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Differences in energy acquisition and storage of farm, wild, and hybrid Atlantic salmon competing in the wild

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Abstract

An understanding of genetic differences in fitness-related traits for farm, wild, and hybrid Atlantic salmon (*Salmo salar*) is key for predicting impacts of aquaculture escapes on wild populations. Here we used lipid and fatty acid (FA) analyses to investigate differences in storage and foraging ability among Atlantic salmon juveniles of three cross types (farm, wild, and F_1 hybrids), at the beginning and end of a common garden experimental release in the Newfoundland wild. We found differences in lipid class and FA profiles among cross types at both release and recapture, with farm fish being the most differentiated at recapture. In addition, low recapture levels of triacylglycerols and certain FAs indicative of freshwater prey suggest the possibility of a feeding disadvantage for farm fish. Overall, we show that lipid and FA profiles in juvenile salmon can change over just a short period of time even under favourable conditions in the wild, and farm fish may have genetic differences affecting energy acquisition and storage that could negatively impact their survival and fitness in the longer term.

Key words: aquaculture escapes, lipid, fatty acid, foraging

Introduction

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

To survive to reproduce, any juvenile organism must balance how they allocate energy to processes such as growth, foraging and predator avoidance, and long-term storage (Post and Parkinson 2001), and therefore diet quality can play a critical role in an organism's success (Orlov et al. 2006). Lipid and fatty acid (FA) analyses can be key tools for assessing the impact of diet and storage on fish health and fitness. Lipids (along with proteins) are the primary macronutrients for fish, providing their main source of metabolic energy (Tocher 2003). Total lipid content is often used as a metric of storage energy for fish (Berg et al. 2000; Finstad et al. 2003), and the assessment of relative percentage of different lipid classes can provide insight into mortality risk (e.g., Finstad et al. 2004). The roles essential fatty acids (EFAs) play in the health of fish are numerous, such as in the structure and function of cell membrane phospholipid (PL) bilayers and in immune response, reproduction, and organ function (reviewed by Tocher 2010). FAs are therefore useful in assessing fish health, and can also provide information about the origin of the food a fish has consumed, since they can be used as diet "signatures" (Bell et al. 1994; Budge et al. 2002, 2012; Heintz et al. 2010).

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

types remains to be determined, especially in a wild environment where differential foraging and competitive abilities (e.g., Orlov et al. 2006) of cross types may also impact their ultimate nutritional states. In this study, we aimed to shed light on differences in storage and foraging ability among farm, wild, and hybrid Atlantic salmon juveniles by comparing lipid and FA content among these cross types before and after an experimental release into a common garden, wild environment. Building upon previous findings (Quinton et al. 2005; Johnston et al. 2006; Powell et al. 2008), we first hypothesized that farm fish are genetically predisposed to have higher fat content than wild fish (with hybrids intermediate), if they have equal and plentiful access to a commercial diet. Should this be the case, farm fish should have a higher total lipid content and higher content of the main storage lipid for fish (triacylglycerols, TAGs) than wild fish before being released into the wild. Secondly, we hypothesized that the different cross types would have differences in their overall energy acquisition and (or) storage in the wild, with farm fish being less capable of obtaining food resources in the wild than wild fish are (e.g., Orlov et al. 2006), and hybrids intermediate. If this is the case, it would be expected that (*i*) cross types would show different patterns of change in their overall lipid/FA profiles from release to recapture and (*ii*) farm fish would exhibit a greater decrease in total lipids, storage lipids, and FAs characteristic of freshwater prey items compared with the other cross types from release to recapture. Ultimately, comparing these traits among cross types in the wild could improve our understanding of mechanisms associated with differences in survival and growth observed among wild, farm, and hybrid juvenile salmon reported in previous studies, and inform predictions of impact of escaped farm salmon on wild salmon populations.

Materials and methods

Cross types, release and recapture

Between 28 November and 21 December 2017, three experimental cross types of Atlantic salmon were generated at the Ocean Sciences Centre of Memorial University (St. John's, Newfoundland, Canada). The three cross types were pure wild (eight families), pure farm (six families), and F_1 hybrids (six families of farm–mother hybrids; denoted "F♀hyb"). Wild parents came from the Garnish River, located on the Burin Peninsula on the south coast of Newfoundland emptying into Fortune Bay (Fig. 1). Farm parents came from the Saint John River strain, which is to date the only farm strain used in Atlantic Canada commercial aquaculture operations. Parent adipose fins were clipped with samples stored in anhydrous ethanol for later use in parentage assignment of offspring.

Embryos (~600–800 per family) were incubated in Heath trays on ambient water (3.1–7.9 °C, pH: 5.7–6.2, dissolved oxygen: 8.0–8.5 mg·L^{-1}) with dead embryos removed every 4–5 days. Pooling of fry by cross type and transfer to 470 L flow-through circular holding tanks (0.90 m diameter × 0.74 m height) occurred on 22 May 2018, shortly after emergence/first feeding. All pooled families were estimated to have at least 100 fry with the exception of one farm and wild family (respectively). Fry were kept in ambient water (8–14 °C) and initially fed a combination of *Artemia* and salmonid starter feed (crumbles (0.5 mm; caloric content: 55% protein and 15% fat), EWOS-Cargill, BC, Canada) for 1 month, after which they were fed pellets only until release. Release occurred on 11 July 2018 at a tributary site of the Garnish River (Fig. 1). Approximately 24 h prior to release, all fry were anaesthetized using MS-222 (Aqua Life TMS, Syndel Laboratories Ltd., Nanaimo, BC, Canada; 50 mg·L^{-1} dosage buffered with an equal amount of sodium bicarbonate) and subsequently had their adipose fins clipped to distinguish them from wild fish upon later recapture. A sample of approximately 20 fish from the three cross types were sacrificed on 10 July 2018 (using an overdose of MS-222 at 400 mg·L^{-1} buffered with sodium bicarbonate) and kept for use as a baseline. Approximately 500 fry per cross type were released, at four locations approximately 50 m apart. Animal use was approved by the Memorial University of Newfoundland Institutional Animal Care Committee following Canadian Council on Animal Care (CCAC) guidelines, under protocol number 18-01-IF.

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

Recaptured fish were euthanized using an overdose of MS-222 (400 mg·L^{-1} buffered with an equal amount of sodium bicarbonate) approximately 2 h post-capture, then weighed (±0.01 g), photographed, and caudal fin-clipped. Fin clips were stored in anhydrous ethanol for later parentage analysis, and whole specimens (minus caudal fin) were frozen at −80 °C.

Genetic analysis

Parentage assignment involved a panel of 25 microsatellite loci (277 alleles), which was a subset of a larger panel of 101 loci for Atlantic Canada *S. salar* (Bradbury et al. 2018). DNA was extracted from fin clips of recaptured offspring and their parents using the DNeasy 96 Blood and Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions for the Purification of Total DNA from Animal Tissues protocol. Microsatellite loci were PCR-amplified following the protocol described by Zhan et al. (2017). Sequencing was run on an Illumina MiSeq and scored using MEGASAT software (Zhan et al. 2017). Recaptured offspring were assigned back to their parents and cross type using COLONY (Jones and Wang 2010). See Crowley et al. (2022) for further details of genetic analysis and COLONY assignment.

Lipid sample preparation

Lipid analyses were performed on the sample of fish sacrificed prior to release in the river, as well as a subsample of those recaptured (see Crowley et al. 2022 for analysis of survival, size, and condition of all fish recaptured). For recaptured fish, ten fish from each of the three cross types were

Fig. 1. Location of tributary site on the Garnish River, Newfoundland, that was used for the release and recapture experiment. Inset shows the island of Newfoundland with black box indicating the general study area. Map created in R using leaflet package (Cheng et al. 2021). Map tiles by Stamen Design, under CC BY 3.0. Data by OpenStreetMap, under ODbL.

randomly chosen for analysis, with at least one fish from each family recaptured. For release samples, ten fish from each of the three cross types were chosen randomly for analysis, as family information for these samples was unknown.

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

Lipid extraction

Lipids were extracted according to the protocol described by Parrish (1999). Briefly, samples were quickly (1–2 min) homogenized in a mixture of 2:1 chloroform:methanol (2 and 8 mL chloroform for release and recapture samples, respectively), followed by a rinse of the homogenizer with 2:1 chloroform:methanol (1 mL release and 4 mL recapture) and chloroform-extracted water (0.5 mL release and 2 mL recapture) into the sample. Samples were then sonicated for 4 min and centrifuged for 2 min to separate layers. The organic layer was subsequently removed using the double pipetting technique and transferred to another lipid-cleaned vial. The double pipetting technique is used to draw up only the bottom (organic) layer of extracted homogenate into the pipette while avoiding contamination from the upper layer. It involves inserting a $5\frac{3}{4}''$ (1 inch = 2.5 cm) glass pipette through the two layers of homogenate while expelling air, then removing the bulb upon reaching the bottom layer, placing a 9″ glass pipette inside the first and drawing up only the organic layer (Parrish and Wells 2021). The sonication–centrifugation–organic layer removal step was repeated an additional three times for each sample, with an addition of chloroform (4 mL release and 12 mL recapture) occurring between each removal step. The organic layer transfers were pooled and then concentrated for transfer to 2 mL vials in two steps (recapture only), the first of which involved evaporation to near-dryness using a flash-evaporator (Buchler Instruments, Fort Lee, NJ, USA), and the second involving evaporation under nitrogen (release samples required only this step due to smaller volumes). Chloroform and methanol (three additions of the former and one of the latter; small volumes just enough to wet sample vials) were added to near-dry samples during each transfer process to ensure transfer of all lipids. Samples were then capped under nitrogen, sealed, and stored at −20 °C.

Total lipids and lipid class analysis

Total lipid and lipid class analyses were done using the Iatroscan Mark V1 TLC-FID (Iatron Laboratories, Tokyo, Japan; Parrish 1999). Chromatograms were analyzed using PeakSimple software (version 4.88, SRI Instruments, Torrance, CA, USA), with each chromatogram visually inspected and peaks cut manually. For 4 out of the total 30 recapture samples, TAG peak areas were close to the blank value for that region of the

chromatogram. For computational purposes, these samples were assigned a TAG value based on the peak area obtained for the lowest calibrant: 1.26 μg. The value for these four samples thus represents their maximum possible TAG value.

Derivatization of lipid extracts to fatty acid methyl esters

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

The FAMEs were analyzed on an HP 6890 gas chromatograph flame ionization detector equipped with a 7683 autosampler. The GC column was a ZB wax+ (Phenomenex, Torrance, CA, USA). Peaks were identified using retention times from standards purchased from Supelco (Bellefonte, PA, USA). Chromatograms were integrated using the Agilent OpenLAB Data Analysis—Build 2.203.0.573. A quantitative standard purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA; product number GLC490) was used to check the GC column about every 300 samples (or once a month) to ensure that the areas returned were as expected.

Size and condition measurements

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

Statistical analyses

All statistical analyses were performed in R (R Core Team 2020, version 4.0.2). For the statistical analyses in this paper, while we do report p values for model parameters, we do not use the dichotomous delineator of $p = 0.05$ to conclude "significance" (or lack thereof), based on the recommendation of Wasserstein et al. (2019). Instead, we report the evidence for the effect of a given explanatory variable using likelihood ratios (LRs) (Royall 1997). LRs indicate the likelihood of the data given two competing models (i.e., given a model including a specific explanatory variable vs. a model lacking it; Glover and Dixon 2004). Using the designations described in Royall (1997), we infer "strong evidence" from LRs > 8 (though note that this designation is not a cut-off threshold). Consistent with avoiding "significance" terminology, we do not use asterisks in figures to indicate differences among groups as implied by p values less than 0.05. Full model results (including F values) can be found in Supplementary Tables S1–S3.

Fish size (weight, length, condition, and TAG:ST) and concentration of various lipid classes and FAs ($\mu g \cdot g^{-1}$ of wet weight of sample for individual lipid classes and FAs; $mg \cdot g^{-1}$ for total lipids) were analyzed using linear and generalized linear models. Comparisons among cross types and across time periods, as well as interaction effects, were made using orthogonal contrasts. This method involves decomposing the degrees of freedom (df) involved with different factors into variables known as contrasts, each with a single degree of freedom and making a single comparison. In this case, there were five contrasts used in the models: contrast 1 tested whether the mean release value (i.e., the mean concentration of a given lipid class) was different from the mean recapture value; contrast 2 tested whether the mean farm value was different from the mean wild value; contrast 3 tested whether the mean hybrid value was different from the average of the means of the pure crosses; contrast 4 tested whether the difference in means between farm and wild fish was the same at both release and recapture; and contrast 5 tested whether the difference between the hybrid mean and the average of the means of the pure crosses was the same at both release and recapture (see Supplementary Table S4 for the coefficients for each contrast used in the models). LRs for evidence of the effect of a given contrast on the concentration of a given lipid/FA were calculated by exponentiating the log likelihoods for competing models (i.e., the model with a given contrast vs. the model without it) and taking their ratio. For example, an LR of 20 for contrast 1 would indicate that the observed data were 20 times more likely given a model including a variable that explicitly codes the means at release vs. recapture as different from one another, vs. a model without this variable. These LRs were then corrected for the number of model parameters using the method of Glover and Dixon (2004).

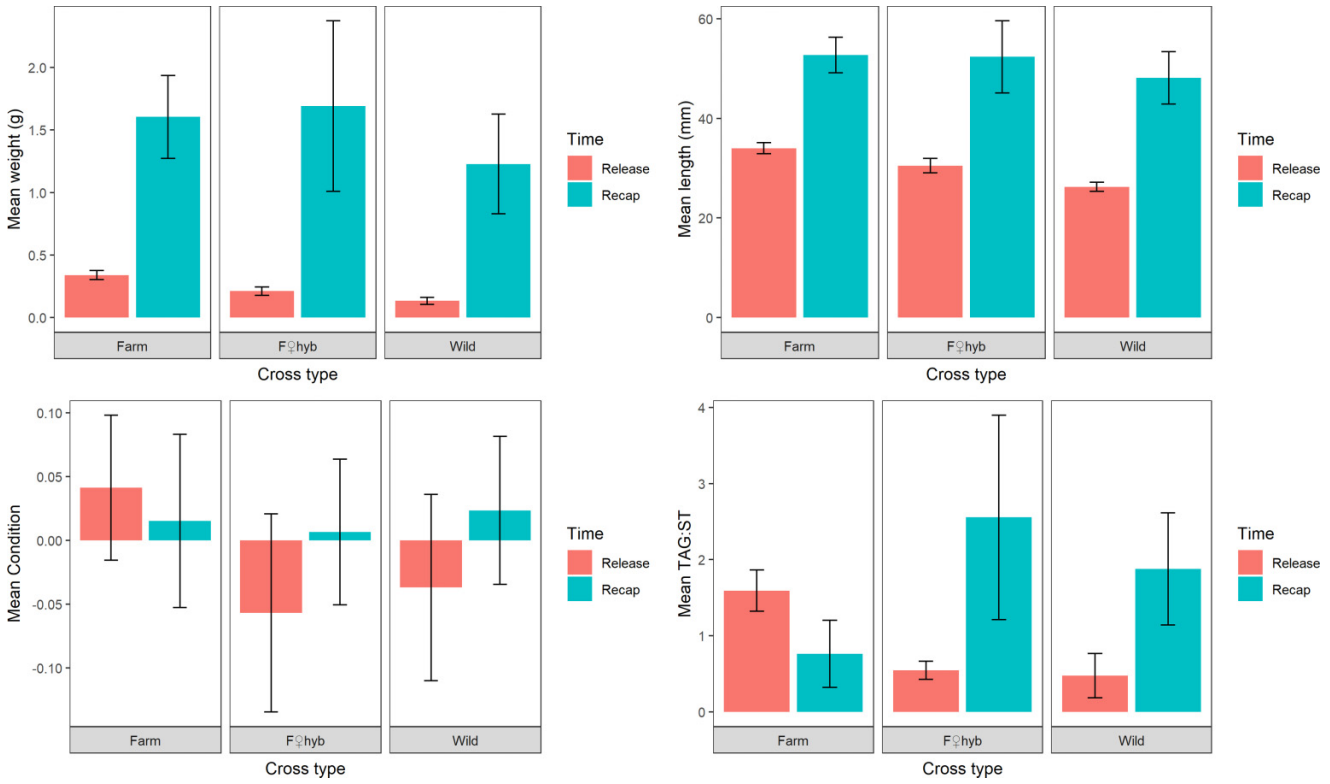
Overall differences in lipid and FA profiles (multivariate percentage data) among cross types were compared using permutational analysis of variance (PERMANOVA) using the package *vegan* (Oksanen et al. 2012) and the adonis() function therein, with 9999 permutations. The model included the same orthogonal contrasts as those used in the concentration models described above. The distance matrix used was generated using the vegdist() function and the Bray–Curtis dissimilarity index. The assumption of homogeneity of group dispersions (variances) was met, as tested with the betadisper() function in *vegan* ($F_{[2,56]} = 1.2564$, LR = 1.15, $p = 0.2926$). LRs for model parameters (orthogonal contrasts) were calculated from sums of squares and corrected for the number of model parameters using the method described in Glover and Dixon (2004). Overall differences in lipid and FA profiles (percentage data) among cross types and across time points were visualized using principal component analysis (PCA), using the PCA() function (scaled to unit variance) in the FactoMineR package (Le et al. 2008), and plotted us-

Table 1. Likelihood ratios (LRs) and *p* values for effects of five different orthogonal contrasts (C1–C5) in linear (condition only) or generalized linear models of weight, length, condition, and TAG:ST ratio among cross types (farm, wild, hybrid) and time points (release vs. recapture).

	C1		C2		C3		C4		C5	
Variable	*p* value	LR	*p* value	LR	*p* value	LR	*p* value	LR	*p* value	LR
Weight	<2.2 e-16	5.31 e+20	0.032	5.6	0.206	1.82	6.75 e-06	58 018	0.655	1.11
Length	<2.2 e-16	6.96 e+17	0.001	172.0	0.409	1.38	0.004	92.74	0.921	1.01
Condition	0.2448	2	0.295	1.77	0.192	2.51	0.186	2.65	0.420	1.44
TAG:ST	0.0006	15.72	0.999	1	0.210	1.41	3.2 e-06	567.4	0.043	3.01

Note: See Supplementary Table S1 for full model output. Condition is the residual from the regression of the natural logarithm of weight on the natural logarithm of length. C1, mean release vs. mean recapture value; C2, mean farm vs. mean wild value; C3, mean hybrid vs. average of means of pure crosses; C4, mean farm vs. wild the same at release and recapture; C5, hybrid mean vs. average of pure cross means the same at release and recapture.

Fig. 2. Comparison of mean weight (g), length (mm), condition, and TAG:ST index (±2 SE) at release and recapture for the three cross types. Condition is the residual from the regression of the natural logarithm of weight on the natural logarithm of length.



ing the fviz_pca_biplot() command in the factoextra package (Kassambara and Mundt 2020).

Results

Growth and condition parameters

Unsurprisingly, there was strong evidence for a difference in mean weight and length at release vs. recapture (contrast 1; Table 1), with fish increasing in mean overall size from release to recapture (Fig. 2). Results also indicated strong evidence for an overall difference in mean length between farm and wild fish (contrast 2), with this difference in means changing from release to recapture (strong evidence for contrast 4)—farm fish were heaviest and wild smallest, though the difference in mean length decreased from release to recapture (Fig. 2). Similarly, there was strong evidence for the difference in mean weight of farm vs. wild fish changing from release to recapture (contrast 4; Table 1). Low LRs for contrast 3 or 5 indicates a lack of evidence for an overall difference between the hybrid mean and the average of the pure crosses' means, as well as a lack of evidence of a change in this comparison from release to recapture (i.e., a lack of evidence that hybrids were not intermediate to the pure crosses). Overall, at recapture the within-cross type variability in size was much greater than at release (Fig. 2).

Farm fish also tended to have higher condition on average than the other two cross types at release, though the within-cross type variability was quite substantial and accordingly there was insufficient evidence for the effect of any contrast

on this measure, with mean values very similar across cross types and time periods (Table 1 and Fig. 2). Lastly, there was strong evidence for a difference in mean TAG:ST index between time points (contrast 1) as well as evidence for the difference in the mean TAG:ST of farm vs. wild fish changing from release to recapture (contrast 4; Table 1). Once again, a lack of evidence for contrast 3 and 5 indicates a lack of evidence for an overall difference between the hybrid mean and the average of the pure crosses' means, as well as a lack of evidence of a change in this comparison from release to recapture (i.e., lack of evidence that hybrids were not intermediate to the pure crosses). Farm fish had the highest mean TAG:ST index at release (with wild and hybrid fish having similar means), while at recapture farm fish had the lowest mean TAG:ST (Fig. 2).

Lipid/FA profile at release and recapture

The results of PERMANOVA indicate strong evidence for a difference in overall lipid/FA profile at release vs. recapture (contrast 1; $F_{[1,53]} = 46.4$, LR = 2.17 e+05, $p = 0.0001$), as well as a change in the difference between the profiles of wild vs. farm fish from release to recapture (contrast 4; $F_{[1,53]} = 17.4$, LR = 557, $p = 0.0001$), and a change in the difference between the hybrid profile vs. the mean of the pure crosses' profiles from release to recapture (contrast 5; $F_{[1,53]} = 6.7$, LR = 9, $p = 0.0021$). These results are visualized in a biplot of a PCA (Fig. 3), which shows clear clustering of samples by time point, as well as the increased overlap of cross types at recapture vs. release.

Concentrations of major lipid classes

There was strong evidence for a difference in mean total lipid concentration at release vs. recapture (contrast 1), and for a change from release to recapture in the difference between the mean hybrid concentration vs. the average of the pure crosses' mean concentrations (contrast 5; Table 2). Low LRs for contrasts 2 and 4 indicate a lack of evidence for an overall difference in mean concentration between wild and farm fish, as well as a lack of evidence for a change in this comparison over time. Pure crosses were similar to one another at release (with hybrids lower), with all cross types decreasing in their concentrations while in the river and being largely similar in their concentrations at recapture (Fig. 4).

The major lipid classes detected were PLs, STs, and TAGs. There was strong evidence for a difference in mean PL concentration at release vs. recapture (contrast 1), but insufficient evidence for an effect of any other contrast (i.e., insufficient evidence for a difference in means among cross types or a change in these differences over time; Table 2). All crosses decreased in their mean concentrations of PL while in the river, with farm fish showing a trend of decreasing the least and wild fish a trend of decreasing the most (Fig. 4). For mean TAG concentration, there was strong evidence for a change in the difference between the farm vs. wild mean over time (contrast 4), and a change in the difference between the hybrid mean and the average of the pure crosses' means over time (contrast 5; Table 2). Farm fish had the highest mean TAG concentration prior to release but the only cross type to decrease while in the river, ultimately having the lowest mean concentration at recapture (Fig. 4). Additionally, there was strong evidence for a difference in mean ST concentration at release vs. recapture (contrast 1), as well as for a difference in means between farm and wild fish overall (contrast 2; Table 2). All cross types decreased in their mean concentration of ST while in the river, and wild fish having a higher mean concentration than farm fish throughout (Fig. 4).

Concentrations of EFAs

There was strong evidence for a difference in mean linoleic acid (LNA) (18:2n-6) concentration at release vs. recapture (contrast 1), as well as strong evidence for a change in the difference between the farm vs. wild mean over time (contrast 4), and a change in the difference between the hybrid mean and the average of the pure crosses' means over time (contrast 5; Table 3). Hybrid and wild mean concentrations of LNA did not change much from release to recapture, but farm fish went from having the highest mean concentration to the lowest, and was the only cross type to show a decrease while in the river (Fig. 5). There was strong evidence for a difference in mean α-linolenic acid (ALA) (18:3n-3) concentration at release vs. recapture (contrast 1) as well as strong evidence for a change in the difference between the farm vs. wild mean over time (contrast 4; Table 3). Farm fish had the highest concentration of ALA at release while wild fish had the lowest, and though all cross types increased in their mean concentrations of ALA while in the river, wild and hybrid fish increased much more than farm fish, who had the lowest concentration of this EFA at recapture (Fig. 5).

There was strong evidence for a difference in mean concentration of ARA (20:4n-6) from release to recapture (contrast 1), but insufficient evidence for the effect of any other contrast (Table 3). All cross types had similar mean concentrations of ARA at release and increased while in the river to have similar mean concentrations to one another at recapture as well (Fig. 5). For concentration of EPA (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3), there was once again strong evidence for a difference in means from release to recapture (contrast 1), as well as evidence for a difference in overall hybrid mean concentration vs. the average of the pure crosses' means (contrast 3; Table 3). For EPA concentration, there was additionally strong evidence for a change in the difference between the hybrid mean vs. the mean of pure crosses from release to recapture (contrast 5; Table 3). All crosses decreased in their mean concentrations of these two EFAs while in the river (Fig. 5).

Discussion

We were able to quantify lipid/FA profile differences among farm, wild, and hybrid juveniles at release and recapture, along with trends of within-river change that are potentially indicative of differential foraging and storage capabilities among cross types. Cross type influenced overall juvenile lipid/FA profiles as well as several major individual lipid classes, EFAs, and FA groups.

The lack of evidence for the models including parameters specifying differences in mean concentration of total lipids

Fig. 3. PCA biplot of lipid class and fatty acid percentages for fish at release and recapture.

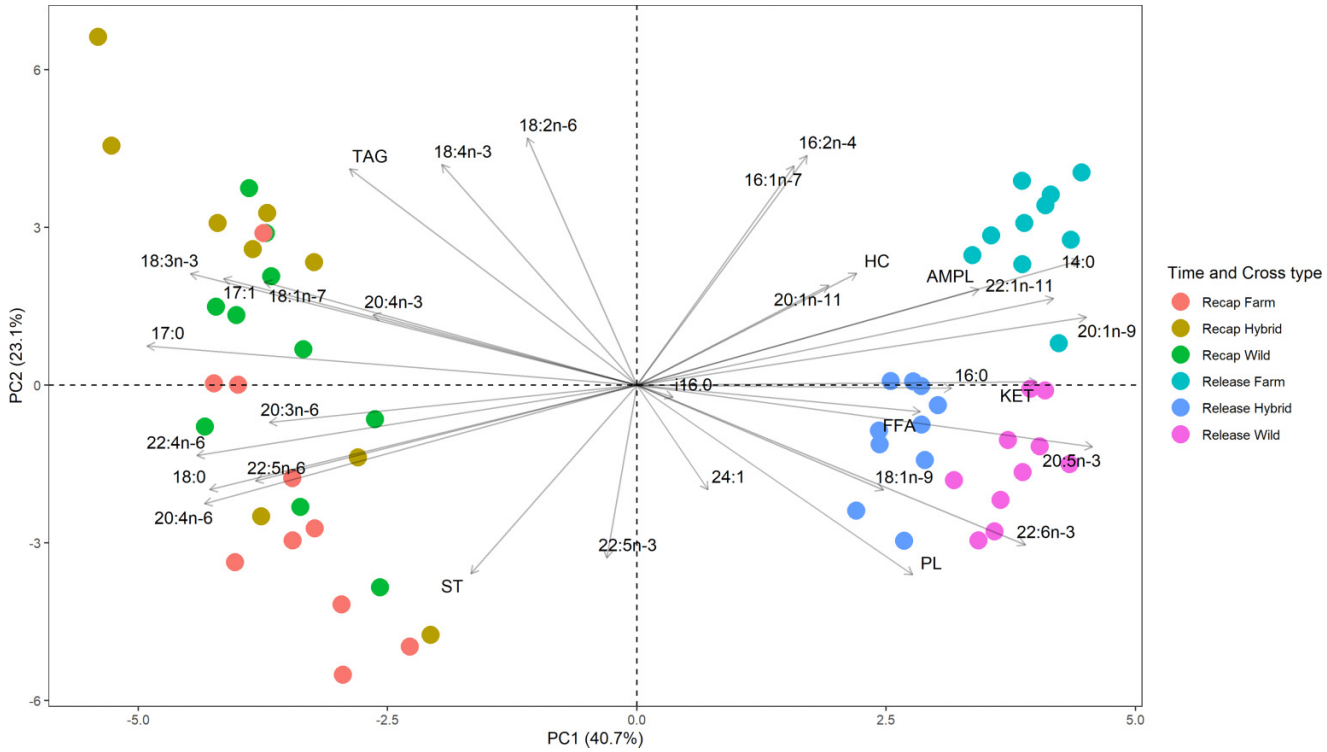


Table 2. Likelihood ratios (LR) and *p* values for effects of five different orthogonal contrasts (C1–C5; see Supplementary Table S4 for more information) in linear or generalized linear models (PL only) of concentration of total lipids (mg·g^{-1} wet weight) or major lipid classes (μg·g^{-1} wet weight) among cross types (farm, wild, hybrid) and time points (release vs. recapture).

	C1		C2		C3		C4		C5	
Variable	*p* value	LR	*p* value	LR	*p* value	LR	*p* value	LR	*p* value	LR
Total lipids	5.682 e-09	1.27 e+07	0.277	1.74	0.083	4.39	0.189	2.4	0.019	21.93
TAG	0.0277	6.08	0.954	1	0.873	1.01	3.29 e-05	5637	0.008	51.08
PL	4.979 e-13	6.8 e+12	0.087	5.55	0.112	4.74	0.175	3.23	0.734	1.08
ST	3.266 e-06	34 142	0.004	82.7	0.403	1.47	0.319	1.74	0.721	1.07

Note: See Supplementary Table S2 for full model output. Abbreviations for lipid classes are as follows: PL, phospholipid; ST, sterol; TAG, triacylglycerol. C1, mean release vs. mean recapture value; C2, mean farm vs. mean wild value; C3, mean hybrid vs. average of means of pure crosses; C4, mean farm vs. wild the same at release and recapture; C5, hybrid mean vs. average of pure cross means the same at release and recapture.

for farm vs. wild fish (overall or changing while in the river) means that our data provide insufficient evidence to corroborate or refute part of our first hypothesis (that farm fish would be predisposed to higher total lipid content than other cross types under ideal conditions, i.e., at release). Instead, results show that pure crosses are similar to one another at release while the hybrids are differentiated with a lower mean concentration of total lipids. On the other hand, the strong evidence for a change in the difference between farm vs. wild mean TAG concentration over time (contrast 4), shows that farm fish did have higher concentrations of this main storage lipid at release than the other cross types, providing support for the second part of hypothesis 1. Specific compositional data on the pre-release diet are not available (the FA profile was not analyzed); however, given the controlled environment and diet prior to release, any differences that existed up until this point in tissue lipids and FAs should not have been due to differences in diet. Instead, differences at release may have been due to (1) lingering maternal effects and (2) a genetic difference in metabolism among cross types, or a combination of these two factors. Ashton et al. (1993) found that Chinook salmon (*Oncorhynchus tshawytscha*) eggs and alevin had differences in their FA profiles that mirrored profile differences in the diets of their parents; however, fish in our experiment were sampled after approximately 6 weeks of external feeding (later than those in Ashton et al. 1993) and therefore perhaps would not have had a lingering maternal influence to the same extent. On the other hand, the time elapsed since first-feeding in our study was shorter than the 14–25 week "wash-out" period thought to be necessary to reset a lipid/FA profile in salmon (e.g., Budge et al. 2011), so it is possible a maternal diet signature could still be partially present. Given the contrast between our results and those of previous work on older fish raised in tank conditions, which

Fig. 4. Comparison of concentrations of total lipids (mg·g^{-1} wet weight) and major lipid classes (μg·g^{-1} wet weight) (±2 SE) at release and recapture for the three cross types. Abbreviations are as follows: PL, phospholipid; ST, sterol; TAG, triacylglycerol.

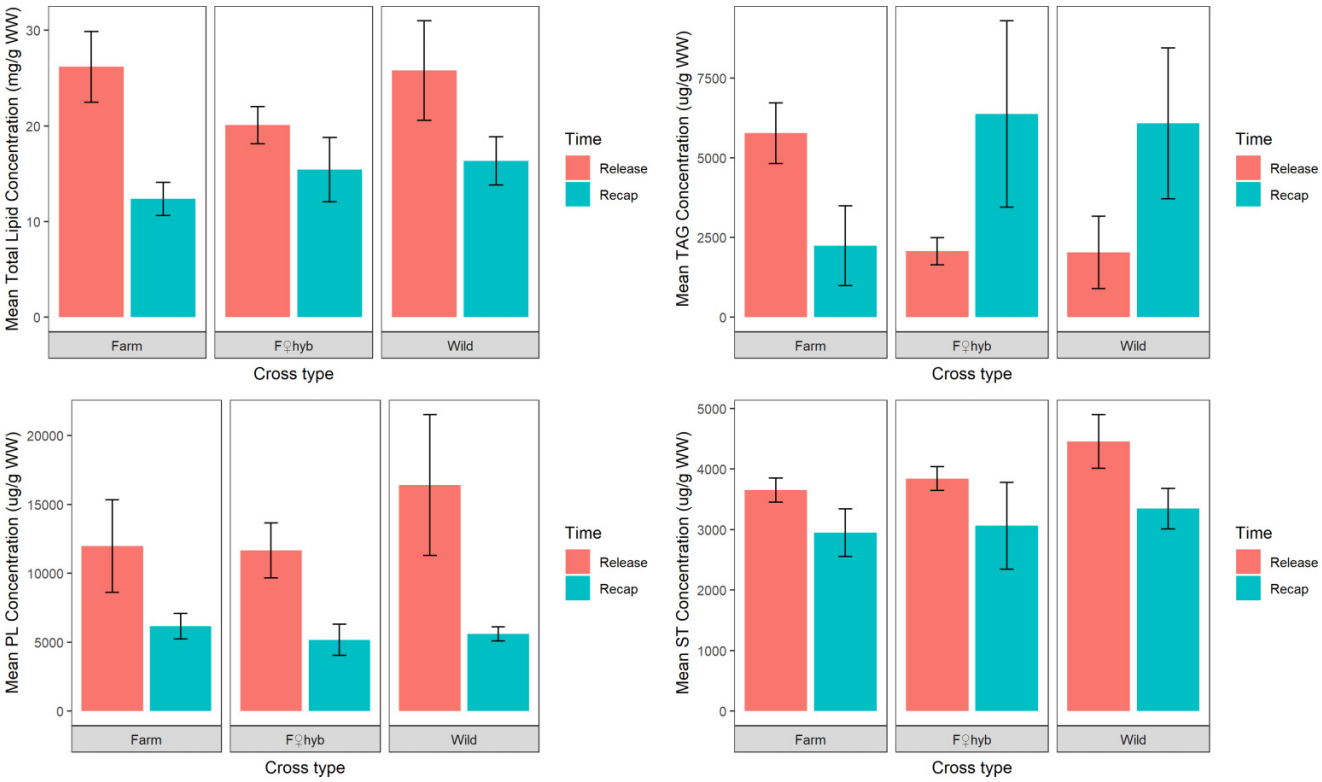


Table 3. Likelihood ratios (LR) and *p* values for effects of five different orthogonal contrasts (C1–C5; see Supplementary Table S4 for more information) in linear (LNA and ARA) or generalized linear models (ALA, EPA, DHA) of concentration of essential fatty acids (EFAs; μg·g^{-1} wet weight) among cross types (farm, wild, hybrid) and time points (release vs. recapture).

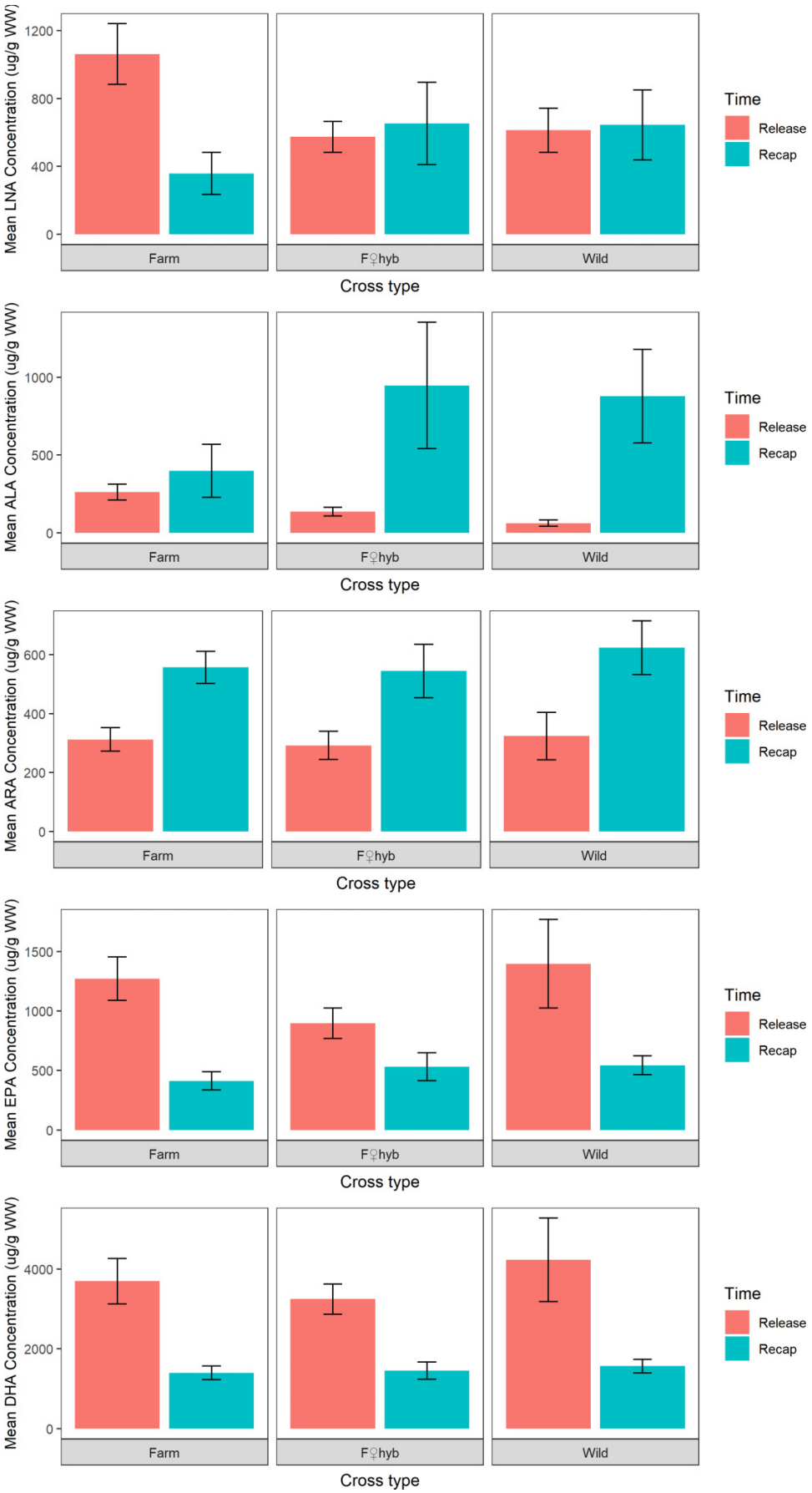
	C1		C2		C3		C4		C5	
Variable	*p* value	LR	*p* value	LR	*p* value	LR	*p* value	LR	*p* value	LR
LNA	0.005	19.32	0.330	1.42	0.388	1.32	4.8 e-05	3700.27	0.007	64.1
ALA	2.098 e-15	6.78 e+10	0.114	2.15	0.059	3.12	1.9 e-08	3.72 e+07	0.307	1.76
ARA	8.406 e-13	9.86 e+11	0.261	1.98	0.250	2.08	0.433	1.41	0.746	1.06
EPA	1.496 e-15	1.13 e+14	0.139	2.86	0.010	36.53	0.131	3.59	0.026	21.54
DHA	<2 e-16	1.29 e+20	0.117	4.1	0.059	8.95	0.609	1.18	0.567	1.23

Note: See Supplementary Table S3 for full model output. Abbreviations for EFAs are as follows: ALA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LNA, linoleic acid. C1, mean release vs. mean recapture value; C2, mean farm vs. mean wild value; C3, mean hybrid vs. average of means of pure crosses; C4, mean farm vs. wild the same at release and recapture; C5, hybrid mean vs. average of pure cross means the same at release and recapture.

found little differentiation in lipid/FA profiles among cross types (Glover et al. 2009), yet evidence of selection for higher total fat content in farm fish (Quinton et al. 2005; Powell et al. 2008), it may be that very young fish have lipid profiles that are not yet determined by their own foraging and storage capabilities and are instead influenced more greatly by maternal contribution. Regardless of the cause for the among-cross type variation in lipid and FA profiles at release, the primary use of the release data was as a baseline with which to compare recapture levels, the main interest of this study and addressed in hypothesis 2.

For our second hypothesis (that cross types would show different energy acquisition and (or) storage capabilities in the wild, with farm fish performing more poorly than wild fish and hybrids) was mainly supported by our results, with evidence for a difference in overall lipid/FA profile from release to recapture, and changes in differences among cross type profiles from release to recapture. On one hand, total lipid concentration (which is commonly used as a measure of storage energy and a potential predictor of survival for fish) was largely similar among cross types at recapture. However, on the other hand it is important to also consider the influence lipid classes may individually have on fish performance (Næsje et al. 2006). The evidence for changes in the difference between cross types in their overall lipid profiles over time, as well as changes in the difference in mean concentrations of several individual lipid classes and EFAs among cross types over time, suggests that there are indeed genetic

Fig. 5. Comparison of concentrations of essential fatty acids ($\mu g \cdot g^{-1}$ wet weight) (± 2 SE) at release and recapture for the three cross types. Abbreviations are as follows: ALA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LNA, linoleic acid.



factors influencing how fish acquire and (or) store food resources in a wild environment. Farm fish were often the most differentiated cross type and often showed a trend of lower concentrations at recapture (except for PL) when compared with the other cross types, while hybrids trended towards being closer to pure wild fish in their levels. While the strength of evidence varied for differences among cross types in their mean recapture levels of different lipids and FAs, the results are suggestive of a possible farm feeding disadvantage and a lack of one for hybrids.

A farm fish feeding disadvantage has indeed been documented before in the wild, with Orlov et al. (2006) finding that farm parr fed less actively, made more false feeding attempts, and had a higher percentage of poor quality prey items in their stomachs than wild parr. Perhaps most importantly, farm fish were the only cross type that did not increase in mean concentration of TAG (the main energy storage lipid class for fishes; Tocher 2003) while in the river, and had the lowest mean concentration of TAG of all cross types at recapture. Simply the fact that farm fish were storing relatively less TAG suggests they were acquiring less and (or) expending more while in the river. Interestingly, the fact that hybrids were more similar to wild than farm fish suggests that hybrids may not face the same severity of a feeding or storage disadvantage when it comes to TAG as pure farm fish. Nonetheless, the large amount of within-cross type variation in concentrations of hybrid TAG at recapture means that uncertainty remains in how these fish perform relative to pure crosses.

The recapture levels and patterns of change among the cross types in their concentrations of various EFAs known to be abundant in freshwater prey also point to a potential farm feeding disadvantage. Farm fish had the lowest mean concentrations of ALA (18:3n-3) and LNA (18:2n-6) at recapture, despite having the highest levels at release, and was the only cross type to decrease in concentration of these EFAs while in the river. Given that LNA is one of the most abundant ω-6 (n-6) FAs and ALA one of the most abundant n-3 FAs in freshwater invertebrates (Bell et al. 1994), the farm decrease and low recapture levels of these two EFAs may again indicate that they were ingesting fewer prey items than other cross types in this environment. Similarω to TAG concentration, hybrids were more similar to wild fish in their levels of these two EFAs, indicating that their energy storage allocation strategy seems to approximate more closely that of wild fish in a natural environment, at least over the summer months.

For concentration of DHA (22:6n-3), an FA that was found to be present only in small levels in freshwater invertebrate prey (Bell et al. 1994), farm fish exhibited a much more similar pattern of change to the other cross types while in the river compared with their changes in other EFAs. All cross types experienced a decrease in DHA from release to recapture, as expected if they were ingesting DHA-lacking prey during this time period. Therefore, their concentration of DHA at recapture would be largely reflective of what was "leftover" from release. Moreover, it makes sense that farm fish would show a pattern of change more similar to that of the other cross types for this particular EFA in the case of a feeding disadvantage, since it would be less representative of prey taken in while in the river. Alternatively, since salmonids do have a limited ability to synthesize long-chain (LC)-PUFA (polyunsaturated fatty acids) from LNA and ALA (Hixson et al. 2014; Katan et al. 2019), it could be that farm fish have a genetic advantage with regards to DHA synthesis even in the face of a feeding disadvantage, which could explain the similar recapture levels among cross types. Indeed, it has been suggested that the high amount of vegetable oils (which are typically lacking in LC-PUFAs) in commercial feed for the past ~20 years has resulted in farm fish being selected for having a higher endogenous LC-PUFA synthesis ability than wild fish when in an EFA-limited environment (Jin et al. 2020).

ARA (20:4n-6) is another one of the most abundant n-6 FAs found in freshwater invertebrates (Bell et al. 1994); however, differences in concentrations of ARA among cross types at recapture (and at release) were small, with a lack of evidence for any cross type or interaction effect. At first glance, this does not seem to be consistent with farm fish having a feeding disadvantage in the river, however interpreting this result in the context of changes in other lipids and EFAs may still provide support for the occurrence of a farm feeding disadvantage. Farm fish having relatively high amounts of ARA may be linked to the fact that they also had similar recapture concentrations of PL as other cross types (Katan et al. 2019), the lipid class that is a fish's main source of essential FAs such as ARA (Tocher et al. 2008). Even in the case of a farm feeding disadvantage resulting in less ARA being acquired through prey, farm fish may in fact be able to better synthesize ARA from n-6 precursors and PLs endogenously. The results of Jin et al. (2020) support this as they found that wild salmon growth was positively influenced by a diet supplemented with PL but the growth of farm salmon was not. Indeed, though there was a lack of evidence for a difference among cross types in mean PL concentration, farm fish did have a slightly higher mean concentration than the other cross types at recapture and exhibited a smaller decrease while in the river than wild fish. Interestingly, with PL and ARA, the within-river trends of hybrids seem to be more similar to those of farm rather than wild fish, though again a lack of evidence for differences among cross types indicates that all cross types were largely similar to one another in these concentrations and the trends observed should be further investigated in the future.

In addition, while EPA was found to be one of the most abundant n-3 FAs in freshwater prey invertebrates (Bell et al. 1994), the fact that concentrations of EPA were similar for all cross types at recapture is perhaps not surprising and does not exclude the potential for a difference in feeding efficiency among cross types. EPA has been suggested to be under stricter physiological control than non-EFAs (Budge et al. 2011) and is the more bioactive EFA when compared to DHA (Horn et al. 2019), so perhaps levels of EPA would vary less among cross types, even when feeding and diet are more variable. The fact that there were larger differences in mean concentration of EPA among cross types at release compared to recapture is perhaps supportive of this idea. In addition, farm fish, if they did indeed have a feeding disadvantage, once again may have been able to compensate for a reduced EPA intake by higher endogenous EPA synthesis (as suggested by

Jin et al. 2020), though farm fish still had the lowest mean concentration at recapture.

Ultimately, if a farm fish feeding disadvantage did indeed exist, the question remains as to why farm fish were not demonstrably smaller than other cross types at recapture (a result that was also reflected in the findings of Crowley et al. (2022) in comparing sizes of all fish recaptured). Farm Atlantic salmon have higher feed consumption and conversion rates than wild fish (Thodesen et al. 1999), which may mean that even if farm fish were less able to capture food in a natural environment than their wild counterparts, their higher feed efficiency ratio may have at least partially made up for this (assuming their higher feed utilization efficiency still occurs under wild conditions). This phenomenon might also explain the results of the hybrid fish, which in this study showed some similarities in lipid/FA profiles to wild fish at recapture and a closer mean size to farm than wild fish. If hybrid fish had a higher feed conversion rate than pure wild fish along with better foraging success than pure farm fish, it is possible this could result in the observed trend towards larger size. To better elucidate this, future work could focus more intensively on hybrid response, with greater sample size at release and recapture to compare hybrid responses to those of pure crosses. In addition, it is important to compare the growth of these farm fish in the wild to their own potential growth under culture conditions. Given that the growth differential between farm and wild fish in a culture environment is typically two to three times or greater (Glover et al. 2018), the much smaller growth differential we observed here may indicate that farm fish had a growth disadvantage while in the river, at least compared to their growth potential in an aquaculture environment. Of course, as there is evidence for selection against the fastest-growing farm fish in the wild (Glover et al. 2018), it is possible that the largest farm fish were removed by selection and were thus not recaptured at all. If this were true, the size of the hybrids must also be considered, as it could be that larger hybrids that were also removed by selection, or alternatively size-based selection may have operated less strongly on hybrids.

In addition to the potential for genetic feeding differences among cross types affecting lipid levels, it is possible that differences in lipid and FA content among cross types may have been at least partially influenced by genetic differences in energy allocation. For young fish, when energy is limited there is a trade-off between allocating energy for storage (to avoid starvation) and allocating energy to growth (to escape gape-limited predators; reviewed by Post and Parkinson 2001). Farm fish have been heavily selected for faster growth, with research showing contradictory results regarding if farm fish store more lipid than wild fish (Johnston et al. 2006; Glover et al. 2018). However until now, whether cross types exhibit differences in lipid storage under common garden conditions has remained untested in a wild environment. If, when energy-limited (whether due to the environment, a feeding disadvantage, or both), farm fish still allocate a relatively higher percentage of energy to growth vs. storage, they would be likely to have relatively lower lipid stores than wild fish—the result we observed here for TAG. Differences in lipid storage among cross types could also be related to adaptation (or lack thereof) to seasonal changes in prey availability. Given that parr in the wild are vulnerable to energy-related mortality during the winter (e.g., Finstad et al. 2004), whereas farm fish have consistent access to food year-round, it may be that selection for high lipid storage before the winter has operated more strongly on wild parr than farm parr. Finally, a study on Norwegian 0^+ Atlantic salmon parr showed that there are differences in lipid storage at both the latitudinal scale and among rivers located in close proximity to one another (Berg et al. 2009), so it is possible that lipid storage of farm fish differed from wild fish due to their ancestral geographic backgrounds (St. John River vs. Garnish River, respectively). Indeed, previous research in fish has shown that at higher latitudes, a shorter growing season combined with the increased importance of faster growth and higher energy storage for overwinter survival can result in selection for faster growth in the warm season (Conover and Present 1990). Though the Garnish River is only at a slightly higher latitude ($\sim 2^\circ$) than the Saint John River, the lower energy storage of farm fish could be in part due to this latitudinal difference and (or) other unexplained geographic/landscape factors. Future work could attempt to disentangle the effects of domestication vs. ancestry by comparing lipids and FAs of wild, farm, and hybrid fish originating from the same river.

The implications of differential patterns of lipid and FA levels among cross types on their performance and survival may present themselves in the short- and (or) long-term. We make the assumption that the subsample of recaptured fish analyzed here for lipid and FA content is reflective of overall cross type patterns for all fish recaptured in the study by Crowley et al. (2022). This assumption seems reasonable given that sampling accounted for within-cross type variation (fish from each family were included in lipid analyses), and in addition, among-cross type comparisons of growth and condition reported here mirror those found for all recaptured fish in Crowley et al. (2022). Thus, it seems reasonable to discuss the lipid and FA results reported here in the context of the survival/recapture results reported in Crowley et al. (2022). They show that for all fish recaptured at site 3 (the study site investigated in this manuscript), farm fish had lower recapture odds than wild fish, though not substantially so. Therefore, it would seem that any disadvantage farm fish had in terms of their lipid and FA intake and (or) storage did not immediately have a great influence on their relative survival over the summer months. Indeed, impacts of lipid and FA storage on survival would likely become most apparent over the harsh winter months. Farm fish with their lower levels of TAG would be at greater risk for energy-related death during the winter period (e.g., Finstad et al. 2004). Also, while parr typically reduce their feeding activity during winter to save energy and avoid predation (Metcalfe and Thorpe 1992), farm fish may be at greater risk of predation during winter if they need to engage in relatively more active feeding periods than wild fish to try and sustain their energy levels. Farm salmon have also been shown to be inherently less risk-averse than wild salmon (Fleming and Einum 1997; Islam et al. 2020; Solberg et al. 2020), potentially compounding this predation risk. Given that farm fish had lower energy storage than wild fish but were not much different in size, allocating more en-

ergy to growth would likely still not confer a large predation-avoidance advantage to this cross type, though they would still have the starvation risk due to lower storage.

While farm fish appear to be the cross type showing the starkest differences in their lipid and FA content in relation to the other cross types, it is likely the hybrids that merit the most consideration in a conservation context given that escaped farm fish exhibit lower reproductive success than wild fish (Fleming et al. 1996; Fleming et al. 2000), and therefore hybrid offspring are more likely to occur than pure feral farm offspring. Our results show that hybrids are often very similar to wild fish in their percentages and concentrations of various lipid classes and FAs at recapture, and can show substantial within-cross type variation. Therefore, results for hybrids do not indicate any obvious disadvantage in terms of feeding, storage, or condition over the summer, though Crowley et al. (2022) show that the hybrid class used in this paper had the lowest recapture odds of all cross types. Once again, it may be that lipid and FA intake and storage for these hybrids does not have a substantial impact on their survival over the short term in favourable conditions. However, given that our results do suggest that farm genes may confer a disadvantage for energy storage and certain essential FAs (LNA, ALA), it may be that these maladaptive genes are only expressed under certain environmental conditions (e.g., Glover et al. 2018) and may become more apparent over the winter. Overall, future work is needed to further investigate comparisons of lipid and FA content among cross types in the wild over longer time scales and a range of environmental conditions.

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Data availability

Data analyzed during this study are available from the Zenodo repository (doi: 10.5281/zenodo.7038047).

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IAF and IRB conceived the study. SEC, IAF, IRB, AMM, SJD, and SSI acquired data. SEC, IAF, IRB, AMM, and CCP contributed to analysis and interpretation. SEC drafted the manuscript, and all authors contributed to critical revisions. All authors have seen and agreed with the contents of the manuscript and have agreed to be listed as authors.

Competing interests

The authors declare there are no competing interests.

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Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjfas-2021-0326.

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