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Comparative transcriptomics reveals domestication-associated features of Atlantic salmon lipid metabolism

Domestication and lipid metabolism in salmon

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Abstract

Domestication of animals imposes strong targeted selection for desired traits but can also result in unintended selection due to new domestic environments. Atlantic salmon was domesticated in the 1970s and has subsequently been selected for faster growth in systematic breeding programmes. More recently, salmon aquaculture has replaced fish oils (FO) with vegetable oils (VO) in feed, radically changing the levels of essential long-chain polyunsaturated fatty acids (LC-PUFA). Our aim was to study the impact of domestication on metabolism and explore the hypothesis that the shift to VO-diets has unintentionally selected for a domestication-specific lipid metabolism. We conducted a 96-day feeding trial of domesticated and wild salmon fed diets based on FO, VO or phospholipids (PL), and compared transcriptomes and fatty acids in tissues involved in lipid absorption (pyloric caeca) and lipid turnover and synthesis (liver). Domesticated salmon had faster growth and higher gene expression in glucose and lipid metabolism compared to wild fish, possibly linked to differences in regulation of circadian rhythm pathways. Only the domesticated salmon increased expression of LC-PUFA synthesis genes when given VO. This transcriptome response difference was mirrored at the physiological level, with domesticated salmon having

higher LC-PUFA but lower 18:3n-3 and 18:2n-6 levels. In line with this, the VO diet decreased growth rate in wild but not domesticated salmon. Our study revealed a clear impact of domestication on transcriptomic regulation linked to metabolism and suggests that unintentional selection in the domestic-environment has resulted in evolution of stronger compensatory mechanisms to a diet low in LC-PUFA.

Keywords Wild salmon; Domestication; Vegetable oil; Circadian regulation; Long-chain polyunsaturated fatty acids; Transcriptomics

1. Introduction

The genetics and physiology of domesticated animals is heavily influenced by the initial domestication process, the captive environment, followed by persistent targeted selection for desirable animal production traits such as faster growth and delayed sexual maturation (Mueller & Diamond, 2001; Zeder, 2015). In addition, domesticated animals evolve "domestication syndromes" linked to unintended selection due to the new domestic environments (Zeder, 2015). One such collateral variable that changes dramatically with domestication is feed and feeding regimes. Unlike wild animals which rely on opportunistic hunting and foraging for different foods, domesticated animals often get standard artificial diets with balanced nutritional levels and regular feeding intervals. This dietary change has likely influenced standard metabolism in domesticated animals (Bicskei, Bron, Glover, & Taggart, 2014; López et al., 2019).

Atlantic salmon was domesticated in 1971 and is considered a pioneer aquaculture species (Harache, 2002). Since its initial domestication, systematic breeding programs has aimed to improve traits such as faster growth, delayed sex maturation, higher feed conversion rate, as well as many other traits important for animal production (Gjedrem, Gjøen, & Gjerde, 1991; Powell, White, Guy, & Brotherstone, 2008; Quinton, McMillan, & Glebe, 2005). And as with most

domesticated animals, unintentional selection is hypothesized to have shaped the physiology of domesticated salmon, especially related to adaptations to new feed composition and feeding regimes.

In the wild, salmon is an opportunistic predator and its diet consists mostly of invertebrates in rivers, and crustaceans and small fish after they migrate to the sea (Hansen & Quinn, 1998; Renkawitz & Sheehan, 2011). Their natural prey, both in freshwater and seawater, often contain substantial amount of long-chain polyunsaturated fatty acids (LC-PUFA) including docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) or arachidonic acid (ARA, 20:4n-6) (Bell, Ghioni, & Sargent, 1994). Domesticated salmon on the other hand have 'unlimited' access to food and their diet is composed of proteins from fish and plant meal, as well as a lipid source. Up until the late 1990s this lipid source was mainly fish oil (FO) from wild fisheries, which contained high levels of LC-PUFA. However, during the last two decades the FO have gradually been substituted with vegetable oils (VO) which naturally devoid of LC-PUFAs. The LC-PUFAs are important for fish because they are key components of cell membranes, they regulate cell membrane fluidity, function as precursors for eicosanoid production, and are important components of neural tissues (Sargent, Tocher, & Bell, 2002; Douglas R. Tocher & Glencross, 2015). This is also reflected in the ability of domesticated salmon to increase endogenous synthesis of LC-PUFA when given VO-rich diets (Datsomor et al., 2019; Stubhaug et al., 2005; Zheng, Tocher, Dickson, Bell, & Teale, 2004). Another major difference between wild and domesticated diets is the levels of dietary phospholipids (PLs). PLs are important for growth and development of salmon especially for early developmental stages (Poston, 1990; Taylor et al., 2015), and dietary PL are more efficient at delivering LC-PUFA into the circulatory system and ultimately the cells compared to neutral lipids such as triacylglycerols (Cahu et al., 2009; Y. Olsen et al., 2014b). The efficiency of utilizing dietary PL could therefore also be different between wild and domesticated fish, however this has never been investigated.

In this study we use a comparative approach to study domestication-associated evolution of transcriptomic and lipid metabolism phenotypes in salmon. Specifically, we hypothesized that the shift to VO-diets has selected for a domestication-specific lipid metabolism phenotype to compensate dietary shortage of LC-PUFA. We approach this question by feeding domesticated and wild salmon contrasting diets either rich in FO, VO or PL, and then perform comparative analyses of transcriptomes and fatty acids in tissues involved in lipid uptake (pyloric caeca) and endogenous synthesis (liver). Our experiment allows us to identify metabolic pathways that respond differently in domesticated compared to wild salmon and reveal novel lipid metabolism features in domesticated salmon putatively linked to unintentional selection and adaptation to a typical domestic VO-diet with low LC-PUFA.

2. Material and methods

2.1 Fish, diets and experimental plan

The domesticated salmon used in this study was a fast-growing strain (AquaGen AS, Trondheim, Norway) which have been selected for faster growth and delayed sexual maturation for 11 generations since 1971. The selective breeding of domesticated salmon has doubled the growth rate and reduced the production cycle for the fish by ~1.5 years compared with the wild origin (Thodesen, Grisdale-Helland, Helland, & Gjerde, 1999). The previous generations of domesticated salmon were always fed standard commercial diets available at the time. This means that the fish were given a freshwater diet with only marine ingredients at early developmental stages but have experienced a gradual switch in seawater diet from FO to VO since the 1990s. The wild salmon stain was purchased from Haukvik Smolt AS, a wild salmon bank used for the preservation of wild Norwegian Atlantic salmon located in Trødelag, Norway. Wild salmon were originally sourced from five independent lines caught in Lærdal river in Norway in 2011 and 2012. Eggs from these fish were grown in the hatchery facility for one or two generations. During this time the

wild fish were kept in outdoor tanks with a transparent roof and water that had same temperature as river. The wild fish were fed a standard "Nutra Sprint" diet (Skretting AS) at fry and early juvenile stage (https://www.skretting.com/en/feeds-services/nutra-sprint/1585246) which satisfied their nutritional requirements. Afterwards, the juvenile the fish was given "Vitalis Røye" diet from Skretting AS (https://www.skretting.com/nb-NO/produkter/vitalis-r-ye/476027), which has an EPA + DHA content of 19-20 % of the fat, and 70 % of the ingredients are of marine origin. Approximately 1300 newly fertilized eggs of domesticated (AquaGen) and 1300 of wild salmon (a mixture of the 2nd and 3rd generation from the five independent lines) were transported to hatching tanks in Ervik hatchery (Frøya, Norway). The water temperature of hatching tanks for domesticated and wild eggs were slightly different to ensure that both strains hatched and start-feed at the same time.

When the yolk sac was depleted, the wild and domestic salmon strains were separated into 12 tanks (2 fish strains x 3 diet treatments x 2 replicate tanks) with 100L water and 200 fish per tank. Feeding was initiated from the next day. The experimental tanks were randomly distributed in the hatchery and the fish of each tank were reared under same temperature, continuous light and fed 24h continuously feed every day. The fish were given three contrasting diets, either a fish oil (FO) diet high in LC-PUFA, or a plant and vegetable oil (VO) enriched diet low in LC-PUFA, or a marine phospholipid (PL) enriched diet with medium level of LC-PUFA but rich in PL (Table 1). All three diets were given to the fish from start feeding up to 94 days. To ensure sufficient DHA and EPA levels the PL used to prepare PL diet was a 50/50 mixture of krill oil (Aker BioMarine AS, Lysaker, Norway) and herring roe oil (kindly provided by Erik Løvaas from Marine BioExploitation AS, Tromsø, Norway). The diets were produced by Sparos AS (Olhão, Portugal). The composition of the diets is shown in Supplementary Table 1. FO diets have higher DHA and ARA than PL diet, while the EPA composition was similar between the two diets (Table 1). VO diet contains higher 18:3n-3 and 18:2n-6 but lower DHA, EPA and ARA compared to the other

two diets. Other components except the lipid source were identical between the three diets (Supplementary Table 1).

Fish weight (n >= 20 from each group) was measured at 0, 48, 65, 78 and 94 days post initial feeding (dpf). The fish were euthanized by exposure to 200 mg/ml Benzoak vet. (ACD Pharmaceuticals AS, Oslo, Norway) before measuring weight. Fish for gene expression and fatty acid measurements were sampled at 94 dpf, when domesticated fish reached an average weight of 4.5g and wild salmon was 2.6g. Fish samples were immediately put in sterile pertri dishes after weight measurement and dissected under a dissecting microscope. The pyloric caeca and liver tissues were immediately transferred into 2mL Eppendorf tubes, and either filled with RNAlater and put on ice for RNA isolation, or frozen in dry ice for lipid extraction. Tissues for RNA isolation were kept at 4°C for 24h to allow sufficient penetration of the solution into the tissues, and then kept at -80 °C until RNA extraction. Tissues for lipid extraction were directly transferred to -80 °C.

2.2 RNA isolation and transcriptomic sequencing

Four individuals per group (n = 4, 2 fish per tank x 2 replicate tanks) were used for RNA isolation. The RNA extraction was performed with the RNeasy Plus Universal Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The concentration and integrity of RNA were determined by a Nanodrop 8000 (Thermo Fisher Scientific, Waltham, USA) and a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA), respectively. All RNA samples had RNA integrity (RIN) values higher than 8, which is sufficient for RNA sequencing. Sequencing libraries were prepared with a TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, USA) according to the manufacturer's protocol. Libraries were sequenced using 100bp single-end mRNA sequencing (RNA-seq) on Illumina Hiseq 2500 (Illumina, San Diego, CA, USA) at the Norwegian Sequencing Centre (Oslo, Norway).

The method for handling RNA-sequencing (RNA-seq) data has been described in detail in previous studies (Gillard et al., 2018; Jin et al., 2018). In brief, read sequences were quality trimmed using Cutadapt (v1.8.1) before being aligned to the salmon genome (ICSASG_v2). Raw gene counts were generated using HTSeq-counts (v0.6.1pl) and the NCBI salmon genome annotation (http://salmobase.org/Downloads/Salmo_salar-annotation.gff3).

2.3 Lipid class separation and fatty acid analysis

Total lipid was extracted from two individual fish from each tank by using the method of Folch, Lees, and Stanley (1957). Extracted total lipid was then applied onto 10 x 10 cm silica plates (Merck, Darmstadt, Germany) and separated by using methyl acetate/isopropanol/chloroform/methanol/0.25% KCl (25:25:25:10:9, by vol) for polar lipids and hexane/diethyl ether/glacial acetic acid (80:20:2, by vol) for neutral lipids (R. E. Olsen & Henderson, 1989). To avoid the oxidation of fatty acids, the plates were exposed to iodine vapor to visualize the lipid class for fatty acids analysis (K. S. Li & Olsen, 2017). Lipid bands of phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn) and triacylglycerol (TAG) were separately scrapped out into 10mL glass tubes. Fatty acid methyl esters (FAME) of each lipid class were prepared by acid-catalyzed transesterification at 50˚C for 16 hours (Christie, 1973) before quantified by a Agilent 7890B gas chromatograph with flame ionization detector (Agilent Technologies, Santa Clara, CA).

2.4 Data analysis

The analysis of RNA-seq data was performed in R (v3.4.1) (Team, 2013). Only genes with a minimum counts level of at least 1 count per million (CPM) in more than 25% of samples from each tissue were kept for differential expression analysis. Differential expression was tested

separately on pyloric caeca and liver using R package edgeR (Robinson, McCarthy, & Smyth, 2010). A full interaction model described in edgeR manual (Diet + Strain + Diet x Strain) was used in each tissue separately to find differential expressed genes (DEGs) between wild and domesticated salmon under any dietary treatments. DEGs were determined if a gene has q value (false discover rate adjusted p value) < 0.05 and absolute log2 fold change (|Log2FC|) > 1 between wild and domesticated salmon. KEGG ontology enrichment analysis (KOEA) was conducted using edgeR. Significant values (p<0.05) were generated based on hypergeometric test where the number of DEGs was compared to total genes annotated to each KO term. A test for enrichments of transcription factor binding sites (TFBS) motifs in the promoter regions (between -1000bp and 100bp from transcription starting sties) of salmon genes was done by using a hypergeometric test in the R package SalMotifDB, which is interacting with a database of transcription factor binding sites for salmonids (https://salmobase.org/apps/SalMotifDB) (Mulugeta et al., 2019).

To further investigate diet specific effect on gene expression between wild and domesticated salmon, samples of different diet were separated to be used for testing differential expression of genes between wild and domesticated salmon under each diet. Same cut-off was used (q<0.05 & |Log2FC| > 1) to identify DEGs. For visualize expression levels between different genes and tissues, normalized counts in the form of transcripts per million (TPM) values were generated. Raw gene counts were first divided by their mRNA length in kilobases to normalize for transcript length, and then divided by the total number of counts from each library to normalize for sequencing depth (Jin et al., 2018).

Statistical analysis of fish weight and fatty acids composition was also performed in R. Two-way ANOVA with Tukey HSD post hoc test was used to test the effect of strain and diet on fish weight and fatty acids composition. Samples of different lipid class, tissue or sampling date were analysed separately. Differences were considered significant when $p < 0.05$.

2.5 Ethical statement

All welfare and use of experimental animals were performed in accordance with the Norwegian Animal Welfare Act 2010. In addition, all personnel involved in rearing, handling and sampling the fish that had undergone training approved by the Norwegian Food Safety Authority.

3. Results

3.1 Growth and development

The domesticated salmon was significantly larger than wild salmon at all sampling times (Figure 1 and Supplementary Table 2). At the end of the trial (94 days), domesticated salmon reached an average weight of 4.5g, while wild salmon had a mean weight of 2.6g. There were no significant differences in weight between domesticated salmon fed FO, VO and PL enriched diets. The growth of wild fish appeared more sensitive to different diets, with VO-fed fish being smaller than PL-fed fish at day 65 (p = 0.03) and day 94 (p = 0.02). FO-fed wild salmon showed intermediate weights, but their weights were not significantly different from either FO or PL diets.

3.2 Transcriptomic differences between domesticated and wild salmon

On average 20 million reads were generated from each sample (min: 12M reads, max:32M reads), with ~85% of the reads mapping to the salmon genome. Out of 81597 annotated loci, 28980 and 24119 genes passed this filtering criteria in pyloric caeca and liver, respectively. Principle component analysis (PCA) on Log2 CPM of the top 1000 most variable genes, identified a clear separation of domesticated and wild salmon in both pyloric caeca and liver (Figure 2).

Differential expression analysis has revealed 187 differential expressed genes (DEGs) in pyloric caeca and 379 DEGs in liver between wild and domesticated salmon (Supplementary Table 3). By mapping DEGs to the KEGG database of metabolic pathways, we have identified 17 pathways that were significantly enriched (p<0.05) in pyloric caeca, while 11 pathways were enriched in liver (Figure 3 A). The DEGs in pyloric caeca were enriched in pathways for glycerophospholipid, glycosphingolipid and glycosaminoglycan metabolism, which are known to be major component of cell membrane (Figure 3 A). A number of cell-signalling pathways were also enriched, including phosphatidylinositol signalling, calcium signalling, apelin signalling, C-type lectin receptor signalling and GnRH signalling pathways. In liver, the DEGs were enriched in metabolic pathways including linoleic acid, glycolysis and gluconeogenesis, fructose and mannose, cysteine and methionine, retinol metabolism pathways (Figure 3 A).

Transcription factor binding site (TFBS) motif enrichment analysis on promoters (-1000bp to 200bp from TSS) of DEGs for each tissue resulted in 16 significant (p<0.005) enriched motifs in pyloric caeca and 128 enriched motifs in liver (Supplementary Table 4). The most enriched motif in pyloric caeca was BHLHB2, which is known to be involved in circadian regulation (Figure 3 B) (Dunlap, 1999). Several other enriched motifs are associated with intestinal development and cell differentiation, including ETV2 (Jedlicka & Gutierrez-Hartmann, 2008), ATOH1 (Shroyer et al., 2007), GR (Lebenthal & Lebenthal, 1999), GATA-1 (Kanki et al., 2017). The most enriched TFBS motif in liver was a CLOCK motif, which is a predicted binding motif for the master regulator of the circadian clock (Dunlap, 1999). Similar to pyloric caeca, BHLHB2 motif was also identified in the top 10 most enriched TFBS motifs in liver. In addition, three lipid metabolism related motifs (RXRA, PPARG, PLAGL2) populated the top-10 enriched TFBS list (Tontonoz, Hu, & Spiegelman, 1994; Van Dyck et al., 2007).

To further investigate differences in expression of genes linked to circadian rhythm between wild and domesticated salmon, we compared the expression of key genes encoding circadian clock

related transcription factors *clock*, *nr1d1*, *bmal1*, *bhlhb2*, *per*, and *cry* (Figure 3 C and Supplementary Figure 1). A systematic difference in circadian clock gene expression was observed between livers of wild and domesticated salmon (Figure 3 C and Supplementary Figure 1), although not all genes were significant regulated at $q<0.05$. Nevertheless, the regulators (*cry2-c*, *nr1d1-a, per1-a, bhlhb2-d* and *nr1d1-a* genes) acting as suppressors of the master regulators of circadian rhythm (CLOCK/BMAL) were consistently lower expressed in domesticated salmon compare to wild (Figure 3 C). However, similar expression levels of *clock* (logFC=0.2, q=0.7) and *bmal1* (logFC=-0.1, q=0.8) genes which encodes master regulator were found between domesticated and wild salmon (Figure 3 C and Supplementary Figure 1). No difference in circadian clock gene expression was observed between pyloric caeca of wild and domesticated salmon.

3.4 Differential regulation of lipid metabolism genes between domesticated and wild salmon

To better understand the effect of diets on gene expression differences between domesticated and wild salmon, we compared gene expression separately between domesticated and wild under each diet. In pyloric caeca, a total number of 230 DEGs were identified between domesticated and wild salmon with FO diet, 164 DEGs were found with VO diet and 689 DEGs were found with PL diet (Supplementary Table 5). Out of these DEGs, only 8 genes were involved in lipid metabolism pathways. This includes *ptdss2* genes of phosphatidylserine synthesis, which was significantly ($q<0.05$ & $|Log2FC|>1$) higher expressed in domesticated salmon regardless of the dietary treatment (Figure 4). Two phosphatidylethanolamine synthesis genes, *pcyt2c-a* and *pcyt2c-b* were both higher expressed in domesticated than wild salmon when fed FO or VO diet, while no difference in gene expression was found when the fish were given PL diet. On the other hand, *etnk2-a* gene involved in phosphatidylethanolamine synthesis, was significantly higher expressed in domesticated compared to wild salmon only when the fish was given PL diet. Feeding with VO induced key genes in LC-PUFA synthesis pathway (*fads2d5* and *fads2d6a,* see Figure 4*)* in both

domesticated and wild salmon. However, no expression difference was observed for these two genes, or any other LC-PUFA synthesis genes between domesticated and wild salmon for any dietary treatment (Figure 4 and Supplementary Table 5).

The number of DEGs between liver of domesticated and wild salmon under each diet was 591 (FO), 179 (VO) and 243 (PL) (Supplementary Table 5). Liver had more DEGs involved in lipid metabolism (28) compared to pyloric caeca (8). Four DEGs in liver had significantly (q<0.05 & |Log2FC|>1) higher expression in domesticated compared to wild salmon under VO diet, but not under FO or PL diet (Figure 5 B). This includes genes with key functions in LC-PUFA synthesis (*fads2d5*, Log2FC = 1 & q = 0.02), gene involved in acyl-CoA synthesis (*acsbg2b-b*, Log2FC = 1.4 & q = 0.03*)*, and fatty acid transport (*fabp7b*, Log2FC = 3.6 & q = 0.02). Although not significant, domesticated salmon fed VO diet also had higher expression of *fads2d6a* (Log2FC = 0.7 & q = 0.2) and *srebp1d* (Log2FC = 0.8 & q = 0.2) compared to wild salmon fed same diet, while the expression difference of the two genes was negligible when the fish was under FO or PL diet (Figure 5 A & B). A key gene involved in conversion of lipids to energy, *cpt1aa* was lower expressed (Log2FC = -1.2 & q = 0.01) in domesticated salmon when fed VO diet. The regulator of fatty acid metabolism *pparg-b* was consistently higher expressed in domesticated compared to wild salmon under all diets, but only significantly different for salmon fed FO diet (Figure 5 A).

In addition to the DEGs of fatty acid metabolism, 5 DEGs involved in phospholipid, cholesterol and triacylglycerol metabolism were found between domesticated and wild salmon (Figure 5 C). This included the *apoa1-b* gene involved in lipoprotein synthesis and lipid transport, which was strongly higher expressed in domesticated salmon than wild, regardless of dietary treatment (Log2 FC > 3 & q<0.001, see Figure 5 C and Supplementary Table 5). A key gene involved in synthesis of bile acid (*cyp7a1-a*), which is responsible for removal of cholesterol in liver, was higher expressed in domesticated salmon when given PL diet. Gene *ptdss2* involved in synthesis of phosphatidylserine, which is a major phospholipid in salmon, was higher expressed in

domesticated salmon than wild fed VO diet (Log2 FC = 1.9, q = 0.0008), though similar trend was also found when the fish were given FO (Log2 FC = 1, q = 0.09) or PL diet (Log2 FC = 0.9, q = 0.2). The expression of *hsl* gene involved in hydrolysing triacylglycerol (stored fat) to diacylglycerol, and diacylglycerol to monoacylglycerol was generally higher expressed in domesticated salmon than wild. On the other hand, the expression of *mgll* involved in hydrolysing monoacylglycerol into free fatty acids was lower expressed in domesticated salmon. (Figure 5 C). In conclusion, the direct comparison of the transcriptomes of domesticated and wild salmon suggests that domestic salmon have boosted expression of genes involved in many aspects of lipid metabolism such as transport, endogenous synthesis and conversion of lipids and fatty acids in both gut and liver (Supplementary table 5).

To further investigate differences in the plasticity of fatty acid metabolism between domesticated and wild salmon, we analysed differences in putative compensatory shifts in gene regulation under diets with low (VO) vs high (FO) levels of LC-PUFA for wild and domesticated salmon separately. These analyses identified 38 DEGs in domesticated and 2 DEGs in wild salmon (Supplementary Table 5). However, only DEGs in domesticated salmon (9 genes) were linked to lipid metabolism, specifically involved in fatty acyl-CoA synthesis (2 genes), LC-PUFA synthesis (2 genes), lipogenesis (2 genes), and transcriptional regulation of lipid metabolism (2 genes) (Table 2).

3.5 Comparison of fatty acid composition between domesticated and wild salmon

The variation in fatty acids composition was generally more driven by diet than strain. About 85% of the fatty acid content in liver and pyloric caeca differed between diets, but only 32% of the fatty acids differed in levels between wild and domesticated salmon (p<0.05, Supplementary Table 6). Both wild and domesticated salmon given the VO diet showed higher levels of 18:3n-3 and 18:2n-6 contents in both liver and pyloric caeca but lower contents of the longer chain fatty acids

(ARA, EPA & DHA) compared to both FO and PL diets (Figure 6). This pattern was consistent for all three lipid classes analysed (PtdCho, PtdEtn, and TAG). Although the differences in fatty acids content were generally small between wild and domesticated salmon fed the same diet, wild fish contained higher content of 18:2n6 (9.1% in wild *versus* 7.3% in domesticated fish, $p = 0.06$) and 18:3n3 (2.3% *versus* 1.5%, $p = 0.006$) in PtdEtn of liver when fed VO diet. Wild salmon also had higher content of 18:3n3 (2.1% *versus* 1.8%, $p = 0.04$) in PtdCho of liver when fed VO diet. On the other hand, wild salmon had significantly lower content of ARA in both PtdCho (1.6 % *versus* 2.1%, $p = 0.02$) and PtdEtn (3.1% *versus* 4.3%, $p = 0.02$) of liver than wild fish when fed VO diet. Wild salmon also contained lower levels of 18:4n-3, 18:3n-6, 18:4n-6, but higher 20:3n-3 levels when a fed VO diet (Supplementary Table 6). No significant differences in DHA and EPA contents were found between domesticated and wild salmon fed the same diets.

4 Discussion

Atlantic salmon provides a unique opportunity to study domestication related evolution because the wild populations that gave rise to domesticated salmon are well-preserved and also accessible in live gene banks in Norway (O'Reilly & Doyle, 2007). Here we took advantage of this and performed a comparative study of domesticated and wild salmon metabolism to test for signatures of unintended selection on lipid metabolism traits in domesticated salmon.

Linking evolution of the domesticated metabolic syndrome with the circadian clock pathway

As demonstrated in other studies, we find that domesticated salmon grew faster than wild salmon (Bicskei et al., 2014; Reid, Armstrong, & Metcalfe, 2012). This reflect 50 years of targeted breeding for fast growth, which has resulted in higher standard metabolic rate, higher feed intake, and improved feed conversion (Thodesen et al., 1999), referred to as the 'domesticated metabolic syndrome' (Bicskei et al., 2014; Tymchuk, Sakhrani, & Devlin, 2009). In line with this, gene

expression differences in liver suggest that energy assimilation and expenditure is higher in domesticated salmon (Figure 3 A), similar to what is found in domesticated pigs (M. Li et al., 2013), chicken (Jackson & Diamond, 1996), and rat (Zeng et al., 2017). The differences in pyloric caeca gene expression between domesticated and wild salmon was associated with regulatory networks controlling intestinal development and cell differentiation (Jedlicka & Gutierrez-Hartmann, 2008; Kanki et al., 2017; Lebenthal & Lebenthal, 1999) (Figure 3 B), which could be linked to higher growth rates and/or feed intake in domesticated fish (Thodesen et al., 1999).

Our results strongly suggest a functional link between evolution of the domesticated metabolic syndrome (i.e. faster growth and higher energy turnover) and regulation of genes through the circadian clock pathway (Figure 3). This is interesting as top regulators (CLOCK/BMAL) are known to impact (directly or indirectly) a multitude of downstream processes including metabolism (Lowrey & Takahashi, 2000; Preitner et al., 2002; Takahashi, 2015). Moreover, the *CLOCK* gene has also been under selection during domestication of rats (Zeng et al., 2017) and is associated to key features of the domestic metabolic syndrome, such as regulation of feed intake, metabolic rates, and glucose and lipid metabolism in both mammals and fish (Esther, Nuria de, Ana, Ángel, & María, 2017; Paschos, 2015; Rudic et al., 2004). Finally, we found that predicted TFBS of the PPAR-RXR heterodimer, a key regulator of glucose (Jones et al., 2005) and lipid (Kliewer et al., 1997) homeostasis, were enriched in promoters of differentially expressed genes between wild and domesticated salmon (Figure 3 B), and that the *pparg* gene was consistently higher expressed in domesticated salmon (Figure 5). This also links to the circadian clock as the *pparg* is known to be under circadian rhythmicity in salmon (Betancor et al., 2014).

Unfortunately, our study was not designed to investigate the connection between differences in circadian oscillations between wild and domestic salmon. However, we are confident that sampling bias related to daily rhythms has not impacted our results. Firstly, all samples used for

the gene expression were sampled between morning and noon within a 2h time period. Secondly, all fish were raised under constant light and continuous feeding in this study. Such rearing conditions are known to abolish daily rhythmicity for both *nr1d1* (Betancor et al., 2014) and *cry-2* (Huang, Ruoff, & Fjelldal, 2010), nevertheless these genes were still expressed lower in domesticated salmon regardless of fish size and age (Supplementary Figure 1).

In conclusion, our results support strong links between the salmon 'domestic metabolic syndrome' and evolution of novel regulation of the circadian clock pathway. We therefore hypothesize that the strong selection on 'fast growers' with high energy metabolism and high appetite target genetic variation linked to regulation of the circadian clock pathway.

Lipid metabolism in domesticated salmon show signatures of unintended selection in the domestic environment

A main aim of this study was to explore the hypothesis that domesticated salmon had undergone unintended selection on lipid metabolism as a response to low levels of LC-PUFA in the domesticated environment. In line with this, we showed that the growth of wild but not domesticated salmon was affected by low LC-PUFA availability in the feed. This suggests that domesticated salmon have evolved more effective lipid absorption, lipid transport, and/or better ability for compensatory endogenous conversion and synthesis of lipids under shortage of essential fatty acids.

In-depth analyses of both transcriptomic and lipid composition data support the notion that all these processes differ between wild and domesticated salmon. Firstly, domesticated fish display higher *apoa1_2* gene transcription, encoding a major component of high-density lipoprotein (HDL) which plays a key role in lipid transport and regulation of cellular cholesterol levels (Otis et al., 2015; Toth et al., 2013). Secondly, the hormone sensitive lipase gene (*hsl*) was also

expressed higher in liver of domesticated salmon compared to wild salmon. This suggests that domesticated salmon has higher ability of hydrolysing triacylglycerol, diacylglycerol and cholesterol ester into monoacylglycerol and free fatty acids (Kraemer & Shen, 2002; Quiroga & Lehner, 2012) which is used for energy production or lipid synthesis. The fact that genes responsible for hydrolysing monoacylglycerol (*mgll*) and transporting fatty acids into the mitochondria for *β*-oxidation (*cpt*) were lower expressed in domesticated salmon compared to wild, support the latter. Third, the growth of wild (but not domesticated), was impacted positively by dietary supplementation of PL which is known to promote absorption and transport of dietary lipids, especially LC-PUFA (R. E. Olsen, Tore Dragnes, Myklebust, & Ringø, 2003; Y. Olsen et al., 2014a; D. R. Tocher, Bendiksen, Campbell, & Bell, 2008). This points towards a higher ability for LC-PUFA absorption and transport in domesticated salmon due to more effective *de-novo* synthesis of PL (Figure 5).

Finally, under dietary shortage of LC-PUFA, domesticated salmon respond with a compensatory increase in gene expression of *fads2d5* and *fads2d6a*, which encode rate limiting enzymes for endogenous synthesis of LC-PUFA (Figure 5). Parallel to this finding, marine stickleback that colonize fresh water environments with lower levels of available dietary DHA evolve a greater endogenous LC-PUFA synthesis ability through increased copy number of the same gene (*fads2*) (Ishikawa et al., 2019). As the ability to perform endogenous synthesis of LC-PUFA is heritable in salmon (Horn, Ruyter, Meuwissen, Hillestad, & Sonesson, 2018), it is likely that the VO based diets in the domestic environment has unintendedly selected for improved ability of LC-PUFA synthesis in the domesticated salmon. The high LC-PUFA levels in domesticated salmon ensures essential requirement for normal growth (Bou et al., 2017), while growth of wild salmon is stunted when fed VO diets due to insufficient synthesis of LC-PUFA. Sterol regulatory binding protein 1 (SREBP-1) transcription factor is likely the key regulator for the differential expression of the *fads2* gene (Figure 5) (Datsomor et al., 2019), but other mechanisms such as epigenetic changes may also contribute to the regulation of gene expression (Clarkson et al., 2017; Vera et al., 2017).

Conclusion

The present study provides evidence for domestication-associated evolution of metabolism in Atlantic salmon, both as a consequence of targeted breeding for fast growth, and as an unintended consequence of adapting to modern aquaculture feed. To further understand causal links between genotype and regulation of metabolism in domesticated salmon future studies should integrate analyses that shed light on the genomic signatures of domestication selection.

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Data Accessibility Statement

- Supplementary files have been deposited to datadryad.org under the accession: https://doi.org/10.5061/dryad.5hqbzkh33
- Raw RNA-Seq fastq. files have been deposited into ArrayExpress Archive under project accession number E-MTAB-8306 (https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-8306/).

Author Contributions

Yang Jin, Yngvar Olsen, Rolf Erik Olsen, Simen Rød Sandve and Olav Vadstein designed and performed the research. Yang Jin and Thomas Nelson Harvey performed the transcriptomic analysis. Yang Jin and Keshuai Li performed the lipid and fatty acid analysis. Jon Olav Vik and Simen Rød Sandve guided the transcriptomic analysis and revised the manuscript. Yngvar Olsen and Rolf Erik Olsen guided the lipid analysis and revised the manuscript. Mari-Ann Østensen and Nina Santi provided input on the experimental design, carried out the experiment and sampling and reviewed the manuscript. Yang Jin drafted the manuscript which was proof-read by all co-authors. Additionally, Yang Jin, Thomas Nelson Harvey and Simen Rød Sandve worked together to revise manuscript. All authors participated in the revision of this paper by providing comments and editing.

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Figure 1 Weight of domesticated and wild salmon fed diets high in fish oil (FO), vegetable oil (VO) or phospholipid oil (PL) during early stages of development. Data are means ± SE (n>100 per group at day 93, n>20 at other days). Different letters indicate significant (p<0.05) different of fish weight between wild fish fed FO, VO and PL diet at day 65 and 94.

Figure 2 Score plot of PCA on log2 count per million (CPM) of the top 1000 most variant genes across all samples (4 replicates x 2 strains x 3 Diets). Two salmon strains (domesticated and wild) were fed either fish oil (FO), vegetable oil (VO) or phospholipid (PL) rich diets from initial feeding. Pyloric caeca and liver samples were taken after 94 days of feeding.

Figure 3 Differential expressed genes (DEGs) between domesticated and wild salmon. A) KEGG enrichment shows significant (p<0.05) enriched pathway, and proportion (%) of up/down regulated DEGs in each pathway. **B)** Motif enrichment analysis shows top 10 most significantly (p<0.005) enriched motifs of transcription factors in promoter regions (-1000bp to 200bp from TSS) of DEGs as compared to all expressed genes in pyloric caeca and liver. Hypergeometric test was applied on both KEGG and motif enrichment analyses, by comparing the number of DEGs to

total genes annotated to each KEGG pathway or each motif. Motif enrichment analysis was done by using SalMotifDB (https://salmobase.org/apps/SalMotifDB). **C)** Expression of key circadian genes in pyloric caeca and liver of domesticated and wild salmon. Gene expression was shown in log2 transcript per million plus one (TPM +1). No statistics was shown for *cry2-c* gene in pyloric caeca, since the gene expression is too low (CPM <1) to be used for differential expression analysis.

Figure 4 Expression of 6 genes involved in lipid metabolism in pyloric caeca of wild and domesticated salmon at day 94 after feeding either fish oil (FO), vegetable oil (VO) or phospholipid (PL) diets. Gene expression was shown as Log2 transcript per million plus one (TPM + 1) which was normalized by library size and mRNA length. Asterisk indicates differential expressed genes (DEGs, q<0.05 & |log2FC| >1) between domesticated and wild salmon under each dietary treatment.

Figure 5 Expression of 14 genes involved in lipid metabolism in liver of wild and domesticated salmon at day 94 after feeding either fish oil (FO), vegetable oil (VO) or phospholipid (PL) diets. Gene expression was shown as Log2 transcript per million plus one (TPM + 1) which was normalized by library size and mRNA length. Asterisk indicates significant (q<0.05 & |Log2FC|>1) different of gene expression between domesticated and wild salmon under each dietary treatment separately.

Figure 6 Percentage of liver fatty acid composition in triacylglycerol (TAG), phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn) of wild and domesticated salmon fed either fish oil (FO), vegetable oil (VO) and phospholipid (PL) rich diets at day 94. A two-way ANOVA was applied to test the fatty acid differences between fish strains and dietary treatment (strain*diet) separately in each lipid class. Tukey's HSD post-hoc test was then applied to test the fatty acid difference between each group. Asterix in the figure indicate

significant (p<0.05) different of fatty acid between domesticated and wild salmon at certain day and dietary treatment. The composition of other fatty acids and their ANOVA test were shown in Supplementary 7.

Table 1 Percent of fatty acids in total fatty acids of three diets rich in fish oil (FO), vegetable and plant oil (VO), or vegetable and marine phospholipid oil (PL).

	FO	VO	PL
14:0	3.6±0.0	0.9±0.0	3.7±0.0
16:0	18±0.2	20±0.2	16±0.2
16:1n-7	4.3±0.0	0.8±0.0	3.1±0.0
18:0	4.4±0.1	3.7±0.1	3.5±0.5
18:1n-9	14±0.1	26±0.3	22±0.1
18:1n-7	2.5±0.0	7.1±0.2	3.2±0.0
18:2n-6	6.8±0.0	15±0.2	11±0.1
18:3n-3	1.2±0.0	11±0.2	3.1±0.0
20:1n-9	2.5±0.0	1.7±0.0	2.4±0.0
20:4n-6	1.3±0.0	0.3±0.0	0.7±0.0
20:5n-3	7.7±0.0	1.7±0.0	7.3±0.1
22:1n-9	2.2±0.0	1.2±0.0	1.8±0.0
22:6n-3	17±0.0	3.7±0.1	11±0.1

Data is shown in mean ± sd (n=2).

Table 2 Log2 fold change and adjusted *p* value (*q*) of lipid gene expression in liver of domesticated/wild salmon feeding vegetable oil (VO) diet compared to fish oil (FO).

	Farm VOvsFO		Wild VOvsFO	
genename	logFC	*q*	logFC	*q*
acsbg2b-b	1.7	0.01	1.0	0.64
acsl1a-a	1.6	0.02	1.0	0.64
agpat3a-b	1.4	0.002	1.2	0.09
agpat3b-a	1.1	0.01	0.5	0.72
fabp7b	6.0	0.001	4.2	0.13
fads2d6a	1.3	0.008	0.8	0.54
fads2d5	1.2	0.01	0.2	1.00
srebp1c	1.4	0.03	0.9	0.62
srebp1d	1.6	0.003	0.5	0.88