Fatty acid composition in salmonid eggs

– a comparative study of Atlantic salmon and brown trout

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Abstract

Atlantic salmon (AS) and brown trout (BT) are part of the salmonid family and are some of the most popular fish species in Western culture. Due to their popularity, there is incentive to understand the varying factors which ensures species survival. This paper aims to investigate the varying roles and relationships of the salmonid eggs fatty acids (FA). Its purpose is to investigate and compare the fatty acid (FA) composition of fertilized eggs from AS and BT spawning in the Dala river and relate the results to egg quality, as well as compare the results to findings in a study from 1998. Specific emphasis is on the important FA arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) as these FA are linked to healthy embryonic development.

The FA composition of the two species were quite similar and mainly differed in the specific FA distribution between the triacylglycerol and phospholipid fractions of the egg total lipids, as well as specific FA ratios. When compared with Pickova (1998), the main difference is found in the n-3/n-6 ratio. No significant differences were found in DHA or AA in between the two species, indicating the general importance of these two FA. EPA was registered in a higher amount in AS, possibly implying a higher presence of dietary EPA and thus reflecting the varying feeding habitats between species. Results indicate that sampled eggs are anadromous and of wild origin. To deepen the understanding of the health condition of the species, future studies should include other molecular- and environmental factors.

Keywords: salmonid eggs, lipids, fatty acids, Atlantic salmon, brown trout, DHA, EPA, AA

Sammanfattning

Atlantlax och öringen är arter i familjen laxfiskar och är några av de mest populära fiskarna i västerländsk kultur. Deras popularitet har påverkat de naturliga bestånden varpå studier nu kartlägger de faktorer som påverkar arternas överlevnad.

Denna studie avser att undersöka de olika rollerna och förhållandena mellan laxäggens fettsyror. Syftet var att undersöka och jämföra fettsyrasammansättningen i befruktade ägg från lax och öring kramade från Dalaälven och koppla resultaten till äggkvalité, samt jämföra resultaten med fynd från en tidigare studie 1998. Särskild tonvikt läggs på de viktiga fettsyrorna arakidonsyran (AA), dokosahexaensyra (DHA) och eikosapentaensyra (EPA).

Fettsyrasammansättningen för de två arterna var relativt lika och skilde sig huvudsakligen i den specifika fettsyrafördelningen mellan triacylglyceroler och fosfolipider, såväl som fettsyrekvoterna. Jämfört med Pickova (1998) återfinns den största skillnaden i förhållandet n-3/n-6. Inga signifikanta skillnader hittades i DHA eller AA mellan arter, vilket indikerar den allmänna betydelsen av dessa två fettsyror. EPA registrerades i en högre mängd i Atlantlax, vilket möjligen antyder en högre förekomst av EPA i kosten och således speglar de varierande födomiljöerna mellan arter. Resultaten indikerar att ägg kommer från anadroma fiskar av vilt ursprung. Framtida studier på laxägg bör inkludera andra molekylära- samt miljö faktorer.

Nyckelord: laxägg, lipider, fettsyror, Atlantlax, öring, DHA, EPA, AA

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Abbreviations

AA	Arachidonic acid
ALA	α-linolenic acid
AS	Atlantic salmon
BT	Brown trout
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EMS	Early mortality syndrome
EPA	Eicosapentaenoic acid
FA	Fatty acids
LA	Linoleic acid
LC-PUFA	Long chain polyunsaturated fatty acids
MUFA	Monounsaturated fatty acids
n-3	Omega 3
n-6	Omega 6
PL	Phospholipids
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
TAG	Triacylglycerols
UFA	Unsaturated fatty acids

1. Introduction

Lipids is a group of macronutrients essential for the development of life. The fatty acid (FA) composition has major health implications for fish, as well as humans, impacting growth, vision, behaviour, membrane fluidity, immune systems and reproduction (Arts and Kohler, 2009). The marine or fresh water ecosystems are distinguished by the high presence of long chain n-3 polyunsaturated fatty acid (PUFA) chains in comparison to their terrestrial counterpart (Bell and Tocher, 2009). A higher intake of these lipids is beneficial for human health as the consumption is associated with decreased risk of cardiovascular disease, stroke or overall mortality (Schwingshackl *et al.*, 2021). Current consumption of n-3 PUFA is considered insufficient in human population, and countermeasures such as fortifying foods or feeding n-3 FA rich diet to livestock are generally not successful as its obstructed by the oxidative deterioration during process and storage (Damodaran, Parkin and Fennema, 2017). This is one of several key health factors making fish a valuable and attractive commodity on the global market.

The valuable qualities linked to FA common in fish has also led to the consumption of fish oil, and thus the production of fish oil and fish meal. Fish meal has usage as feed for aquaculture and also cattle. The two products are made in the same process and it takes approximately 100 kg of fish to manufacture 21 kg of meal and 3-6 kg of oil (European Market Observatory for Fisheries and Aquaculture Products (EUMOFA), 2021).

The natural occurrence of Atlantic salmon (AS) and brown trout (BT) has decreased in recent years due to reasons such as overfishing, loss of natural habitat, acidic rains, pollution and dam constructions to name a few (Cornet *et al.*, 2021). Out of all the fish consumed in the EU ,74 % is of wild origin but salmon only represents 0.16 % of that and trout an additional 2.05 %. A majority of the salmonids consumed in the EU are consequently farmed (European Market Observatory for Fisheries and Aquaculture Products (EUMOFA), 2020). To maintain the wild salmonid population, it’s imperative to understand the varying factors that ensures survival. Two fundamental factors underpinning this is the reproductive health and the egg quality of the fish respectively.

1.1. Purpose

The eggs nutritional status is a vital component for any egg-laying animal and key to ensure the survival of the species. This study aims to investigate the eggs fatty acid composition and examine if and what the fatty acid differences are between the species Atlantic salmon and brown trout from the Dala river, and also in comparison with eggs collected in 1996 to reveal possible influences on the environmental factors since then.

2. Background

2.1. Atlantic salmon and brown trout

AS (*Salmo salar*) and BT (*Salmo trutta*) are both part of the salmonid family and are identified by their long silver coloured bodies with spotting along their sides. They are anadromous species which allows them to move between fresh and salt waters and are present in the entire Atlantic region in the northern hemisphere (Barton, 1996). The presence of salmonids is dated 50 million years back and has evolved ever since. Although closely related, there are phenotypic and ecological differences between the two species. AS are better adapted to live in strong current waters and withstand long migrations in open waters between feeding grounds and reproductive sites. The BT is smaller and will more often stay in freshwater habitats. Some groups will migrate between lakes and rivers, others between lakes and streams. The BT anadromous populations will migrate to coastal seas but rarely seek open waters (Jonsson and Jonsson, 2011b). Salmonids habitats range in temperatures from 0-30°C. Varying temperatures is the habitual component that has greatest impact on the development of salmonids; both on behaviours and physiological reactions such as metabolic rate, feeding, swimming, growth and reproduction (Jonsson and Jonsson, 2011a).

BT inhabit deeper waters which entails colder temperatures and would be a motivation to incorporate more fat. It also offers a different feeding habitat than that of the AS. BT exhibits growth within a temperature range of 5-23°C and AS between 6-25°C. 25°C is the upper lethal limit for BT whereas it is 28°C for AS. As temperature increases, oxygen solubility decreases which makes for a bad habitat for salmonids which rely on high oxygen content. (Jonsson and Jonsson, 2011a). With climate change and increasing temperatures, one can hypothesize the detrimental effects on various species.

2.2. Farmed salmonids

Farmed salmon is a major global seafood commodity. According to FAO (2019), Atlantic salmon as a species is ranked two by value and 15 by quantity. There is no data specifically for brown trout. However, in a group analysis, including salmons, trouts and smelts from the Osmeridae family, they are ranked 3 by value and 9 by quantity, producing 3.5 million tonnes yearly. AS stands for 68 % of that production (FAO, 2019). Due to the variating life cycles, feeding strategies and diets between farmed and wild salmon, the overall lipid content and FA composition varies between the two groups (Jensen *et al.*, 2020). This domestication has created phenotypic differences between the two groups. When escaped farmed salmon enter the ecosystem of the wild salmon, these will breed and potentially put the wild salmon at risk (Gross, 1998).

2.3. Fatty acids

A common denominator for the group of compounds called lipids, is that they are soluble in organic solvents. They can be nonpolar or polar, solid or liquid at room temperature. These classifications indicate the lipids' solubility and functional properties (Damodaran, Parkin and Fennema, 2017). FA is the major group of lipids, characterized by a carboxylic acid group with an aliphatic chain that commonly contains between 14-24 carbons. They exist in three main classes; triacylglycerol (TAG), phospholipids (PL) and cholesterol and are generally classified as saturated or unsaturated (Ibid.).

2.3.1. Saturation

Saturated fatty acids (SFA) have no double bonds on their carbon chain in comparison to unsaturated fatty acids (UFA). UFA contain double bonds between carbons and naturally have a *cis* configuration, meaning that the carbon chains extend to the same side of the double bond. FA with one double bond is known as monounsaturated fatty acids (MUFA). The more double bonds the molecule contains, the more bent it will become. These are known as polyunsaturated fatty acids (PUFA). This means that *cis* PUFA take up a lot of space i.e., they are non-linear, in comparison to PUFAs with *trans* bonds that extend to the opposite side, which enables these molecules to pack closer together. This causes *cis* fats to have a lower melting point than *trans* fats. Another contributor to lower melting point is a high amount of double bonds. To indicate where these double bonds are located on the FA, the omega system classifies from the first double bond at the end of the free methyl group, i.e. α-linolenic acid (ALA; 18:3n-3), see figure 1 (Damodaran, Parkin and Fennema, 2017). These molecular differences have a great impact on

health for both fish and mammals. A PUFA has a melting point around -50°C and SFA range from 58°C for myristic acid (12:0) and +77°C for arachidic acid (18:0) (Arts and Kohler, 2009; Schwingshackl *et al.*, 2021). Current evidence is encouraging dietary intake for humans to limit SFA and increase consumption of MUFA and PUFA (Schwingshackl *et al.*, 2021).

2.3.2. Triacylglycerols and phospholipid

TAG consist of three molecules of FA esterified onto a glycerol backbone, which stabilizes the disruptive nature of FA due to their ability to react with cell membranes. Glycerol being a three-carbon molecule, enables three possible anchor points for the FA. Generally, SFA and MUFA will be located on the terminal carbons of the glycerol. PUFAs on the other hand will be found on the center carbon (Sargent, Tocher and Bell, 2003; Damodaran, Parkin and Fennema, 2017). Either a single FA can be esterified on all three anchor points or a variety of two or three different FA. The TAG are nonpolar lipids and are the main form in which lipids are stored in adipose tissue (Tocher, 2003).

PL are derivations of TAG; instead of three FA chains, one of the terminal FA has been replaced by a phosphate group. This makes the molecule polar with two hydrophobic FA chains and a hydrophilic phosphate group. These properties make them a key component for cell membranes. In addition, there is a relatively high percentage of unsaturated FAs in membrane PL to maintain fluid form and avoid crystallization when temperatures decrease. The FA chain on the center carbon of the glycerol will in this case be more saturated than the FA on the terminal carbon (Damodaran, Parkin and Fennema, 2017). Phospholipids in animal cell membranes rarely contain other SFA than 16:0 and 18:0, but these are a vital component as they contribute with some rigor as the PUFA contribute with fluidity and flexibility (Arts and Kohler, 2009). Due to their chemical attributes, the amount of SFA will decrease and UFA will increase with lower temperatures. The proportions between SFA and PUFA are therefore of great importance.

2.3.3. Essential & vital fatty acids, DHA, EPA & AA

Essential FA are those that cannot be synthesized by the organism but are essential for their survival. For most vertebrates this is at a minimum linoleic acid (LA; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3) (Sargent, Tocher and Bell, 2003). Three long chain FAs have been identified to be especially vital for the health and wellbeing of fish and other vertebrates; the n-3 PUFAs docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3) and the n-6 FA arachidonic acid (AA; 20:4n-6) (Sargent *et al.*, 1999). The chemical structure of these five FA can be seen in Figure 1.

The reasons for the importance of EPA, DHA and AA are that they play a vital role as precursors for hormones as well as maintaining the integrity of cell membranes (Sargent *et al.*, 1999). DHA has six double bonds and is as such the longest and most unsaturated FA commonly found in nature. Due to the amount of saturation, it maintains flexibility and fluidity even in low temperatures, which enables membrane function, ion transport and enzymatic activity (Arts and Kohler, 2009). EPA is an important building block for cell membranes, and may be beneficial due to its ability to increase the anti-inflammatory response (Torniainen *et al.*, 2017). AA is a precursor for vital C20 metabolites known as eicosanoids. These are hormone-like substances such as prostaglandins, thromboxanes and leukotrienes. This FA is mainly found in the PL fraction of egg and liver (Gunstone, 1999). EPA is also a precursor for eicosanoids, however, AA is the preferred precursor as derivates from this FA are more biologically active than from EPA (Czesny *et al.*, 2009).

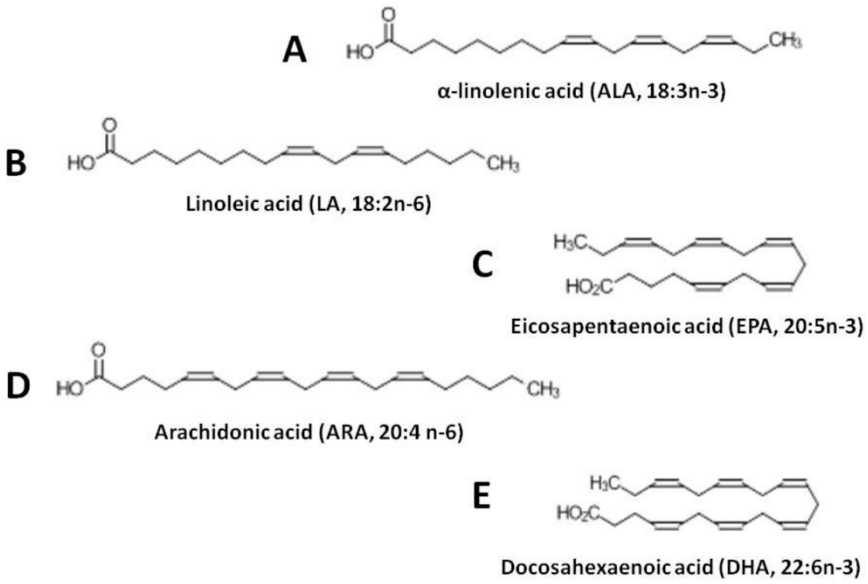

Figure 1 The chemical structure of the essential PUFA. A. α-linolenic acid, B. linoleic acid, C. EPA, D, ARA, E. DHA. Figure from (Schiller Vestergren, 2012:12)

2.4. Lipid metabolism

FA catabolism, the process of generating ATP, predominantly takes place in the adipose tissue in fish. Lipolysis initiates the process by removing FA with lipase from the glycerol molecule in the TAG. The free FA are released into the bloodstream from the adipose tissue, attached to an albumin protein due to their hydrophobic nature (Tocher, 2003).

Lipogenesis is the process of synthesizing FA which can occur in two main pathways. Either through dietary lipids with elongation and desaturation or through *de novo* lipogenesis meaning that they are made anew from acetyl-CoA derived from carbohydrates or protein (Gunstone, 1999). The liver and adipose tissue are the two main sites of FA synthesis in eukaryotes. The liver mainly produces FA for TAG intended for very low density lipoproteins which are secreted into the bloodstream, whereas the adipose tissue synthesize FA for triacylglycerol to be stored in the adipose tissue (Sul and Smith, 2008). In salmonids, the liver is the main site for FA synthesis (Pickova, 1998).

2.4.1. Biosynthesis of PUFA

As mentioned, LA and ALA are dietary essential FA. Through a four-step process of elongation, each supported by a specific enzyme, these FA can be converted into either DHA, EPA or AA, visualized in Figure 2. Elongating a long chain-PUFA (LC-PUFA) from ALA; (18:3n-3) or LA; (18:2n-6) is an alternating process of reactions with elongases and desaturases. Elongation initiates by carboxylating acetyl-CoA into the two-carbon malonyl-CoA with acetyl-CoA carboxylase enzyme. Malonyl-CoA then reacts with the precursor FA, LA or ALA, which produces a b-ketoacyl which through a three step process will be hydrogenated (Bell and Tocher, 2009). Further desaturation of LC-PUFA is an aerobic reaction which occurs in the endoplasmic reticulum. The fatty acid desaturases enzymes add a double bond in the *cis*-confirmation to the FA through an electron transport chain; electrons from NADPH are transferred to the enzyme cytochrome b_5 reductase which is then reduced into cytochrome b_5 and further reacts with oxygen and fatty acyl-CoA desaturase (Tocher, 2003).

The desaturases are commonly classified with an Δ, which indicates the position of the new double bond from the carboxyl end of the FA, i.e. Δ-9 desaturases indicates that the double bond is located between the 9^{th} and 10^{th} carbon (Bell and Tocher, 2009).

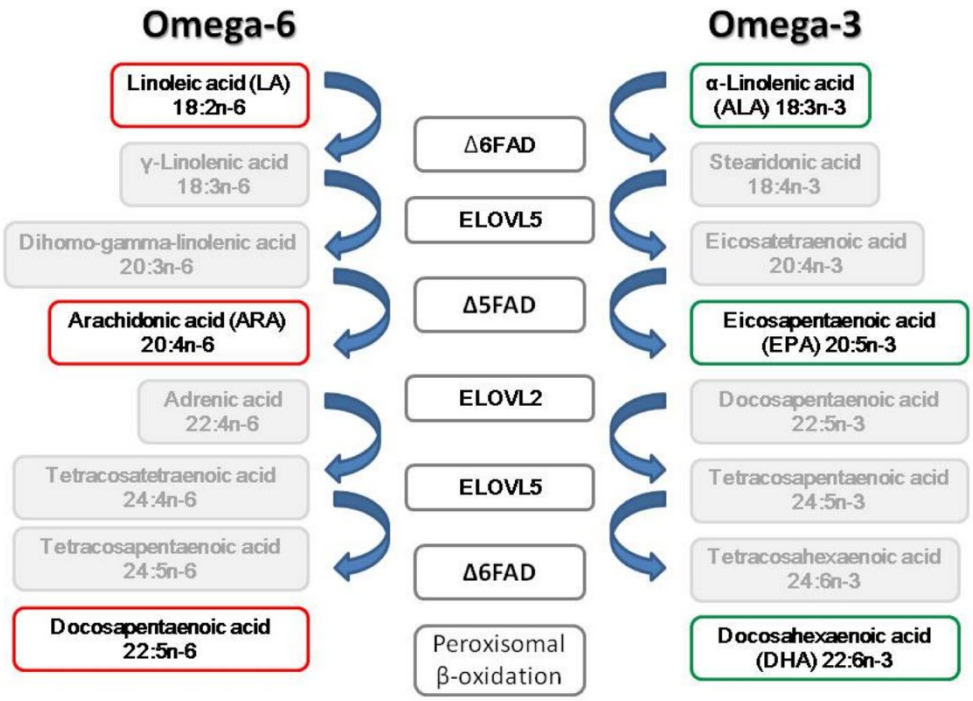

Figure 2 - Elongation and desaturation of n-6 LA (18:2n-6) into AA as well as the n-3 ALA (18:3n-3) into EPA and DHA. Figure from (Schiller Vestergren, 2012:27)

2.4.2. Oxidation and antioxidants

UFA are vulnerable to attack by oxygen free radicals which in turn lead to chain reactions in lipid oxidation (Cowey *et al.*, 1985). Free radicals are molecules or atoms with unpaired electrons and lipid oxidation is the process where these radicals react with FA and oxygen, leading to lipid degradation (Damodaran, Parkin and Fennema, 2017). The number of double bonds in a FA, as well as the length of the chain, will increase the reactivity and thus the risk for oxidation through attack (Pickova, 1998).

Free radicals have a varying amount of energy which affects their qualities and reactive abilities. A free radical with high energy, for instance the hydroxyl radical (*OH), can oxidize most molecules. The benefit of antioxidants, is that they form radicals with low energy that are incapable of attacking molecules such as UFA, hence oxidative stability can be enhanced through antioxidants by controlling free radicals and thus slow down the rate of oxidation (Damodaran, Parkin and Fennema, 2017).

Astaxanthin is a carotenoid which is naturally produced in algae and commonly present in salmon eggs. Due to the molecular structure containing a benzene ring, it can donate an e- to the radical and yet remain stable itself. Experiments where salmons were fed supplements of antioxidants, increased their tissue levels of antioxidants and in turn their growth rate (Pickova, 1998).

2.5. Aquatic fatty acids

A majority of the lipids in marine fish originate from phytoplankton and zooplankton (Tocher, 2003). Aquatic dietary long chain n-3 PUFA are mainly produced by either heterotrophic prokaryotes such as bacteria, or photosynthetic organisms like algae. Commonly the bacteria will produce either EPA or DHA (Bell and Tocher, 2009). The FA produced by these organisms then travel through the food web into all living fish (Sargent, Tocher and Bell, 2003).

2.5.1. Dietary fatty acids and *de novo* synthesis

Some vertebrate species, including the Atlantic salmon and brown trout, have limited *de novo* synthesis of LC-PUFA. However, even if a species can synthesize these FA, the conversion rate is generally low. There are specific dietary requirements for syntheses to occur as the n-3 and n-6 FAs compete for the same enzymes. The ratio of the n-3/n-6 FA are therefore a vital component, i.e. if LA is more abundant in the diet, AA will be synthesized more abundantly, hence DHA and EPA will increasingly become dietary essential FA (Sargent *et al.*, 1999; Sargent, Tocher and Bell, 2003). Top predator marine fish will seldom synthesize fatty acids *de novo,* but consume their nutritional needs through diet (Tocher, 2003). There is thus an intrinsic relationship between *de novo*- and dietary lipid synthesis.

Different FA impact each other in varying ways, so the composition is a delicate balance. For instance, the presence of one FA can promote the oxidation in another i.e. linoleic acid promotes the oxidation of oleic acid as linoleic acid is easily oxidized (Gunstone, 1999).

The FA composition of the diet has numerous times been shown to influence the FA composition of the fish. In the salmon flesh, lipid content of wild fish has been recorded to 6.0 %. In reared salmon, the total lipid content in the flesh is currently around 18 %, a 12 % increase in 8 years and a reflection of changes in the fishes' feed (Jensen *et al.*, 2020). It has been shown that the non-polar lipids are susceptible to dietary changes to a higher degree than the polar lipids, i.e. phospholipids (Jobling and Bendiksen, 2003). There is a relationship between the FA composition and the water temperature, colder temperature increases the ratio between UFA and SFA (Jobling and Bendiksen, 2003).

2.6. Life cycle of salmonids

The salmonids are synchronous spawners meaning all of the eggs are developed homogeneously (Pickova, 1998). Spawning usually takes place in autumn or early winter in freshwater, mainly rivers or streams. The spawning behavior amongst the two species are somewhat similar. The female builds a nest by digging with her fin

in the gravel, this ensures water flow and thus an oxygenated environment. She will build nests in a row about 0.5-1 m apart and spawn her eggs in these different nests over a period of 1-7 days for AS and 1-3 days for BT. As the BT is smaller, it will spawn fewer eggs per nest. The eggs are fertilized externally and directly after spawning, the female will dig in front of the nest to cover the eggs in a protective layer of gravel. The nests are a barrier for predation and protects from other spawning females until they hatch the following spring (Jonsson and Jonsson, 2011c).

Spawning time is affected by water temperatures to ensure optimal circumstances for the initial feeding of the juveniles when they swim up. Time of spawning is also influenced by the competitive nature within the population for nest sites and increasing survival chances for their offspring. Early spawners risk nest destruction by females who spawn later but give their offspring the advantage of emerging earlier and thus getting a head start in developing territories and initiate feeding earlier (Aas, 2010). In captivity, the females and males are hand stripped and eggs hatched in hatching trays after mixing the two gamets.

Fertilized eggs can be seen in Figure 3. The eggs hatch and become alevins which are attached to a yolk-sac which they initially depend on for nutrition. Alevins will remain in the protective environment of the nest until it has depleted the yolk-sac. Once it has developed the capability of feeding itself outside the nest it is referred to as fry and its main source of nutrition will be plankton and small invertebrates. In captivity, fry feed is fed to the fry. Parr is the name of the final stage of the juvenile phase in which it will fully develop scales, fins and start to consume other fish (Jonsson and Jonsson, 2011b). The juvenile stages of the salmonids are spent in the spawning freshwaters, an approximate of 1-4 years. The parr then undergoes smoltification, a physiological adaptation to living in seawater. When complete the smolt migrates to feeding grounds for a growth period where the salmon matures, to later return to the original birth waters to spawn (Pickova, 1998; Elliott and Elliott, 2010).

Fish that feed in lakes and coastal areas will have a higher growth rate than fish who stay in rivers. This can especially be seen in BT females that grow faster as they travel to rich feeding waters while their male counterparts tend to stay in nursery rivers, a safer environment due to less predators but with poorer feeding opportunities (Jonsson and Jonsson, 2011a). Fresh and salt water have varying osmotic pressures which also affects growth rate. Anadromous fish will grow faster in saltwater than fresh. BT has a better growth rate than AS in freshwater, indicating it is better adapted to freshwater. In turn, AS is more adjusted to salt water (Jonsson and Jonsson, 2011a)

Spawning is a period of fasting for the female. For months she will cease to eat as she approaches spawning rivers, living off of her muscle lipid reserves for both her own metabolism as well as egg development (Torniainen *et al.*, 2017).

Figure 3 Salmon eggs in varying stages of development. Top left has developed eyes and a lens. Far right with visible blood vessels. Photographer Uwe Kils (2005) CC-BY-SA 3.0

2.6.1. Maternal influence and egg development

A variety of maternal factors influence the egg quality and offspring survival chance; spawning time, egg size, dietary intake and spawning location, her endocrine/hormonal status when ovulating as well as her genetic status to name a few (Aas, 2010).

The female salmon devotes about 6 times more energy in her reproduction organs than her male counterpart (Fleming, 1996). The ovary produces oocytes, immature egg cells, which through the process of vitellogenesis will deposit nutrients in the oocyte to form a yolk. This process is influenced by environmental factors such as temperature, salinity and light as well as available nutrition (Pickova, 1998). Eggs vary in size depending on the female's length and body mass. As the female grows larger, so do her eggs and her ability to produce more eggs per season. The size of the female thus increases her fecundity i.e., the ability to produce offspring. Larger eggs produce larger spawn which results in a lifelong competitive advantage. However, the larger female will produce fewer eggs in relation to her size compared with smaller females, which suggests a trade-off in fecundity; fewer eggs but larger vs more eggs but smaller (Jonsson and Jonsson, 2011a; Jonsson and Jonsson, 2011c).

During maturation, but not limited to this specific process, EPA is favored over DHA in catabolic processes when generating energy for gonad production. This could contribute to a higher ratio of DHA:EPA in the formatted eggs as the female would have consumed more EPA from the adipose tissue, leaving lesser amounts to deposit in the egg (Sargent, Tocher and Bell, 2003).

There are Atlantic salmon reared in landlocked habitats, known as non-anadromous as they never leave fresh waters. Studies of these show a very different n-3/n-6 ratio in their eggs when compared to their wild counterparts. The main difference between these two stocks is the feed they consume, indicating the influence and importance of the females' diet (Pickova, 1998).

A high diet of UFA comes with the risk of an increased prooxidant stress on the fish which can cause oxidative diseases to arise, such as M74 (Sargent, Tocher and Bell, 2003). M74 is a lethal syndrome developing during the yolk stage and is caused by a deficiency in thiamine vitamin (B1) as well as an antioxidant shortage and imbalanced FA composition (Pickova, 1998). These oxidative chain reactions can to some extent be counterbalanced through an increased consumption of antioxidants. But there is generally an antioxidant deficiency in diseased fish. M74 is thus a result of an unbalanced FA diet, stressing the importance of a healthy ratio of n-3/n-6 dietary FA (Sargent, Tocher and Bell, 2003; Torniainen *et al.*, 2017).

2.6.2. Salmonid eggs

The major component of salmonid eggs is water, approximately two thirds of the total composition. Protein contributes with the second largest component, approximately 20 % of the wet weight (Barton, 1996). The lipid content among freshwater fish eggs, as well as anadromous fish such as the Atlantic salmon and brown trout, is between 2,5-10% of the wet weight. Two subclasses can be seen with lipid levels: either <5 % or >5 %. Salmonids belong to the high lipid content group with levels around 10 %. In addition to the phospholipid-rich yolk, these eggs have higher concentrations of neutral lipids stored as oil globules. In size the eggs measure around 0.5 cm in diameter (Sargent, Tocher and Bell, 2003; Jonsson and Jonsson, 2011c). The high lipid content is thought to be due to the lengthy time between spawning and hatching (Torniainen *et al.*, 2017).

2.6.3. Fatty acids in egg and egg quality

Gondoic acid (20:1n-9) is a common TAG MUFA in adipose tissue of the salmon but present, if at all, in very low quantities in eggs. This FA contributes with energy in the formation of eggs, hence little remains to store in the eggs (Sargent, Tocher and Bell, 2003).

Salmon eggs are rich in PUFA as to remain normal cell and cell membrane function in the cold winter waters as well as provide the precursors required for growth and development (Pickova, 1998). Eicosanoids are biological active molecules which AA, EPA and docosapentaenoic acid (DPA) (22:5, n-3) are precursors for. Prostaglandins are an example of important eicosanoids which are involved in the maturation of eggs. In fish, prostaglandins formed from n-6 AA, are the preferred substrate over n-3 EPA, as these have a higher biological activity. The dietary ratio of EPA/AA is thus important, as the eicosanoids from both FA compete for the same receptors (Pickova, 1998). It has also been shown that higher levels of AA has been linked to increased egg quality, disease resistance and growth (Czesny *et al.*, 2009).

To study egg quality in relation to successful development in egg-fry-parr, ratios of AA, EPA and DHA have been used as indicators. As AA is the preferred eicosanoid precursor, a lower EPA:AA ratio can indicate wholesome embryonic development. The DHA:EPA ratio in the PL fraction of the yolk is another common indicator for healthy embryonic development. This ratio is commonly 2:1. Some findings on another species in the salmonid family, the brook trout, found that a higher DHA:EPA ratio was linked to early mortality syndrome (EMS) which is synonymous with the M74 syndrome in the Atlantic salmon (Torniainen *et al.*, 2017). The quality of Atlantic salmon eggs have been shown to be influenced by the ratio between the overall n-3 FA and AA as well as the DHA:EPA ratio (Sargent, Tocher and Bell, 2003).

Other physical changes in the environment also affect the quality of the egg and thus their survival chances, such as hydropower operations which can increase the oxygen levels of the water thus increasing survival chances (Casas-Mulet *et al.*, 2016). Nota bene that this singular positive effect by no means weighs up for overall decidedly detrimental impact of hydropower on fish by means of habitat loss, sound disturbance, vibrations etc.

2.7. Analytical methods

In order to analyze the fatty acid composition in a sample, firstly they are dissolved in hexane and homogenized for short bursts of time to minimize the risk of overheating the sample which can cause oxidation of the UFA. Aqueous sodium sulfate $(Na_2SO_4)_{aq}$ is added to the solution which ensures to remove nonlipid contaminants (Hara and Radin, 1978)

Dry methanol is a vital component as it ensures no presence of moisture during the methylation. During humid weather, an increase of dry methanol might be necessary. The Lewis acid, boron trifluoride, catalyzes the electron reaction which

will methylate the esters, thus creating fatty acid methyl ester (FAME) (Gunstone, 1999).

Chromatography is the process of separating a mixture into their individual components through a stationary- and a mobile phase. With thin layer chromatography (TLC), a sample is added to silica gel coated on a sheet of glass, the stationary phase. The sheets are then placed in a beaker with the mobile phase and covered with a lid to ensure complete saturation of the solvent vapor. The mobile phase then carries the different components upwards through the stationary phase, and as different fractions will have different densities they will separate. To visualize the separation, iodine is used to stain the dried plates as it binds to the double bonds of the FA.

Gas chromatography works on the same principle as TLC. Here the sample is heated and due to the varying volatility of the individual compounds, they will separate and be identified with different retention times with a flame ionization detector. It is a solid tool to analyze and quantify the variety and amount of individual FA.

3. Materials and methods

The fish eggs used in this study were retrieved from the Dala river in Sweden. Where the fish originated from is however difficult to determine; they could be wild, farmed fish that have escaped or artificially reared until smoltification and then released.

3.1. Materials

Fertilized frozen salmon eggs were provided by Älvkarleby laboratory, Department of Aquatic Sciences, SLU. They were retrieved in 2018 from the Dala river, frozen in CO_2 ice and then stored in -80° C in plastic vials. The difference between AS1 and AS2 is that the fish has been stripped at different dates, this difference is however not the basis of the study.

3.2. Methods

3.2.1. Lipid extraction

Approximately 1 g of whole eggs for each egg sample were homogenized in a Potter-Elvhjem homogenizer. The lipids were extracted from tissue with a total of 15 mL hexane:isoproponal, (3:2, v/v) (HIP) and homogenized 3 times à 30 sec. 7 mL of 6,67 % aqueous sodium sulfate $(Na_2SO_4)_{aq}$ were added followed by stirring. The second step ensures to remove nonlipid contaminants (Hara and Radin, 1978).

3.2.2. Preparation of fatty acid methyl esters

20 mg of oil per sample were dissolved in 0.5 mL hexane in a test tube. 2 mL 0.01 NaOH in dry methanol were added to the tube which was then closed with a clip and incubated on a Grant BT3 block heater at 60° C for 10 min. 3 ml of BF_3 reagent was added and then incubated for another 10 min at 60° C to then be cooled under a running tap of water. 2 ml of 20 % NaCl and 2 ml hexane were added to the tube and then vortexed vigorously. The tubes were then placed to rest in a fridge for the

layers to separate. The hexane fraction was transferred by glass pipette to a small tube. If needed, more hexane was added to ensure complete removal of lipids. The small tubes were then evaporated with N_2 in a fume cupboard *with the lights turned off*. 100 + 100 µl hexane was added, and the tubes were vortexed. The samples were sealed with teflon tape and stored in a freezer for 2 days.

3.2.3. Control of FAME with thin layer chromatography

Prior to running the samples through capillary column gas chromatography (GC), the methylation of the samples was controlled through the process of thin layer chromatography (TLC).

Three µl of sample was pipetted as a dot, two centimeters from the boarder of precoated silica gel plates. A reference standard No 18:1C accompanied the samples. The silica plates were then placed in a development chamber with 100 ml of mobile phase containing hexane / diethyl ether / acetic acid (85:15:1, v/v/v) for approx. 45 minutes. The plates were then left to dry before they were developed for 20 minutes in a staining chamber with iodine vapor to visualize the different classes and check that FAME were obtained.

3.2.4. Capillary column gas chromatography

100 µl of sample was placed in two milliliter GC vials. One microliter of fatty acid methyl esters was injected in a CP-3800 GC with a flame ionization detector (FID). The column was BPX 70, 50 m × 0.22 mm silica capillary coated with 0.25 µm film thickness and the mobile phase was helium. The injector temperature was set at 230°C and the column temperature to 158°C. The program initiated with five minutes at 158°C and then increased 2°C/minute to 220°C where the temperature was held for 8 minutes. The flame ionization detector (FID) was set to 250°C.

A standard with a mixture of FAME was run in the same program. The relative retention times and elution pattern of the FAME samples were compared to this known reference mixture to identify the compounds and response factors.

3.2.5. TAG and PL separation through TLC

Separation of the extracted lipids into TAG and PL fractions was done through TLC on the same basis as explained in 2.2.3. Instead of a dot, a two mg sample was spread in a two cm line on the precoated silica gel plates with two cm in between each sample. Following the staining, the dried plates were divided into rectangles through ruler and pencil, separating the PL from the TAG fractions from other lipids. The rectangles containing TAG and PL fraction, were scraped off

individually and the powder was resuspended. The TAG fraction was extracted with 2 mL chloroform-methanol (1:1, v/v) and twice with 2mL chloroform. The PL fraction was extracted with 2 mL chloroform-methanol (1:2, v/v) and 2 mL of chloroform. The solvent was then evaporated with nitrogen gas and the remaining lipids suspended in 0.5 mL hexane.

The TAG and PL fractions were methylated according to 2.2.2 and analyzed through the same procedure as described in 2.2.4.

3.2.6. Statistical analysis

A statistical comparison was carried out between the sample groups Atlantic salmon 1 (AS1) and brown trout (BT) through the Ttest function in Excel with uneven tails as the sample groups were of different sizes. These two groups were thought to be in similar developmental stages.

P-values ≤ 0.05 are regarded with high significance, ≤ 0.09 slightly significant and ≥ 0.10 non-significant.

4. Results

4.1. Total lipid

The amount of total lipid was on average 5.4 % and 5.8 % of the wet weight between the two sample groups of the Atlantic salmon. The brown trout had an average of 5.9 %. There was no significant difference between the groups.

Table 1 Mean of total lipid content (in %) of the sampled salmon and trout eggs

Mean % of total lipids					
Atlantic salmon				Brown trout	
(n=8)		*(n=4)*		*(n=4)*	
AS 1	STDEV	AS 2	STDEV	BT	STDEV
5.42	0.95	5.85	0.62	5.92	0.98

4.2. Fatty acid composition in total lipid

Shown in Table 2, the content of EPA (20:5n-3) differed significantly between the samples from AS1 and BT in the total lipid fraction ($p < 0.0003$). AA (20:4n-6) had no significant difference ($p < 0.126$) and DHA (22:6n-3) had a slight difference ($p < 0.089$) between the two species. Both Linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) differed significantly (($p < 0.0005$) and ($p < 0.028$) respectively).

Table 3 presents summaries and ratios between fatty acids in the total lipid fraction. No difference was recorded in total SFA. A significantly higher amount of MUFA was found in the BT ($p < 0.0021$) and thus a significantly higher amount of PUFA in AS1 ($p < 0.005$). Significantly higher values of both PUFA n-3 ($p < 0.008$). and n-6 ($p < 0.043$) was seen in AS1. However, no difference was recorded in the n-3/n-6 ratio.

The DHA/EPA ratio was significantly higher in brown trout ($p < 0.005$) and the EPA/AA ratio was significantly higher in Atlantic salmon 1 ($p < 0.0005$).

Table 2 Fatty acid composition of total lipid content (in %) in Atlantic salmon and brown trout eggs

Fatty acid composition means of total lipids in %						
	Atlantic salmon				Brown trout	
	(n=8)		(*n=4*)		(*n=4*)	
Fatty acid	AS 1	STDEV	AS 2	STDEV	BT	STDEV
C14:0	1.20**	0.19	1.09	0.14	1.62**	0.30
C14:1	n/a	n/a	0.05	0.10	n/a	n/a
C16:0	12.7**	0.45	12.7	0.08	13.7**	0.27
C16:1(n-7)	3.53***	0.14	3.74	0.21	4.28***	0.35
C17:0	0.37*	0.19	0.37	0.07	0.07*	0.15
C17:1	0.31	0.15	0.28	0.04	0.16	0.24
C18:0	5.82*	0.47	6.15	0.47	5.00*	0.93
C18:1(n-9)	23.8	1.67	24.3	1.19	24.2	1.41
C18:1(n-7)	3.27***	0.35	3.62	0.51	4.60***	0.06
C18:2(n-6)	3.69***	0.34	3.64	0.24	2.77***	0.30
C18:3(n-3)	2.30*	0.19	2.22	0.33	2.04*	0.20
C20:1(n-9)	0.80***	0.17	0.70	0.15	1.76***	0.14
C20:2(n-6)	0.65*	0.15	0.71	0.18	0.87*	0.15
C20:4(n-6)	1.46	0.15	1.48	0.15	1.58	0.18
C20:3(n-3)	0.32*	0.27	0.57	0.09	0.67*	0.05
C20:4(n-3)	2.52*	0.37	2.58	0.40	2.02*	0.48
C20:5(n-3)	8.77***	0.65	8.13	0.69	6.97***	0.43
C22:5(n-3)	6.09*	0.29	6.38	0.58	6.60*	0.43
C22:6(n-3)	22.5	1.14	21.37	1.61	21.1	2.13

Stars in a row indicate significant difference between AS1 and BT: * p<0.05; **p<0.01; ***p<0.001

Table 3 - Summary of total lipids (in %)

Summaries and ratios between fatty acids in total lipds						
	Atlantic salmon				Brown trout	
	(n=8)		(n=4)		(n=4)	
Fatty acid	AS 1 %	STDEV	AS 2 %	STDEV	BT %	STDEV
SFA	20.1	0.87	20.3	0.24	20.4	0.69
MUFA	31.7**	1.54	32.6	1.47	35.0**	1.31
PUFA	48.2**	1.89	47.1	1.65	44.6**	1.79
PUFA (n-3)	42.4**	1.68	41.3	1.62	39.4**	1.80
PUFA (n-6)	5.80*	0.53	5.83	0.39	5.22*	0.41
n-3/n-6	7.36	0.68	7.10	0.60	7.58	0.72
DHA/EPA	2.57**	0.19	2.64	0.24	3.04**	0.34
EPA/AA	6.02***	0.51	5.56	0.97	4.45***	0.67

Abbreviations: SFA=Saturated fatty acid, MUFA=Monounsaturated fatty acids, PUFA= Polyunsaturated fatty acid, DHA=Docosahexaenoic acid, EPA=Eicosapentaenoic acid, AA=Arachidonic acid

Stars in a row indicate significant difference between AS1 and BT: * p<0.05; **p<0.01; ***p<0.001

4.3. Fatty acid composition in triacylglycerols

In table 4 the FA composition of TAG is presented. The content of EPA (20:5n-3) differed significantly between Atlantic salmon 1 and Brown trout in the TAG fraction ($p < 0.0005$). No relevant difference could be seen in AA ($p < 0.25$) but a slight difference in DHA ($p < 0.086$). Both LA (18:2n-6) and ALA (18:3n-3) differed significantly between the two species ($P < 0.0003$ and $P < 0.01$).

Brown trout have significantly higher amount of both SFA ($P < 0.006$) and MUFA ($P < 0.01$) as shown by table 5. Atlantic salmon 1 has higher amounts of PUFA ($P < 0.0006$) which is seen in both the n-3 ($P < 0.0016$) and n-6 ($P < 0.0021$), 34.9 vs 30.8 and 7.08 vs 5.5. Brown trout had a significant higher n-3:n-6 ratio ($P < 0.04$), 5.6 vs 4.9 in AS1 as well as a higher DHA:EPA ($P < 0.02$), 2.5 vs 2.0. EPA:AA was significantly higher in Atlantic salmon ($P < 0.005$), 8.3 vs 6.6 in BT.

Table 4 - Fatty acid composition (in %) of triacylglycerols in Atlantic salmon and brown trout eggs

	Fatty acid composition means of triacylglycerols in %					
	Atlantic salmon				Brown trout	
	(n=8)		(n=4)		(n=4)	
Fatty acid	AS 1	STDEV	AS 2	STDEV	BT	STDEV
C14:0	1.68***	0.17	1.67	0.25	2.29***	0.26
C14:1	0.04	0.08	0.04	0.08	0.03	0.07
C16:0	10.8**	0.77	11.0	0.75	12.6**	0.78
C16:1(n-7)	5.46**	0.52	5.94	0.38	6.36**	0.50
C17:0	0.11	0.16	0.16	0.19	0.11	0.13
C17:1	0.43*	0.05	0.41	0.05	0.35*	0.04
C18:0	2.98	0.51	3.12	0.20	3.06	0.12
C18:1(n-9)	31.9	1.67	33.2	1.93	32.3	1.50
C18:1(n-7)	3.64***	0.37	4.13	0.46	4.97***	0.47
C18:1(n-5)	0.26	0.14	0.29	0.03	0.21	0.07
C18:2(n-6)	5.44***	0.62	5.38	0.27	3.80***	0.34
C18:3(n-3)	3.22*	0.39	3.15	0.44	2.64*	0.20
C20:1(n-9)	0.66***	0.13	0.66	0.06	1.35***	0.16
C20:2(n-6)	0.58***	0.05	0.64	0.09	0.78***	0.05
C20:3(n-6)	0.04	0.12	0.07	0.14	0.00	0.00
C20:4(n-6)	1.02	0.12	0.98	0.11	0.96	0.18
C20:3(n-3)	0.58*	0.12	0.62	0.16	0.70*	0.06
C20:5(n-3)	8.42***	0.79	7.77	1.11	6.18***	0.84
C22:5(n-3)	5.81	0.36	5.76	0.70	5.83	0.58
C22:6(n-3)	16.9	1.42	14.9	1.02	15.5	1.89

Stars in a row indicate significant difference between AS1 and BT: * p<0.05; **p<0.01; ***p<0.001

Table 5 - Summary of triacylglycerols (in%)

Summaries and ratios between fatty acids in triacylglycerols						
	Atlantic salmon				Brown trout	
	(n=8)		(n=4)		(n=4)	
Fatty acid	AS 1 %	STDEV	AS 2 %	STDEV	BT %	STDEV
SFA	15.5**	1.45	15.9	0.95	18.1**	1.07
MUFA	42.5**	1.50	44.7	1.64	45.6**	2.43
PUFA	42.0***	1.49	39.3	2.29	36.4***	3.05
PUFA n-3	34.9**	1.19	32.3	2.26	30.8**	2.60
PUFA n-6	7.08**	0.75	7.07	0.39	5.54**	0.47
n3/n6	4.99*	0.58	4.58	0.41	5.57*	0.16
DHA/EPA	2.03*	0.31	1.95	0.23	2.54*	0.40
EPA/AA	8.29**	0.63	8.01	1.59	6.55**	1.32

Abbreviations: SFA= Saturated fatty acid, MUFA=Monounsaturated fatty acids, PUFA= Polyunsaturated fatty acid, DHA= Docosahexaenoic acid, EPA=Eicosapentaenoic acid, AA=Arachidonic acid

Stars in a row indicate significant difference between AS1 and BT: * p<0.05; **p<0.01; ***p<0.001

4.4. Fatty acid composition of phospholipids

The content of EPA was found to differ significantly in the PL fraction ($P < 0.034$) shown in table 6, with higher values in Atlantic salmon 1 vs Brown trout; 7.6 vs 6.9. Neither AA ($P < 0.12$) nor DHA *(*$P < 0.26$) differed significantly. LA was present in significantly higher amount in Atlantic salmon ($P < 0.026$) but no difference was seen in ALA ($P < 0.43$).

SFA was present in a significantly higher amount in Atlantic salmon ($P < 0.0003$) but MUFA was significantly higher in brown trout ($P < 0.0024$). Total PUFA and n-3 PUFA on the other hand did not differ, only the n-6 fraction where Brown trout had higher levels ($P < 0.046$). Atlantic salmon had slightly higher ratios of n-3:n-6, 8.7 vs 8 ($P < 0.069$). The DHA:EPA ratio was slightly higher in brown trout, 3.8 vs 3.5 ($P < 0.076$) and the EPA:AA was significantly higher in the Atlantic salmon 1, 3.1 vs 2.6 ($P < 0.024$).

Table 6 - Fatty acid composition (in %) of phospholipids in Atlantic salmon and brown trout eggs

Fatty acid composition means of phospholipids in %						
	Atlantic salmon				Brown trout	
	(n=8)		*(n=4)*		*(n=4)*	
Fatty acid	AS 1	STDEV	AS 2	STDEV	BT	STDEV
C14:0	0.74*	0.12	0.63	0.06	0.88*	0.09
C16:0	19.1	1.60	18.0	0.68	18.2	0.36
C16:1(n-7)	1.27***	0.13	1.25	0.20	1.62***	0.12
C17:0	0.35*	0.21	0.39	0.26	0.08*	0.17
C18:0	13.1***	0.46	13.3	0.22	8.96***	0.55
C18:1(n-9)	14.7	2.90	15.2	1.43	15.3	0.93
C18:1(n-7)	3.50***	0.50	3.68	0.40	5.07***	0.09
C18:1(n-5)	0.13	0.18	n/a	n/a	0.16	0.31
C18:2(n-6)	1.44*	0.12	1.42	0.16	1.25*	0.20
C18:3(n-3)	0.55	0.26	0.73	0.10	0.56	0.38
C20:1(n-9)	1.01***	0.26	0.96	0.09	2.89***	0.28
C20:2(n-6)	0.86**	0.23	0.87	0.15	1.29**	0.23
C20:4(n-6)	2.47	0.28	2.66	0.30	2.68	0.22
C20:5(n-3)	7.65*	0.62	7.48	0.43	6.91*	0.52
C22:5(n-3)	6.23***	0.22	6.57	0.67	7.65***	0.56
C22:6(n-3)	26.9	1.07	26.9	2.08	26.5	1.14

Stars in a row indicate significant difference between AS1 and BT: * p<0.05; **p<0.01; ***p<0.001

Table 7 - Summary of phospholipids (in %)

Summaries and ratios between fatty acids in phospholipids						
	Atlantic salmon				Brown trout	
	(n=8)		(n=4)		(n=4)	
Fatty acid	AS 1 %	STDEV	AS 2 %	STDEV	BT %	STDEV
SFA	33.3***	2.02	32.3	0.85	28.1***	0.67
MUFA	20.6**	2.36	21.1	1.61	25.1**	0.91
PUFA	46.1	1.52	46.6	2.11	46.8	0.64
PUFA n-3	41.4	1.45	41.6	2.15	41.6	0.77
PUFA n-6	4.77*	0.42	4.94	0.44	5.22*	0.30
n3/n6	8.72	0.80	8.48	1.03	8.00	0.55
DHA/EPA	3.54	0.30	3.59	0.22	3.86	0.41
EPA/AA	3.12*	0.37	2.83	0.27	2.60*	0.39

Abbreviations: SFA= Saturated fatty acid, MUFA=Monounsaturated fatty acids, PUFA= Polyunsaturated fatty acid, DHA= Docosahexaenoic acid, EPA=Eicosapentaenoic acid, AA=Arachidonic acid

Stars in a row indicate significant difference between AS1 and BT: * p<0.05; **p<0.01; ***p<0.001

5. Discussion

5.1. Total lipids

The total lipid content was in accordance with what other authors (Sargent, Tocher and Bell, 2003; Jonsson and Jonsson, 2011c) have previously stated in regards to salmonid eggs having a high lipid content of > 5 %. A high lipid content is possibly contributing to supporting the embryonic and alevin development, as the young salmonid depends on the nutrition the yolk-sac provides until the young fish becomes fry and starts feeding itself. The result is however not close to the common 10 % total lipid content suggested by these other authors. This could be a result of FA being catabolized to provide energy for varying processes, indicating the eggs are in development.

5.2. SFA

The salmonid eggs contained 20 % SFA in all sample groups, the majority located in the PL fraction where its main function is to maintain rigor in the cell membranes. The most common saturated fatty acid in fish are palmitic acid (16:0) and stearic acid (18:0) (Tocher, 2003) which in accordance to these results is also accurate for fish eggs. Myristic acid (14:0) and margaric acid (17:0) are the other two saturated FA present in the samples, but in very low quantities. These FA are commonly found in plants (14:0) and cattle (17:0) (Gunstone, 1999) so these low levels are to be expected.

The SFA are distributed with significant differences between the TAG and PL fraction among AS and BT. It is within the PL fraction that environmental changes in temperature can be seen to affect the FA composition (Jobling and Bendiksen, 2003). Lower temperatures lead to decreased amounts of SFA and increased UFA. As the brown trout had significantly lower amounts of SFA in the PL fraction than the Atlantic salmon, it verifies the literature stating that the BT inhabits colder waters than the AS. As climate change is altering the temperatures of the Atlantic to milder, with more precipitation and less ice-covered periods (Jonsson and

Jonsson, 2009), this variation in the FA composition could also be an effect partly attributed to environmental exposure. If temperatures continue to rise, this could further affect the FA composition and would thus need to be continuously monitored. Based solely on this conducted study, the differences in the SFA can't be explained, further investigation is warranted.

Table 8 - Comparison with Atlantic salmon, Baltic stock of M74 afflicted eggs and healthy eggs from Pickova (1998) of the essential fatty acid composition in the triacylglycerol- and phospholipid fraction

	Atlantic salmon, Baltic stock, Dalälven				Atlantic salmon mean		Brown trout	
	(n=4)		*(n=4)*		*(n=12)*		(*n=4)*	
Fatty acid	M74	STDV	NonM74	STDV	AS %	STDV	BT%	STDEV
Triacylglycerol								
C20:4(n-6)	0.9	0.18	1.0	0.07	1.01	0.11	0.96	0.18
C20:5(n-3)	9.7	2.21	8.9	0.80	8.20	0.91	6.18	0.84
C22:5(n-3)	5.4	0.33	5.4	0.42	5.80	0.47	5.83	0.58
C22:6(n-3)	16.3	0.22	15.3	1.0	16.3	1.57	15.5	1.89
SFA	13.9	1.07	15.3	2.33	15.7	1.28	18.1	1.07
MUFA	40.6	2.86	38.6	0.59	43.2	1.83	45.6	2.43
PUFA n-3	37.6	1.66	36.2	1.74	34.0	2.00	30.8	2.60
PUFA n-6	5.2	1.05	6.0	0.57	7.07	0.63	5.54	0.47
n3/n6	7.4	1.38	6.0	0.73	4.85	0.55	5.57	0.16
DHA/EPA	1.8	0.37	1.7	0.10	2.00	0.28	2.54	0.40
Phospholipids								
C20:4(n-6)	1.2	0.08	1.5	0.19	2.54	0.29	2.68	0.22
C20:5(n-3)	8.2	1.63	8.7	0.35	7.60	0.55	6.91	0.52
C22:5(n-3)	6.0	0.76	6.3	0.52	6.34	0.43	7.65	0.56
C22:6(n-3)	29.8	1.71	29.2	1.17	26.9	1.39	26.5	1.14
SFA	30.2	0.97	29.0	0.33	32.9	1.74	28.1	0.67
MUFA	18.1	0.8	18.1	1.06	20.7	2.08	25.1	0.91
PUFA n-3	45.5	1.12	46.1	1.27	41.4	1.62	41.6	0.77
PUFA n-6	2.2	0.21	3.0	0.47	4.83	0.42	5.22	0.30
n3/n6	20.7	1.95	15.6	2.6	8.64	0.84	8.00	0.55
DHA/EPA	3.8	1.17	3.4	0.25	3.56	0.26	3.86	0.41

5.3. MUFA

Fish can desaturase the SFA palmitic acid (16:0) into palmitoleic acid (16:1, n-7) as well as stearic acid (18:0) into oleic acid (18:1, n-9) (Tocher, 2003) which means that these FA are heavily intertwined. The unsaturated carbon of the MUFA denotes that the FA has a considerably lower melting point than its saturated precursor, which provides regulatory qualities, such as improved viscosity to cell membranes (Tocher, 2003). Oleic acid is the most abundant FA and MUFA in fish eggs in all categories (total lipid, TAG and PL), and is present to a higher extent than stearic acid. Reasons being that oleic acid is the favored MUFA incorporated as structural lipids in fish eggs (Czesny *et al.*. 2009). Other MUFA, like palmitoleic acid (16:1, n-7) and vaccenic acid (18:1, n-7) is used for catabolic processes as MUFA mainly functions as energy reserves. The high amount of oleic acid is thus an indication that this FA is being preserved for structural use. There are increased levels of MUFA in both TAG and PL compared to Pickova (1998), see Table 8.

5.4. PUFA

In total lipids, the PUFA content is 48 % in AS and 44 % in BT. High PUFA levels are necessary as they are essential building blocks for development, on the contrary high PUFA levels also increase the risk for lipid oxidation. This is generally balanced through adequate levels of antioxidants, such as astaxanthin, in the fish's diet. Knowing the astaxanthin levels would thus provide a greater insight into whether the current PUFA levels are potential drivers for risk of disease.

In this study, the LA (18:2, n-6) proportion is around 3 %, which is low. High level of LA (18:2, n-6), around 10 %, indicates a diet rich in vegetable oils which is commonly seen in farmed salmonids. The inclusion of vegetable oils has increased steadily the last decades as marine-based feed has become costly. A higher LA (18:2, n-6) content can also be seen in escaped fish (Jensen *et al.*, 2020), as well as in wild fish from brackish- or fresh-water habitats as LA is common in freshwater microalgae (Torniainen *et al.*, 2017). The low LA content suggests, in our study, that the eggs are of wild origin or from fish feeding on natural prey during fattening before spawning.

A study investigating early mortality syndrome in lake trout found a positive correlation with the presence of docosapentaenoic acid (DPA) (22:5, n-3) in PL (Czesny *et al.*. 2009). Both the BT and AS have similar levels of DPA to that of the lake trout, indicating an increased risk for early mortality syndrome (EMS) in the swim up stage of the fish. There can also be an unknown factor, not related to the EMS. DPA in moderate amounts is an important FA for the bioactive molecules in prostaglandin and eicosanoid synthesis and the hormonal reactions these molecules instigate.

DHA is the second most abundant FA in total, following oleic acid, and the most abundant PUFA with levels around 20 %. The lack of significant differences in DHA and AA in all fractions indicates the general importance of these FA. In PL, the n-3 precursor for DHA and EPA, ALA (18:3, n-3), had no difference and was present in very low amounts, 0.55 % in AS1 and 0.56 % in BT. This further strengthens the argument that DHA is the preferred FA in PL. The DHA level in the TAG fraction is considerably lower than in the PL fraction which could indicate that the majority of DHA was directed to the PL as it's a vital component of the cell membrane, leaving little excess available for the TAG. Comparing results to Pickova (1998), the PL DHA has decreased and the AA has had a substantial increase. This could be a result of a dietary change or warmer temperatures, or any other reason not investigated in this study.

As expected, AA was found in higher levels in the PL fraction than TAG as it is commonly found in the PL bilayer with no significant difference between the species. Its role as a precursor for vital hormone-like substances indicates that the FA is utilized fully in both AS and BT. The AA proportion is seemingly stock specific, Pickova (1998) and others suggest that landlocked stocks will exhibit higher levels of this FA. These findings indicate that the sampled eggs are of wild anadromous origin.

Of the investigated FA, EPA is the one that differs most between species with significantly higher levels found in Atlantic salmon. A possible explanation would be that the AS have had a diet richer in EPA and thus had greater reserves of this important FA in adipose tissue of the fish.

As the literature has stated, AA is the preferred FA as precursors for eicosanoids (Czesny *et al.*, 2009). A higher amount of EPA is therefore not naturally beneficial, as it's the overall composition and ratio which is important. EPA and AA contribute with anti-inflammatory activity and the eicosanoid and prostaglandin synthesis, being the bases of this. These FA must be in a balance to sustain the correct inflammation response suitable for the fish.

5.5. General discussion

There was no difference in the n-3/n-6 ratio between AS and BT in total lipids, but a significant difference in the TAG ($P < 0.042$). Here, levels were found between 4.6 and 5.0 in AS and 5.6 in the BT. However, when comparing the PL n-3/n-6 ratio with Pickova (1998) the differences are greater, see Table 8. In AS eggs from 1998, the ratio was 20.7 in M74 affected fish and 15.6 in healthy fish compared to 8.6 in AS and 8.0 in BT. The main change in the FA composition is an increase in the amount of n-6 PUFA as well as a decrease in n-3. The ratio affects the reproductive function as there is a delicate balance where the interplay and competition between FA will impact the embryonic development and future larvae survival. A different

n-3/n-6 ratio could be grounds for new diseases and challenges for the species but could also be a result of a variety of other factors.

For healthy embryonic development, a ratio of 2:1 is preferred between DHA:EPA (Torniainen et al., 2017). This ratio was slightly elevated in all sample groups, and expectedly higher in PL than TAG; 3.5 in AS1 and 3.9 in BT and 2.0 and 2.5 respectively. The functional PL fraction is protected to a further extent from lipid degradation, this fraction thus gives a better insight in the FA composition prior to the female initiated fasting for the spawning. One can speculate if the brown trout has had a diet with a FA profile richer in DHA and thus lower in EPA in comparison to the Atlantic salmon. As EPA is primarily targeted over DHA for catabolic processes, such as gonad production, a low EPA diet would consequently result in lower EPA reserves in the adipose tissue, with little possibility to deposit EPA in the eggs. The results make an argument that there is a dietary ratio improvement to be made between DHA and EPA. A higher ratio in DHA:EPA than 2:1, which is especially seen in the brown trout, has consequences. In lake trout, ratio levels > 3 in PL and > 1.5 in TAG was associated with EMS (Czesny et al., 2009). The ratio indicates potential unhealthy conditions for normal embryonic development and thus an increased risk for juvenile mortality. Pickova (1995) concluded that increased levels of DHA linked with low thiamine levels was present in M74 infected eggs. As no measurements of thiamine was conducted in this study, and with no status of the health condition of the maternal fish, this comparison can't be made in full.

AS has a higher ratio of EPA:AA than BT. A high EPA:AA ratio could be grounds for increased risk of disease, these levels are however similar to those of the Baltic salmon eggs in Torninainen (2017) which argue that the levels are adequate for wholesome embryonic development.

To also investigate the astaxanthin in the eggs would be a good way to elucidate the antioxidative protection in the different stocks, respectively.

6. Conclusion

The purpose of this study was to investigate the FA composition and identify possible differences between Atlantic salmon and brown trout as well as comparing the results with findings from 1998.

Comparing the FA composition between AS and BT, differences were found in distribution between the TAG and PL fractions. BT had lower amounts of SFA indicating its habitats are colder. No significant differences were found in AA or DHA. EPA was found in significant higher amounts in AS, indicating a dietary habitat richer in EPA. Partly due to this, PUFA ratios differed between species. As an increased ratio of DHA:EPA is linked to EMS, the sampled salmonid eggs could suffer from poor hatching success. Both the AA level and the low LA (18:2, n-6) indicate that the sampled eggs are anadromous and of wild origin.

In 23 years, the FA composition is quite similar besides from the n-3/n-6 ratio that has shifted. FA ratios are complex as there are many intertwining factors, external factors such as climate and temperatures could be an equal contributing factor to as well as diet. As the n3/n6 ratio of the egg has changed in relation to both healthy and M74 affected fish from Pickova (1998), it may have affected the egg quality indicating that the fish could be facing potential new diseases and challenges with this new FA composition.

The FA composition that the egg has will have direct effect on the survival chances as the lipids support the nutritional needs essential for healthy embryonic development. The dietary FA ratios is thus a vital component for healthy animals and fish stock as all the n-3 and n-6 FA compete biochemically yet have so diverse biological roles that they are not exchangeable. With the BT having even higher DHA:EPA ratios than that of the AS, the health and thus egg quality of the BT might be facing more severe challenges than the AS.

Continued monitoring of the AS and BT with research linking habitat, temperatures and diets to the FA composition would contribute to a greater understanding of the health condition of the species.

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And to Kåra, my main motivator. It's all for you.

Popular scientific summary

The popular science summary can be found on the following pages.

Does the lipid composition in salmonid eggs matter?

And is there a difference amongst related species?

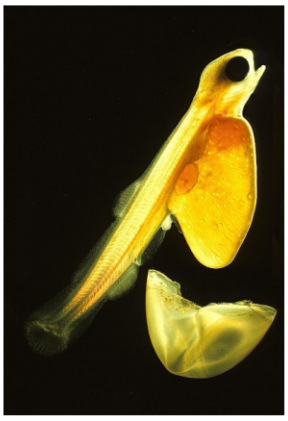
Introduction

Atlantic salmon and brown trout are found in the entire Atlantic of the Northern Hemisphere. They are part of the salmonid family and unique as they move between fresh and salt water. They are popular fish on the global market due to their nutritional profile with a high fat content including high amounts of the healthy omega-3 fatty acids. Most salmonid consumed today originate from farmed sources, as wild salmonids have decreased in populations caused by the water power plants in the rivers important for migration.

The embryonic development lays the foundation for the life to be, the building blocks available in this stage of life will determine the embryonic development. To increase the knowledge on the species requirements for survival, studies are carried out to increase the understanding of what the specific building blocks are, their roles and how they relate to each other.

Background

Fats are one of these main building blocks and more specifically fatty acids. The fats qualities as well as function will be determined by the variety and relationship of the individual fatty acids, which decides the size and the amount of double bonds within the molecule. Saturated fatty acids have no double bonds, monounsaturated fatty acids have one and polyunsaturated fatty acids (PUFA) have several. Some fats, mainly PUFA, are essential to consume through diet as the body either can't produce them or will do so in very low quantities. DHA, EPA and AA are three known long chain PUFA that are of specific importance which contribute to healthy embryonic development in salmonids. Amongst other things, these three PUFA take part in maintaining normal cell function and act as precursors for vital hormones, so imbalances in these fatty acids can cause malformations and mortalities.



A recently hatched salmon alevin with a yolk sac Photographer: Uwe Kils (2005) CC-BY-SA 3.0.

About the study

This study was a master thesis project at the Department of Molecular Sciences at SLU, Uppsala. The purpose of the study was to look at the fatty acid composition of fertilized eggs from Atlantic salmon and brown trout from the Dala river and to reveal potential fatty acid differences between species but also differences to eggs collected in 1996. To enable this, the fats from the eggs were extracted, separated and analyzed.

Linnéa Appert

A popular science summary of the master thesis "Fatty acid composition of salmonid eggs – A compartive study of Atlantic salmon and brown trout

SCIENCE AND EDUCATION FOR SUSTAINABLE LIFE

Does the lipid composition in salmonid eggs matter?

And is there a difference amongst related species?

Results

The fatty acid composition tells us that the eggs are of wild origin or have at least fed on natural prey before spawning.

The majority of the saturated fats were found in the eggs cell membrane. Saturated fats have an important structural role in the membranes as these fatty acids are more rigorous than unsaturated fats. The latter contain double bonds which better maintains the fluidity in colder temperatures, higher amounts of unsaturation will increasingly contribute to maintaining cell membrane function as temperature decreases. Compared to the salmon, the trout had lower amount of saturated- and higher amounts of unsaturated fats in the cell membrane which tells us that it lives in colder waters. The presence of some unsaturated fats indicates a higher risk for disease and mortality in the young fish's life. DHA was an abundant fatty acid, but the content had decreased compared to the 1998 study, some fatty acids ratios of fatty acids had also changed. This difference could be due to a warmer climate or a dietary change of the fish. The levels of DHA and AA didn't differ between the species, the similarity indicate that these fatty acids are important regardless of species. EPA differed the most between the salmon and trout, possibly due to varying feeding habitats where the trout could have access to prey more abundant in EPA.

Salmon egg in varying stages of development.
Photographer: Uwe Kils (2005) CC-BY-SA 3.0.

Conclusion

The purpose of the study was to compare the fatty acid composition between salmon and trout eggs and compare with results from 1998. The differences between species are small but present. The lipid composition of the eggs is similar to what they were 23 years ago. This tells us that the eggs are resilient to change and that the fish, to its best abilities, will allocate necessary resources to the egg. A shift in ratios between certain fatty acids could have an effect on the embryonic health. Future research linking diet, habitat and climate to the FA composition will increase the understanding of the fish's condition.

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SCIENCE AND EDUCATION FOR SUSTAINABLE LIFE