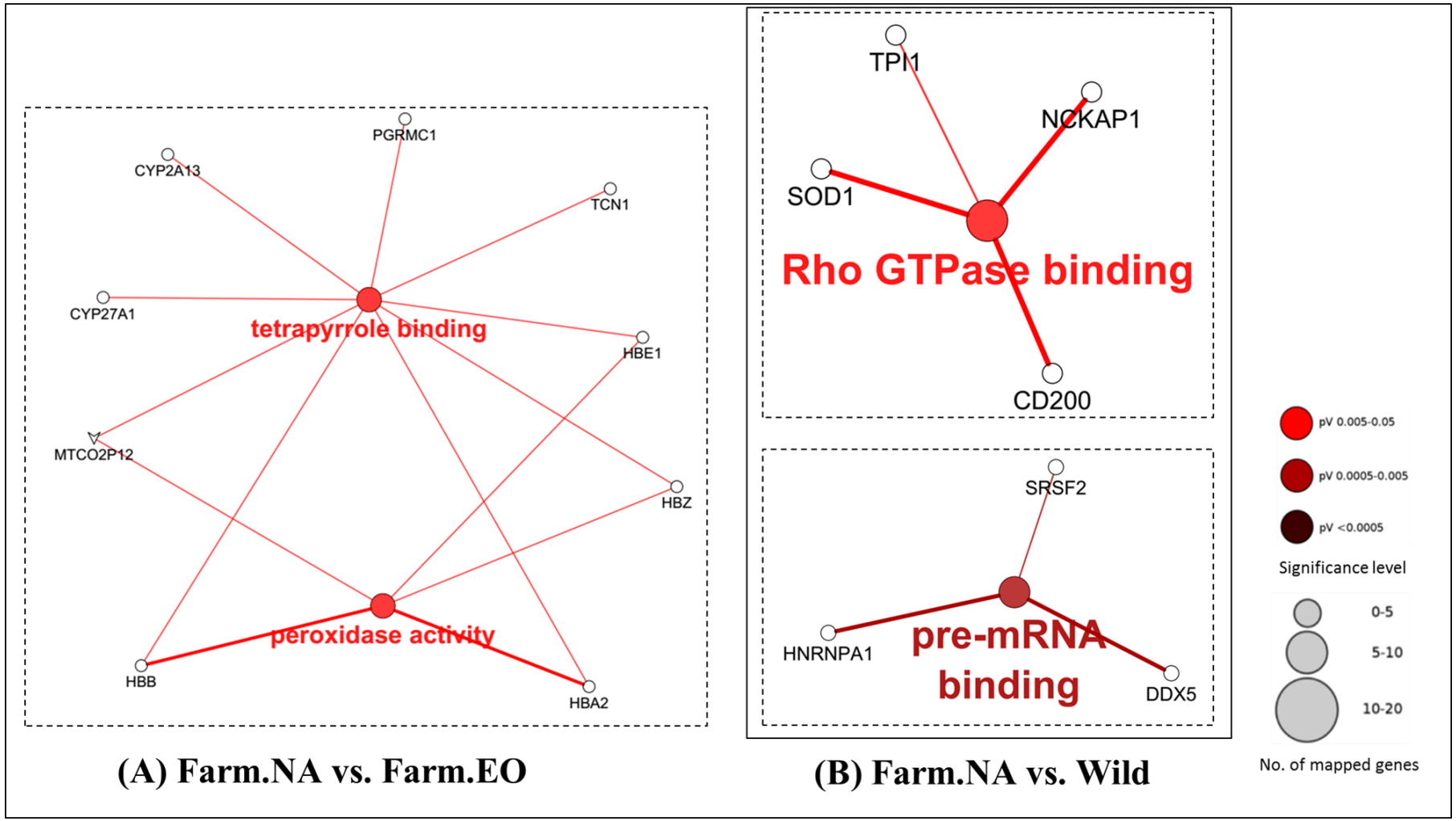
The microarray and qPCR fold-change between Hyb.$NA_{f♀}$ vs. Farm.NA, Hyb.$EO_{w♀}$ vs. Farm.EO, and Hyb.$EO_{f♀}$ vs. Farm.EO were not identified by RP & SAM



Supplementary Figure S3.1: A pair-wise cross type comparison of the number of differentially expressed transcripts identified by RP (Percentage of False Prediction < 0.1). Panel A represents Farm.NA vs. Wild, Farm.EO vs. Wild, and Farm.NA vs. Farm.EO pair-wise comparisons, panel B shows Hyb.NA$_{f♀}$ vs. Wild, Hyb.EO$_{f♀}$ vs.Wild, and Hyb.EO$_{w♀}$ vs. Wild, and panel C represents Hyb.NA$_{f♀}$ vs. Farm.NA, Hyb.EO$_{w♀}$ vs.Farm.EO, and Hyb.EO$_{f♀}$ vs. Farm.EO.



Supplementary Figure S3.2: Hierarchical clustering analyses of 36 samples among 6 different cross types using 396 DEPs from RP analysis (after removing redundancy from 532 DEPs). Top coloured panel indicates all 36 biological replicates of 6 different cross types: blue-Farm.NA, yellow-Hyb.NA♀f, grey-Wild, light blue-Hyb.EO♀w, red-Hyb.EO♀f, and black-Farm.EO.



Supplementary Figure S3.3 (A-B): Enriched molecular function (MF) gene ontology (GO) term networks in different cross types comparisons [(A) Farm.NA vs. Farm.EO, and (B) Farm.NA vs. Wild]. GO terms visualized by ClueGO plugin of Cytoscape. The nodes (round shape) represent GO terms, node color represents the level of significance as indicated in the legend, while node size reflects the number of genes in each enriched GO term.

CHAPTER 4

Linking dominance, growth and survival: fitness consequences of hybridization of divergent European and North American farmed with wild Newfoundland Atlantic salmon populations

Preface

The research described in Chapter 4 has been submitted to the journal Aquaculture Environment Interactions as: Islam, S. S., B. W. Wringe, C. M. Conway, I. R. Bradbury, and I. A. Fleming. Linking dominance, growth and survival: fitness consequences of hybridization of divergent European and North American farmed with wild Newfoundland Atlantic salmon populations; see Co-authorship statement on page XXIV-XXV.

4.1 Abstract

Multi-generation domestication selection and geographic and ancestral relationships have resulted in genetic divergence between farmed and wild Atlantic salmon. This raises concerns about potential negative fitness consequences for native populations from interbreeding with farmed salmon. In Newfoundland (NF), Canada, while the majority of aquaculture sites use North American (NA) Saint John River strain salmon, site-specific permission has been granted to farm a European origin (EO) strain. I designed two complementary experiments to compare juvenile fitness-related traits. The first experiment tested differences in dominance status among EO and NA farmed strains, NF wild, and F_1 hybrid fish. The second assessed the effect of competition on the NF wild fish by comparing growth and survival in allopatry with that in sympatry with EO and NA farmed and F_1 hybrid fish across contrasting tank and semi-natural stream environments. Farm.NA fish were more dominant and less subordinate than NF wild conspecifics, with hybrids being intermediate, not differing from wild fish. Farm.EO fish also tended to dominate NF wild fish, though the difference was not significant. Competition with farmed and hybrid fish did not affect the growth of wild fish in sympatry versus allopatry in the tank environment; however, in the stream environment, where wild fish in sympatry with Farm.NA and hybrids outgrew those in allopatry. Within sympatric treatments, both EO and NA farmed fish similarly outgrew wild fish in the tank environment, but not necessarily always in the stream environment (e.g., Farm.NA). F_1 hybrids tended to display intermediate growth performance relative to farmed and wild fish both in tank and stream environments. No survival differences were detected among farmed, wild, and F_1 hybrid juveniles both in

tank and stream environments. These findings suggest that multi-generation domestication selection has generated fitness differences among farmed and wild fish and their related hybrids that may have effects on productivity and viability for local NF populations.

4.2 Introduction

Predicting and assessing the fitness consequences of intraspecific hybridization has long been a two-sided debate in ecology and evolution. On one side, hybridization may lead to adaptive potential for populations by increasing heterozygosity, creating new genetic combinations, and masking deleterious alleles (Anderson & Stebbins, 1954; Lynch & Walsh, 1998; Frankham, 2015; Chan et al., 2019). On the other side, hybridization between two reproductively isolated populations may result in the breakup of co-adapted gene complexes, and/or disrupt local adaptation, which leads to outbreeding depression, a mechanism of fitness reduction (Dobzhansky, 1940; Allendorf et al., 2001; Edmands, 2007; Hamilton & Miller, 2016). Consequently, such outbreeding depression is expected when hybridization involves more genetically distant populations (Allendorf &Waples, 1996; Edmands & Timmerman, 2003). The resulting progeny may not be well adapted locally, as an allele that is advantageous in one environment or genetic background may be disadvantageous to overall fitness in another. Thus, there is a growing need to understand the fitness implications of hybridization among divergent populations for the conservation and management of intraspecific biodiversity.

Given rapid climate change and anthropogenic influences on the exploitation of wild living resources (Hutchings, 2000; Myers & Worm, 2003; Thiault et al., 2019), captive production intuitively represents an alternative means of food security (Gering et al., 2019;

Houston et al., 2020). Although, compared with other livestock species which have been domesticated as a source of food for thousands of years (e.g., pigs, poultry, sheep, goats, and cattle were domesticated 8,000 to 10,000 years before present; Craig, 1981), domestication is less complete in many fish species, such as Atlantic salmon (*Salmo salar* L) (Gjedrem, 2000). Atlantic salmon, an ecologically and culturally significant fish species, have been intensively farmed since 1970 and have undergone directed selection for economically important traits (e.g., faster growth, delayed maturation, disease resistance through phenotypic and family-based selection; Gjedrem, 2010). Consequently, this species is regarded as one of the most domesticated aquaculture species globally for food (Teletchea & Fontaine, 2014).

Behavioural traits, which often underlie growth and survival, are among the first fitness traits affected by the domestication process, where unintentional and relaxed selection alter phenotypes (e.g., aggressive, dominance, and antipredator behaviours) (Metcalfe et al., 2003; Huntingford, 2004). Therefore, domesticated fish species, such as commercially bred farmed Atlantic salmon, may behave in a manner that results in a competitive advantage over their wild conspecifics in a culture environment (Einum & Fleming, 1997; Fleming & Einum, 1997). Moreover, social interactions and hierarchies can be influenced by body size and affect the outcome of resource competition, providing faster-growing cultured salmon with a further advantage (Abrahams &Sutterlin, 1999; Biro et al., 2004, 2006). In general, bigger, bolder, and dominant fish get better access to food and territories than smaller, shy, and submissive fish (Sundstróm et al., 2004). However, due to various natural selective pressures in the wild environment, cultured fish do not always show better performance in all situations (Fleming et al., 2000; McGinnity et al.,

2003; Skoglund et al., 2011). To date, limited research has been devoted to assessing dominance status among domesticated, wild, and related hybrid fish in the context of distinct geographical and ancestral relationships and domestication selection among divergent populations.

One of the largest and most consistent differences in phenotypic trait expression between domesticated and wild salmon is growth, an important component of fitness (Solberg et al., 2013a; Harvey et al., 2016a; Glover et al., 2018). Recent evidence indicates that farmed salmon can display a growth rate that is over two to three-fold higher than that of wild conspecifics when reared under identical culture environments (Solberg et al., 2013b; Harvey et al., 2016b; Glover et al., 2018). In contrast, in the natural environment, the growth of farmed salmon is only marginally higher than that of wild counterparts (Glover et al., 2018; Skaala et al., 2019). This begs the question, why do farmed salmon outgrow wild salmon extensively in the culture environment while not in the wild? This difference may be a plastic response driven by divergent energy budgets between the two environments. For example, from the start of exogenous feeding, juveniles in captivity are fed high-energy commercial diets (Harvey et al., 2016b), whereas those in nature feed on a diet of natural prey. The natural prey of wild fish can vary substantially in type, form, and nutrient composition (Jonsson & Jonsson, 2011). Moreover, farmed salmon are less effective at catching prey in the wild, expend more energy in their search, and are vulnerable to starvation (Solberg et al., 2020). Also, there can be a trade-off between energetic gain and mortality ("high-risk, high gain"; Biro & Stamps, 2008), where farmed juveniles displaying the highest growth potential are more susceptible to predation in the wild than those exhibiting lower growth potential (Solberg et al., 2020). Such a selection

mechanism (growth-potential mortality) may therefore result in more similar growth among surviving farmed and wild juveniles in a natural environment. Thus, it is becoming essential to examine growth and survival response among multi-generation domesticated farmed strains, wild populations, and related F_1 hybrids across the contrasting culture and natural environments.

Genetic and competitive interactions between escaped farmed and wild salmon have been documented where the two coexist in Atlantic Canada (Keyser et al., 2018; Wringe et al., 2018; Sylvester et al., 2019). Currently, salmon aquaculture practices in Atlantic Canada use farmed strains that originate from the Saint John River, New Brunswick (NB). In Newfoundland (NF), permission has also been recently granted to import a strain of European (hereafter "EO") aquaculture salmon (StofnFiskur, Iceland), domesticated from wild Norwegian populations, to be farmed as triploids. The triploidization process is not completely effective (Benfey, 2015), and among any farmed salmon that may escape, a proportion may be non-triploid EO. Non-triploid escapees will be able to breed successfully and interact genetically and ecologically with wild populations (O'Reilly et al., 2006). This raises concerns that introgressive hybridization of EO origin farmed strains into NF wild populations could be a greater threat than hybridization between NA farmed and wild salmon. Effects will depend on the distinct geographical and ancestral relationships among the populations as well as commonalities in the effects of domestication selection. A compelling body of evidence indicates that EO farmed salmon (Norwegian) and North American farmed salmon (Saint John River strain; hereafter "NA") are highly divergent genetically ($F_{st} > 0.40$; S.J. Lehnert, pers. comm. based on data from Jeffery et al., 2018). Although phenotypic and genetic differences exist

among wild salmon populations within NF (e.g., F_{st} = 0.12, between Garnish and Northeast Placentia River populations; S.J. Lehnert, pers. comm.), the divergence between NF wild and NA farmed salmon populations is larger (F_{st} = 0.14 - 0.20; Bradbury et al., 2018).

Here, using two complementary experiments designed to investigate (a) differences in dominance status; and (b) growth and survival differences among divergent EO and NA farmed, NF wild, and related hybrids across contrasting tank and semi-natural stream environments. I tested the hypotheses that: (i) EO and NA farmed will be more dominant than NF wild salmon, and given that genetic differences between wild and farmed salmon are typically additive (McGinnity et al., 2003; Fraser et al., 2010), F_1 hybrids will be intermediate; (ii) the growth and survival of wild fish in allopatry will be higher than that of those in sympatry (i.e., competing with farmed and related hybrids); (iii) both EO and NA farmed and F_1 hybrids will display higher growth and survival than wild fish in sympatry; and (iv) multi-generation domestication selection and the geographic and ancestral relationships will be reflected in the growth, survival, and dominance status among divergent EO and NA farmed, NF wild, and related hybrids.

4.3 Material and Methods

4.3.1 Parental populations

The complementary dominance and growth and survival experiments were conducted across two years (2016 and 2017) with two cohorts of fish. The first cohort was generated in 2015 using three base populations. Farm.EO, which was a Norwegian farmed strain, produced in an Icelandic facility (StofnFiskur). Gametes were obtained from Iceland and transported by air to St John's, NF. Farm.NA, are Atlantic Canada's principal

aquaculture strain, originally derived from the Saint John River, NB. Farm.NA gametes were obtained from Northern Harvest Sea Farms, a local aquaculture company based on the south coast of NF. Wild.NA with a signal of EO ancestry (hereafter "Wild.NA_{EO}"), were collected as adults from the Northeast Placentia River, NF (Lat: 47.2408 °N, Lon: 53.9566 °W) on July 27, 2015, and transported to the Ocean Science Centre (OSC, Memorial University of Newfoundland), where they were held in broodstock tanks until crossing in the Fall. This wild population was included in this study as it showed evidence of EO introgression when glaciers retreated, and salmon recolonized (~10,000 years before present; see Bradbury et al., 2015). The second cohort was generated in 2016 using two base populations: again, Farm.EO gametes were collected from the Icelandic facility; and Wild.NA, were collected as adults from the Garnish River, NF (Lat: 47.2348 °N; Lon: 55.3615 °W) on August 9, 2016, and transported to the OSC, and held in broodstock tanks until performing the crosses in the Fall (for cross details, see chapter two).

Each year following yolk sac absorption, families were pooled by cross type. Each family consisted of ca. 200-400 juveniles based on the number of families per cross type to maintain similar densities and transferred into 470-liter flow-through circular holding tanks (0.9 m diameter x 0.5 m high).

4.3.2 Dominance experiment

Approximately four weeks following the onset of exogenous feeding, a dominance experiment was conducted with the juvenile fish from the 2015 and 2016 cohorts between 20 June – 30 August 2016, and 25 June - 4 September 2017, respectively. In 2016, I tested a total of 175 fish (35 from each of the five cross types: Farm.NA, Hb.NA (Farm.NA(♀)-x-Wild.NA_{EO}(♂) hybrid), Wild.NA_{EO}, Hb.EO (Farm.EO(♀)-x-Wild.NA_{EO}(♂) hybrid), and

Farm.EO), and in 2017, I tested a total of 140 fish (35 from each of the four cross types: Wild.NA, $Hb_{W♀}$ (Wild.NA(♀)-x-Farm.EO(♂) hybrid), $Hb_{f♀}$ (Farm.EO(♀)-x-Wild.NA(♂) hybrid), and Farm.EO). Thirty-five trials were conducted each year. Experimental protocols and conditions were the same both years, and all observations took place between 09:00 and 17:00 hours. Two trials were conducted per day using two experimental aquaria (70 x 45 x 36 cm) (Fig. 4.1). Three sides of the aquaria were covered externally to minimize disturbance, and data were recorded both manually and by video (VIXIA HF R60 HD Digital Camcorder, Canon, USA, Inc.). Before conducting each trial, experimental fish were anaesthetized with AQUALIFE TMS (MS-222; 400 mgl^{-1}, Syndel Laboratories Ltd, Nanaimo, BC, Canada), saline buffered with 0.1 M sodium bicarbonate, and measured for wet weight (mg) to minimize the influence of size differences (fish within 25 mg of each other) on their dominance status. To identify each individual during the trials, each fish was marked by injecting a small amount of different coloured visible implant elastomer (Northwest Marine Technology, WA, USA) below the dorsal fin (both sides). The fish were deprived of food for a day before dominance trials, and there was no tag loss or mortality while the fish were in the experimental aquaria.

Approximately 24 hours after acclimatisation in the experimental aquaria, the fish (one per cross type, n = 5 in 2016 and n = 4 in 2017) were tested for their dominance status. Removal tests (adopted from Adriaenssens & Johnsson, 2010) were conducted to assign dominance status among the different cross types. Dominance status was calculated by a combined index using the three indicators: (i) spatial upstream position in the aquarium; (ii) feeding

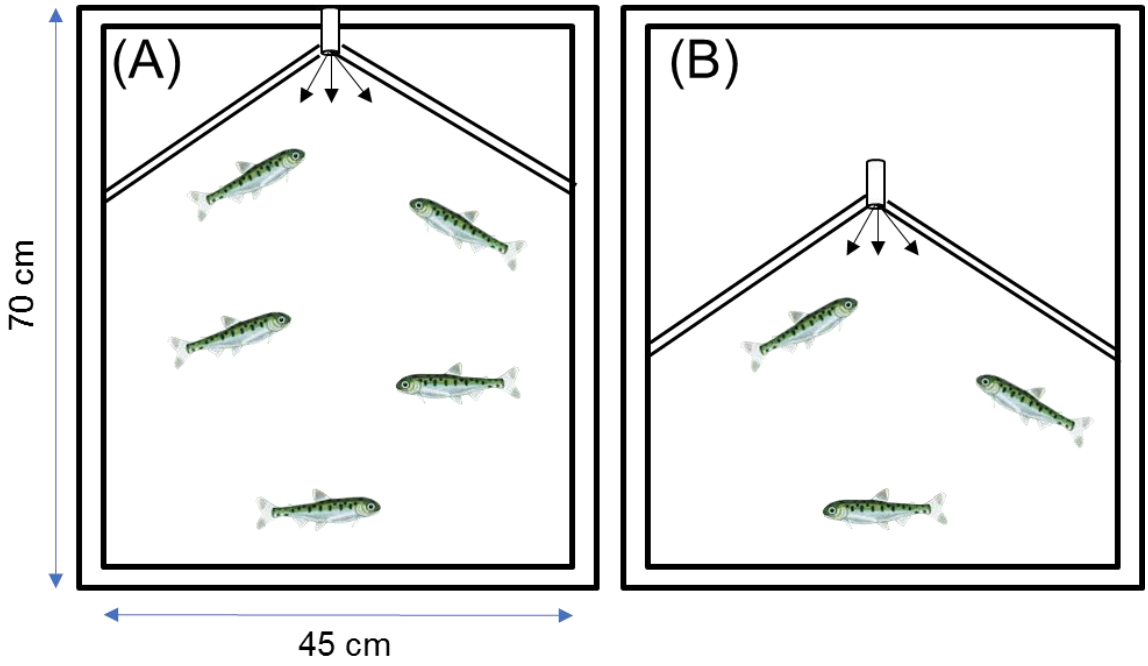


Figure 4.1: A schematic representation of the experimental aquaria used for the dominance experiments in 2016 and 2017. Prior to (A) and after removal (B) of the dominant individual (s) when the experimental area was reduced to minimize the density effects.

attempts and success; and (iii) aggressive interactions. Each of 5 observations was scored for 2 min every 15 min. At the beginning of each observation, 10-15 pellets (size: 1 mm) were delivered, and spatial position was recorded (1 point for upstream). A score of 2 points was given for a feeding attempt and 1 point for consuming a pellet. Agonistic behaviours were also recorded; individuals were given 2 points for an aggressive interaction (e.g., chasing). After 5 observations, all scores were summed, and the fish receiving the highest score (total score was > 3 than that of other individuals) was considered dominant. The dominant individual (s) was/were removed from the tank, and the remaining individuals were left to recover for 2 h. The procedure was then repeated with the remaining fish until dominance status (intermediate and subordinate) had been determined for all individuals.

To reduce the behavioural effects of decreasing density (Adriaenssens & Johnsson, 2010), the tank area was reduced after the removal of dominant individuals (Fig. 4.1).

4.3.3 Growth and survival experiments in tank and stream environments

Approximately four weeks after the start of exogenous feeding and following pooling by cross type, farmed, wild, and hybrid fry were placed in either a tank environment consisting of 36 rectangular tanks (.32 m x .24 m x .16 m), each with an independent continuous flow-through water supply or a semi-natural stream environment consisting of 36 mesocosms (1.2 m x 0.22 m x 0.15 m) (Table 4.1). In the latter case, the mesocosms were constructed inside 9 raceways (2.7 m x 0.45 m x 0.30 m) that contained gravel substrates (5-10 cm). Each raceway had a similar flow-through water supply (10-15 $cm.s^{-1}$), and adjacent mesocosms were separated by a double-screened buffer zone. Fish were selected haphazardly from rearing tanks, anaesthetised with buffered AQUALIFE TMS (MS-222), and measured for wet weight (mg) and fork length (cm). To identify individuals to cross type, fish were marked by injecting a small amount of coloured visible implant elastomer (Northwest Marine Technology, WA, USA) below the dorsal fin. In both the tank and semi-natural stream environments in 2016, fish were placed into one of the three forms of competitive treatments: (i) Allopatric Wild.NA_{EO}: 12 replicates, 30 individuals/replicate, (ii) Sympatric NA: 12 replicates; 30 individuals/replicate (10 Wild.NA_{EO}, 10 Hb.NA, 10 Farm.NA), and (iii) Sympatric EO: 12 replicates; 30 individuals/replicate (10 Wild.NA_{EO}, 10 Hb.EO, 10 Farm.EO) (Table 1). Similarly, in 2017, three competitive treatments were examined: (i) Allopatric Wild.NA: 12 replicates, 30 individuals/replicate, (ii) Sympatric-I: 12 replicates; 30 individuals/replicate (10

Wild.NA, 10 $Hb_{W♀}$, 10 Farm.EO), and (iii) Sympatric-II: 12 replicates; 30 individuals/replicate (10 Wild.NA, 10 $Hb_{F♀}$, 10 Farm.EO) (Table 4.1).

In the tank environment, fish were provided with a diet of commercial salmonid starter dry pellets (EWOS-Cargill, BC, Canada) four times per day. Feeding rates and caloric content were standardized: 75 mg of pellet in each replicate during each feeding (pellet size: crumbles 0.5 mm; composition: 55% protein and 15% fat, EWOS-Cargill, BC, Canada) for the first 40 days. During the last 40 days (day 41 to 80), the feeding rate was increased to double with 150 mg of pellet in each replicate per feeding event (pellet size: crumbles 0.7 mm; composition: 54% protein and 16% fat, EWOS-Cargill, BC, Canada). In the stream environment, fish were provided a combination of live brine shrimp (*Artemia* spp.) and frozen blood worm (*Chironomidae* spp., commercial fish food supplier) as semi-natural feed. *Artemia* concentration was standardized by growing 1 000 000 cysts/L and suspending the live *Artemia* from a batch into 2L of water prior to feeding. The *Artemia* were enriched with Ori-one (0.3 g/million) and Ori-green (0.8 g/million); algae-based enrichments with highly unsaturated fatty acids and vitamins (Skretting, NB, Canada).

Table 4.1: Overview of the experimental design of growth and survival experiments in tank and semi-natural stream environments (2016 and 2017). The initial number of fish per replicate, total number of fish at initial stage for each cross type, and the final number of surviving fish sampled at termination for each cross type in allopatric and sympatric environments are indicated. All comparisons of initial mass and length within environment were significant ($P < 0.001$) and different letters denote the significant mean trait differences among cross types.

Growth and survival experiment (2016)														
Environment	**Tank**							**Semi-natural stream**						
Competitive treatments (n=replicates)	Allo-Patric (n=12)	Sympatric (NA) (n=12)			Sympatric (EO) (n=12)			Allo-patric (n=12)	Sympatric (NA) (n=12)			Sympatric (EO) (n=12)		
Cross-types	Wild. NA_{EO}	Wild. NA_{EO}	Hb. NA	Farm. NA	Wild. NA_{EO}	Hb. EO	Farm. EO	Wild. NA_{EO}	Wild. NA_{EO}	Hb. NA	Farm. NA	Wild. NA_{EO}	Hb. EO	Farm. EO
Initial fish per replicate	30	10	10	10	10	10	10	30	10	10	10	10	10	10
Total fish at initial stage	360	120	120	120	120	120	120	360	120	120	120	120	120	120
Initial mass (mg)	142.8^{a}	131.3^{b}	118^{cd}	122^{bd}	122^{bd}	144.1^{a}	146.5^{a}	143.5^{a}	143.0^{a}	123.7^{b}	126.9^{b}	138.3^{a}	162.1^{c}	147.4^{a}
Initial length (cm)	2.66^{a}	2.62^{a}	2.50^{b}	2.53^{b}	2.53^{b}	2.67^{a}	2.56^{b}	2.69^{a}	2.68^{a}	2.47^{b}	2.52^{b}	2.71^{a}	2.79^{c}	2.68^{a}
Sampled at termination	276	94	95	103	92	98	89	278	108	106	104	97	91	79
Diet	Pellet							Semi-natural feed						
Duration	80 days							80 days						
Growth and survival experiment (2017)														
Environment	**Tank**							**Semi-natural stream**						
Competitive treatments (n=replicates)	Allo-Patric (n=12)	Sympatric-I (n=12)			Sympatric-II (n=12)			Allo-patric (n=12)	Sympatric-I (n=12)			Sympatric-II (n=12)		
Cross-types	Wild. NA	Wild. NA	$Hb_{W♀}$	Farm. EO	Wild. NA	$Hb_{F♀}$	Farm. EO	Wild. NA	Wild. NA	$Hb_{W♀}$	Farm. EO	Wild. NA	$Hb_{F♀}$	Farm. EO
Initial fish per replicate	30	10	10	10	10	10	10	30	10	10	10	10	10	10
Total fish at initial stage	360	120	120	120	120	120	120	360	120	120	120	120	120	120
Initial mass (mg)	160.1^{a}	158.0^{a}	136.2^{b}	152.3^{a}	156.7^{a}	180.6^{c}	155.8^{a}	143.9^{a}	157^{bd}	148^{ad}	154^{bd}	147^{ad}	192.9^{c}	161.2^{b}
Initial length (cm)	2.85^{ab}	2.85^{ab}	2.84^{ab}	2.89^{b}	2.83^{a}	2.97^{c}	2.85^{a}	2.76^{a}	2.86^{bc}	2.80^{ad}	2.83^{bd}	2.77^{a}	3.04^{e}	2.91^{c}
Sampled at termination	154	48	52	52	71	68	58	319	75	78	96	93	98	100
Diet	Pellet							Semi-natural feed						
Duration	80 days							80 days						

The caloric contents of frozen blood worm were 6% crude protein; 0.5% crude fat; 0.9% crude fiber; and 89% moisture (J & L Aquatics, BC, Canada). Feeding rates were standardized at 15 ml of semi-natural feed (80% of Artemia and 20% of blood worm) in each replicate for the first 40 days and doubled (30 ml) for the last 40 days. The feeding frequency was the same as for the tank environment, and both experiments were carried out simultaneously for 80 days (between 15 June - 2 September 2016, and 21 June - 8 September 2017). At the termination of the experiments (day 80), the fish were weighed (mg) and photographed for subsequent fork length (cm) measurement. There were some differences in initial size (body mass and length) among the cross types across the different treatments (allopatric vs. sympatric) within an environment (tank or semi-natural stream) (Table 4.1). In 2016, Wild.NA_{EO} were typically larger than Farm.NA and Hb.NA, but similar in size to Farm.EO and smaller than Hb.EO. In 2017, Wild.NA were similar in size to Farm.EO (except in sympatric-II stream), and typically smaller than $Hb_{F♀}$ and larger than $Hb_{W♀}$.

The fish in both the tank and stream environments were reared under common environmental conditions (ambient water temperature = 15-17 ºC; pH = 5.7-6.2, dissolved oxygen = 8.0-8.5 $mg \cdot L^{-1}$) and the photoperiod was maintained at a 12L: 12D schedule. All animals were treated following the guidelines provided by the Canadian Council on Animal Care during holding and experimentation, and approval was granted by the Memorial University Animal Care Committee (15-21-IF).

4.3.4 Statistical analyses

All statistical analyses were performed in R version 4.0.5 (R Core Team, 2021). Statistical significance was inferred if $P < 0.05$ after sequential Bonferroni adjustment (Rice, 1989). All data were checked visually using Q-Q plots and histograms to examine normality. To check the constancy of variance, homoscedasticity was checked visually using residuals vs. fitted values (Crawley, 2005).

To assess the dominance status, logistic regression with a binomial generalized linear model (GLM) was used. Tukey adjusted pairwise contrasts were carried out using the *emmeans* package (Lenth et al., 2018) to estimate the dominance status (dominant, intermediate, and subordinate) between cross types. This test reported parameter estimates, *z* - values (for binary data), and *P* – values (see Supplementary table S4.1).

Specific growth rates in terms of mass (SGR_{mass}) and length (SGR_{length}) were calculated as 100 × [ln (body mass or length at termination) – ln (body mass or length at the beginning)]/ time. The final Fulton's body condition factor was calculated as $K = (mass/length^3) \times 100$.

SGR_{mass}, SGR_{length}, and condition were analysed using linear mixed effects (LME) models with the *lme4* package (Bates et al., 2014). Treatment (allopatry, sympatry) and/or cross type and environment (tank, stream) were included as categorical fixed effects. Replicate and final density (nested within replicate) were included as a random intercept. Mass data were log_{10}-transformed. The final model was selected from the full model with the *lmerTest* package, which allowed for automatic model selection (Kuznetsova et al., 2017). The *step* function performed backwards selection, where non-significant random covariates were removed first. Non-significant interaction terms were eliminated before the fixed effects, and if significant interaction terms were found, all fixed covariates were

included in the final model, nonetheless of their significance level. The *P* - values for random effects were estimated using likelihood ratio tests (LRTs), whereas the significance values for the fixed effects were obtained using a *F* - test based on Satterthwaite's approximation. The final model was confirmed by using plots of the model residuals, and the normality of the fitted model residuals was confirmed visually using histograms.

Survival was analysed using a generalized linear mixed-effects model (GLMM) with a binomial distribution (logit-link function) using the *glmer* function again in the *lme4* package (Bates et al., 2014) using Laplace approximation to the likelihood. Again, treatment and/or cross type and environment were included as fixed covariates, and replicate and final density nested within replicate were included as random covariates. Non-significant interaction terms and covariates were removed backwards stepwise using LRTs. The model residual plots and normality were confirmed for the final model, as for the growth data.

Estimated marginal means (see Supplementary Table S4.2) and Tukey adjusted post hoc multiple comparisons (using Kenward-Roger's degrees-of-freedom method) were again carried out using the *emmeans* package (Lenth et al., 2018). This test estimated all pairwise cross type contrasts (see Supplementary Tables S4.3 and S4.4), and reported parameter estimates, *t* - values (for normally distributed data), *z* - values (for binary data), and *P* - values.

4.4Results

4.4.1 Dominance status

Significant differences in dominance status were observed among EO and NA farmed strains, NF wild populations, and related hybrids in the 2016 and 2017 experiments. Farm.NA fry were more dominant and less subordinate than Wild.NA_{EO} fry in the 2016 experiment ($P < 0.05$), with Hb.NA and Hb.EO being intermediate and not differing from any other cross type ($P > 0.05$; Fig. 4.2A, Supplementary Table S4.1). There were no differences in intermediate status among cross types ($P > 0.05$). In 2017, Farm.EO fry were more dominant and less subordinate than $Hb_{f♀}$ ($P < 0.05$) but did not differ significantly from Wild.NA and $Hb_{w♀}$ ($P > 0.05$; Fig. 4.2B, Supplementary Table S4.1). No differences in intermediate status were observed.

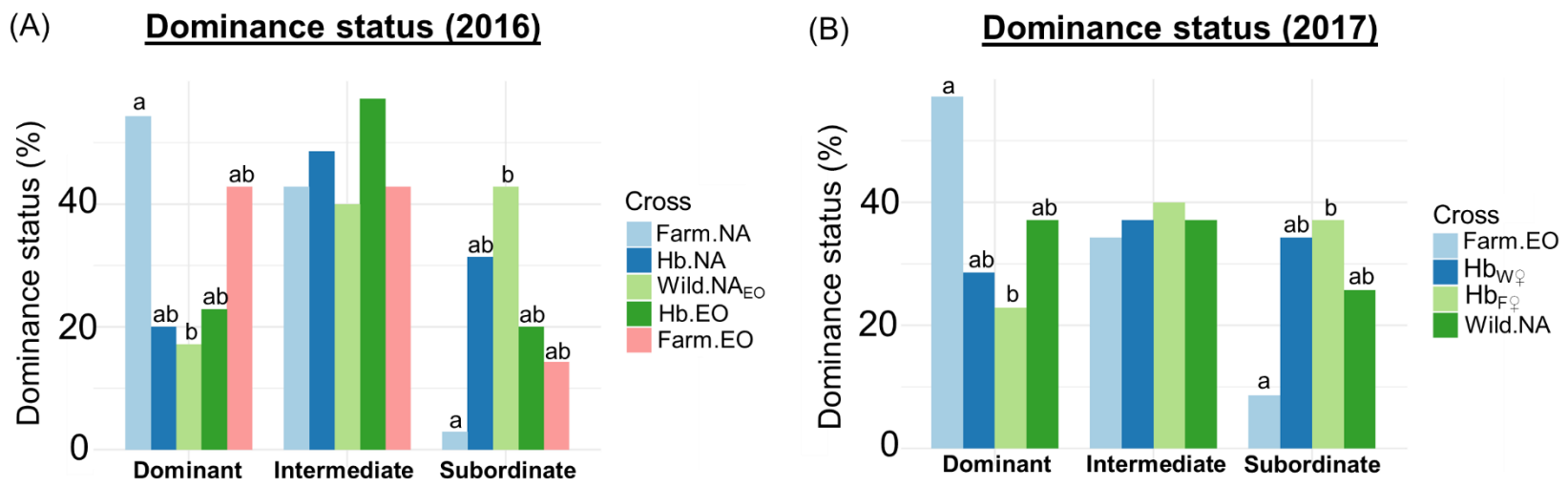


Figure 4.2: Dominance status (%) among different cross types in the (A) 2016 and (B) 2017 dominance experiments. Different letters indicate significant differences in dominance status among cross types. See Table S1 for Tukey-adjusted pairwise contrasts of different cross types for the estimation of dominance status. Cross types: Farm.NA; Hb.NA; Wild.NA$_{EO}$; Hb.EO; and Farm.EO from the 2016 dominance experiment. Cross types: Farm.EO; Hb$_{w♀}$; Hb$_{f♀}$; and Wild.NA from the 2017 dominance experiment.

4.4.2 Growth and survival in tank and stream environments

Significant differences in growth (SGR_{mass} and SGR_{length}) and body condition were observed among EO and NA farmed strains, the NF wild population, and related hybrids in both the tank and stream environments in 2016 (Table 4.2, 4.3, Fig. 4.3A-F). In order to assess the effect of competition on the wild fish, I compared the performance of wild fish in allopatry to those in sympatry with farmed and hybrid fish and found that $SGR_{mass}$and SGR_{length} were best described by models that included treatment ($P < 0.0001$ and $P < 0.01$, respectively), environment ($P < 0.0001$) and their two-way interaction ($P < 0.001$; Table 2). For condition, only the fixed covariate environment was significant ($P < 0.0001$), and therefore retained in the final model (Table 4.2). More specifically, in the tank environment, SGR_{mass} and SGR_{length}, of $Wild.NA_{EO}$ fish in allopatry did not differ from those in either sympatric treatment (Table 4.3, Fig. 4.3A-B). However, in the stream environment, $Wild.NA_{EO}$fish in the Sympatric NA treatment had higher SGR_{mass} and SGR_{length} than those in allopatry, while those in the Sympatric EO treatment did not differ between themselves (Table 4.3, Fig. 4.3D-E). By contrast, no differences in condition were observed between allopatric and sympatric $Wild.NA_{EO}$fish in both the tank and stream environments (Table 4.3, Fig. 4.3C and 4.3F).

In the Sympatric NA treatment, both SGR_{mass} and SGR_{length} varied significantly by environment ($P < 0.0001$) and cross-x-environment interaction ($P < 0.0001$, $P < 0.001$, respectively) (Table 4.2), and therefore retained after model selection. As the higher-order interaction term was significant, the lower-order non-significant fixed term cross type was also retained in the final model. For condition, only the fixed term environment was

Table 4.2: Statistical summary of LME model selection for examining growth variation (SGR_{mass}, SGR_{length}, and condition) between treatments (T), environments (E), and cross types (C) from the 2016 and 2017 growth experiments. Final density nested within replicate were included as random intercepts.

	Growth experiment (2016)[a]					**Growth experiment (2017)[b]**				
Treatment	**Variable**	**Sum Sq**	**d.f**	**F**	***P***	**Variable**	**Sum Sq**	**d.f.**	**F**	***P***
Allopatric Wild	*SGR_{mass}*					*SGR_{mass}*				
vs.	T	0.67	2,55	16.35	**<0.0001**	T	0.07	2,66	4.43	**<0.05**
Sympatric Wild	E	8.71	1,55	424.5	**<0.0001**	E	5.78	1,66	94.3	**<0.0001**
	T x E	0.28	2,55	6.71	**<0.001**	T x E	0.17	2,66	1.42	0.25
	SGR_{length}					*SGR_{length}*				
	T	0.05	2,55	7.06	**<0.01**	T	0.00	2,66	4.29	**<0.05**
	E	0.76	1,55	213.0	**<0.0001**	E	0.45	1,66	109.2	**<0.0001**
	T x E	0.06	2,55	7.86	**<0.001**	T x E	0.01	2,66	1.02	0.37
	Condition					*Condition*				
	T	0.02	2,55	2.37	0.08	T	0.01	2,66	0.55	0.58
	E	0.10	1,55	27.52	**<0.0001**	E	0.13	1,66	11.4	**<0.01**
	T x E	0.00	2,55	0.05	0.95	T x E	0.13	2,66	1.09	0.43
Sympatric NA[a]	*SGR_{mass}*					*SGR_{mass}*				
/Sympatric-I[b]	C	0.11	2,48.61	2.46	0.09[†]	C	1.93	2,66	15.80	**<0.0001**
	E	9.60	1,51.12	436.1	**<0.0001**	E	7.29	1,66	119.1	**<0.0001**
	C x E	0.58	2,44.78	13.29	**<0.0001**	C x E	0.10	2,66	0.80	0.45
	SGR_{length}					*SGR_{length}*				
	C	0.01	2,57.92	1.80	0.17[†]	C	0.10	2, 44.87	22.89	**<0.0001**
	E	0.62	1,60.06	280.1	**<0.0001**	E	0.15	1,65.31	63.53	**<0.0001**
	C x E	0.04	2,53.55	9.94	**<0.001**	C x E	0.04	2,51.17	7.71	**<0.01**
	Condition					*Condition*				
	C	0.01	2,49.61	1.52	0.23	C	0.02	2,66	0.57	0.57
	E	0.08	1,52.17	47.96	**<0.0001**	E	0.34	1,66	18.19	**<0.0001**
	C x E	0.00	2,46.15	0.12	0.89	C x E	0.07	2,66	1.77	0.18
Sympatric EO[a]	*SGR_{mass}*					*SGR_{mass}*				
/Sympatric-II[b]	C	0.77	2,55	14.83	**<0.0001**	C	3.24	2,53.17	55.82	**<0.0001**

E	9.46	1,55	365.4	**<0.0001**	E	6.40	1,61.66	220.3	**<0.0001**
C x E	0.08	2,55	1.52	0.22	C x E	1.23	2,47.85	21.25	**<0.0001**
SGR_{length}					SGR_{length}				
C	0.08	2,51.56	11.37	**<0.0001**	C	0.20	2,55	33.74	**<0.0001**
E	0.82	1,52.62	226.6	**<0.0001**	E	0.69	1,55	234.1	**<0.0001**
C x E	0.02	2,51.67	2.84	0.07	C x E	0.12	2,55	20.64	**<0.0001**
Condition					*Condition*				
C	0.12	2,66	14.71	**<0.0001**	C	0.07	2,66	1.33	0.12
E	0.16	1,66	37.76	**<0.0001**	E	0.08	1,66	9.35	**<0.01**
C x E	0.00	2,66	0.40	0.67	C x E	0.04	2,66	2.17	0.12

Notes. Significant fixed effects in bold were retained in the final model. †Insignificant fixed effects were also retained in the final model as the interaction terms were significant. Significant random effects (final density nested within replicate) were also retained in the final model. Sum Sq, = sum of squares; d.f. = degrees of freedom based on Sattherwaithe's approximations.

Table 4.3: Cross type effects on fitness-related trait between competitive treatments in tank and stream environments (2016 and 2017) using mixed-effects models (controlled for random intercept). Significant values after sequential Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, NS: Not Significant). Different letters denote significant differences in estimated marginal mean traits among cross types.

Growth and survival experiment (2016)																
Environment	**Tank**								**Semi-natural stream**							
Competitive treatments	Allo-patric	Sympatric (NA)			Sympatric (EO)			*P*	Allo-patric	Sympatric (NA)			Sympatric (EO)			*P*
Cross-types	Wild. NA_{EO}	Wild. NA_{EO}	Hb. NA	Farm. NA	Wild. NA_{EO}	Hb. EO	Farm. EO		Wild. NA_{EO}	Wild. NA_{EO}	Hb. NA	Farm. NA	Wild. NA_{EO}	Hb. EO	Farm. EO	
Final mass (mg)	814^{ad}	771^{ab}	784^{ad}	890^{cd}	676.7^{b}	798^{ad}	940.5^{c}	****	453^{ac}	562^{b}	393^{a}	442^{ac}	398^{a}	533^{bc}	549^{b}	****
Final length (cm)	4.63^{a}	4.50^{ab}	4.49^{ab}	4.66^{a}	4.37^{b}	4.49^{ab}	4.64^{a}	***	3.94^{ad}	4.15^{b}	3.68^{c}	3.80^{cd}	3.78^{cd}	4.06^{ab}	4.05^{ab}	****
SGR_{mass}	2.17^{a}	2.22^{ac}	2.38^{bc}	2.48^{b}	2.14^{a}	2.13^{a}	2.31^{bc}	****	1.43^{ac}	1.70^{b}	1.44^{ac}	1.55^{ab}	1.32^{c}	1.46^{ac}	1.63^{ab}	****
SGR_{length}	0.69^{ac}	0.68^{ac}	0.73^{bc}	0.76^{b}	0.70^{ac}	0.65^{a}	0.74^{b}	****	0.47^{ac}	0.55^{b}	0.50^{ab}	0.51^{ab}	0.41^{c}	0.47^{ac}	0.51^{ab}	****
Condition ($g.cm^{-3}$)	0.82^{a}	0.85^{a}	0.87^{ab}	0.88^{ab}	0.82^{a}	0.87^{ab}	0.93^{b}	****	0.75^{a}	0.78^{ab}	0.79^{ab}	0.80^{ab}	0.74^{a}	0.78^{ab}	0.82^{b}	**
Survival (%)	96.4	93.7	91.2	93.4	90.1	92.5	89.9	NS	92.4	94.3	93.6	92.4	89.2	92.8	89.1	NS
Growth and survival experiment (2017)																
Environment	**Tank**								**Semi-natural stream**							
Competitive treatments	Allo-patric	Sympatric-I			Sympatric-II			*P*	Allo-patric	Sympatric-I			Sympatric-II			*P*
Cross-types	Wild. NA	Wild. NA	$Hb_{W♀}$	Farm. EO	Wild. NA	$Hb_{F♀}$	Farm. EO		Wild. NA	Wild. NA	$Hb_{W♀}$	Farm. EO	Wild. NA	$Hb_{F♀}$	Farm. EO	
Final mass (mg)	1107^{ab}	1032^{ac}	1158^{ab}	1309^{b}	911^{a}	1287^{bc}	1326^{b}	****	577^{a}	622^{ab}	733^{b}	850^{c}	599^{a}	594^{a}	903^{c}	****
Final length (cm)	4.79^{a}	4.83^{a}	4.86^{ac}	5.12^{bc}	4.69^{a}	5.23^{b}	5.13^{bc}	****	4.14^{a}	4.16^{a}	4.48^{b}	4.72^{c}	4.11^{a}	4.17^{a}	4.70^{c}	****
SGR_{mass}	2.37^{ac}	2.32^{a}	2.65^{bc}	2.68^{b}	2.18^{a}	2.44^{ab}	2.67^{bc}	****	1.71^{a}	1.70^{a}	1.91^{ab}	2.09^{b}	1.76^{a}	1.39^{c}	2.15^{b}	****
SGR_{length}	0.65^{ab}	0.66^{ab}	0.67^{ab}	0.72^{b}	0.63^{a}	0.71^{ab}	0.73^{b}	***	0.50^{a}	0.47^{a}	0.58^{b}	0.64^{b}	0.49^{a}	0.39^{c}	0.60^{b}	****
Condition ($g.cm^{-3}$)	1.0	0.91	1.02	0.97	0.87	0.90	0.98	NS	0.80	0.86	0.82	0.80	0.87	0.81	0.87	NS
Survival (%)	89.3	95.3	97.2	88.9	88.1	92.5	77.3	NS	98.6	96.7	91.0	94.2	94.6	96.0	92.3	NS

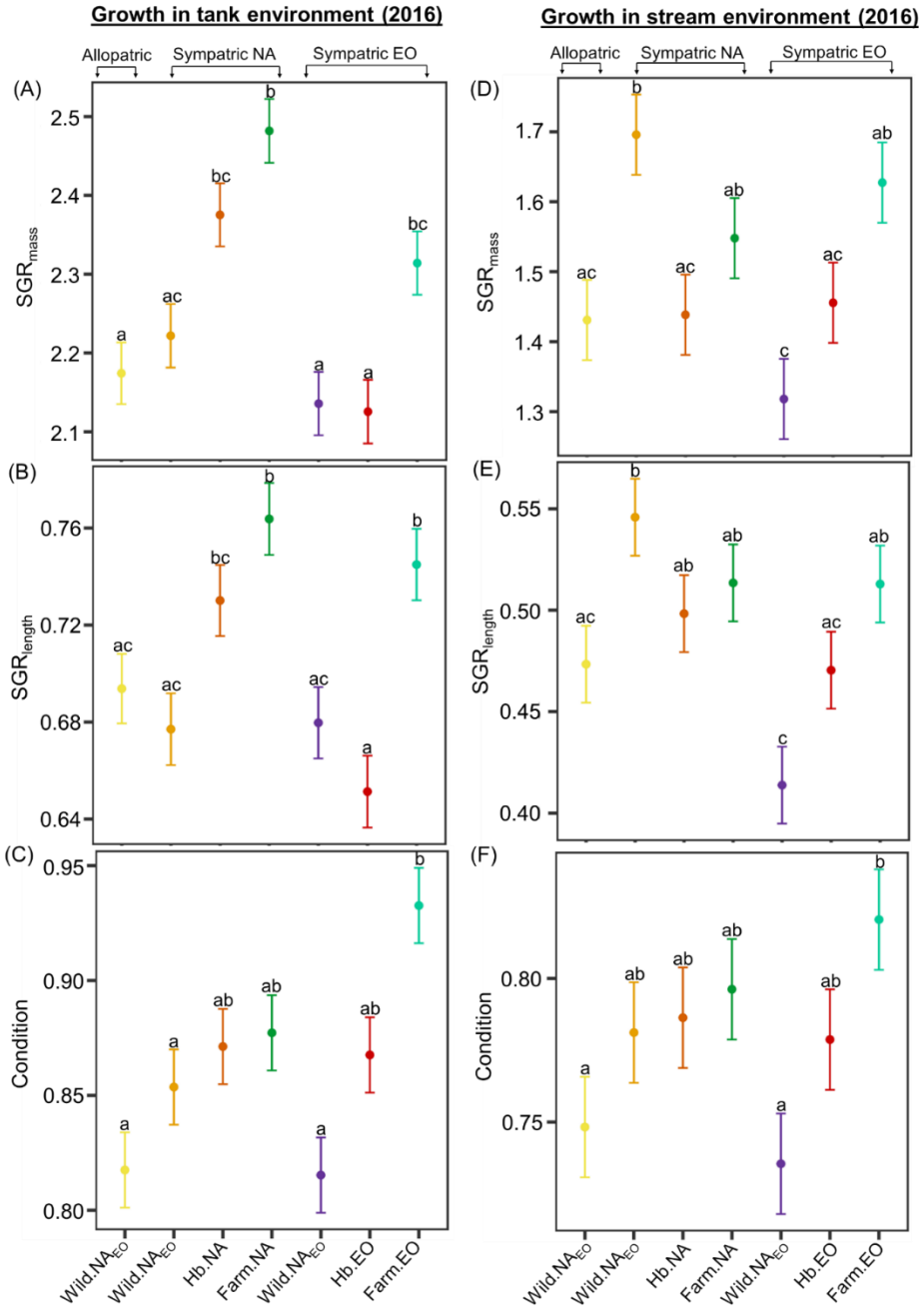


Figure 4.3 (A-F): Cross type effects on growth differences in tank and stream environments (2016) using mixed-effects models. Displayed are marginal means and standard errors. Different letters denote significant differences in estimated marginal mean traits among cross types. See Supplementary Table S4.2 for estimated marginal means, and Supplementary Tables S4.3 for Tukey-adjusted pairwise contrasts among cross types fitted in the final models. Cross types: Farm.NA; Hb.NA; Wild.NA_{EO}; Hb.EO; and Farm.EO from the 2016 growth experiment.

significant ($P < 0.0001$), and therefore retained after model selection (Table 4.2). In particular, for both SGR_{mass} and SGR_{length}, in the tank environment, Farm.NA had a higher growth rate than $Wild.NA_{EO,}$ with Hb.NA being intermediate and not differing from the other two (Table 4.3, Fig. 4.3A-B). However, in the stream environment, $Wild.NA_{EO}$ exhibited higher SGR_{mass} than Hb.NA, with Farm.NA being intermediate and not differing from the other two, while there was no difference in SGR_{length} among cross types (Table 4.3, Fig. 4.3D-E). There were also no differences in condition observed among cross types within the Sympatric NA treatment for either the tank or stream environments (Table 4.3, Fig. 4.3C and 4.3F).

In the Sympatric EO treatment, SGR_{mass}, SGR_{length}, and condition varied significantly by cross type ($P < 0.0001$) and environment ($P < 0.0001$) and therefore retained after model selection (Table 4.2). Specifically, in the tank environment, Farm.EO had higher growth performance than both $Wild.NA_{EO}$ and Hb.EO, which did not differ between themselves (Table 4.3, Fig. 4.3A-B). In the stream environment, however, Farm.EO exhibited a higher growth performance than only $Wild.NA_{EO}$, with Hb.EO being intermediate and not differing from the other two. $Wild.NA_{EO}$ had lower condition than Farm.EO, with Hb.EO being intermediate in both environments.

Significant differences in growth (SGR_{mass} and SGR_{length}) were observed among the EO farmed strain, NF wild population, and related hybrids in both the tank and stream environments in 2017, but not in terms of body condition (Table 4.2, 4.3, Fig. 4.4A-F). When assessing the effect of competition on the wild fish by comparing their performance

in allopatry with that in sympatry with farmed and hybrid fish (i.e., Allopatric vs. Sympatric Wild), I found SGR_{mass} and SGR_{length} to vary significantly by treatment ($P < 0.05$) and environment ($P < 0.0001$) which were therefore retained in the final model (Table 4.2). For condition, only the fixed term environment was significant ($P < 0.01$), and therefore, retained after model selection. In terms of wild fish in allopatry vs. sympatry, there were no differences in growth performance (SGR_{mass} and SGR_{length}) or condition in either the tank or stream environments (Table 4.3, Fig. 4.4).

In the Sympatric-I treatment (Wild, $Hb_{W♀}$, Farm.EO), both SGR_{mass} and SGR_{length} varied significantly as a function of cross type ($P < 0.0001$) and environment ($P < 0.0001$), with a significant interaction term also present in the SGR_{length} model ($P < 0.01$) (Table 4.2). In the tank environment, Farm.EO and $Hb_{W♀}$ had higher SGR_{mass} than Wild.NA and the pattern was similar in terms of SGR_{length}, except that $Hb_{W♀}$ was intermediate (Table 4.3, Fig. 4.4A, B). In the stream environment, Farm.EO had higher SGR_{mass} than Wild.NA, with $Hb_{W♀}$ being intermediate (Table 4.3, Fig 4.4D). The pattern was similar for SGR_{length}, except that $Hb_{W♀}$ had higher growth than Wild.NA (Fig. 4.4E). There were no differences in condition among cross types in both the tank and stream environments (Table 4.3, Fig. 4.4C and 4.4F).

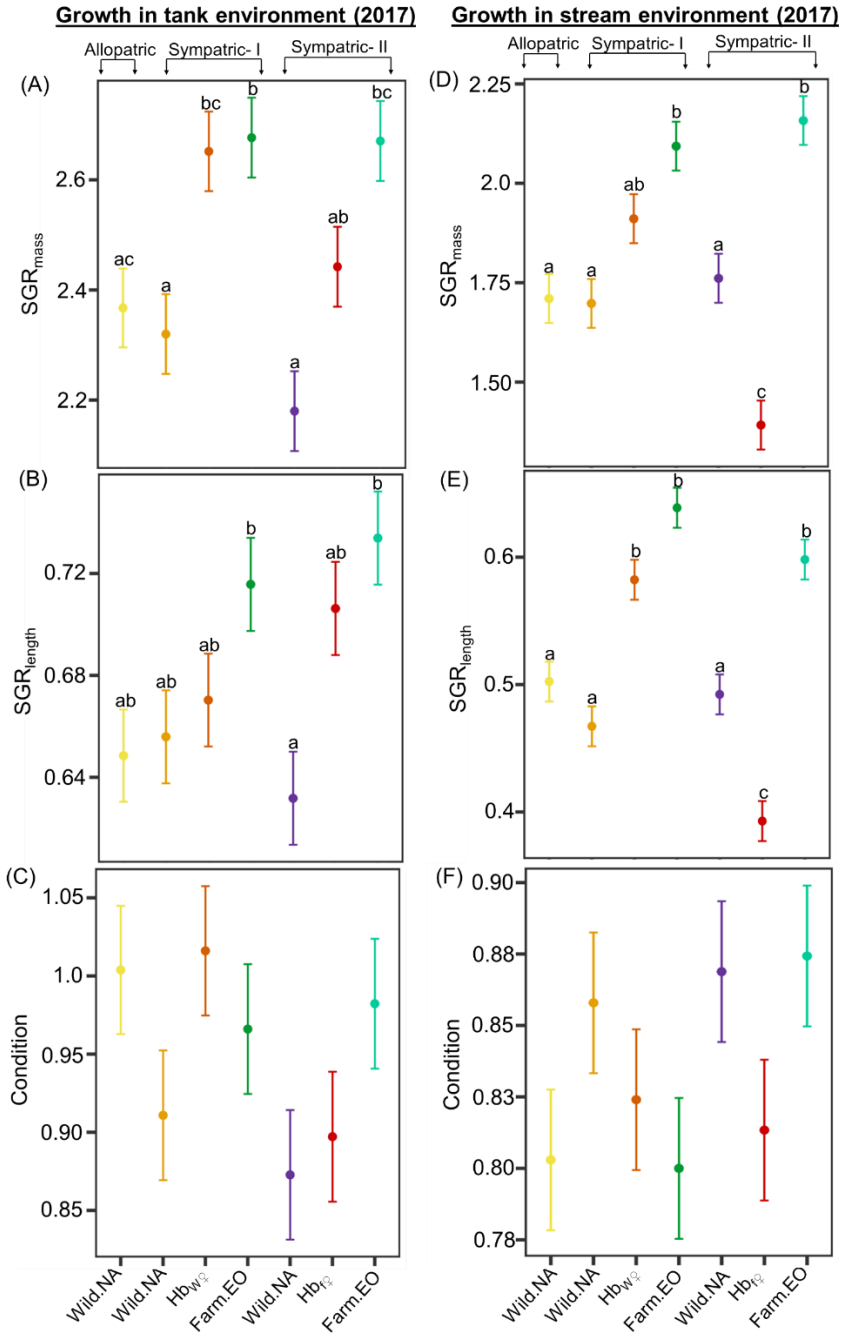


Figure 4.4 (A-F): Cross type effects on growth differences in tank and stream environments (2017) using mixed-effects models. Displayed are marginal means and standard errors. Different letters denote significant differences in estimated marginal mean traits among cross types. See Supplementary Table S4.2 for estimated marginal means, and Supplementary Tables S4.3 for Tukey-adjusted pairwise contrasts among cross types fitted in the final models. Cross types: Farm.EO; $Hb_{w♀}$; $Hb_{f♀}$; and Wild.NA from the 2017 growth experiment.

In the Sympatric-II treatment (Wild, $Hb_{f♀}$, Farm.EO), growth performance (SGR_{mass} and SGR_{length}) varied significantly by cross ($P < 0.0001$), environment ($P < 0.0001$) and their interaction ($P < 0.0001$), and therefore, retained in the final model (Table 4.2). For condition, the only significant term was environment ($P < 0.01$), and thus, retained after model selection. In the tank environment of Sympatric-II, Farm.EO had higher growth performance (SGR_{mass} and SGR_{length}) than Wild.NA, with $Hb_{f♀}$ having intermediate performance that did not differ from the other two (Table 4.3, Fig. 4.4A-B). In the stream environment, the pattern was similar, except that the intermediate growth performance of $Hb_{f♀}$ now differed significantly from that of both the farmed strain and wild population (Table 4.3, Fig. 4.4D-E). No differences in condition were detected among cross types both in the tank and stream environments (Table 4.3, Fig. 4.4C and 4.4F).

Significant interactions of competitive treatment or cross type with environment were detected in both 2016 and 2017 (Table 4.2), indicative of treatments/cross types responding differently to environments in a plastic manner, resulting in differing growth reaction norms (Fig. 4.5A-D). In terms of slopes in SGR_{mass} between environments (2016), $Wild.NA_{EO}$ in the Sympatric NA treatment displayed a flatter reaction norm slope than those in the Allopatric and Sympatric EO treatments (Fig. 4.5A). For SGR_{length}, slopes for $Wild.NA_{EO}$ were similar among Allopatric, Sympatric NA and EO (Fig. 4.5B). Within the Sympatric NA treatment (2016), the SGR_{mass} and SGR_{length} slopes being flatter for $Wild.NA_{EO}$ than that

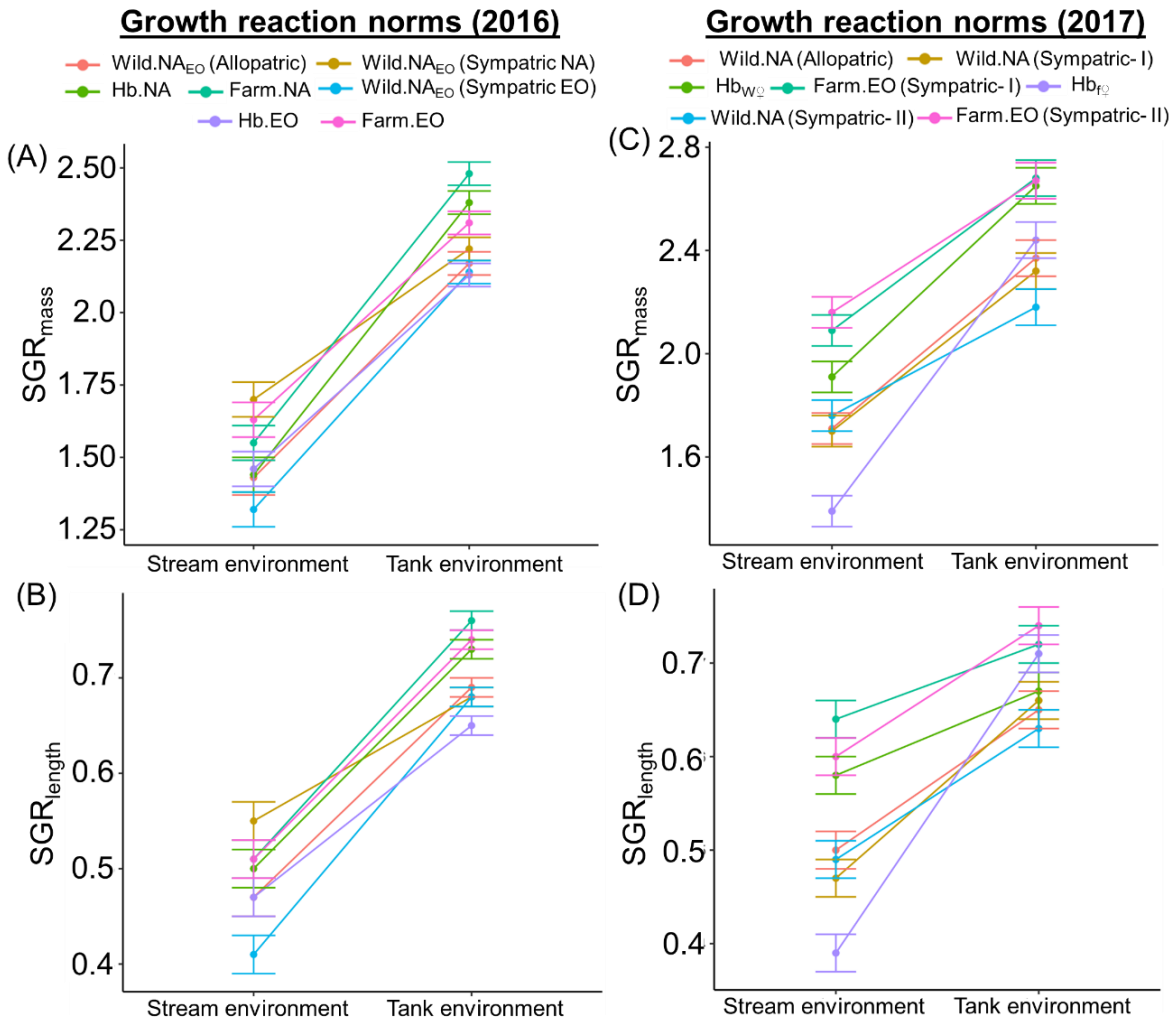


Figure 4.5 (A-D): Growth reaction norms between the environments (tank environment with pellet feed and stream environment with semi-natural feed) among cross types in 2016 and 2017 growth experiments. Displayed are marginal means and standard errors.

for Farm.NA and Hb.NA. However, within the Sympatric EO treatment (2016), in terms of SGR_{mass} and SGR_{length}, similar plastic responses/ growth reaction norms were observed across the environments. By contrast, similar growth reaction norms across environments were observed for the Allopatric vs. Sympatric Wild treatments in 2017 (Table 4.2, Fig 4.5C-D). Within the Sympatric-I treatment of 2017, SGR_{mass} reaction norms were similar

among cross types across the environments, while there was significant a cross-x-environment interaction for SGR_{length}, indicative of differing reaction norms across the environments. In the latter case, Wild.NA showed a steeper slope than $Hb_{w♀}$ and Farm.EO. Within the Sympatric-II treatment of 2017, for both SGR_{mass} and SGR_{length}, Wild.NA and Farm EO showed flatter slopes than $Hb_{f♀}$.

Intriguingly, no significant differences in survival were observed among EO and NA farmed strains, NF wild populations, and related hybrids in the tank and stream environments in 2016 and 2017 ($P > 0.05$; Table 4.4, Fig. 4.6A-D). In the Allopatric Wild vs. Sympatric Wild treatments in 2016 and 2017, the only model term that was significant and retained in the final model was environment ($LR = 7.65$, $P < 0.05$ and $LR = 9.76$, $P < 0.01$, respectively; Table 4.4). The only case of a significant effect of environment was for the Sympatric-II treatment in 2017 ($LR = 4.40$, $P < 0.05$; Table 4.4), which was therefore retained after model selection.

Table 4.4: Statistical summary of the GLMM models selection for investigating the difference in survival using likelihood ratio tests (LRTs) between treatments (T), environments (E), and cross types (C) from the 2016 and 2017 survival experiments. Final density nested within replicate were included as random intercepts.

Treatment	Model No.	Terms included in GLMM model	Term tested	Versus model No.	Log likelihood	AIC	d.f.	χ^2	*P*
Survival experiment (2016)									
Allopatric Wild vs. Sympatric Wild	*Survival*								
	0	T + E + T: E			-88.51	193.01	8		
	1†	T + E	T: E	0	-88.78	188.32	6	0.55	0.76
	2	T	**E**	1	-89.16	189.56	5	7.65	**<0.05**
	3	E	T	1	-92.60	193.21	4	0.76	0.38
Sympatric NA	*Survival*								
	0	C + E + C: E			-66.11	148.21	8		
	1	C + E	C: E	0	-66.21	144.43	6	0.22	0.90
	2	C	E	1	-66.22	142.44	5	0.01	0.92
	3†	E	C	1	-66.53	141.06	4	0.63	0.73
	4	Intercept only	C + E	1	-66.53	139.06	3	0.62	0.89
Sympatric EO	*Survival*								
	0	C + E + C: E			-80.11	176.21	8		
	1	C + E	C: E	0	-80.26	172.53	6	0.31	0.86
	2†	C	E	1	-80.27	170.54	5	0.02	0.90
	3	E	C	1	-82.41	172.83	4	4.30	0.12
	4	Intercept only	C + E	1	-82.42	170.84	3	4.32	0.23
Survival experiment (2017)									
Allopatric Wild vs. Sympatric Wild	*Survival*								
	0	T + E + T: E			-70.64	153.34	8		
	1†	T + E	T: E	0	-68.67	153.28	6	3.94	0.14
	2	T	**E**	1	-75.52	161.04	5	9.76	**<0.01**

	3	E	T	1	-71.33	150.65	4	1.38	0.50
Sympatric-I	*Survival*								
	0	C + E + C: E			-49.63	112.10	8		
	1	C + E	C: E	0	-48.05	111.27	6	3.17	0.21
	2	C	E	1	-49.64	109.29	5	0.02	0.90
	3†	E	C	1	-49.84	107.69	4	0.41	0.81
	4	Intercept only	C + E	1	-49.85	105.70	3	0.43	0.93
Sympatric-II	*Survival*								
	0	C + E + C: E			-74.19	164.38	8		
	1†	C + E	C: E	0	-74.49	160.98	6	0.60	0.73
	2	C	**E**	1	-76.69	163.38	5	4.40	**<0.05**
	3	E	C	1	-77.80	163.60	4	0.52	0.76

Notes. †Retained final model. AIC = Akaike Information Criterion; d.f. =, degrees of freedom.

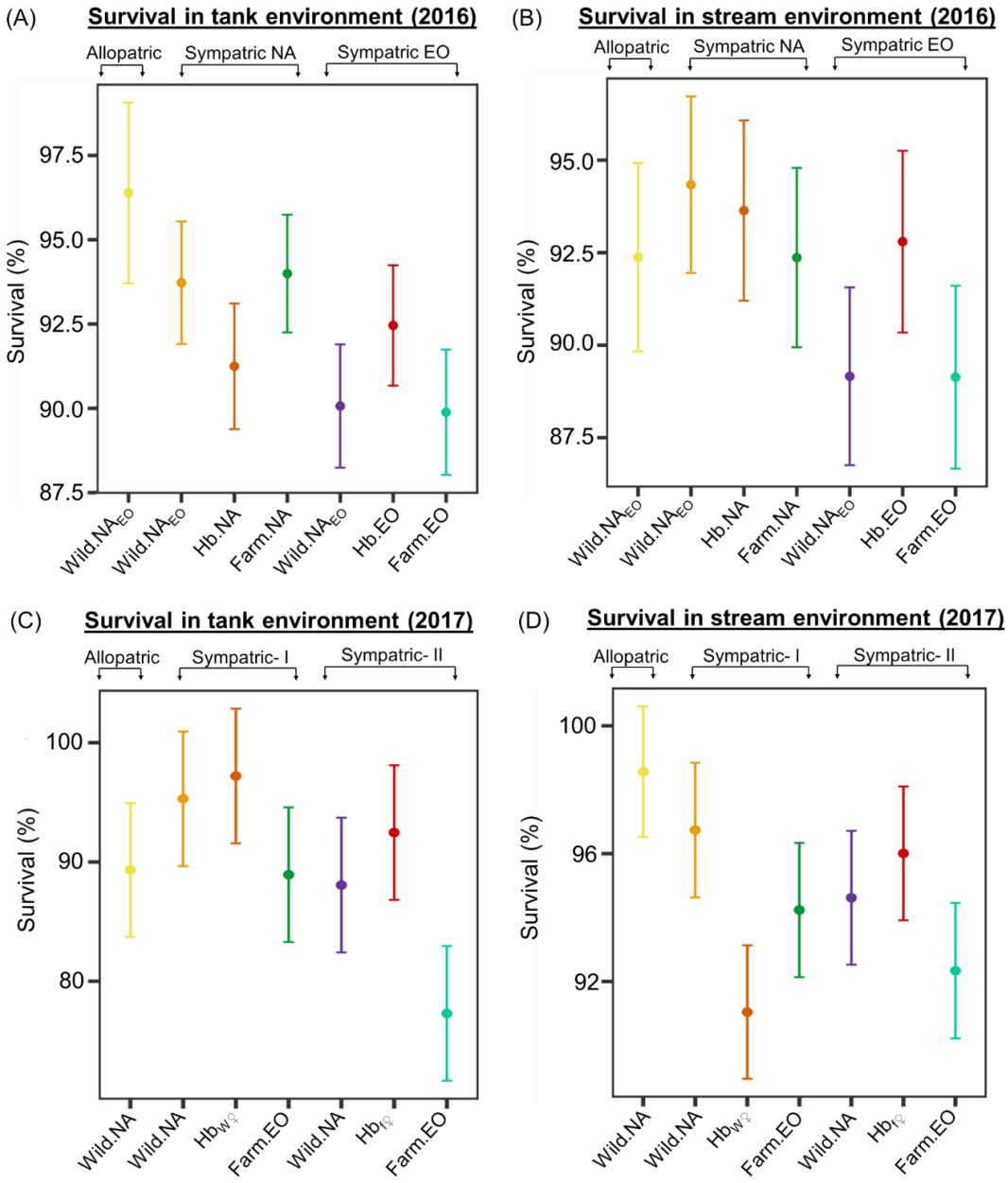


Figure 4.6 (A-D): Cross type effects on survival differences in tank and stream environments (2016 and 2017 experiments) using mixed-effects models. Displayed are marginal means and standard errors. See supplementary Table S4.2 for estimated marginal means, and supplementary Tables S4.3 for Tukey-adjusted pairwise contrasts among cross types fitted in the final models. Cross types: Farm.NA; Hb.NA; Wild.NA$_{EO}$; Hb.EO; and Farm.EO from the 2016 survival experiment. Cross types: Farm.EO; Hb$_{w♀}$; Hb$_{f♀}$; and Wild.NA from the 2017 survival experiment.

4.5 Discussion

The present study has demonstrated fitness-related trait (dominance, growth, and survival) differences among divergent EO and NA farmed strains, NF wild populations, and related F_1 hybrid salmon and provided insight into the fitness effect of hybridization. The main findings can be summarized in five key points: (i) Farm.NA fish were more dominant and less subordinate than NF wild conspecifics, with hybrids being intermediate, not differing from wild fish; and Farm.EO fish also tended to dominate NF wild fish, though the difference was not significant; (ii) competition with farm and hybrid fish did not affect the growth of wild fish in sympatry versus allopatry in the tank environment; however, that was not the case in one instance in the stream environment where wild fish in Sympatry NA outgrew those in allopatry; (iii) Within Sympatric treatments, farmed fish outgrew wild fish in the tank environment, but not necessarily always in the stream environment (e.g., Sympatric NA); (iv) F_1 hybrids tended to exhibit intermediate growth performance relative to those of pure farmed strains and native wild populations both in tank and stream environments; and (v) no survival difference was detected among EO and NA farmed, NF wild and F_1 hybrids both in tank and stream environments. These findings suggest that multi-generation domestication selection has generated fitness differences among farmed, wild fish and their related hybrids, which may have potential effects on production and viability for the local NF wild populations due to increased competition.

4.5.1 Differences in dominance status

I found that farmed fish frequently tended to dominant wild fish and hybrids, but showed no significant difference in intermediate status, which is consistent with the observations of earlier farmed-wild dominance studies (Einum & Fleming, 1997; Fleming & Einum, 1997; Houde et al., 2010). This is not surprising as behavioural traits (e.g., dominance) are believed to be among the first traits to respond to domestication (Huntingford, 2004; Price, 1984). Moreover, both Farm.NA and Farm.EO fish showed similar patterns in dominance status, suggesting common domestication effects as observed previously in terms of exploratory behaviour, novel object response, boldness under predation risk, and aggression (Islam et al., 2020). These effects are likely to be at least partly genetic, given that the fish were reared and tested under common environmental conditions.

4.5.2 Growth and survival differences in tank and stream environments

Interestingly, I found no growth difference of wild fish (Wild.NA_{EO} and Wild.NA) when in allopatry versus sympatry in the tank environment, but in the stream environment, Wild.NA_{EO} in sympatry (Sympatric NA; i.e., competing with Farm.NA and Hb.NA) outgrew their counterparts in allopatry. Given the difference in dominance, one would expect a growth difference between allopatric and sympatric wild in the tank environment, as the tank environment is somewhat more benign than that of the stream environment, but the inter-strain competition for food remains in the sympatric treatment. Moreover, social interaction and hierarchies are well documented among salmon in tank environments (Huntingford, 2004; Koebele, 1985). However, my results indicate that intra-strain competition for wild fish has no different effect than that of inter-strain competition with

farmed and hybrid fish, a pattern that has also been observed with Norwegian populations (Solberg et al., 2013b). There was just one difference in growth between wild fish in allopatry and sympatry in the stream environment; where wild fish in Sympatry NA (i.e., with Farm.NA and Hb.NA), but not in Sympatry EO (i.e., with Farm.EO and Hb.EO), outgrew those in allopatry. This is indicative of intra-strain competition for Wild.NA_{EO} fish being greater than the inter-strain competition with Farm.NA and Hb.NA. It suggests that the different genetic origins of the farmed strains and other factors associated with semi-natural stream conditions may influence the outcome of competition between wild fish in allopatry and sympatry in the stream environment.

Not surprisingly, within sympatry, both EO and NA farmed fish outgrew wild fish in the tank environment, which was not unexpected based on previous observations of other domesticated salmonid populations (Einum & Fleming, 1997; Glover et al., 2018; Harvey et al., 2016a; Solberg et al., 2013a). In this study, the two farmed strains used are historically genetically divergent but have likely experienced similar domestication selection; thus an outstanding question that remained was whether these two farmed strains would display fitness-related trait (e.g., growth) differences between each other. In this study, both Farm.NA and Farm.EO fish equally outgrew Wild.NA_{EO} and Wild.NA in the tank environment. It has been suggested previously that domesticated Atlantic salmon display increased consumption, metabolism, and potentially feed conversion efficiency when presented with excess and high energy diet that results in two to three-fold greater growth than wild conspecifics under farming conditions (Glover et al., 2018; Harvey et al., 2016b; Solberg et al., 2013b). However, this was not necessarily the same in the stream

environment, as at least in one instance (i.e., Sympatric NA) Wild.NA_{EO} outgrew Hb.NA and tended to do so relative to Farm.NA as well, though non significantly. This suggests that the impact of competition with Farm.NA and associated hybrid are less than that imposed by the Farm.EO strain. In the present study, F_1 hybrids tended to have intermediate growth performance relative to those of pure farmed strains and native wild populations both in the tank and stream environments; though not in all cases, which was consistent with earlier studies (Einum & Fleming, 1997; Fleming et al., 2000; Houde et al., 2010a; McGinnity et al., 1997; 2003).

In the present study, I found that growth was consistently better in the tank than stream environment, yet both treatments and cross types responded somewhat differently to the two contrasting environments in a plastic manner, resulting in different growth reaction norms. As it has been suggested in earlier studies that the plasticity is mediated through the lack of available energy (Glover et al., 2018) or the accessibility/use of more energy (brown trout; Sundt-Hansen et al., 2009), leading to size-selective mortality (Glover et al., 2018; Solberg et al., 2013b) in the natural environment. It is possible that fish were unable to obtain and utilize the correct balance of nutrients to maximize growth in the stream environment. Although, unlike true natural environment with live prey and the presence of predators, in my stream study, we provided a semi-natural diet of a combination of brine shrimp and frozen blood worm in the absence of predation, where they had to seek food in a horizontally flowing environment where establishment and defence of territories may be important. I, therefore, tentatively suggested that growth in the semi-natural stream may be influenced mainly by competition for food and nutrients.

I found no survival difference among EO and NA farmed, NF wild, and F_1 hybrids both in the tank and stream environments. Although some previous studies suggested higher survival of wild fish in natural environments (Fleming et al., 2000; McGinnity et al., 1997, 2003; Skaala et al., 2012, 2019; Sylvester et al., 2019), the differences in viability may be most pronounced at the earliest life stages, and after reaching a certain size (e.g., parr), differences in survival may be less apparent (Einum & Fleming, 1997; Fleming et al., 2000). Moreover, this current study was conducted in the predator-free tank and stream environments, so I am less likely to see size-selective mortality (Biro et al., 2006; Glover et al., 2018; Solberg et al., 2015) or that associated with differences in risk sensitivity (Einum & Fleming, 1997; Fleming & Einum, 1997; Houde et al., 2010b; Solberg et al., 2020). Furthermore, the experiments were undertaken for a short period of time (less than three months), which may have made it difficult to identify survival differences.

4.5.3 Implications

Every year, millions of farmed salmon escape into the wild (Glover et al., 2017; Bradbury et al., 2020; Føre & Thorvaldsen, 2021), and introgressive hybridization of escaped farmed with wild fish has been documented throughout salmon farming regions in Atlantic Canada (O'Reilly et al., 2006; Keyser et al., 2018; Wringe et al., 2018). A persistent concern of successful breeding of escaped farmed salmon with local wild populations and the resultant hybridization is the fitness impacts and consequent threat to local adaptation. It was unknown, however, whether the competitive and fitness consequences posed by escaped EO farmed fish will be greater than that of escaped NA farmed fish for local NF wild populations. The fitness-related trait (dominance and growth)

differences that were observed in the current study validate these concerns of the potential threat posed by escaped farmed fish, whether they be NA and EO in origin, to local NF wild fish. Moreover, intermediate trait expression (with a few exceptions) between F_1 hybrids and wild fish infer that farm-wild hybridization can lead to the breakdown of local adaptation (Fraser et al., 2011; Garcia de Leaniz et al. 2007). As offspring of escaped farmed salmon have already been detected in 17 out 18 wild salmon rivers in Southern NL, Canada (Wringe et al., 2018), it is likely that a high number of hybrids will alter the trait expression patterns relative to wild fish (Sylvester et al., 2019). For example, there has been a decline in the abundance of wild salmon in southern NL of ~45% between 1996-2010, particularly near the main farming area (e.g., ~70% decline in the Conne River), and these wild populations have been designated as threatened (COSEWIC, 2010). In our study, we have found that the performance and consequence of hybridization was similar for NA and EO farmed strains, except in the stream environment where Farm.NA and the related hybrid were outperformed by the wild fish, which may indicate that escape of Farm.EO presents more concerns than that of Farm.NA.

4.6 Concluding remarks

In general, the present study has demonstrated that multi-generation domestication selection has generated fitness-related trait differences among divergent EO and NA farmed, NF wild, and F_1 hybrids. Briefly, common unintentional domestication effects appear to be responsible for both Farm.NA and Farm.EO fish tending to dominate their NF wild conspecifics in the tank environment. Similarly, such effects are likely responsible for both farm strains outgrowing their NF wild conspecifics in the tank environment, but this

was not always the same scenario in the stream environment. Also, we found F_1 hybrids tended to exhibit intermediate performance while competing with farmed and wild juveniles. Moreover, extensive interbreeding of escaped farmed salmon with wild populations can influence lifetime fitness and threaten native populations (Fleming et al., 1996, 2000; McGinnity et al., 1997, 2003; Skaala et al., 2012, 2019). Our findings suggest that escaped EO and NA farmed, and resultant hybrid juveniles may influence the productivity of NF wild populations due to altered competition. Thus understanding the effect of hybridization and, consequently, fitness-related trait differences among divergent farmed, wild and F_1 hybrid populations can provide valuable insight for the conservation and management of Atlantic salmon.

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4.8 Appendices

Supplementary Table S4.1: Tukey-adjusted pairwise contrasts of different cross types from the 2016 and 2017 dominance experiments for the estimation of dominance status (dominant, intermediate and subordinate) using logistic regression with binomial GLM model. Estimate, parameter estimate.SE, standard errors.z, Wald z-statistics.

Dominance experiment (2016)					**Dominance experiment (2017)**				
Pairwise contrasts	**Estimate**	**±SE**	**z**	***P***	**Pairwise contrasts**	**Estimate**	**±SE**	**z**	***P***
Dominant					*Dominant*				
Wild.NA$_{EO}$- Farm.NA	-1.75	0.56	-3.11	**<0.05**	Wild.NA- Hb$_{W♀}$	0.39	0.51	0.76	0.87
Wild.NA$_{EO}$- Farm.EO	-1.29	0.56	-2.28	0.15	Wild.NA- Hb$_{f♀}$	0.69	0.53	1.29	0.57
Wild.NA$_{EO}$- Hb.NA	-0.36	0.60	-0.60	0.98	Wild.NA- Farm.EO	-0.81	0.49	-1.66	0.34
Wild.NA$_{EO}$- Hb.EO	-0.35	0.60	-0.59	0.97	Hb$_{W♀}$- Hb$_{f♀}$	0.30	0.55	0.54	0.95
Farm.NA- Farm.EO	0.46	0.48	0.95	0.88	Hb$_{W♀}$- Farm.EO	-1.20	0.51	-2.38	0.08
Farm.NA- Hb.NA	1.39	0.53	2.64	0.06	Hb$_{f♀}$- Farm.EO	-1.50	0.53	-2.85	**<0.05**
Farm.NA - Hb.EO	1.38	0.52	2.63	0.07					
Farm.EO- Hb.NA	0.93	0.53	1.76	0.40					
Farm.EO- Hb.EO	0.92	0.52	1.75	0.39					
Hb.NA- Hb.EO	0.0	0.57	0.0	1.0					
Intermediate					*Intermediate*				
Wild.NA$_{EO}$- Farm.NA	-0.12	0.49	-0.24	1.0	Wild.NA- Hb$_{W♀}$	0.0	0.50	0.0	1.0
Wild.NA$_{EO}$- Farm.EO	-0.11	0.48	-0.23	0.99	Wild.NA- Hb$_{f♀}$	-0.12	0.49	-0.24	0.99
Wild.NA$_{EO}$- Hb.NA	-0.35	0.48	-0.72	0.95	Wild.NA- Farm.EO	0.12	0.50	0.25	1.0
Wild.NA$_{EO}$- Hb.EO	-0.69	0.49	-1.43	0.61	Hb$_{W♀}$- Hb$_{f♀}$	-0.12	0.49	-0.25	0.99
Farm.NA- Farm.EO	0.0	0.48	0.0	1.0	Hb$_{W♀}$- Farm.EO	0.12	0.50	0.25	1.0
Farm.NA- Hb.NA	-0.23	0.48	-0.48	0.98	Hb$_{f♀}$- Farm.EO	0.25	0.50	0.49	0.96
Farm.NA - Hb.EO	-0.58	0.48	-1.19	0.76					
Farm.EO- Hb.NA	-0.23	0.48	-0.48	0.99					
Farm.EO- Hb.EO	-0.57	0.48	-1.19	0.76					
Hb.NA- Hb.EO	-0.35	0.48	-0.72	0.95					
Subordinate					*Subordinate*				
Wild.NA$_{EO}$- Farm.NA	3.0	1.07	2.80	**<0.05**	Wild.NA- Hb$_{W♀}$	-0.41	0.53	-0.78	0.86
Wild.NA$_{EO}$- Farm.EO	1.27	0.60	2.12	0.21	Wild.NA- Hb$_{f♀}$	-0.54	0.52	-1.03	0.73
Wild.NA$_{EO}$- Hb.NA	0.12	0.50	0.25	0.99	Wild.NA- Farm.EO	1.31	0.72	1.82	0.26

Wild.NA$_{EO}$- Hb.EO	1.05	0.57	1.85	0.35	$Hb_{W♀}$- $Hb_{f♀}$	-0.12	0.50	-0.25	0.99
Farm.NA- Farm.EO	-1.74	1.12	-1.54	0.53	$Hb_{W♀}$- Farm.EO	1.72	0.70	2.45	0.70
Farm.NA- Hb.NA	-2.88	1.08	-2.67	0.06	$Hb_{f♀}$- Farm.EO	1.84	0.70	2.64	**<0.05**
Farm.NA - Hb.EO	-1.95	1.11	-1.76	0.40					
Farm.EO- Hb.NA	-1.14	0.60	-1.90	0.32					
Farm.EO- Hb.EO	-0.22	0.66	-0.33	1.0					
Hb.NA- Hb.EO	0.93	0.57	1.62	0.49					

Supplementary Table S4.2: Estimated marginal means of different cross types from the growth and survival experiments (2016 and 2017) in tank and stream environments derived from the fitted final models for final mass (mg) and length (cm), SGR_{mass}, SGR_{length}, condition, and survival (%). SE, standard errors. Lower. CL, lower confidence limit.Upper.CL, upper confidence limit.

Cross types	**Estimated marginal mean**	**±SE**	**Lower. CL**	**Upper. CL**
Final mass in tank environment (2016)				
Wild.NA_{EO} (Allopatric)	813.97	28.48	757.16	870.78
Wild.NA_{EO} (Sympatric NA)	771.40	28.04	715.41	827.38
Hb.NA	784.28	28.20	728.04	840.53
Farm.NA	890.41	27.87	834.70	946.13
Wild.NA_{EO} (Sympatric EO)	676.66	28.10	620.57	732.75
Hb.EO	797.98	27.96	742.12	853.84
Farm.EO	940.48	28.13	884.35	996.61
Final length in tank environment (2016)				
Wild.NA_{EO} (Allopatric)	4.63	0.05	4.53	4.74
Wild.NA_{EO} (Sympatric NA)	4.50	0.05	4.39	4.61
Hb.NA	4.49	0.05	4.38	4.60
Farm.NA	4.66	0.05	4.56	4.77
Wild.NA_{EO} (Sympatric EO)	4.37	0.05	4.26	4.47
Hb.EO	4.49	0.05	4.39	4.60
Farm.EO	4.64	0.05	4.54	4.75
SGRmass in tank environment (2016)				
Wild.NA_{EO} (Allopatric)	2.17	0.04	2.10	2.25
Wild.NA_{EO} (Sympatric NA)	2.22	0.04	2.14	2.30
Hb.NA	2.38	0.04	2.30	2.45
Farm.NA	2.48	0.04	2.40	2.56
Wild.NA_{EO} (Sympatric EO)	2.14	0.04	2.06	2.22
Hb.EO	2.13	0.04	2.05	2.21
Farm.EO	2.31	0.04	2.23	2.39
SGRlength in tank environment (2016)				
Wild.NA_{EO} (Allopatric)	0.69	0.01	0.67	0.72
Wild.NA_{EO} (Sympatric NA)	0.68	0.01	0.65	0.71
Hb.NA	0.73	0.01	0.70	0.76
Farm.NA	0.76	0.01	0.73	0.79
Wild.NA_{EO} (Sympatric EO)	0.68	0.01	0.65	0.71
Hb.EO	0.65	0.01	0.62	0.68

Farm.EO	0.74	0.01	0.72	0.77
Condition in tank environment (2016)				
Wild.NA$_{EO}$ (Allopatric)	0.82	0.02	0.78	0.85
Wild.NA$_{EO}$ (Sympatric NA)	0.85	0.02	0.82	0.89
Hb.NA	0.87	0.02	0.84	0.90
Farm.NA	0.88	0.02	0.84	0.91
Wild.NA$_{EO}$ (Sympatric EO)	0.82	0.02	0.78	0.85
Hb.EO	0.87	0.02	0.83	0.90
Farm.EO	0.93	0.02	0.90	0.97
Survival (%) in tank environment (2016)				
Wild.NA$_{EO}$ (Allopatric)	96.39	2.68	91.02	101.75
Wild.NA$_{EO}$ (Sympatric NA)	93.73	1.82	90.03	97.42
Hb.NA	91.25	1.87	87.47	95.03
Farm.NA	94.00	1.75	90.44	97.55
Wild.NA$_{EO}$ (Sympatric EO)	90.07	1.83	86.35	93.78
Hb.EO	92.46	1.78	88.83	96.09
Farm.EO	89.88	1.86	86.12	93.65
Final mass in stream environment (2016)				
Wild.NA$_{EO}$ (Allopatric)	452.55	25.22	401.96	503.14
Wild.NA$_{EO}$ (Sympatric NA)	561.62	25.22	511.03	612.21
Hb.NA	392.47	25.22	341.88	443.06
Farm.NA	441.78	25.22	391.19	492.37
Wild.NA$_{EO}$ (Sympatric EO)	398.17	25.22	347.58	448.76
Hb.EO	532.69	25.22	482.10	583.28
Farm.EO	548.55	25.22	497.96	599.14
Final length in stream environment (2016)				
Wild.NA$_{EO}$ (Allopatric)	3.93	0.05	3.82	4.03
Wild.NA$_{EO}$ (Sympatric NA)	4.15	0.05	4.04	4.25
Hb.NA	3.68	0.05	3.57	3.79
Farm.NA	3.80	0.05	3.70	3.91
Wild.NA$_{EO}$ (Sympatric EO)	3.78	0.05	3.67	3.89
Hb.EO	4.06	0.05	3.96	4.17
Farm.EO	4.05	0.05	3.94	4.15
SGRmass in stream environment (2016)				
Wild.NA$_{EO}$ (Allopatric)	1.43	0.06	1.32	1.55
Wild.NA$_{EO}$ (Sympatric NA)	1.70	0.06	1.58	1.81
Hb.NA	1.44	0.06	1.32	1.55
Farm.NA	1.55	0.06	1.43	1.66
Wild.NA$_{EO}$ (Sympatric EO)	1.32	0.06	1.20	1.43

Hb.EO	1.46	0.06	1.34	1.57
Farm.EO	1.63	0.06	1.51	1.74
SGRlength in stream environment (2016)				
Wild.NA$_{EO}$ (Allopatric)	0.47	0.02	0.44	0.51
Wild.NA$_{EO}$ (Sympatric NA)	0.55	0.02	0.51	0.58
Hb.NA	0.50	0.02	0.46	0.54
Farm.NA	0.51	0.02	0.48	0.55
Wild.NA$_{EO}$ (Sympatric EO)	0.41	0.02	0.38	0.45
Hb.EO	0.47	0.02	0.43	0.51
Farm.EO	0.51	0.02	0.47	0.55
Condition in stream environment (2016)				
Wild.NA$_{EO}$ (Allopatric)	0.75	0.02	0.71	0.78
Wild.NA$_{EO}$ (Sympatric NA)	0.78	0.02	0.75	0.82
Hb.NA	0.79	0.02	0.75	0.82
Farm.NA	0.80	0.02	0.76	0.83
Wild.NA$_{EO}$ (Sympatric EO)	0.74	0.02	0.70	0.77
Hb.EO	0.78	0.02	0.74	0.81
Farm.EO	0.82	0.02	0.79	0.86
Survival (%) in stream environment (2016)				
Wild.NA$_{EO}$ (Allopatric)	92.38	2.55	87.30	97.46
Wild.NA$_{EO}$ (Sympatric NA)	94.34	2.39	89.56	99.11
Hb.NA	93.64	2.44	88.77	98.50
Farm.NA	92.37	2.42	87.52	97.21
Wild.NA$_{EO}$ (Sympatric EO)	89.16	2.40	84.35	93.96
Hb.EO	92.80	2.46	87.90	97.70
Farm.EO	89.13	2.47	84.21	94.06
Final mass in tank environment (2017)				
Wild.NA (Allopatric)	1107.35	61.88	984.14	1230.56
Wild.NA (Sympatric- I)	1032.47	62.56	907.90	1157.05
Hb$_{W♀}$	1158.19	62.35	1034.04	1282.33
Farm.EO (Sympatric- I)	1309.45	62.61	1184.77	1434.12
Wild.NA (Sympatric- II)	911.39	62.54	786.84	1035.93
Hb$_{f♀}$	1287.36	62.66	1162.58	1412.13
Farm.EO (Sympatric- II)	1326.16	62.66	1201.39	1450.94
Final length in tank environment (2017)				
Wild.NA (Allopatric)	4.79	0.07	4.65	4.93
Wild.NA (Sympatric- I)	4.83	0.07	4.69	4.96
Hb$_{W♀}$	4.86	0.07	4.72	5.00
Farm.EO (Sympatric- I)	5.12	0.07	4.98	5.26

Wild.NA (Sympatric- II)	4.69	0.07	4.56	4.83
$Hb_{f♀}$	5.23	0.07	5.10	5.37
Farm.EO (Sympatric- II)	5.13	0.07	5.00	5.27
SGRmass in tank environment (2017)				
Wild.NA (Allopatric)	2.37	0.07	2.22	2.51
Wild.NA (Sympatric- I)	2.32	0.07	2.18	2.46
$Hb_{w♀}$	2.65	0.07	2.51	2.80
Farm.EO (Sympatric- I)	2.68	0.07	2.53	2.82
Wild.NA (Sympatric- II)	2.18	0.07	2.04	2.32
$Hb_{f♀}$	2.44	0.07	2.30	2.59
Farm.EO (Sympatric- II)	2.67	0.07	2.53	2.82
SGRlength in tank environment (2017)				
Wild.NA (Allopatric)	0.65	0.02	0.61	0.68
Wild.NA (Sympatric- I)	0.66	0.02	0.62	0.69
$Hb_{w♀}$	0.67	0.02	0.63	0.71
Farm.EO (Sympatric- I)	0.72	0.02	0.68	0.75
Wild.NA (Sympatric- II)	0.63	0.02	0.60	0.67
$Hb_{f♀}$	0.71	0.02	0.67	0.74
Farm.EO (Sympatric- II)	0.73	0.02	0.70	0.77
Condition in tank environment (2017)				
Wild.NA (Allopatric)	1.00	0.04	0.92	1.09
Wild.NA (Sympatric- I)	0.91	0.04	0.83	0.99
$Hb_{w♀}$	1.02	0.04	0.93	1.10
Farm.EO (Sympatric- I)	0.97	0.04	0.88	1.05
Wild.NA (Sympatric- II)	0.87	0.04	0.79	0.96
$Hb_{f♀}$	0.90	0.04	0.81	0.98
Farm.EO (Sympatric- II)	0.98	0.04	0.90	1.06
Survival (%) in tank environment (2017)				
Wild.NA (Allopatric)	89.32	5.62	78.11	100.53
Wild.NA (Sympatric- I)	95.29	5.65	84.02	106.57
$Hb_{w♀}$	97.21	5.64	85.94	108.47
Farm.EO (Sympatric- I)	88.93	5.65	77.65	100.21
Wild.NA (Sympatric- II)	88.06	5.65	76.78	99.34
$Hb_{f♀}$	92.46	5.65	81.18	103.75
Farm.EO (Sympatric- II)	77.29	5.65	66.01	88.57
Final mass in stream environment (2017)				
Wild.NA (Allopatric)	577.17	28.30	520.77	633.56
Wild.NA (Sympatric- I)	622.45	28.30	566.05	678.84
$Hb_{w♀}$	732.55	28.30	676.15	788.94

Farm.EO (Sympatric- I)	850.19	28.30	793.79	906.59
Wild.NA (Sympatric- II)	599.15	28.30	542.75	655.55
$Hb_{f♀}$	593.86	28.30	537.46	650.26
Farm.EO (Sympatric- II)	903.43	28.30	847.04	959.83
Final length in stream environment (2017)				
Wild.NA (Allopatric)	4.14	0.05	4.03	4.24
Wild.NA (Sympatric- I)	4.16	0.05	4.05	4.27
$Hb_{w♀}$	4.48	0.05	4.37	4.58
Farm.EO (Sympatric- I)	4.72	0.05	4.61	4.82
Wild.NA (Sympatric- II)	4.11	0.05	4.00	4.22
$Hb_{f♀}$	4.17	0.05	4.06	4.28
Farm.EO (Sympatric- II)	4.70	0.05	4.59	4.80
SGRmass in stream environment (2017)				
Wild.NA (Allopatric)	1.71	0.06	1.59	1.83
Wild.NA (Sympatric- I)	1.70	0.06	1.58	1.82
$Hb_{w♀}$	1.91	0.06	1.79	2.03
Farm.EO (Sympatric- I)	2.09	0.06	1.97	2.22
Wild.NA (Sympatric- II)	1.76	0.06	1.64	1.88
$Hb_{f♀}$	1.39	0.06	1.27	1.51
Farm.EO (Sympatric- II)	2.16	0.06	2.04	2.28
SGRlength in stream environment (2017)				
Wild.NA (Allopatric)	0.50	0.02	0.47	0.53
Wild.NA (Sympatric- I)	0.47	0.02	0.44	0.50
$Hb_{w♀}$	0.58	0.02	0.55	0.61
Farm.EO (Sympatric- I)	0.64	0.02	0.61	0.67
Wild.NA (Sympatric- II)	0.49	0.02	0.46	0.52
$Hb_{f♀}$	0.39	0.02	0.36	0.42
Farm.EO (Sympatric- II)	0.60	0.02	0.57	0.63
Condition in stream environment (2017)				
Wild.NA (Allopatric)	0.80	0.02	0.75	0.85
Wild.NA (Sympatric- I)	0.86	0.02	0.81	0.91
$Hb_{w♀}$	0.82	0.02	0.77	0.87
Farm.EO (Sympatric- I)	0.80	0.02	0.75	0.85
Wild.NA (Sympatric- II)	0.87	0.02	0.82	0.92
$Hb_{f♀}$	0.81	0.02	0.76	0.86
Farm.EO (Sympatric- II)	0.87	0.02	0.83	0.92
Survival (%) in stream environment (2017)				
Wild.NA (Allopatric)	98.56	2.04	94.49	102.63
Wild.NA (Sympatric- I)	96.74	2.11	92.54	100.94

$Hb_{W♀}$	91.04	2.09	86.88	95.20
Farm.EO (Sympatric- I)	94.24	2.10	90.05	98.42
Wild.NA (Sympatric- II)	94.62	2.09	90.45	98.79
$Hb_{f♀}$	96.01	2.09	91.84	100.18
Farm.EO (Sympatric- II)	92.34	2.12	88.11	96.56

Supplementary Table S4.3: Tukey-adjusted pairwise contrasts of different cross types from the growth experiments (2016 and 2017) in tank and stream environments derived from the fitted final models for final mass and length, SGR_{mass}, SGR_{length}, and condition. Estimate, parameter estimate.SE, standard errors.t, t-value.

Pairwise contrasts	**Estimate**	**±SE**	**t**	***P***
Final mass in tank environment (2016)				
Wild.NA_{EO} (Allopatric)- Wild.NA_{EO} (Sympatric NA)	42.6	39	1.09	0.93
Wild.NA_{EO} (Allopatric)- Hb.NA	29.7	39.1	0.76	0.99
Wild.NA_{EO} (Allopatric)- Farm.NA	-76.4	38.9	-1.97	0.45
Wild.NA_{EO} (Allopatric)- Wild.NA_{EO} (Sympatric EO)	137.3	39.1	3.52	<0.05
Wild.NA_{EO} (Allopatric)- Hb.EO	16	39	0.41	0.99
Wild.NA_{EO} (Allopatric)- Farm.EO	-126.5	39.1	-3.24	<0.05
Wild.NA_{EO} (Sympatric NA)- Hb.NA	-12.9	38.7	-0.33	0.99
Wild.NA_{EO} (Sympatric NA)- Farm.NA	-119	37.3	-3.19	<0.05
Wild.NA_{EO} (Sympatric NA)- Wild.NA_{EO} (Sympatric EO)	94.7	36.9	2.57	0.16
Wild.NA_{EO} (Sympatric NA)- Hb.EO	-26.6	36.9	-0.72	0.99
Wild.NA_{EO} (Sympatric NA)- Farm.EO	-169.1	37.1	-4.56	<0.001
Hb.NA- Farm.NA	-106.1	36.2	-2.94	0.07
Hb.NA- Wild.NA_{EO} (Sympatric EO)	107.6	37.6	2.86	0.08
Hb.NA- Hb.EO	-13.7	38	-0.36	0.99
Hb.NA- Farm.EO	-156.2	38.2	-4.09	<0.01
Farm.NA- Wild.NA_{EO} (Sympatric EO)	213.8	37.9	5.64	<.0001
Farm.NA- Hb.EO	92.4	36	2.57	0.16
Farm.NA- Farm.EO	-50.1	37.4	-1.34	0.83
Wild.NA_{EO} (Sympatric EO)- Hb.EO	-121.3	37.3	-3.25	<0.05
Wild.NA_{EO} (Sympatric EO)- Farm.EO	-263.8	38.2	-6.92	<.0001
Hb.EO- Farm.EO	-142.5	37.5	-3.80	<0.01
Final length in tank environment (2016)				
Wild.NA_{EO} (Allopatric)- Wild.NA_{EO} (Sympatric NA)	0.13	0.07	1.78	0.57
Wild.NA_{EO} (Allopatric)- Hb.NA	0.14	0.07	1.93	0.47
Wild.NA_{EO} (Allopatric)- Farm.NA	-0.03	0.07	-0.41	0.99
Wild.NA_{EO} (Allopatric)- Wild.NA_{EO} (Sympatric EO)	0.27	0.07	3.56	<0.05
Wild.NA_{EO} (Allopatric)- Hb.EO	0.14	0.07	1.87	0.51
Wild.NA_{EO} (Allopatric)- Farm.EO	-0.01	0.07	-0.14	1.0
Wild.NA_{EO} (Sympatric NA)- Hb.NA	0.01	0.08	0.14	1.0
Wild.NA_{EO} (Sympatric NA)- Farm.NA	-0.16	0.08	-2.16	0.33
Wild.NA_{EO} (Sympatric NA)- Wild.NA_{EO} (Sympatric EO)	0.13	0.08	1.76	0.58
Wild.NA_{EO} (Sympatric NA)- Hb.EO	0.01	0.08	0.09	1.0

Wild.NA$_{EO}$ (Sympatric NA)- Farm.EO	-0.14	0.07	-1.91	0.48
Hb.NA- Farm.NA	-0.17	0.08	-2.31	0.25
Hb.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.12	0.08	1.62	0.67
Hb.NA- Hb.EO	0.00	0.08	-0.05	1.0
Hb.NA- Farm.EO	-0.15	0.08	-2.03	0.40
Farm.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.30	0.08	3.89	<0.01
Farm.NA- Hb.EO	0.17	0.08	2.26	0.28
Farm.NA- Farm.EO	0.02	0.08	0.27	1.0
Wild.NA$_{EO}$ (Sympatric EO)- Hb.EO	-0.13	0.08	-1.66	0.64
Wild.NA$_{EO}$ (Sympatric EO)- Farm.EO	-0.28	0.08	-3.65	<0.01
Hb.EO- Farm.EO	-0.15	0.08	-1.99	0.43
SGRmass in tank environment (2016)				
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric NA)	-0.05	0.06	-0.85	0.98
Wild.NA$_{EO}$ (Allopatric)- Hb.NA	-0.20	0.06	-3.59	<0.05
Wild.NA$_{EO}$ (Allopatric)- Farm.NA	-0.31	0.06	-5.46	<.0001
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric EO)	0.04	0.06	0.69	0.99
Wild.NA$_{EO}$ (Allopatric)- Hb.EO	0.05	0.06	0.87	0.98
Wild.NA$_{EO}$ (Allopatric)- Farm.EO	-0.14	0.06	-2.49	0.18
Wild.NA$_{EO}$ (Sympatric NA)- Hb.NA	-0.15	0.06	-2.68	0.12
Wild.NA$_{EO}$ (Sympatric NA)- Farm.NA	-0.26	0.06	-4.55	<0.001
Wild.NA$_{EO}$ (Sympatric NA)- Wild.NA$_{EO}$ (Sympatric EO)	0.09	0.06	1.52	0.73
Wild.NA$_{EO}$ (Sympatric NA)- Hb.EO	0.10	0.06	1.70	0.62
Wild.NA$_{EO}$ (Sympatric NA)- Farm.EO	-0.09	0.06	-1.64	0.66
Hb.NA- Farm.NA	-0.11	0.06	-1.88	0.50
Hb.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.24	0.06	4.23	<0.01
Hb.NA- Hb.EO	0.25	0.06	4.38	<0.001
Hb.NA- Farm.EO	0.06	0.06	1.08	0.93
Farm.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.35	0.06	6.04	<.0001
Farm.NA- Hb.EO	0.36	0.06	6.26	<.0001
Farm.NA- Farm.EO	0.17	0.06	2.94	0.06
Wild.NA$_{EO}$ (Sympatric EO)- Hb.EO	0.01	0.06	0.18	1.0
Wild.NA$_{EO}$ (Sympatric EO)- Farm.EO	-0.18	0.06	-3.13	<0.05
Hb.EO- Farm.EO	-0.19	0.06	-3.32	<0.05
SGRlength in tank environment (2016)				
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric NA)	0.02	0.02	0.81	0.98
Wild.NA$_{EO}$ (Allopatric)- Hb.NA	-0.04	0.02	-1.77	0.57
Wild.NA$_{EO}$ (Allopatric)- Farm.NA	-0.07	0.02	-3.39	<0.05
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric EO)	0.01	0.02	0.68	0.99
Wild.NA$_{EO}$ (Allopatric)- Hb.EO	0.04	0.02	2.06	0.39

Wild.NA$_{EO}$ (Allopatric)- Farm.EO	-0.05	0.02	-2.49	0.18
Wild.NA$_{EO}$ (Sympatric NA)- Hb.NA	-0.05	0.02	-2.53	0.16
Wild.NA$_{EO}$ (Sympatric NA)- Farm.NA	-0.09	0.02	-4.14	<0.01
Wild.NA$_{EO}$ (Sympatric NA)- Wild.NA$_{EO}$ (Sympatric EO)	0.00	0.02	-0.13	1.0
Wild.NA$_{EO}$ (Sympatric NA)- Hb.EO	0.03	0.02	1.24	0.88
Wild.NA$_{EO}$ (Sympatric NA)- Farm.EO	-0.07	0.02	-3.29	<0.05
Hb.NA- Farm.NA	-0.03	0.02	-1.62	0.67
Hb.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.05	0.02	2.43	0.20
Hb.NA- Hb.EO	0.08	0.02	3.77	<0.01
Hb.NA- Farm.EO	-0.01	0.02	-0.71	0.99
Farm.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.08	0.02	4.01	<0.01
Farm.NA- Hb.EO	0.11	0.02	5.39	<.0001
Farm.NA- Farm.EO	0.02	0.02	0.90	0.97
Wild.NA$_{EO}$ (Sympatric EO)- Hb.EO	0.03	0.02	1.36	0.82
Wild.NA$_{EO}$ (Sympatric EO)- Farm.EO	-0.07	0.02	-3.13	<0.05
Hb.EO- Farm.EO	-0.09	0.02	-4.50	<0.001
Condition in tank environment (2016)				
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric NA)	-0.04	0.02	-1.57	0.70
Wild.NA$_{EO}$ (Allopatric)- Hb.NA	-0.05	0.02	-2.34	0.24
Wild.NA$_{EO}$ (Allopatric)- Farm.NA	-0.06	0.02	-2.60	0.14
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric EO)	0.00	0.02	0.10	1.0
Wild.NA$_{EO}$ (Allopatric)- Hb.EO	-0.05	0.02	-2.18	0.32
Wild.NA$_{EO}$ (Allopatric)- Farm.EO	-0.12	0.02	-5.01	<0.0001
Wild.NA$_{EO}$ (Sympatric NA)- Hb.NA	-0.02	0.02	-0.77	0.99
Wild.NA$_{EO}$ (Sympatric NA)- Farm.NA	-0.02	0.02	-1.03	0.95
Wild.NA$_{EO}$ (Sympatric NA)- Wild.NA$_{EO}$ (Sympatric EO)	0.04	0.02	1.67	0.64
Wild.NA$_{EO}$ (Sympatric NA)- Hb.EO	-0.01	0.02	-0.61	0.99
Wild.NA$_{EO}$ (Sympatric NA)- Farm.EO	-0.08	0.02	-3.44	<0.05
Hb.NA- Farm.NA	-0.01	0.02	-0.26	1.0
Hb.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.06	0.02	2.44	0.20
Hb.NA- Hb.EO	0.00	0.02	0.16	1.0
Hb.NA- Farm.EO	-0.06	0.02	-2.67	0.12
Farm.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.06	0.02	2.70	0.11
Farm.NA- Hb.EO	0.01	0.02	0.42	0.99
Farm.NA- Farm.EO	-0.06	0.02	-2.41	0.21
Wild.NA$_{EO}$ (Sympatric EO)- Hb.EO	-0.05	0.02	-2.28	0.27
Wild.NA$_{EO}$ (Sympatric EO)- Farm.EO	-0.12	0.02	-5.11	<0.0001
Hb.EO- Farm.EO	-0.07	0.02	-2.83	0.08
Final mass in stream environment (2016)				

Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric NA)	-109.07	30.40	-3.59	<0.05
Wild.NA$_{EO}$ (Allopatric)- Hb.NA	60.08	30.40	1.98	0.44
Wild.NA$_{EO}$ (Allopatric)- Farm.NA	10.77	30.40	0.36	0.99
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric EO)	54.37	30.40	1.79	0.56
Wild.NA$_{EO}$ (Allopatric)- Hb.EO	-80.15	30.40	-2.64	0.13
Wild.NA$_{EO}$ (Allopatric)- Farm.EO	-96.00	30.40	-3.16	<0.05
Wild.NA$_{EO}$ (Sympatric NA)- Hb.NA	169.15	30.40	5.57	<.0001
Wild.NA$_{EO}$ (Sympatric NA)- Farm.NA	119.84	30.40	3.95	<0.01
Wild.NA$_{EO}$ (Sympatric NA)- Wild.NA$_{EO}$ (Sympatric EO)	163.44	30.40	5.38	<.0001
Wild.NA$_{EO}$ (Sympatric NA)- Hb.EO	28.93	30.40	0.95	0.96
Wild.NA$_{EO}$ (Sympatric NA)- Farm.EO	13.07	30.40	0.43	0.99
Hb.NA- Farm.NA	-49.31	30.40	-1.62	0.66
Hb.NA- Wild.NA$_{EO}$ (Sympatric EO)	-5.71	30.40	-0.19	1.0
Hb.NA- Hb.EO	-140.22	30.40	-4.62	<0.001
Hb.NA- Farm.EO	-156.08	30.40	-5.14	<0.0001
Farm.NA- Wild.NA$_{EO}$ (Sympatric EO)	43.60	30.40	1.44	0.78
Farm.NA- Hb.EO	-90.92	30.40	-3.00	0.06
Farm.NA- Farm.EO	-106.77	30.40	-3.52	<0.05
Wild.NA$_{EO}$ (Sympatric EO)- Hb.EO	-134.52	30.40	-4.43	<0.001
Wild.NA$_{EO}$ (Sympatric EO)- Farm.EO	-150.37	30.40	-4.95	<0.0001
Hb.EO- Farm.EO	-15.85	30.40	-0.52	0.99
Final length in stream environment (2016)				
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric NA)	-0.22	0.07	-3.24	<0.05
Wild.NA$_{EO}$ (Allopatric)- Hb.NA	0.25	0.07	3.62	<0.001
Wild.NA$_{EO}$ (Allopatric)- Farm.NA	0.12	0.07	1.78	0.57
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric EO)	0.15	0.07	2.14	0.34
Wild.NA$_{EO}$ (Allopatric)- Hb.EO	-0.14	0.07	-2.05	0.40
Wild.NA$_{EO}$ (Allopatric)- Farm.EO	-0.12	0.07	-1.80	0.56
Wild.NA$_{EO}$ (Sympatric NA)- Hb.NA	0.47	0.07	6.86	<0.0001
Wild.NA$_{EO}$ (Sympatric NA)- Farm.NA	0.34	0.07	5.02	<0.0001
Wild.NA$_{EO}$ (Sympatric NA)- Wild.NA$_{EO}$ (Sympatric EO)	0.37	0.07	5.38	<0.0001
Wild.NA$_{EO}$ (Sympatric NA)- Hb.EO	0.08	0.07	1.19	0.89
Wild.NA$_{EO}$ (Sympatric NA)- Farm.EO	0.10	0.07	1.45	0.78
Hb.NA- Farm.NA	-0.13	0.07	-1.84	0.53
Hb.NA- Wild.NA$_{EO}$ (Sympatric EO)	-0.10	0.07	-1.48	0.76
Hb.NA- Hb.EO	-0.39	0.07	-5.66	<0.0001
Hb.NA- Farm.EO	-0.37	0.07	-5.41	<0.0001
Farm.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.02	0.07	0.37	0.99
Farm.NA- Hb.EO	-0.26	0.07	-3.82	<0.001

Farm.NA- Farm.EO	-0.24	0.07	-3.57	<0.05
Wild.NA$_{EO}$ (Sympatric EO)- Hb.EO	-0.29	0.07	-4.19	<0.01
Wild.NA$_{EO}$ (Sympatric EO)- Farm.EO	-0.27	0.07	-3.94	<0.001
Hb.EO- Farm.EO	0.02	0.07	0.25	1.0
SGRmass in stream environment (2016)				
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric NA)	-0.26	0.07	-4.00	<0.001
Wild.NA$_{EO}$ (Allopatric)- Hb.NA	-0.01	0.07	-0.11	1.0
Wild.NA$_{EO}$ (Allopatric)- Farm.NA	-0.12	0.07	-1.77	0.57
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric EO)	0.11	0.07	1.71	0.61
Wild.NA$_{EO}$ (Allopatric)- Hb.EO	-0.02	0.07	-0.37	0.99
Wild.NA$_{EO}$ (Allopatric)- Farm.EO	-0.20	0.07	-2.97	0.06
Wild.NA$_{EO}$ (Sympatric NA)- Hb.NA	0.26	0.07	3.89	<0.001
Wild.NA$_{EO}$ (Sympatric NA)- Farm.NA	0.15	0.07	2.24	0.29
Wild.NA$_{EO}$ (Sympatric NA)- Wild.NA$_{EO}$ (Sympatric EO)	0.38	0.07	5.71	<0.0001
Wild.NA$_{EO}$ (Sympatric NA)- Hb.EO	0.24	0.07	3.63	<0.001
Wild.NA$_{EO}$ (Sympatric NA)- Farm.EO	0.07	0.07	1.03	0.94
Hb.NA- Farm.NA	-0.11	0.07	-1.66	0.64
Hb.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.12	0.07	1.82	0.54
Hb.NA- Hb.EO	-0.02	0.07	-0.26	1.0
Hb.NA- Farm.EO	-0.19	0.07	-2.86	0.08
Farm.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.23	0.07	3.48	<0.05
Farm.NA- Hb.EO	0.09	0.07	1.40	0.80
Farm.NA- Farm.EO	-0.08	0.07	-1.20	0.89
Wild.NA$_{EO}$ (Sympatric EO)- Hb.EO	-0.14	0.07	-2.08	0.38
Wild.NA$_{EO}$ (Sympatric EO)- Farm.EO	-0.31	0.07	-4.68	<0.0001
Hb.EO- Farm.EO	-0.17	0.07	-2.60	0.14
SGRlength in stream environment (2016)				
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric NA)	-0.07	0.02	-3.09	<0.05
Wild.NA$_{EO}$ (Allopatric)- Hb.NA	-0.02	0.02	-1.06	0.94
Wild.NA$_{EO}$ (Allopatric)- Farm.NA	-0.04	0.02	-1.71	0.61
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric EO)	0.06	0.02	2.54	0.16
Wild.NA$_{EO}$ (Allopatric)- Hb.EO	0.00	0.02	0.13	1.0
Wild.NA$_{EO}$ (Allopatric)- Farm.EO	-0.04	0.02	-1.68	0.63
Wild.NA$_{EO}$ (Sympatric NA)- Hb.NA	0.05	0.02	2.03	0.41
Wild.NA$_{EO}$ (Sympatric NA)- Farm.NA	0.03	0.02	1.38	0.8
Wild.NA$_{EO}$ (Sympatric NA)- Wild.NA$_{EO}$ (Sympatric EO)	0.13	0.02	5.63	<0.0001
Wild.NA$_{EO}$ (Sympatric NA)- Hb.EO	0.08	0.02	3.21	<0.05
Wild.NA$_{EO}$ (Sympatric NA)- Farm.EO	0.03	0.02	1.40	0.80
Hb.NA- Farm.NA	-0.02	0.02	-0.65	0.99

Hb.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.08	0.02	3.60	<0.05
Hb.NA- Hb.EO	0.03	0.02	1.19	0.90
Hb.NA- Farm.EO	-0.01	0.02	-0.62	0.99
Farm.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.10	0.02	4.25	<0.01
Farm.NA- Hb.EO	0.04	0.02	1.83	0.53
Farm.NA- Farm.EO	0.00	0.02	0.02	1.0
Wild.NA$_{EO}$ (Sympatric EO)- Hb.EO	-0.06	0.02	-2.41	0.21
Wild.NA$_{EO}$ (Sympatric EO)- Farm.EO	-0.10	0.02	-4.22	<0.01
Hb.EO- Farm.EO	-0.04	0.02	-1.81	0.55
Condition in stream environment (2016)				
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric NA)	-0.03	0.02	-1.56	0.71
Wild.NA$_{EO}$ (Allopatric)- Hb.NA	-0.04	0.02	-1.80	0.55
Wild.NA$_{EO}$ (Allopatric)- Farm.NA	-0.05	0.02	-2.27	0.28
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric EO)	0.01	0.02	0.60	0.99
Wild.NA$_{EO}$ (Allopatric)- Hb.EO	-0.03	0.02	-1.44	0.78
Wild.NA$_{EO}$ (Allopatric)- Farm.EO	-0.07	0.02	-3.41	<0.05
Wild.NA$_{EO}$ (Sympatric NA)- Hb.NA	-0.01	0.02	-0.25	1.0
Wild.NA$_{EO}$ (Sympatric NA)- Farm.NA	-0.02	0.02	-0.71	0.99
Wild.NA$_{EO}$ (Sympatric NA)- Wild.NA$_{EO}$ (Sympatric EO)	0.05	0.02	2.16	0.33
Wild.NA$_{EO}$ (Sympatric NA)- Hb.EO	0.00	0.02	0.12	1.0
Wild.NA$_{EO}$ (Sympatric NA)- Farm.EO	-0.04	0.02	-1.86	0.51
Hb.NA- Farm.NA	-0.01	0.02	-0.47	0.99
Hb.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.05	0.02	2.40	0.21
Hb.NA- Hb.EO	0.01	0.02	0.36	0.99
Hb.NA- Farm.EO	-0.03	0.02	-1.62	0.67
Farm.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.06	0.02	2.87	0.07
Farm.NA- Hb.EO	0.02	0.02	0.83	0.98
Farm.NA- Farm.EO	-0.02	0.02	-1.15	0.91
Wild.NA$_{EO}$ (Sympatric EO)- Hb.EO	-0.04	0.02	-2.04	0.40
Wild.NA$_{EO}$ (Sympatric EO)- Farm.EO	-0.09	0.02	-4.02	<0.001
Hb.EO- Farm.EO	-0.04	0.02	-1.97	0.44
Final mass in tank environment (2017)				
Wild.NA (Allopatric)- Wild.NA (Sympatric- I)	74.90	88.10	0.85	0.98
Wild.NA (Allopatric)- Hb$_{W♀}$	-50.80	87.90	-0.58	0.99
Wild.NA (Allopatric)- Farm.EO (Sympatric- I)	-202.10	88.10	-2.29	0.26
Wild.NA (Allopatric)- Wild.NA (Sympatric- II)	196.00	87.60	2.24	0.29
Wild.NA (Allopatric)- Hb$_{f♀}$	-180.00	87.70	-2.05	0.39
Wild.NA (Allopatric)- Farm.EO (Sympatric- II)	-218.80	87.90	-2.49	0.18
Wild.NA (Sympatric- I)- Hb$_{W♀}$	-125.70	88.20	-1.43	0.79

Wild.NA (Sympatric- I)- Farm.EO (Sympatric- I)	-277.00	88.30	-3.14	<0.05
Wild.NA (Sympatric- I)- Wild.NA (Sympatric- II)	121.10	88.70	1.37	0.82
Wild.NA (Sympatric- I)- $Hb_{f♀}$	-254.90	88.50	-2.88	0.08
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- II)	-293.70	88.60	-3.31	<0.05
$Hb_{w♀}$- Farm.EO (Sympatric- I)	-151.30	88.10	-1.72	0.61
$Hb_{w♀}$- Wild.NA (Sympatric- II)	246.80	88.40	2.79	0.09
$Hb_{w♀}$- $Hb_{f♀}$	-129.20	88.70	-1.46	0.77
$Hb_{w♀}$- Farm.EO (Sympatric- II)	-168.00	88.70	-1.89	0.49
Farm.EO (Sympatric- I)- Wild.NA (Sympatric-II)	398.10	88.90	4.48	<0.0001
Farm.EO (Sympatric- I)- $Hb_{f♀}$	22.10	88.70	0.25	1.0
Farm.EO (Sympatric- I)- Farm.EO (Sympatric- II)	-16.70	88.50	-0.19	1.0
Wild.NA (Sympatric- II)- $Hb_{f♀}$	-376.00	88.40	-4.26	<0.01
Wild.NA (Sympatric- II)- Farm.EO (Sympatric- II)	-414.80	88.30	-4.70	<0.001
$Hb_{f♀}$- Farm.EO (Sympatric- II)	-38.80	87.70	-0.44	0.99
Final length in tank environment (2017)				
Wild.NA (Allopatric)- Wild.NA (Sympatric- I)	-0.04	0.10	-0.37	0.99
Wild.NA (Allopatric)- $Hb_{w♀}$	-0.07	0.10	-0.75	0.99
Wild.NA (Allopatric)- Farm.EO (Sympatric- I)	-0.33	0.10	-3.45	<0.05
Wild.NA (Allopatric)- Wild.NA (Sympatric- II)	0.10	0.09	1.02	0.95
Wild.NA (Allopatric)- $Hb_{f♀}$	-0.44	0.09	-4.69	<0.0001
Wild.NA (Allopatric)- Farm.EO (Sympatric- II)	-0.34	0.10	-3.61	<0.05
Wild.NA (Sympatric- I)- $Hb_{w♀}$	-0.04	0.09	-0.38	0.99
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- I)	-0.30	0.09	-3.14	<0.05
Wild.NA (Sympatric- I)- Wild.NA (Sympatric- II)	0.13	0.10	1.38	0.81
Wild.NA (Sympatric- I)- $Hb_{f♀}$	-0.41	0.10	-4.27	<0.01
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- II)	-0.31	0.10	-3.22	<0.05
$Hb_{w♀}$- Farm.EO (Sympatric- I)	-0.26	0.09	-2.76	0.10
$Hb_{w♀}$- Wild.NA (Sympatric- II)	0.17	0.10	1.76	0.58
$Hb_{w♀}$- $Hb_{f♀}$	-0.37	0.10	-3.87	<0.001
$Hb_{w♀}$- Farm.EO (Sympatric- II)	-0.27	0.10	-2.83	0.09
Farm.EO (Sympatric- I)- Wild.NA (Sympatric-II)	0.43	0.10	4.42	<0.0001
Farm.EO (Sympatric- I)- $Hb_{f♀}$	-0.11	0.10	-1.19	0.90
Farm.EO (Sympatric- I)- Farm.EO (Sympatric- II)	-0.01	0.10	-0.14	1.0
Wild.NA (Sympatric- II)- $Hb_{f♀}$	-0.54	0.10	-5.68	<0.0001
Wild.NA (Sympatric- II)- Farm.EO (Sympatric- II)	-0.44	0.10	-4.62	<0.0001
$Hb_{f♀}$- Farm.EO (Sympatric- II)	0.10	0.09	1.08	0.93
SGRmass in tank environment (2017)				
Wild.NA (Allopatric)- Wild.NA (Sympatric- I)	0.05	0.10	0.47	0.99
Wild.NA (Allopatric)- $Hb_{w♀}$	-0.28	0.10	-2.82	0.08

Wild.NA (Allopatric)- Farm.EO (Sympatric- I)	-0.31	0.10	-3.06	<0.05
Wild.NA (Allopatric)- Wild.NA (Sympatric- II)	0.19	0.10	1.87	0.50
Wild.NA (Allopatric)- $Hb_{f♀}$	-0.07	0.10	-0.75	0.99
Wild.NA (Allopatric)- Farm.EO (Sympatric- II)	-0.30	0.10	-3.01	0.05
Wild.NA (Sympatric- I)- $Hb_{W♀}$	-0.33	0.10	-3.30	<0.05
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- I)	-0.36	0.10	-3.55	<0.05
Wild.NA (Sympatric- I)- Wild.NA (Sympatric- II)	0.14	0.10	1.38	0.81
Wild.NA (Sympatric- I)- $Hb_{f♀}$	-0.12	0.10	-1.21	0.89
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- II)	-0.35	0.10	-3.46	<0.05
$Hb_{W♀}$- Farm.EO (Sympatric- I)	-0.02	0.10	-0.25	1.0
$Hb_{W♀}$- Wild.NA (Sympatric- II)	0.47	0.10	4.68	<0.0001
$Hb_{W♀}$- $Hb_{f♀}$	0.21	0.10	2.06	0.39
$Hb_{W♀}$- Farm.EO (Sympatric- II)	-0.02	0.10	-0.19	1.0
Farm.EO (Sympatric- I)- Wild.NA (Sympatric-II)	0.50	0.10	4.88	<0.0001
Farm.EO (Sympatric- I)- $Hb_{f♀}$	0.23	0.10	2.31	0.25
Farm.EO (Sympatric- I)- Farm.EO (Sympatric- II)	0.01	0.10	0.06	1.0
Wild.NA (Sympatric- II)- $Hb_{f♀}$	-0.26	0.10	-2.60	0.14
Wild.NA (Sympatric- II)- Farm.EO (Sympatric- II)	-0.49	0.10	-4.86	<0.0001
$Hb_{f♀}$- Farm.EO (Sympatric- II)	-0.23	0.10	-2.30	0.27
SGRlength in tank environment (2017)				
Wild.NA (Allopatric)- Wild.NA (Sympatric- I)	-0.01	0.02	-0.30	0.99
Wild.NA (Allopatric)- $Hb_{W♀}$	-0.02	0.02	-0.89	0.97
Wild.NA (Allopatric)- Farm.EO (Sympatric- I)	-0.07	0.02	-2.73	0.11
Wild.NA (Allopatric)- Wild.NA (Sympatric- II)	0.02	0.02	0.69	0.99
Wild.NA (Allopatric)- $Hb_{f♀}$	-0.06	0.02	-2.37	0.23
Wild.NA (Allopatric)- Farm.EO (Sympatric- II)	-0.09	0.02	-3.48	<0.05
Wild.NA (Sympatric- I)- $Hb_{W♀}$	-0.01	0.02	-0.59	0.99
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- I)	-0.06	0.02	-2.46	0.19
Wild.NA (Sympatric- I)- Wild.NA (Sympatric- II)	0.02	0.02	0.98	0.96
Wild.NA (Sympatric- I)- $Hb_{f♀}$	-0.05	0.02	-2.04	0.40
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- II)	-0.08	0.02	-3.16	<0.05
$Hb_{W♀}$- Farm.EO (Sympatric- I)	-0.05	0.02	-1.87	0.51
$Hb_{W♀}$- Wild.NA (Sympatric- II)	0.04	0.02	1.57	0.70
$Hb_{W♀}$- $Hb_{f♀}$	-0.04	0.02	-1.45	0.77
$Hb_{W♀}$- Farm.EO (Sympatric- II)	-0.06	0.02	-2.57	0.15
Farm.EO (Sympatric- I)- Wild.NA (Sympatric-II)	0.08	0.02	3.38	<0.05
Farm.EO (Sympatric- I)- $Hb_{f♀}$	0.01	0.02	0.38	0.99
Farm.EO (Sympatric- I)- Farm.EO (Sympatric- II)	-0.02	0.02	-0.74	0.98
Wild.NA (Sympatric- II)- $Hb_{f♀}$	-0.07	0.02	-3.04	<0.05

Wild.NA (Sympatric- II)- Farm.EO (Sympatric- II)	-0.10	0.02	-4.16	<0.01
$Hb_{f♀}$- Farm.EO (Sympatric- II)	-0.03	0.02	-1.15	0.91
Condition in tank environment (2017)				
Wild.NA (Allopatric)- Wild.NA (Sympatric- I)	0.09	0.06	1.59	0.69
Wild.NA (Allopatric)- $Hb_{W♀}$	-0.01	0.06	-0.21	1.0
Wild.NA (Allopatric)- Farm.EO (Sympatric- I)	0.04	0.06	0.65	0.99
Wild.NA (Allopatric)- Wild.NA (Sympatric- II)	0.13	0.06	2.26	0.28
Wild.NA (Allopatric)- $Hb_{f♀}$	0.11	0.06	1.83	0.53
Wild.NA (Allopatric)- Farm.EO (Sympatric- II)	0.02	0.06	0.37	0.99
Wild.NA (Sympatric- I)- $Hb_{W♀}$	-0.11	0.06	-1.80	0.55
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- I)	-0.06	0.06	-0.94	0.96
Wild.NA (Sympatric- I)- Wild.NA (Sympatric- II)	0.04	0.06	0.65	0.99
Wild.NA (Sympatric- I)- $Hb_{f♀}$	0.01	0.06	0.23	1.0
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- II)	-0.07	0.06	-1.21	0.88
$Hb_{W♀}$- Farm.EO (Sympatric- I)	0.05	0.06	0.86	0.97
$Hb_{W♀}$- Wild.NA (Sympatric- II)	0.14	0.06	2.45	0.19
$Hb_{W♀}$- $Hb_{f♀}$	0.12	0.06	2.02	0.41
$Hb_{W♀}$- Farm.EO (Sympatric- II)	0.03	0.06	0.58	0.99
Farm.EO (Sympatric- I)- Wild.NA (Sympatric-II)	0.09	0.06	1.58	0.69
Farm.EO (Sympatric- I)- $Hb_{f♀}$	0.07	0.06	1.17	0.90
Farm.EO (Sympatric- I)- Farm.EO (Sympatric- II)	-0.02	0.06	-0.28	1.0
Wild.NA (Sympatric- II)- $Hb_{f♀}$	-0.02	0.06	-0.42	0.99
Wild.NA (Sympatric- II)- Farm.EO (Sympatric- II)	-0.11	0.06	-1.87	0.51
$Hb_{f♀}$- Farm.EO (Sympatric- II)	-0.09	0.06	-1.46	0.76
Final mass in stream environment (2017)				
Wild.NA (Allopatric)- Wild.NA (Sympatric- I)	-45.28	38.30	-1.18	0.90
Wild.NA (Allopatric)- $Hb_{W♀}$	-155.38	38.30	-4.06	<0.001
Wild.NA (Allopatric)- Farm.EO (Sympatric- I)	-273.02	38.30	-7.13	<0.0001
Wild.NA (Allopatric)- Wild.NA (Sympatric- II)	-21.98	38.30	-0.57	0.99
Wild.NA (Allopatric)- $Hb_{f♀}$	-16.69	38.30	-0.44	0.99
Wild.NA (Allopatric)- Farm.EO (Sympatric- II)	-326.27	38.30	-8.52	<0.0001
Wild.NA (Sympatric- I)- $Hb_{W♀}$	-110.10	38.30	-2.88	0.08
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- I)	-227.74	38.30	-5.95	<0.0001
Wild.NA (Sympatric- I)- Wild.NA (Sympatric- II)	23.30	38.30	0.61	0.99
Wild.NA (Sympatric- I)- $Hb_{f♀}$	28.59	38.30	0.75	0.99
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- II)	-280.99	38.30	-7.34	<0.0001
$Hb_{W♀}$- Farm.EO (Sympatric- I)	-117.64	38.30	-3.07	<0.05
$Hb_{W♀}$- Wild.NA (Sympatric- II)	133.40	38.30	3.48	<0.05
$Hb_{W♀}$- $Hb_{f♀}$	138.69	38.30	3.62	<0.001

$Hb_{W♀}$- Farm.EO (Sympatric- II)	-170.89	38.30	-4.46	<0.0001
Farm.EO (Sympatric- I)- Wild.NA (Sympatric-II)	251.04	38.30	6.56	<0.0001
Farm.EO (Sympatric- I)- $Hb_{f♀}$	256.33	38.30	6.69	<0.0001
Farm.EO (Sympatric- I)- Farm.EO (Sympatric- II)	-53.25	38.30	-1.39	0.81
Wild.NA (Sympatric- II)- $Hb_{f♀}$	5.29	38.30	0.14	1.0
Wild.NA (Sympatric- II)- Farm.EO (Sympatric- II)	-304.29	38.30	-7.95	<0.0001
$Hb_{f♀}$- Farm.EO (Sympatric- II)	-309.57	38.30	-8.08	<0.0001
Final length in stream environment (2017)				
Wild.NA (Allopatric)- Wild.NA (Sympatric- I)	-0.03	0.07	-0.36	0.99
Wild.NA (Allopatric)- $Hb_{W♀}$	-0.34	0.07	-4.84	<0.0001
Wild.NA (Allopatric)- Farm.EO (Sympatric- I)	-0.58	0.07	-8.23	<0.0001
Wild.NA (Allopatric)- Wild.NA (Sympatric- II)	0.03	0.07	0.39	0.99
Wild.NA (Allopatric)- $Hb_{f♀}$	-0.03	0.07	-0.47	0.99
Wild.NA (Allopatric)- Farm.EO (Sympatric- II)	-0.56	0.07	-7.95	<0.0001
Wild.NA (Sympatric- I)- $Hb_{W♀}$	-0.31	0.07	-4.47	<0.0001
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- I)	-0.55	0.07	-7.87	<0.0001
Wild.NA (Sympatric- I)- Wild.NA (Sympatric- II)	0.05	0.07	0.76	0.99
Wild.NA (Sympatric- I)- $Hb_{f♀}$	-0.01	0.07	-0.11	1.0
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- II)	-0.53	0.07	-7.59	<0.0001
$Hb_{W♀}$- Farm.EO (Sympatric- I)	-0.24	0.07	-3.39	<0.05
$Hb_{W♀}$- Wild.NA (Sympatric- II)	0.37	0.07	5.23	<0.0001
$Hb_{W♀}$- $Hb_{f♀}$	0.31	0.07	4.37	<0.0001
$Hb_{W♀}$- Farm.EO (Sympatric- II)	-0.22	0.07	-3.11	<0.05
Farm.EO (Sympatric- I)- Wild.NA (Sympatric-II)	0.61	0.07	8.62	<0.0001
Farm.EO (Sympatric- I)- $Hb_{f♀}$	0.55	0.07	7.76	<0.0001
Farm.EO (Sympatric- I)- Farm.EO (Sympatric- II)	0.02	0.07	0.28	1.0
Wild.NA (Sympatric- II)- $Hb_{f♀}$	-0.06	0.07	-0.86	0.98
Wild.NA (Sympatric- II)- Farm.EO (Sympatric- II)	-0.59	0.07	-8.34	<0.0001
$Hb_{f♀}$- Farm.EO (Sympatric- II)	-0.53	0.07	-7.48	<0.0001
SGRmass in stream environment (2017)				
Wild.NA (Allopatric)- Wild.NA (Sympatric- I)	0.01	0.09	0.14	1.0
Wild.NA (Allopatric)- $Hb_{W♀}$	-0.20	0.09	-2.31	0.25
Wild.NA (Allopatric)- Farm.EO (Sympatric- I)	-0.38	0.09	-4.41	<0.0001
Wild.NA (Allopatric)- Wild.NA (Sympatric- II)	-0.05	0.09	-0.59	0.99
Wild.NA (Allopatric)- $Hb_{f♀}$	0.32	0.09	3.66	<0.001
Wild.NA (Allopatric)- Farm.EO (Sympatric- II)	-0.45	0.09	-5.17	<0.0001
Wild.NA (Sympatric- I)- $Hb_{W♀}$	-0.21	0.08	-2.54	0.16
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- I)	-0.40	0.09	-4.66	<0.0001
Wild.NA (Sympatric- I)- Wild.NA (Sympatric- II)	-0.06	0.08	-0.76	0.99

Wild.NA (Sympatric- I)- $Hb_{f♀}$	0.31	0.09	3.52	<0.05
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- II)	-0.46	0.08	-5.51	<0.0001
$Hb_{w♀}$- Farm.EO (Sympatric- I)	-0.18	0.09	-2.15	0.34
$Hb_{w♀}$- Wild.NA (Sympatric- II)	0.15	0.09	1.72	0.61
$Hb_{w♀}$- $Hb_{f♀}$	0.52	0.09	6.02	<0.0001
$Hb_{w♀}$- Farm.EO (Sympatric- II)	-0.25	0.09	-2.88	0.08
Farm.EO (Sympatric- I)- Wild.NA (Sympatric-II)	0.33	0.09	3.87	<0.001
Farm.EO (Sympatric- I)- $Hb_{f♀}$	0.70	0.08	8.26	<0.0001
Farm.EO (Sympatric- I)- Farm.EO (Sympatric- II)	-0.06	0.08	-0.76	0.99
Wild.NA (Sympatric- II)- $Hb_{f♀}$	0.37	0.09	4.29	<0.01
Wild.NA (Sympatric- II)- Farm.EO (Sympatric- II)	-0.40	0.08	-4.75	<0.0001
$Hb_{f♀}$- Farm.EO (Sympatric- II)	-0.77	0.08	-9.43	<0.0001
SGRlength in stream environment (2017)				
Wild.NA (Allopatric)- Wild.NA (Sympatric- I)	0.04	0.02	1.68	0.63
Wild.NA (Allopatric)- $Hb_{w♀}$	-0.08	0.02	-3.83	<0.001
Wild.NA (Allopatric)- Farm.EO (Sympatric- I)	-0.14	0.02	-6.53	<0.0001
Wild.NA (Allopatric)- Wild.NA (Sympatric- II)	0.01	0.02	0.48	0.99
Wild.NA (Allopatric)- $Hb_{f♀}$	0.11	0.02	5.25	<0.0001
Wild.NA (Allopatric)- Farm.EO (Sympatric- II)	-0.10	0.02	-4.59	<0.0001
Wild.NA (Sympatric- I)- $Hb_{w♀}$	-0.12	0.02	-5.51	<0.0001
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- I)	-0.17	0.02	-8.21	<0.0001
Wild.NA (Sympatric- I)- Wild.NA (Sympatric- II)	-0.03	0.02	-1.20	0.89
Wild.NA (Sympatric- I)- $Hb_{f♀}$	0.07	0.02	3.56	<0.05
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- II)	-0.13	0.02	-6.27	<0.0001
$Hb_{w♀}$- Farm.EO (Sympatric- I)	-0.06	0.02	-2.70	0.11
$Hb_{w♀}$- Wild.NA (Sympatric- II)	0.09	0.02	4.31	<0.001
$Hb_{w♀}$- $Hb_{f♀}$	0.19	0.02	9.07	<0.0001
$Hb_{w♀}$- Farm.EO (Sympatric- II)	-0.02	0.02	-0.76	0.99
Farm.EO (Sympatric- I)- Wild.NA (Sympatric-II)	0.15	0.02	7.01	<0.0001
Farm.EO (Sympatric- I)- $Hb_{f♀}$	0.25	0.02	11.78	<0.0001
Farm.EO (Sympatric- I)- Farm.EO (Sympatric- II)	0.04	0.02	1.94	0.46
Wild.NA (Sympatric- II)- $Hb_{f♀}$	0.10	0.02	4.76	<0.0001
Wild.NA (Sympatric- II)- Farm.EO (Sympatric- II)	-0.11	0.02	-5.07	<0.0001
$Hb_{f♀}$- Farm.EO (Sympatric- II)	-0.21	0.02	-9.83	<0.0001
Condition in stream environment (2017)				
Wild.NA (Allopatric)- Wild.NA (Sympatric- I)	-0.05	0.03	-1.61	0.68
Wild.NA (Allopatric)- $Hb_{w♀}$	-0.02	0.03	-0.62	0.99
Wild.NA (Allopatric)- Farm.EO (Sympatric- I)	0.00	0.03	0.09	1.0
Wild.NA (Allopatric)- Wild.NA (Sympatric- II)	-0.07	0.03	-1.93	0.47

Wild.NA (Allopatric)- $Hb_{f♀}$	-0.01	0.03	-0.31	0.99
Wild.NA (Allopatric)- Farm.EO (Sympatric- II)	-0.07	0.03	-2.09	0.37
Wild.NA (Sympatric- I)- $Hb_{W♀}$	0.03	0.03	0.99	0.95
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- I)	0.06	0.03	1.69	0.62
Wild.NA (Sympatric- I)- Wild.NA (Sympatric- II)	-0.01	0.03	-0.32	0.99
Wild.NA (Sympatric- I)- $Hb_{f♀}$	0.04	0.03	1.30	0.85
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- II)	-0.02	0.03	-0.48	0.99
$Hb_{W♀}$- Farm.EO (Sympatric- I)	0.02	0.03	0.70	0.99
$Hb_{W♀}$- Wild.NA (Sympatric- II)	-0.04	0.03	-1.31	0.84
$Hb_{W♀}$- $Hb_{f♀}$	0.01	0.03	0.31	0.99
$Hb_{W♀}$- Farm.EO (Sympatric- II)	-0.05	0.03	-1.47	0.76
Farm.EO (Sympatric- I)- Wild.NA (Sympatric-II)	-0.07	0.03	-2.01	0.42
Farm.EO (Sympatric- I)- $Hb_{f♀}$	-0.01	0.03	-0.39	0.99
Farm.EO (Sympatric- I)- Farm.EO (Sympatric- II)	-0.07	0.03	-2.17	0.32
Wild.NA (Sympatric- II)- $Hb_{f♀}$	0.06	0.03	1.62	0.67
Wild.NA (Sympatric- II)- Farm.EO (Sympatric- II)	-0.01	0.03	-0.16	1.0
$Hb_{f♀}$- Farm.EO (Sympatric- II)	-0.06	0.03	-1.78	0.57

Supplementary Table S4.4: Tukey-adjusted pairwise contrasts of different cross types from the survival experiments (2016 and 2017) in tank and stream environments derived from the fitted final models for survival (logits). Estimate, parameter estimate.SE, standard errors. z, Wald z-value.

Pairwise contrasts	**Estimate**	**±SE**	z	***P***
Survival (logits) in tank environment (2016)				
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric NA)	0.36	0.67	0.53	1.00
Wild.NA$_{EO}$ (Allopatric)- Hb.NA	0.80	0.61	1.30	0.85
Wild.NA$_{EO}$ (Allopatric)- Farm.NA	0.37	0.64	0.57	1.00
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric EO)	1.17	0.57	2.06	0.37
Wild.NA$_{EO}$ (Allopatric)- Hb.EO	0.46	0.64	0.71	0.99
Wild.NA$_{EO}$ (Allopatric)- Farm.EO	1.39	0.57	2.45	0.18
Wild.NA$_{EO}$ (Sympatric NA)- Hb.NA	0.44	0.69	0.64	1.00
Wild.NA$_{EO}$ (Sympatric NA)- Farm.NA	0.01	0.70	0.01	1.00
Wild.NA$_{EO}$ (Sympatric NA)- Wild.NA$_{EO}$ (Sympatric EO)	0.81	0.63	1.29	0.85
Wild.NA$_{EO}$ (Sympatric NA)- Hb.EO	0.10	0.70	0.14	1.00
Wild.NA$_{EO}$ (Sympatric NA)- Farm.EO	1.03	0.64	1.61	0.68
Hb.NA- Farm.NA	-0.43	0.62	-0.69	0.99
Hb.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.37	0.57	0.66	0.99
Hb.NA- Hb.EO	-0.34	0.65	-0.52	1.00
Hb.NA- Farm.EO	0.59	0.58	1.03	0.95
Farm.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.80	0.60	1.34	0.83
Farm.NA- Hb.EO	0.09	0.65	0.14	1.00
Farm.NA- Farm.EO	1.02	0.59	1.73	0.60
Wild.NA$_{EO}$ (Sympatric EO)- Hb.EO	-0.71	0.61	-1.17	0.91
Wild.NA$_{EO}$ (Sympatric EO)- Farm.EO	0.22	0.54	0.40	1.00
Hb.EO- Farm.EO	0.93	0.61	1.53	0.73
Survival (logits) in stream environment (2016)				
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric NA)	-0.39	0.51	-0.76	0.99
Wild.NA$_{EO}$ (Allopatric)- Hb.NA	-0.12	0.47	-0.26	1.00
Wild.NA$_{EO}$ (Allopatric)- Farm.NA	-0.08	0.47	-0.17	1.00
Wild.NA$_{EO}$ (Allopatric)- Wild.NA$_{EO}$ (Sympatric EO)	0.51	0.41	1.24	0.88
Wild.NA$_{EO}$ (Allopatric)- Hb.EO	-0.05	0.49	-0.11	1.00
Wild.NA$_{EO}$ (Allopatric)- Farm.EO	0.42	0.44	0.94	0.97
Wild.NA$_{EO}$ (Sympatric NA)- Hb.NA	0.26	0.58	0.46	1.00
Wild.NA$_{EO}$ (Sympatric NA)- Farm.NA	0.31	0.58	0.54	1.00

Wild.NA$_{EO}$ (Sympatric NA)- Wild.NA$_{EO}$ (Sympatric EO)	0.89	0.53	1.68	0.63
Wild.NA$_{EO}$ (Sympatric NA)- Hb.EO	0.33	0.60	0.56	1.00
Wild.NA$_{EO}$ (Sympatric NA)- Farm.EO	0.81	0.56	1.43	0.79
Hb.NA- Farm.NA	0.05	0.55	0.08	1.00
Hb.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.63	0.50	1.27	0.87
Hb.NA- Hb.EO	0.07	0.57	0.12	1.00
Hb.NA- Farm.EO	0.54	0.53	1.02	0.95
Farm.NA- Wild.NA$_{EO}$ (Sympatric EO)	0.58	0.49	1.19	0.90
Farm.NA- Hb.EO	0.02	0.57	0.04	1.00
Farm.NA- Farm.EO	0.50	0.53	0.94	0.97
Wild.NA$_{EO}$ (Sympatric EO)- Hb.EO	-0.56	0.52	-1.08	0.93
Wild.NA$_{EO}$ (Sympatric EO)- Farm.EO	-0.09	0.48	-0.18	1.00
Hb.EO- Farm.EO	0.47	0.55	0.86	0.98
Survival (logits) in tank environment (2017)				
Wild.NA (Allopatric)- Wild.NA (Sympatric- I)	-0.96	0.87	-1.10	0.93
Wild.NA (Allopatric)- Hb$_{W♀}$	-1.61	1.04	-1.56	0.71
Wild.NA (Allopatric)- Farm.EO (Sympatric- I)	-0.17	0.80	-0.22	1.00
Wild.NA (Allopatric)- Wild.NA (Sympatric- II)	-0.08	0.68	-0.12	1.00
Wild.NA (Allopatric)- Hb$_{f♀}$	-0.95	0.72	-1.32	0.84
Wild.NA (Allopatric)- Farm.EO (Sympatric- II)	0.65	0.66	0.99	0.96
Wild.NA (Sympatric- I)- Hb$_{W♀}$	-0.65	1.15	-0.57	1.00
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- I)	0.78	0.97	0.81	0.98
Wild.NA (Sympatric- I)- Wild.NA (Sympatric- II)	0.88	0.88	1.00	0.95
Wild.NA (Sympatric- I)- Hb$_{f♀}$	0.01	0.89	0.01	1.00
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- II)	1.60	0.82	1.95	0.45
Hb$_{W♀}$- Farm.EO (Sympatric- I)	1.44	1.08	1.33	0.84
Hb$_{W♀}$- Wild.NA (Sympatric- II)	1.53	1.07	1.44	0.78
Hb$_{W♀}$- Hb$_{f♀}$	0.66	1.08	0.61	1.00
Hb$_{W♀}$- Farm.EO (Sympatric- II)	2.26	1.03	2.19	0.30
Farm.EO (Sympatric- I)- Wild.NA (Sympatric-II)	0.09	0.86	0.11	1.00
Farm.EO (Sympatric- I)- Hb$_{f♀}$	-0.78	0.91	-0.86	0.98
Farm.EO (Sympatric- I)- Farm.EO (Sympatric- II)	0.82	0.83	0.99	0.96
Wild.NA (Sympatric- II)- Hb$_{f♀}$	-0.87	0.79	-1.10	0.93
Wild.NA (Sympatric- II)- Farm.EO (Sympatric- II)	0.73	0.71	1.03	0.95
Hb$_{f♀}$- Farm.EO (Sympatric- II)	1.60	0.66	2.44	0.18
Survival (logits) in stream environment (2017)				
Wild.NA (Allopatric)- Wild.NA (Sympatric- I)	1.16	0.77	1.50	0.75

Wild.NA (Allopatric)- $Hb_{W♀}$	2.11	0.63	3.38	0.01
Wild.NA (Allopatric)- Farm.EO (Sympatric- I)	1.63	0.66	2.48	0.17
Wild.NA (Allopatric)- Wild.NA (Sympatric- II)	1.46	0.68	2.14	0.33
Wild.NA (Allopatric)- $Hb_{f♀}$	1.17	0.72	1.63	0.66
Wild.NA (Allopatric)- Farm.EO (Sympatric- II)	1.85	0.62	2.98	0.05
Wild.NA (Sympatric- I)- $Hb_{W♀}$	0.95	0.70	1.37	0.82
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- I)	0.47	0.72	0.65	1.00
Wild.NA (Sympatric- I)- Wild.NA (Sympatric- II)	0.30	0.75	0.40	1.00
Wild.NA (Sympatric- I)- $Hb_{f♀}$	0.01	0.78	0.01	1.00
Wild.NA (Sympatric- I)- Farm.EO (Sympatric- II)	0.69	0.69	1.00	0.95
$Hb_{W♀}$- Farm.EO (Sympatric- I)	-0.49	0.56	-0.87	0.98
$Hb_{W♀}$- Wild.NA (Sympatric- II)	-0.66	0.59	-1.12	0.92
$Hb_{W♀}$- $Hb_{f♀}$	-0.94	0.63	-1.50	0.75
$Hb_{W♀}$- Farm.EO (Sympatric- II)	-0.26	0.52	-0.50	1.00
Farm.EO (Sympatric- I)- Wild.NA (Sympatric-II)	-0.17	0.62	-0.28	1.00
Farm.EO (Sympatric- I)- $Hb_{f♀}$	-0.46	0.66	-0.69	0.99
Farm.EO (Sympatric- I)- Farm.EO (Sympatric- II)	0.23	0.56	0.40	1.00
Wild.NA (Sympatric- II)- $Hb_{f♀}$	-0.29	0.69	-0.42	1.00
Wild.NA (Sympatric- II)- Farm.EO (Sympatric- II)	0.40	0.59	0.68	0.99
$Hb_{f♀}$- Farm.EO (Sympatric- II)	0.68	0.63	1.09	0.93

CHAPTER 5

Behavioural variation among divergent European and North American farmed and wild Atlantic salmon (*Salmo salar*) populations

Preface

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5.1 Abstract

Animals often display consistent differences in behaviours across situations and contexts. However, little is known about how behavioural traits might vary in a context-dependent manner, with selection favouring correlated sets of behaviours in particular types of environments. Comparative studies of behavioural trait differences and associations among different populations can provide valuable insights into how this might operate. For example, farmed Atlantic salmon (*Salmo salar*) often escape into the wild and may produce hybrid and feral offspring that interact with wild conspecifics. Interactions between these offspring types may be mediated by differences in behaviour reflective of their different selective histories, which may, in turn, affect their relative fitness. In Newfoundland, Canada, site-specific permission has been granted to farm a strain of European origin (EO) salmon in addition to the current North American (NA) Saint John River strain. However, because these two farmed strains are genetically divergent, there is concern that behavioural trait differences will exist that affect interactions with native wild populations. I thus designed a common-garden experiment to compare the behaviour of individual fish from NA and EO farm strains in four different contexts (exploration, response to a novel object, boldness under predation risk, and aggression) relative to that of wild fish and hybrid conspecifics. NA and EO farm fish did not differ in their behaviour ($P > 0.05$), but both were more explorative ($P < 0.001$ in 2016; $P < 0.05$ in 2017), responsive to a novel object ($P < 0.05$, both years), bold ($P < 0.05$, both years), and aggressive ($P < 0.001$, both years) than wild fish and related hybrids. I found the presence of behavioural correlations in some circumstances suggesting that behavioural syndromes covary consistently with aspects of

the selective environment. Overall, these results suggest that the variation in the behavioural traits studied among divergent EO and NA farmed and NA wild populations largely reflect domestication selection and less so the geographic and ancestral relationships.

5.2 Introduction

Intraspecific variation in behavioural traits can affect the strength of interaction and is expected to have a number of other effects at the population and community levels (Des Roches et al., 2018). While numerous empirical studies of behaviour have looked at variation among individuals within a population (Dall et al., 2004; Reale et al., 2007; Dingemanse et al., 2010; Wolf & Weissing, 2012), only recently has the concept of consistent differences in behavioural traits and behavioural correlations (i.e., behavioural syndrome, Sih et al., 2004, 2012; Bell, 2005, 2007) among populations received attention. Such studies can provide ecological insights into how behavioural differences among populations influence a species' ability to adapt to environmental conditions and mediate inter-population interactions, as well as how such consistent differences might have evolved.

Recent studies have documented behavioural variation in a broad range of 'non-model organisms' where fish have been among the most studied taxa (Conrad et al., 2011; Mittelbach et al., 2014; Johnsson & Naslund, 2018). These studies have shown fish, like many other vertebrates, exhibit consistent behavioural variation regardless of their behavioural plasticity. Among fish, Salmonid (Order Salmoniformes, Familiy Salmonidae) are an ecologically important and extensively studied group. Despite their importance and

the fact that they are relatively well studied, an understanding of the ecological consequences of behavioural differences and syndromes in salmonids, particularly in the context of domestication, remains relatively weak.

Atlantic salmon (*Salmo salar*) is the most economically important salmonids species, with global aquaculture production currently estimated at more than 2 million tonnes per annum (ICES WGNAS, 2018). Associated with this scale of farming, a substantial number of farmed salmon escape aquaculture facilities at all life stages (Naylor et al., 2005; Thorstad et al., 2008; Glover et al., 2013). The total number of salmon that escape into the North Atlantic has been estimated at more than 2 million individuals annually (Yeates et al., 2014; Keyser et al., 2018). These escapes lead to persistent concerns about negative ecological (Fleming et al., 2000; Jonsson & Jonsson, 2006; Nylund et al., 2019) and genetic interactions with wild populations (McGinnity et al., 2003; Skaala et al., 2012; Bolstad et al., 2017; Wringe et al., 2018) that may affect the latter's viability (Hindar et al., 2006; Glover et al.,2017; Castellani et al., 2018; Sylvester et al., 2019). It has been hypothesized that these interactions between wild and farmed salmon may be at least in part mediated by behavioural differences. For example, farmed and hybrid salmon offspring are less risk-averse (Fleming & Einum, 1997; Johnsson et al., 2001; Houde et al., 2010; Solberg et al., 2015), more aggressive (Fleming & Einum, 1997) and demonstrate increased competitive abilities (Einum & Fleming, 1997; Houde et al.,2009) relative to their wild counterparts. Such behavioural differences are thought to arise in part due to markedly different selective pressures in aquaculture (i.e., directed for production-related traits, inadvertent selection, and relaxed natural selection) versus the wild (Fleming &

Einum, 1997; Huntingford, 2004; Huntingford & Adams, 2005; Debes & Hutchings, 2014; Solberg et al., 2015). Thus, the extent of subsequent risk to wild populations mediated by behavioural interaction will be dependent on the relationship between wild and farm strains resulting from both selection during domestication and the ancestral relationship of farm strains to wild populations.

The degree of genetic divergence between farmed and local wild salmon will depend on the farm strain used (local or non-local in origin) and degree and form of domestication. When farm strains of distant geographic origin are used, concerns are raised as to whether the genetic and ecological risks posed by escaped farm fish will be greater than if more locally derived strains are used (Baskett & Waples, 2013). Currently, the dominant farmed strain in the Northwest Atlantic originates from the Saint John River, New Brunswick. While phenotypic and genetic differences exist among salmon populations on the Island of Newfoundland (F_{st} = 0.12, between Garnish and Northeast Placentia River populations; S.J. Lehnert, pers. comm. based on data from Jeffery et al. 2018), the divergence between Newfoundland and mainland North American salmon populations is larger (F_{st} = 0.14 - 0.20; Bradbury et al., 2018; S.J. Lehnert, pers. comm.).At the largest geographical scales, most North American (hereafter "NA") and European (hereafter "EO") salmon populations have been separated for ~1,670,000 years (Rougemont and Bernatchez, 2018; but also see, > 1,000,000 years, Nilsson et al., 2001; 600,000-700,000 years, King et al., 2007). The prolonged reproductive isolation between NA and EO Atlantic salmon has allowed chromosomal numbers to diverge: EO salmon generally have 29 chromosome pairs with 74 arms, where NA salmon have 27 chromosome

pairs with 72 arms (Lubieniecki et al., 2010; Brenna-Hansen et al., 2012; Cauwelier et al., 2012; Lehnart et al., 2019). Like their wild counterparts, a compelling body of evidence shows that EO farmed salmon (Norwegian) and NA wild salmon (native stocks in Newfoundland) are highly divergent genetically ($F_{st} > 0.40$; S.J. Lehnert, pers. comm.). In Newfoundland, permission has been granted to import a strain of EO aquaculture salmon (StofnFiskur), originally derived from wild Norwegian populations, to be farmed as triploids in the near future (by Grieg NL, a subsidiary of the Norwegian-based Grieg Seafood). Existing knowledge indicates that if a proportion of farmed EO salmon are non-triploid and escape, it is likely that they will be able to breed successfully and interact ecologically with wild populations. Whether the resultant impacts would be greater than those that would occur with the use of NA farm strains is uncertain ($F_{st} > 0.44$, between EO farm and NA farm; S.J. Lehnert, pers. comm.) and will depend on the specific nature of trait differences between the respective farm strains and local wild populations. Adding another layer of complexity to the situation is the fact that the NA/EO separation has not been complete in the study area and historical trans-Atlantic straying and colonization have resulted in some salmon populations of the Northwest Atlantic, particularly in southeastern Newfoundland and Labrador, Canada showing evidence of EO introgression (~10,000 years before present; Lehnert et al., 2019).

On these considerations, I designed a common-garden experiment to measure how individual fish from NA and EO farm strains behaved relative to NA wild fish and conspecific hybrids in four different contexts: exploration in an unfamiliar environment, response to a novel object, boldness under predation risk and levels of aggression. To my knowledge, this is the first experiment with salmon aimed specifically to understand both

(i) variation in behavioural traits across individuals among populations and (ii) behavioural correlations (syndromes) within populations. I tested the hypotheses that: (i) multi-generation domestication selection in both EO and NA farm fish has resulted in similar directions of behavioural trait differences relative to wild fish; (ii) the geographic and ancestral relationships of the fish will be reflected in behavioural trait differences; (iii) interbreeding will cause hybrids to display altered behaviours relative to wild and farm fish; and (iv) behavioural syndromes within populations will differ reflective of their different selective histories (wild vs. domesticated).

5.3 Methods

5.3.1 Study populations

Crosses of pure NA and EO farmed and NA wild salmon, and their hybrids were generated in the fall 2015 and 2016. The four base populations were Wild NA, Farm NA, Wild NA with a signature of historic EO ancestry (Wild.NA_{EO}; Lehnert et al., 2019) and Farm EO. Wild.NA were derived from the Garnish River (Lat: 47.2348 °N; Lon: 55.3615 °W), Newfoundland, and Wild.NA_{EO} derived from the Northeast Placentia River (Lat: 47.2408 °N, Lon: 53.9566 °W), Newfoundland. Atlantic Canada's principal aquaculture strain, originally derived from the Saint John River, New Brunswick, was used to produce the Farm.NA. Farm.EO were produced using the EO origin farm strain (StofnFiskur) recently approved for culture at sites on the south coast of Newfoundland, Canada. The StofnFiskur strain itself derives from Norwegian strains, and is produced in an Icelandic facility, from where the gametes were garnered for the crosses. All crosses were generated

and reared at Memorial University of Newfoundland's Ocean Sciences Centre (see cross details in chapter two).

Following yolk sac absorption (i.e., at the start of exogenous feeding), 64 families (Farm.NA: 20, Wild.NA_{EO}: 11, Hb.NA: 13, Farm.EO: 10, and Hb.EO: 10) in 2015 and 40 families (Farm.EO: 10, Wild.NA: 10, $Hb_{W♀}$: 10; and $Hb_{F♀}$: 10) in 2016 were pooled and reared together by cross-type (each family consisting of ca. 200-400 juveniles depending on the number of families per cross-type to maintain similar densities) and transferred into 470-liter flow-through circular holding tanks (0.9mdiamter x 0.5m high).They were fed ad libitum with a combination of live brine shrimp (*Artemia* spp.), frozen blood worm (*Chironomidae* spp., commercial fish food supplier), and salmonid starter dry feed (EWOS-Cargill, BC, Canada). The temperature was ambient (8-17 °C) and photoperiod was maintained at a 12L: 12D schedule during holding and experimentation. All animals were treated following the guidelines provided by the Canadian Council on Animal Care during holding and experimentation, and approval was granted by the Memorial University Animal Care Committee (15-21-IF).

5.3.2 Experimental Protocol

Experiments were undertaken to quantify behaviour in four different contexts: exploration in an unfamiliar environment; response to a novel object; boldness under predation risk; and aggression. These experiments were conducted with the age-0 fish between 2 October – 13 December 2016, and 11 September - 29 October 2017. Experimental conditions and protocols were the same between years, and all behavioural observations took place between 09:00 and 17:00 hours. Two trials were run per day, using

four experimental aquaria: two aquaria (90 x 45 x 36 cm) for exploration, novel object and boldness tests; and two aquaria (50 x 30 x 20 cm) for aggression tests (Fig. 5.1). The aquaria were covered externally on three sides to minimize disturbance, and data for all four contexts were recorded both manually and by video recording (VIXIA HF R60 HD Digital Camcorder, Canon, USA, Inc.). To increase intra-observer reliability, all data measurements were assessed using both manual and digital video recordings. Specific modifications to the experimental aquaria for each of the four behavioural contexts studied are described below.

Before each trial, fish were anaesthetised with AQUALIFE TMS (MS-222; Syndel Laboratories Ltd, Nanaimo, BC, Canada) and measured for wet weight (g) and fork length (cm). To enable individual recognition during the trials, each fish was marked uniquely by injecting a small amount of coloured visible implant elastomer (Northwest Marine Technology, WA, USA) below the dorsal fin (both sides). The exploration, novel object and boldness tests were conducted first, and the fish were placed into a refuge section (30 x 20 x 36 cm) within one of the large aquaria, which was separated from the remainder of the aquarium by transparent partition equipped with a sliding door. Small pieces of PVC pipe were placed in the section for shelter. The fish were deprived of food one day before behavioural observation. While they were in the experimental aquaria, there was no tag loss or mortality.

5.3.3 Behavioural observations

In 2016, I tested a total of 175 fish (35 individuals from each of the five cross types), and in 2017, I tested a total of 140 fish (35 individuals from each of the four cross types).

There were 35 trials in each of the two years. All tests were carried out in the same order. Exploration, novel object, and boldness tests were carried out successively, with exploration and novel object tests being conducted first as they were undertaken in the absence of food, whereas during the boldness test the fish were offered food to assess foraging under predation risk. To minimize stress associated with transfer to a different aquarium, aggression tests were conducted last and after a 48 hours acclimation period.

5.3.3.1 Exploration of an unfamiliar environment

Approximately 24 hours after acclimatisation in the refuge section of the experimental aquaria, individuals (one of each cross-type, n = 5 in 2016 and n = 4 in 2017) were tested for their willingness to explore in an unfamiliar environment (Fig. 5.1a; Jones and Godin, 2010). At the onset of this test, the sliding door of the refuge section was raised to allow the fish to enter and swim freely throughout the remaining open area of the aquarium. Each fish was given 10 minutes to exit the refuge section and explore the novel environment. I recorded the number of seconds that elapsed until the fish exited from the refuge ("latency to emerge"). I then quantified the total time fish spent active or holding without moving ("swimming vs. holding"), and the numbers of different areas (n = 9) in the tank used by the fish ("areas used"). Individuals with a long latency time to emerge or short time spent in the novel area were considered to be a less explorative. Fish that did not emerge from the refuge during the 10-minute trial period were assigned a latency to exit score of 10 minutes. Fish were then left undisturbed for 20 minutes until the onset of the novel object experiment.

5.3.3.2 Response to a novel object

During this trial, I measured the individual's tendency to interact with a novel object (Fig. 5.1b; Wilson et al., 1993). Fish were kept in the same experimental aquaria. The refuge section was removed prior to the 20 minutes acclimation period and replaced by an opaque divider at the centre of the tank to separate the experimental fish from the novel object. The novel object consisted of a washer attached to a fishing line. The experiment began by lowering the novel object gently to the centre of the separated section, and then the divider was taken out. I then measured the latency to explore the novel object, measured as the time until the first approach to within 5 cm of the novel object, and the number of approaches towards the object. Each individual was scored for 15 minutes.

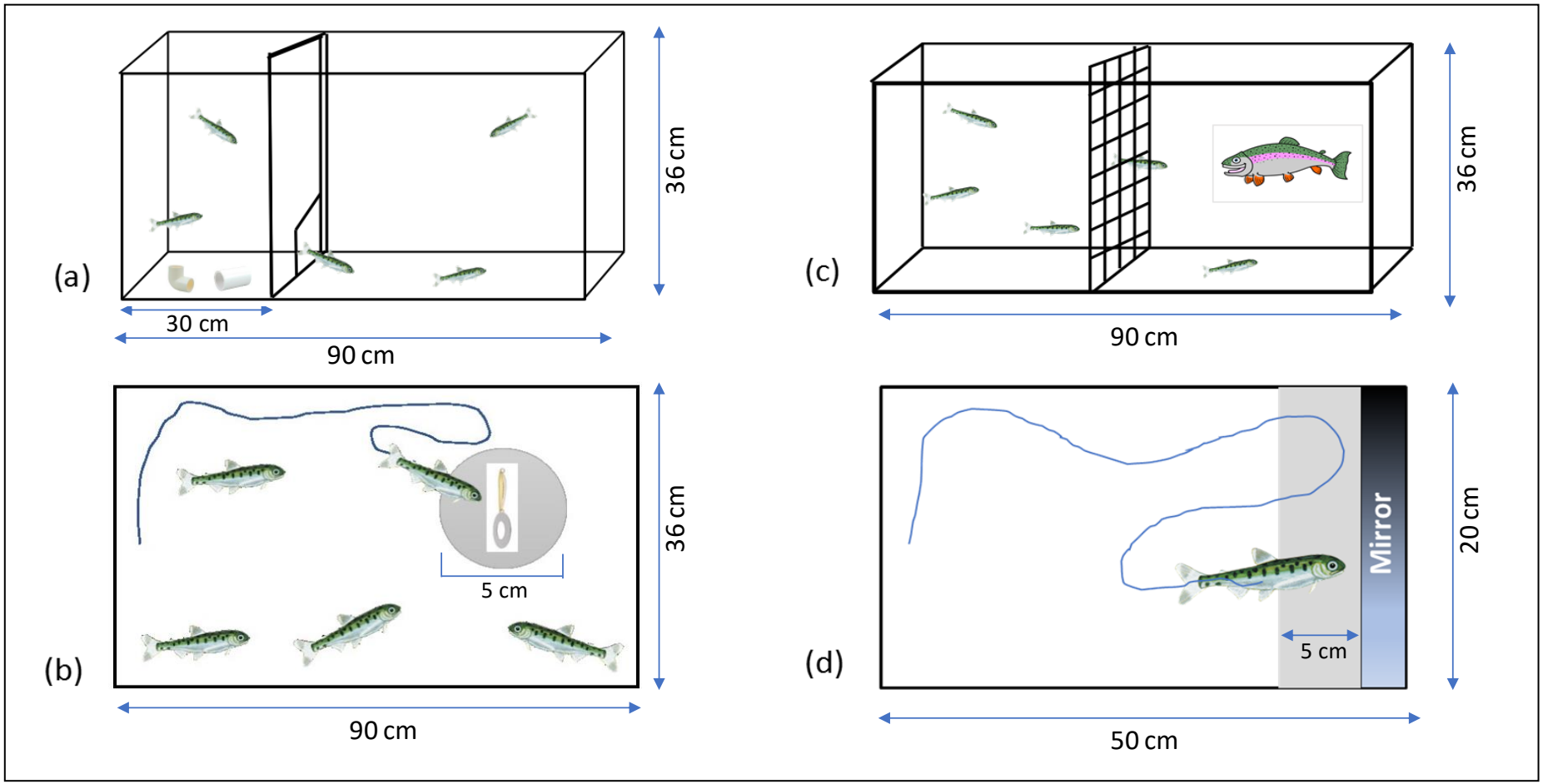

Figure 5.1: Schematic diagram of the four different contexts in which behaviour was scored: (a) Exploration in an unfamiliar environment (b) Novel-object test, (c) Boldness under predation risk test, and (d) Aggression test. Grey areas (2b and 2d) represent areas where fish were scored as being close to the novel object (2b) and close to the mirror (2d).

5.3.3.3 Boldness under predation risk

At least an hour following the novel object trial, I scored the willingness of the fish to forage under predation risk with a live rainbow trout (*Oncorhynchus mykiss*), a predator of juvenile salmon (Fig. 5.1c; Johnsson, 1993). The same aquaria were used and divided into two areas with a pass-through mesh screen, as well as a solid divider at the centre. One area was a safer zone for the experimental fish and the other was a risky zone with the predator. The rainbow trout was held in a transparent plexiglass box with a fine mesh screen at each end to prevent actual predation. At the beginning of this test, the divider was removed to allow fish access via the mesh screen to forage in the risky zone, where feed (salmonid starter dry feed) was delivered. Four grams of feed was delivered three times at 5 minute intervals. Fish were allowed 15 minutes to pass through the screen to the risky zone to forage. Once the experimental fish passed the screen, two measures were taken: latency to forage under risk and time spent in the risky zone. If the experimental fish did not pass through the screen to forage within 15 minutes, they were assigned a latency to forage score of 15 minutes. The fish were then transferred to a small aquarium to acclimate for 48 hr for the next experiment (aggression test).

5.3.3.4 Aggression

A mirror-image stimulation test (MIS) was used to quantify individual aggression (Fig. 5.1d). MIS is a method by which to standardize the opponent, with the mirror image showing an equally sized contestant mimicking the behaviour of the focal individual (Gallup, 1968; Adriaenssens & Johnsson, 2013). MIS is effective in eliciting aggression in juvenile salmonids (Johnsson & Naslund, 2018). For this test, small aquaria (50 x 30 x 20

cm) were used with a mirror (30 x 20 cm) carefully inserted into the aquarium at approx. 3 cm from one of the ends. A minute following mirror insertion, the behaviour of the fish was scored for 10 minutes. I recorded the latency to first attack (s), number of approaches (swim against the mirror), number of attacks (fish attacks its image), and total time spent near the mirror (within 5 cm). If the experimental fish did not approach the mirror within 10 minutes, it was assigned a latency to first attack of 10 minutes.

5.3.4 Statistical analyses

All statistical analyses were performed using R version 3.5.2 (R Core Team, 2018). To summarize the behavioural measures, principal component analyses (PCA) followed by varimax rotation were used, using the Factoextra R packages. Data were standardized (i.e., rescaling the distribution of values) before conducting the PCA. All variables measured for the four behavioural contexts were collapsed first, and then variables within a single behavioural context were collapsed into a single component score for subsequent analysis. The first principal components (PC1) of the context-specific PCAs explained the greatest total variance (68-95%) and all had eigenvalues greater than one (Kaiser-Guttman criterion). By performing statistical analyses on a component score rather than many different variables, the issue of multiple comparisons was avoided, and differences in the scale among behavioural measurements are standardised.

To measure the behavioural differences among the populations, the Kruskal-Wallis test (non-parametric alternative to one-way ANOVA) was used. Separate Wilcoxon signed-rank tests (non-parametric alternative to t-test) were performed post hoc to compare the behavioural differences between two populations.

Lastly, to measure the correlations between behavioural contexts (i.e., syndromes) across individuals within a population, Spearman rank correlations were used. A Shapiro-Wilk's test was applied, as the data were not expected to be normally distributed. Statistical significance was inferred if $P < 0.05$ after sequential Bonferroni adjustment (Rice, 1989).

5.4 Results

5.4.1 Axes of Behaviour

Neither body length nor weight were correlated with any of the behavioural traits ($P > 0.05$). A PCA conducted across all variables measured for the four behavioural contexts revealed among cross-type differences along the PC1 (Kruskal-Wallis, $P < 0.001$) but not PC2 ($P > 0.05$; Fig. 5.2, Table 5.1) during the 2016 and 2017 experiments. In 2016, Farm.NA and Farm.EO strains did not differ in PC1 scores from each other ($P > 0.05$), but differed significantly from the Wild.NA_{EO} and hybrid populations ($P < 0.001$). Wild.NA_{EO} fish differed significantly from Hb.EO ($P < 0.05$), but did not differ from Hb.NA ($P > 0.05$) (Fig. 5.2A). In 2017, Farm.EO fish differed in PC1 scores from both hybrids ($P < 0.001$) and Wild.NA fish ($P < 0.05$), which did not differ among themselves ($P > 0.05$) (Fig. 5.2B). Separate PCAs for each of the four behavioural contexts then specified that PC1 described much of the variation in behaviour within a context (68-95%). Thus, for all subsequent analyses of context-specific differences, I used the PCA scores extracted from the first component for the trials both in 2016 and 2017.

The first behavioural context described "exploration in an unfamiliar environment", where the latency to emerge from the refuge section loaded positively on the component, whereas total time spent active (s) and the number of the different areas explored in the

aquarium loaded negatively (Table 5.1). Fish that had a short latency time to emerge (i.e., fast at emerging from the refuge section), spent more time active and explored more areas of the aquarium. PC1 scores both years had eigenvalues larger than 1 (2.84 in 2016; 2.04 in 2017) and explained 94.7% of the exploration context variance in 2016 and 68.2% in 2017.

The second behavioural context described "response to a novel object", where latency to novel object interaction loaded positively, and the number of approaches towards object loaded negatively (Table 5.1). Thus, fish that had a short latency to approach the novel object generally also approached it more often. The eigenvalues for the PC1 scores were 1.83 in 2016 and 1.77 in 2017, with the novel object context variance explained being 91.6% and 88.6%, respectively.

The third behavioural context described "boldness under predation risk", where the latency to forage under risk loaded positively, and the total time spent in the risky zone loaded negatively (Table 5.1). Therefore, fish that began foraging sooner (i.e., exhibiting a short latency time to forage) and spent more time in the risky zone were considered bold. The eigenvalues for the PC1 scores were 1.79 in 2016 and 1.66 in 2017, with the boldness context variance explained being 89.4% and 82.8%, respectively.

Table 5.1: PC1 loadings of behavioural measures recorded across all four behavioral contexts: exploration in an unfamiliar environment, novel object test, boldness under predation risk, and aggression test. See text for details of eigenvalues and percentages of total variance explained for each behavioural context.

Behavioural Variables	**Loadings (PC1)**	
	2016 (30%)	**2017 (27.5%)**
Latency to emerge from refuge (s)	0.32	0.17
Total time spent active (s)	-0.36	-0.19
No. of different areas used in tank	-0.37	-0.14
Latency to novel object search (s)	0.11	0.10
No. of approaches towards object	-0.12	-0.06
Latency to forage under risk (s)	0.15	0.09
Total time spent in risky zone (s)	-0.18	-0.07
Latency to 1st attack (s)	0.29	0.43
No. of approaches (Swim against mirror)	-0.41	-0.50
No. of attacks (fish attacks its image)	-0.41	-0.50
Total time spent near mirror (5 cm) (s)	-0.36	-0.45

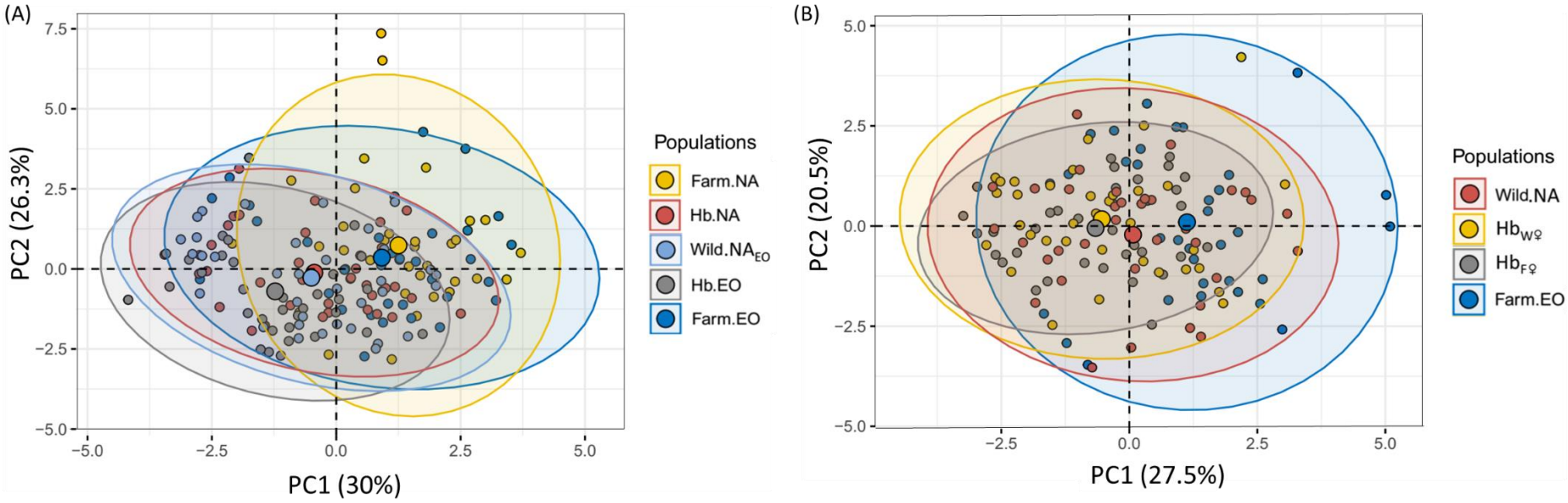

Figure 5.2: PC analysis across all variables measured for the four behavioral contexts [exploration in an unfamiliar environment, novel object test, boldness under predation risk, and aggression test] during the (A) 2016 and (B) 2017 experiments.

For the last context “aggression”, latency to the first attack of the mirror image loaded positively, whereas the number of approaches, number of attacks, and total time spent near the mirror loaded negatively (Table 5.1). Thus, fish that took less time before their first attack of the mirror image, approached and attacked it more frequently as well as spent more time close to the mirror were considered aggressive. The eigenvalues for the PC1 scores were 2.86 in 2016 and 3.06 in 2017, with the aggression context variance explained being 71.42% and 76.38%, respectively.

5.4.2 Behavioural trait variation among populations

In 2016, there were among cross-type differences (Kruskal-Wallis, $P < 0.001$) in exploration in an unfamiliar environment (Fig. 5.3A). Both Farm.NA and Farm.EO fish were more explorative than related hybrids and Wild.NA_{EO} fish. Individuals from both farmed populations had shorter latency time to emerge from the refuge section, were more active and explored more areas. There was no significant difference in exploration between hybrids and Wild.NA_{EO} fish. However, in 2017, there was no difference in exploration between Farm.EO and Wild.NA fish (Fig. 5.3B). Individuals from Wild.NA were more explorative than both hybrids ($Hb_{W♀}$ and $Hb_{F♀}$), whereas Farm.EO were more explorative than $Hb_{F♀}$, but not $Hb_{W♀}$. There was no statistical difference in exploration between $Hb_{W♀}$ and $Hb_{F♀}$.

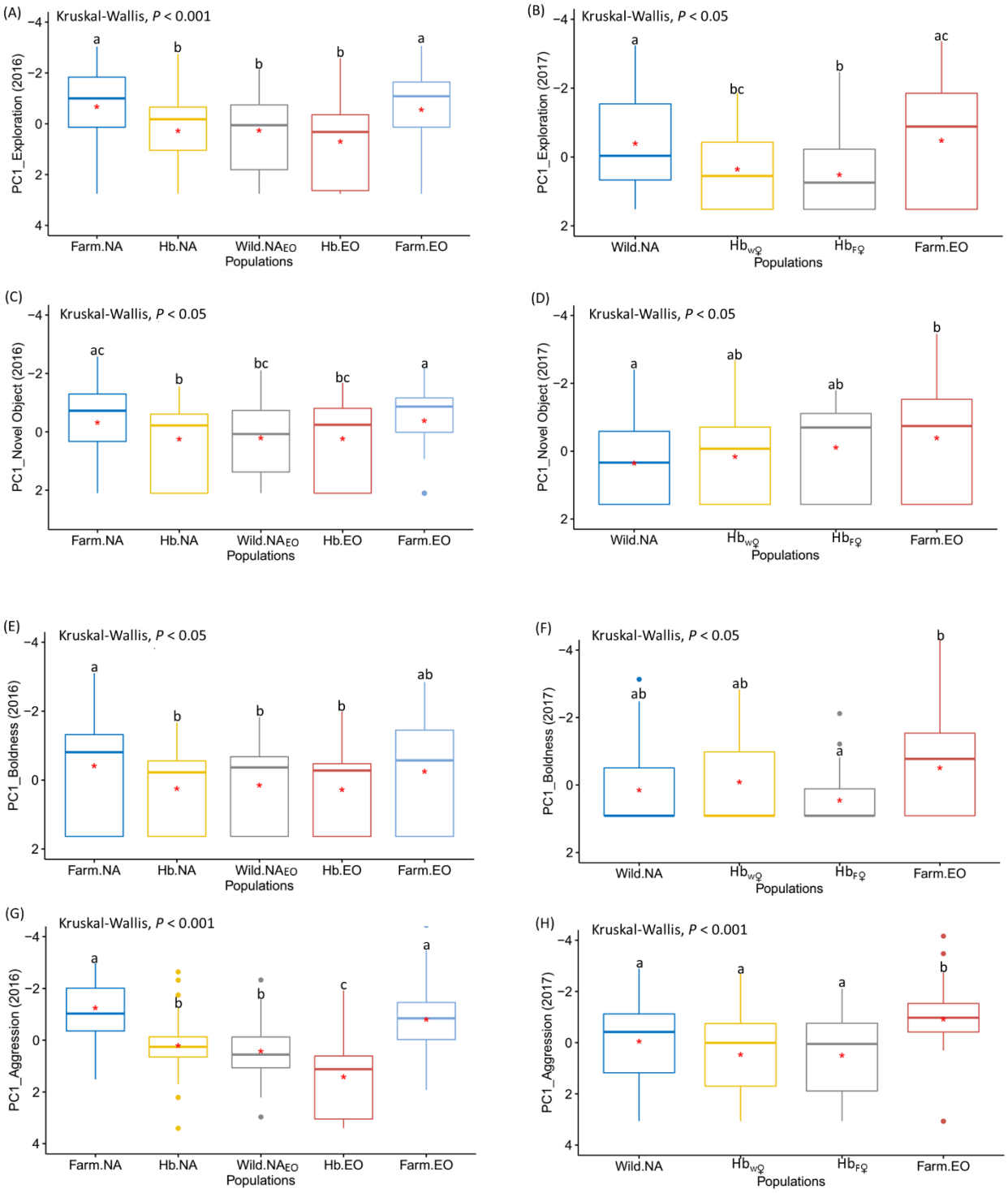

Figure 5.3 (A-H): Boxplot of behavioural trait variation among populations. The first principal components scores of behaviours associated with (A) Exploration 2016 and (B) 2017; (C) Novel object search 2016 and (D) 2017; (E) Boldness under predation risk 2016 and (F) 2017;

(G) Aggression 2016 and (H) 2017. Bold line represents median, boxes 25 and 75 % quartiles, whiskers 95% confidence interval, dots outliers and red asterisk (*) mean.

In the response to a novel object experiment, both Farm.NA and Farm.EO showed greater searching behaviour than Hb.NA in 2016 (Fig. 5.3C), but no difference was observed between the two farmed populations. Farm.EO also differed significantly from Hb.EO and Wild.NA_{EO} cross types, while Farm.NA did not. Similarly, in 2017, Farm.EO searched more when exposed to a novel object than Wild.NA fish, but there was no significant difference with $Hb_{W♀}$ and $Hb_{F♀}$. (Fig. 5.3D).

With regards to the boldness under predation risk test, individuals from Farm.NA were bolder and foraged for a longer time in the presence of a live predator than hybrids and Wild.NA_{EO}fish in 2016 (Fig. 5.3E). However, I did not observe any difference in foraging activity among Farm.EO, hybrids, and Wild.NA_{EO}. I did not observe any significant difference in foraging under predation risk between Farm.EO, $Hb_{W♀}$, and Wild.NA fish in 2017, but Farm.EO fish were bolder than $Hb_{F♀}$ fish (Fig. 5.3F).

Both Farm.NA and Farm.EO fish were more aggressive towards their mirror image than related hybrids and Wild.NA_{EO} fish (Fig. 5.3G). Individuals from both farmed populations had a shorter latency period to attack its image, undertook more attacks, and spent more time close to the mirror (5 cm) than the other groups. There was also a significant difference in aggression between Hb.NA and Hb.EO populations. Wild.NA_{EO} fish were more aggressive than Hb.EO, but did not differ from Hb.NA. In 2017, Farm.EO fish were more aggressive than related hybrids and Wild.NA conspecifics, which did not differ among themselves (Fig. 5.3H).

5.4.3 Variation in Behavioural Syndrome

In 2016, both Farm.NA and Farm.EO fish behaviour in one context was frequently correlated to the individual's behaviour in a different context (Table 5.2). For example, both Farm.NA and Farm.EO individuals showed positive correlations between boldness and aggression. As well, Farm.NA fish exhibited negative correlations between exploration and boldness as well as aggression. Among Farm.EO fish, novel object search was positively associated with aggression but negatively associated with boldness.

Within the hybrid cross types there was only one significant correlation between behavioural traits; a positive correlation between boldness and novel object search within Hb.EO (Table 5.2). For Wild.NA_{EO}, aggression was significantly negatively correlated with boldness, which contrasts with the positive correlations seen within Farm.EO and Farm.NA.

Table 5.2: Behavioural syndromes as indicated by across-context Spearman correlation coefficients between PC1 scores for the four behavioral contexts within populations during the 2016 experiments. Significant values after sequential Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Population	Variables	Novel Object	Boldness	Aggression
Farm.EO	Exploration	-0.149	-0.177	0.010
	Novel Object		**-0.258****	**0.256****
	Boldness			**0.212***
Farm.NA	Exploration	-0.097	**-0.247****	**-0.206***
	Novel Object		0.102	-0.073
	Boldness			**0.224***
$Wild.NA_{EO}$	Exploration	0.177	-0.012	-0.118
	Novel Object		-0.065	**-0.353*****
	Boldness			**-0.248****
Hb.EO	Exploration	0.045	0.023	0.025
	Novel Object		**0.219***	0.077
	Boldness			-0.058
Hb.NA	Exploration	0.029	-0.048	-0.026
	Novel Object		0.046	0.085
	Boldness			-0.145

In 2017, the only significant correlation observed was within Wild.NA fish involving boldness and novel object search, which was similar to that observed within Farm.EO fish in 2016 (Table 5.3). Farm.EO fish showed no significant behavioural correlations in 2017 in contrast to the previous year.

Table 5.3: Behavioural syndromes as indicated by across-context Spearman correlations between PC1 scores for the four behavioral contexts within populations during the 2017 experiments. Significant values after sequential Bonferroni correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Population	Variables	Novel Object	Boldness	Aggression
Farm.EO	Exploration	0.100	-0.082	-0.173
	Novel Object		0.215	0.143
	Boldness			0.056
Wild.NA	Exploration	-0.151	0.125	0.159
	Novel Object		**-0.235***	0.204
	Boldness			0.061
$Hb_{F♀}$	Exploration	-0.060	0.025	0.093
	Novel Object		0.152	-0.049
	Boldness			0.071
$Hb_{W♀}$	Exploration	0.147	-0.052	-0.164
	Novel Object		-0.189	-0.166
	Boldness			0.083

5.5 Discussion

While studies often examine variation in behavioural traits among populations, few couple this with an investigation of behavioural syndromes. Herein, I demonstrated behavioural trait variation and correlations among divergent farmed, F_1 hybrids, and wild salmon populations to provide insight into their behavioural patterns from an ecological perspective. The main findings can be summarized as: (i) NA and EO farm fish did not differ in their behaviour, and both were more explorative, bold and aggressive than wild fish and related hybrids; and (ii) the behavioural correlations among behavioural contexts were either positively or negatively correlated or independent of each other and differed

among populations. These findings suggest that both intentional and unintentional selection may have generated behavioural differences among farmed, wild fish and their related hybrids, and the behavioural correlations vary among populations given that different behavious may be advantageous in different contexts.

5.5.1 Behavioural trait variation

I found farmed fish to be more explorative, bold, and aggressive than wild fish, and conspecific hybrids across different behavioural contexts. This is consistent with observations of other domesticated salmonid populations (Einum & Fleming, 1997; Fleming & Einum, 1997; Biro et al., 2004, 2007; Sundstrom et al., 2004; Adriaenssens & Johnsson, 2010, 2011; Houde et al., 2010; Debes & Hutchings, 2014; Solberg et al., 2015). In addition to the degree of domestication, the extent of behavioural trait differences between a given farmed and a given wild population may also depend on their historic geographic separation (Weber & Fausch, 2003). For example, when farm strains of distant geographical origins are used, genetic differences related to isolation by distance and differing local environmental conditions are of consequence in addition to changes that have arisen through domestication. In this study, along with hybrid and wild fish, I examined the behaviour of Farm.NA and Farm.EO populations. As these two farmed populations are historically genetically divergent but have likely experienced similar domestication selection, an outstanding question that remains is whether these two farmed populations would display behavioural differences between each other. The findings indicated no differences in the behaviour of Farm.NA and Farm.EO, both of which tended to be similarly more explorative (though Farm.EO ≥ Wild.NA in 2017 but not significant),

bold (though Farm.EO ≥ Wild.NA$_{EO}$ (2016)/Wild.NA (2017) but not significant), and aggressive than wild fish and related hybrids. Both farm strains have undergone multiple generations of domestication selection (Farm.EO 10-12 generations; Farm.NA 5-7 generations). Although, they originated from different, genetically distinct regions, it appears that domestication selection has similarly shaped the behaviours studied as I did not observe any behavioural trait differences between these two farmed populations. Domestication selection can be detected already within the first cultured generation, which means that fish bred in a hatchery environment can quickly diverge behaviourally from their wild conspecifics (Christie et al., 2012, 2016; Horreo et al.,2018). Likewise, in the context of boldness, aggression and novel object search, both local wild populations, Wild.NA$_{EO}$ and Wild.NA, were behaviourally similar relative to Farm.EO. Furthermore, hybrids in both years showed similar levels of behavioural expressions to each other (except in aggression, Hb.NA > Hb.EO) and relative to the two wild populations (except in exploration, hybrids < wild) than to the two farm strains. These results suggest that the behaviours of native/farmed hybrids may be more similar to the locally adapted native fish than to farm fish.

5.5.2 Population variation in behavioural syndromes

These results suggest that the behavioural correlations within populations vary because selection has favoured different suites of correlated behaviours. The adaptive hypothesis for the existence of behavioural syndromes predicts that the behavioural correlation should evolve only in populations where they are favoured by selection (Dingemanse et al., 2007). According to this hypothesis, I may not see the same patterns

among populations *per se*, because selection might favour decoupling of behaviours (Bell & Sih, 2007; Bell et al., 2010; Dingemanse et al., 2007; Stamps, 2007). The results presented here (Tables 2 and 3) support this prediction as behavioural correlations among exploration of an unfamiliar environment, response to a novel object, boldness under predation risk and aggression were either positively or negatively correlated or independent of each other and differed among populations, likely reflective of the differing selective environments experienced. For example, in this study, boldness under predation risk was positively correlated with aggression across individuals in both Farm.NA and Farm.EO (2016). However, exploration was negatively associated with boldness and aggression in Farm.NA but not in Farm.EO.While novel object search was negatively related to boldness and positively related to aggression in Farm.EO but not Farm.NA. Across Wild.NA_{EO} individuals, both novel object search and boldness were negatively associated with aggression.

Moreover, it is worth pointing out that populations are not necessarily consistent in their behavioural syndromes across years. For example, in 2016, Farm.EO fish exhibited both positive and negative associations between behaviours, but intriguingly, I did not see any significant correlations between behaviours in 2017, though the directions of several relationships were similar (i.e., negative or positive). One of the possible reasons for the lack of across-year consistency is that the differences are indeed fixed, but the environments in which the fish were reared differed somewhat between years, and thus, the correlation of behaviours were not expressed the same way. Additionally, I can not entirely exclude the possibility of a seasonal timing effect, as all behavioural trials in 2016 were

conducted between October and December, whereas in 2017 they were conducted between September and October. Moreover, during the period of acclimatisation, they might develop some social relationships, which could impact behavioural syndromes seen between years.

In conclusion, the differences in the behavioural traits examined among divergent NA and EO farmed and wild populations largely reflect the influence of domestication selection and less so geographic (ancestral) origin. Both deliberate selection for traits such as fast growth and unintentional selection for fish that flourish in intensive aquaculture systems likely have generated the behavioural differences between farmed (Farm.NA and Farm.EO), their related hybrids and wild fish (Wild.NA_{EO} and Wild.NA). The variation in the presence of behavioural syndromes (correlations) within the populations also suggests that different behavioural phenotypes may be advantageous in different contexts, reflecting different selectional environments.

5. 6 References

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CHAPTER 6: Conclusion

6.1 Conclusion and future direction

Successful interbreeding of escaped farmed salmon with local wild populations and the resultant hybridization that poses fitness impacts which threaten local adaptation remains a persistent management and conservation concern. It was unknown, however, whether the genetic and competitive fitness consequences for local NF wild populations posed by escaped EO farmed fish will be greater than that of escaped NA farmed fish (Baskett & Waples, 2013). Overall, the results of my thesis discuss several key findings regarding the escaped farmed-wild salmon ecological and genetic interaction paradigm. The early-life fitness-related trait differences (development time, size, growth, survival) reported in chapter two, and subsequent juvenile fitness-related trait differences (e.g., growth survival, dominance, exploration, novel object search, boldness under predation risk, aggression) reported in chapters four and five of my thesis, show that both escaped EO and NA farmed fish will pose similar threat to the local NF wild fish. Furthermore, the findings from chapter three suggest that the interbreeding of escaped EO/NA farmed and NF wild populations would alter gene transcription, however, the potential consequences of hybridization could be greater from escaped EO than NA farmed salmon to the local NF wild salmon populations.

Firstly, my thesis emphasizes the effect of hybridization on key fitness-related traits of salmon at early-life stages, which is crucial because they can have knock-on effects for important fitness-related traits (e.g., growth and survival) at the later stages. More specifically, my experimental results showed that early-life fitness-related traits among

divergent EO and NA farmed, NF wild, and F_1 hybrid groups seem to be influenced mainly by geographic and ancestral relationships of the farmed strains and maternal effects rather than domestication selection. I did not find consistent early-life trait differences of EO and NA farmed strains relative to NF wild populations, and indicated that egg size is the primary maternal effect trait influencing offspring phenotypes. My results highlight the integral role egg size plays in the contemporary evolution of salmon early-life history traits. However, one caveat of this study is that due to the crossing design, it was not possible to assess the direct genetic effect (i.e., additive genetic variance). Therefore, future studies are needed to better understand the genetic effect shaping egg size and offspring traits among divergent populations. My results also found few trait differences between F_1 hybrids and respective maternal strains. We have known for a while that the farmed females displayed a greater relative spawning success than farmed males (Fleming et al., 1996, 2000), which will increase the relative frequency of hybrid as opposed to pure farmed offspring. Therefore, the maternal effect of farm females will be important in understanding the early-life fitness consequences of hybridization. It has been increasingly evident that the interbreeding of escaped farmed strains and wild populations will have fitness consequences (behaviour, growth, survival) at juvenile and adult life stages, influencing lifetime success and potentially jeoparadizing native wild populations (McGinnity et al., 1997, 2003; Fleming et al., 2000, Skaala et al., 2019). However, whether the offspring of farmed or hybrid salmon that will live their entire lives in the wild will always have lower reproductive fitness than wild salmon remains unclear and worthy of further study.

My thesis further highlights the impact of hybridization on gene expression differences of salmon at early-life stages, which is crucial to local NF wild salmon conservation and management. I showed that Farm.EO expressed a larger number of differentially expressed genes than Farm.NA relative to the wild population. This raises concern that the escape of non-triploid fish of the genetically distinct EO farmed strain may pose a greater genetic threat to NF wild populations than those of the local Farm.NA strain. Among the ecologically relevant comparisons, my results found the largest gene expression differences to be between Farm.EO and Farm.NA, which suggests that the difference in geographical/ancestral origins contributed to the gene expression differences between these two farmed strains originated from two different continents. However, I cannot rule out that the difference in the form and number of generations of domestication selection may also have contributed. Though, one would think that there might have been commonalities due to domestication. My results indicate fewer gene transcript differences between F_1 hybrids and domesticated/wild maternal strains, suggesting maternal effects at the early-life stage, which is concordant with the findings of the early-life fitness trait study. The findings of this gene expression study suggest that the genetic consequences of hybridization could be greater from escaped EO farmed than NA farmed salmon, resulting in potential effects on the fitness of NF wild populations (Baskett & Waples, 2013). Therefore, there is a further need to use molecular-genetic markers to quantify introgression in populations. Introgression of NA farmed salmon has already been documented, and site-specific EO farmed salmon aquaculture has recently been started on the south coast of NF, but its introgression remains unquantified. Using molecular markers to quantify introgression, and accurately compute individual admixture depends upon markers being

diagnostic for farmed fish. This may be affected by factors such as the ancestry of the specific farmed strains and wild populations involved. A better understanding of the genomic basis of domestication would help to identify better markers.

The genetic differences between escaped EO and NA farmed and NF wild salmon that affect fitness need to be better understood to predict the impact of introgression. As not all trait differences may influence fitness in the wild, there is a need to identify which traits have the most negative impact in any given population subject to introgression. My thesis highlights fitness-related trait differences (dominance, growth and survival) among EO and NA farmed strains, NF wild populations and related F_1 hybrid juvenile salmon. My experimental findings showed similar patterns in dominance status between Farm.NA and Farm.EO, suggesting common domestication effects. However, my results did not find growth differences between wild fish in allopatry and sympatry in the tank environment, suggesting that intra-strain competition for wild fish has a similar effect relative to inter-strain competition. Although, this pattern was not the same in the stream environment as my results showed that wild fish in the sympatric NA treatment outgrew wild fish in allopatry, while no difference was observed between allopatry and the sympatric EO treatment. My results thus indicate that intra-strain competition for Wild.NA_{EO} fish was greater than that of inter-strain competition with Farm.NA and Hb.NA, suggesting that the different genetic origins of the farmed strains and other associated factors of the semi-natural stream conditions may influence the outcome of the competition. My findings also showed that both farmed strains similarly outgrew wild conspecifics in the tank environment, again suggesting common domestication effects. The dominance and growth

experiments showed that F_1 hybrids tended to exhibit intermediate performance while competing with farmed and wild juveniles, suggesting that escaped EO and NA farmed, and resultant hybrid juveniles may influence the productivity of NF wild populations due to altered competition. My study did not find any survival differences among cross types both in the tank and stream environments. Differences in viability may be most evident at early stages, and after reaching the parr stage, differences in survival may be less obvious (Einum & Fleming, 1997, Fleming et al., 2000). Further studies are needed on the fitness of farmed, admixed and wild salmon in different NF rivers, by monitoring offspring with the comibation of genetic and ecological measurements following spawning intrusions, and on selective change.

Finally, my thesis emphasizes how juvenile salmon from NA and EO farmed strains behaved relative to NF wild fish and conspecific hybrids. The experimental findings showed that both EO and NA farmed fish were equally more explorative, bold, and aggressive than wild fish and related hybrids, which was consistent with the dominance experiment findings in chapter four. It appears that deliberate selection for traits such as fast growth and unintentional selection for fish that flourish in intensive aquaculture systems likely have generated the behavioural differences between farmed, their related hybrid and wild fish. Also, the findings showed that the behavioural correlations among behavioural contexts differed among populations, suggesting that different behaviours may be advantageous in different contexts, reflecting different selectional environments.

Predicting the genetic and ecological impacts along the south coast of NF remains difficult because of sparse information on the state of wild Atlantic salmon populations and

the degree to which they are affected by genetic and ecological interactions with aquaculture. There has been a decline in the abundance of wild salmon in southern NL of ~45% between 1996-2010, particularly near the main farming area (e.g., ~70% decline in the Conne River), and these wild populations have been designated as threatened. A recent study has detected offspring of escaped farmed salmon in 17 out 18 wild salmon rivers in Southern NL, Canada (Wringe et al., 2018); as such, concern exists that the escape of fertile fish of the genetically distinct EO farmed strain may present further issues. There are considerable data and knowledge gaps that exist regarding possible ecological and genetic impacts of farmed EO origin Atlantic salmon. In addition to the lack of data to assess the health of wild stocks on the south coast, the lack of monitoring activities targeted to assessing the current extent of escaped farmed salmon in the region means that the impacts of EO farmed salmon interactions are less understood compared to other regions in the world. Inferring impacts to EO farmed Atlantic salmon is made even more difficult by the absence of controlled experiments that compare ecological traits (e.g., disease resistance) and performance (lifetime survival) of strains of farmed salmon under conditions reflective of the south coast of NF. Not only does this limit assessments of specific impacts but also the ability to conduct risk and cost-benefit assessments related to introducing foreign strains.

Existing knowledge suggests that if genetic introgression occurs, the genotypic and phenotypic changes are frequently expected to be maladaptive (Fleming et al., 2000; McGinnity et al., 2003), and take several generations to dissipate under favourable conditions of natural selection (Hindar et al., 2006; Bradbury et al., 2020). Selection during

domestication, whether intentional (e.g., growth) or unintentional (e.g., dominance, aggression), may impart an advantage to hybrid salmon when competing with wild juveniles. This could have greater population-level impacts due to increasing hybrid abundance. Assessing the likelihood of hybridization is made difficult by the context-dependant nature of these interactions and the dynamic nature of conditions along the south coast of NF. As mentioned above, wild stocks along the south coast are in flux and are expected to continue to decline (Bradbury et al., 2018), increasing their vulnerability to genetic and ecological interactions. With proper management of farming operations, escapement rates may remain low but still create impacts if the scale of the aquaculture industry continues to expand. Furthermore, changing environmental conditions have the potential to alter the fitness of both farmed and wild fish and alter genetic and ecological interactions.

The long-term biological and evolutionary consequences of non-native EO farmed strain invasions for native NF populations can be expected to lead to changes in life-history traits, reduced population productivity and decreased resilience to future impacts such as climate change (i.e., less fish and more fragile stocks). Conducting research on various aspects of the genetic and ecological interactions among EO and NA farmed escapees and NF wild conspecifics is crucial to quantify impacts, determine resiliency, and estimate the recuperative potential of NF wild populations. Such research will, however, not solve the problem. This requires additional research into impact avoidance or mitigation strategies that can hinder or stop further erosion of genetic integrity. Finally, it is important to make it unequivocally clear that only a substantial or complete reduction in the number of escapees in rivers, and/or creating a reproductive barrier through sterilization of EO and

NA farmed salmon, will represent a solution to the challenge. Therefore, my PhD thesis could serve as a primer for better understanding the genetic and ecological effect of hybridization among divergent EO and NA farmed and NF wild fish, and can provide valuable insight for the conservation and management of Atlantic salmon.

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