

Assessing the quality of Atlantic salmon (*Salmo salar*) eggs

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TABLE OF CONTENT

List of abbreviations.....	4
I. FOREWORD	5
II. LITERATURE SURVEY	7
1. Worldwide fish egg production and consumption.....	7
2. Salmonids	11
2.1. Atlantic salmon (<i>Salmo salar</i>)	11
2.2. Other salmonids	19
3. Fatty acids.....	20
3.1. Definition	20
3.2. Polyunsaturated fatty acids and omega-3 long chain polyunsaturated fatty acids.....	20
3.3. Poly-unsaturated fatty acids importance and dietary requirements.....	22
3.4. Human sources of n-3 polyunsaturated fatty acids	23
4. Atlantic salmon eggs	25
4.1. Fish egg composition	25
4.2. Fish egg quality to improve the survival rate	26
5. Sensory analysis	28
III. OBJECTIVES AND EXPERIMENTAL STRATEGY	30
IV. ATLANTIC SALMON EGGS FOR A BETTER SPAWNING SUCCESS	31
1. Material and methods	31
1.1. Egg and feed sampling	31
1.2. Analytical methods	32
1.3. Statistical methods	32
2. Results	34
3. Discussion.....	45
V. ATLANTIC SALMON EGGS FOR HUMAN CONSUMPTION.....	52
1. Material and methods	52
1.1. Egg and feed sampling	52
1.2. Analytical methods	54
1.3. Sensory test	54
1.4. Statistical methods	55
2. Results	57

2.1. Chemical analyses.....	57
2.2. Sensory test	70
3. Discussion.....	75
3.1. Chemical analyses.....	75
3.2. Sensory test	78
VI. GENERAL DISCUSSION AND PERSPECTIVES.....	81
Bibliography	85

LIST OF ABBREVIATIONS

AA	Arachidonic acid (20:4n-6)
ALA	α -linolenic acid (18:3n-3)
AOAC	Association of Official Analytical Chemists
CNSS	Conservatoire National du Saumon Sauvage
CoSMos	Conservatoire du Saumon Mosan
DHA	Docosahexaenoic acid (22:6n-3)
DM	Dry matter
EPA	Eicosapentaenoic acid (20:5n-3)
FAME	Fatty acid methyl ester
FM	Fresh matter
GC	Gas chromatography
HUFA	Highly unsaturated fatty acid(s)
ISO	International Organisation for Standardisation
LA	Linoleic acid (18:2n-6)
LC-PUFA	Long chain polyunsaturated fatty acid(s)
MUFA	Monounsaturated fatty acid(s)
n-3	Omega-3
n-6	Omega-6
n-9	Omega-9
PUFA	Polyunsaturated fatty acid(s)
SFA	Saturated fatty acid(s)
TFA	Total identified fatty acids

I. FOREWORD

Fish eggs are famous around the world, thanks to the authentic caviar derived from sturgeon (*Acipenseridae*). These little black pearls are rare and purchased at outrageous prices (ranging on average from 100 to 490 euros per 50 g). Therefore, we mostly find them during festive celebrations. Nowadays, a large range of fish eggs are commercially available at reasonable prices (at an average of 10 euros per 50 g), like trout (*Oncorhynchus spp* – *Salmo*) eggs and Pacific salmon (*Oncorhynchus spp*) eggs. We can find them as decoration on dishes or on toasts for aperitives, but they still remain food item for special occasions.

One of the reasons for the growing interest in fish eggs, in our countries, is their high content in health-promoting omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA). Indeed, fish eggs may represent an additional source of n-3 LC-PUFA to meet the increasing human demand.

The reason why we do find Pacific salmon eggs on the market and seldomly from Atlantic salmon (*Salmo salar*) remains an open question. Is this because the Atlantic salmon eggs are of poor quality and taste? This is a key question to answer to before we can further explore if egg production could become a valid opportunity to give an added value to the Atlantic salmon farming.

Currently, most existing fish farms have as purpose to produce Atlantic salmon for the flesh or smolts production. Apparently, none has developed the opportunity to produce and commercialise Atlantic salmon eggs for human consumption. The reason for this, remains unclear and could possibly be linked to organoleptic aspects. An intensive production of Atlantic salmon to collect their eggs for human consumption, would require, among others, a high egg nutritional quality maintained over time.

In Belgium, the Atlantic salmon farming has developed techniques allowing the production of significant volume of Atlantic salmon eggs. One fish farm in Wallonia has set up a system to breed Atlantic salmons and collect eggs from farmed and semi-wild Atlantic salmons in order to perform artificial reproduction aiming to restock the rivers of the Meuse basin. Moreover, this farm can maintain sexually mature females and collect good quality eggs from the same fish for several years. However, the survival rate of juveniles (alevins and parrs), at farm and possibly after their release in the rivers, is still low. Evaluating the reason of this low survival rate is a key success factor to improve the Meuse river restock programme. This might be due to the egg lipid composition that could vary, according to different factors such as, the growth area and the number of years spent at sea for the semi-wild adults of the Loire-Allier origin captured in the Meuse, or the artificial diet fed and ration given to farm-born captive F1 broodstocks. These are some of the questions that the “Saumon Meuse” programme tries to answer to, in particular with the help of the fish farm named “Conservatoire du Saumon Mosan” (CoSMos, Erezée, Belgium).

The goal of this master thesis is to contribute answering to the two following questions:

- Is there a link between the egg composition and the survival rate of juvenile Atlantic salmon in CoSMos, which would in term influence the efficiency in the restocking of the Mosan basin rivers?
- Is there a nutritional and organoleptic interest in launching an Atlantic salmon egg production for human consumption?

These questions will be addressed through comparisons of different types of fish eggs from different fish species, from farmed or semi-wild animals, from Belgium (Meuse) or from France (Loire-Allier), etc.. For more clarity, this work will be divided into two parts, each, focussing on one of the two questions.

The present master thesis will first review information found in the literature to provide a better understanding of the subject, before to develop the objectives and the experimental strategy, aiming at addressing the two distinct questions of this work. Then, the practical part will be divided in two sections, each developing the fish egg sampling strategy and the comparisons selected to respond to the question. Proximate composition and fatty acid profile of the samples will be determined. In addition, a sensory test will be conducted to evaluate the appreciation of the Atlantic salmon eggs by potential consumers. This master thesis will end up with a general discussion, gathering the two aspects of the work, and proposing further research perspectives.

II. LITERATURE SURVEY

1. Worldwide fish egg production and consumption

Fish eggs are already well known on the market and are worldwide consumed. The most prestigious ones on the market, but not affordable for everyone, remain caviar, which are issued from sturgeon. Currently, we can also find in our market eggs from lump (*Cyclopterus lumpus*), trout, Pacific salmon and many other fish species.

The edible fish eggs are issued from the mature ovaries of the females and can also be called roe. They present themselves attached to one another in connective tissues and not as an individual distinct egg like chicken eggs. In the case of sturgeon eggs, we can only define them as caviar once they are split thanks to the salt added (Cordain, 2015).

Beluga sturgeon (*Huso huso*) roe of the Caspian Sea, is regarded as the true caviar. Caviar also exists from other types of sturgeon but those are considered as imitations. Beluga is the largest sturgeon worldwide. It can reach up a length of five meters and a weight of two tonnes, producing 10 to 15 kg of caviar per mature female (Monfort, 2002). Beluga eggs have a diameter of 3.5 mm and a colour ranging from light to dark grey (Monfort, 2002). Caviar can be sold as fresh product or pasteurised. Due to overfishing, sturgeon has been on the path to extinction, notably in the Caspian Sea. Therefore, its capture fishery is protected since 1998 by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (FRC magazine, 2010-2011). Most of the caviar is now originating from farmed fish (approximately 90% of the global production), which enables an increased caviar supply, but also leads to a different egg taste (Planetoscope, 2012). In 2002, the production of caviar from farmed fish in Europe was estimated at about 15 tonnes (Monfort, 2002). In 2012, this number reached 260 tonnes for the world caviar production from farmed fish (Bronzi & Rosenthal, 2014). The main sturgeon species raised for caviar production are the Siberian sturgeon (*Acipenser baerii*) and the white sturgeon (*Acipenser transmontanus*) (Monfort, 2002). When the production of caviar from wild sturgeon was still authorised, the main producers were Russia and Iran. Now that aquaculture has been strongly developed, Italy, France (Aquitaine), Belgium, Germany, Spain and China appear to be the main producers (Monfort, 2002; Nebot, 2018). However, new farms rapidly grow in other countries, which will lead to an increase of global production and may lead to a decrease of the price (Planetoscope, 2012; Nebot, 2018). The main consumers of caviar are first located in Russia, then, in Iran, in the EU (mainly France, Germany and Spain) and in USA (Bronzi et al., 2011). Beluga caviar can be purchased at prices ranging from 4,500 to 5,500 euros per kilo, whereas French caviar, produced from the farmed sturgeon *Acipenser baerii*, can cost between 1,400 and 1,800 euros per kilo (Monfort, 2002).

Currently, the eggs of more than 38 fish species are used to produce substitutes to the true caviar (Bronzi & Rosenthal, 2014). These other fish roes are more affordable. The most popular in Europe, among these fish roe substitutes, are the eggs from lump originating from the North Atlantic Ocean and Baltic Sea. Lumpfish caviar is mainly

produced in Canada, Greenland, Denmark, Sweden and Iceland (Johannesson, 2006; Ethic Ocean, 2017). Each lumpfish can produce 700 g of roe (Monfort, 2002). A paper of the FAO fisheries department in 2006, reported that between 3,000 and 4,000 tonnes of lumpfish caviar were sold annually, and that the main consumers were located in France, Germany and USA (Johannesson, 2006). The same paper gave an estimation of the lumpfish caviar consumption per country, as a percentage of the total world market. Europe would be the main market for lumpfish caviar with 80% of the world consumption (France 30%, Germany 20%, Spain 10%, Italy 10% and others 10%), followed by USA, with 15% of the world lumpfish caviar consumption. The remaining 5% corresponds to the consumption of Asian and other non-European countries (Johannesson, 2006). The lumpfish eggs are, generally, extremely salty (10%) and contain a high level of artificial dye (mainly of azo nature) in order to obtain their red or dark colour, since they are naturally grey (Monfort, 2002; Simon, 2016). The lump eggs are known as the most cost-effective alternative to caviar but induce a high environmental cost since females are often thrown overboard once eggs have been collected, considering their poor flesh value (FRC magazine, 2010). More importantly, there is no specific management of the lump fishery and we currently cannot know the stock levels. Their consumption should thus be avoided (WWF, 2018). Lumpfish roes can be purchased at prices ranging from 20 to 30 euros per kilo (Monfort, 2002).

The production of the rainbow trout (*Oncorhynchus mykiss*) eggs, is mainly located in European fish farms, mostly represented by France and Denmark (Ethic Ocean, 2017). Trout roes can be purchased at prices ranging from 50 to 80 euros per kilo (Monfort, 2002).

The Pacific salmon eggs, also known as the red caviar, are mainly originating from Japan, Russia, Canada and USA (Bledsoe et al., 2003). Those Pacific salmon eggs are primarily derived from salmon species of the north-eastern Pacific Ocean (near Alaska during summer and along Washington State during fall). Amongst the five different species of Pacific salmon, three are more common for their egg consumption: the Coho salmon, *Oncorhynchus kisutch* (silver), the Pink salmon, *Oncorhynchus gorbusha* (humpback) and the Chum salmon, *Oncorhynchus keta* (dog) (Bledsoe et al., 2003). The King salmon, *Oncorhynchus tshawytscha* (chinook) and the Sockeye salmon, *Oncorhynchus nerka* (red) are less popular for their egg consumption among producers and consumers. These Pacific salmon eggs differ in their size and in their pigmentation. The Pink salmon roe with a middle size of 7 mm are the most popular. Besides, the caviar colour can range from light orange for the Chum salmon to dark red for the Sockeye salmon. The flavor can also vary between species, from a rich taste to a sweeter flavor (Nikitenko, 2016). The Pacific salmon eggs are more commonly consumed in Japan, known as "ikura", with an estimated consumption of 4,000 tonnes in 2002, and in Eastern Europe, mostly represented by Germany, followed by France, where they are called "ikra", with an estimated consumption of 300 to 400 tonnes in 2002 (Monfort, 2002; Nikitenko, 2016). Data from 1976 already reported that the majority of the Pacific salmon roe production of the west coast of North America was consumed in Japan (Tsuyuki et al., 1976). Besides, until 2002, France, Spain, Germany, Taiwan, Holland, China, Korea and

Scandinavia were also importing large amounts of Pacific salmon roe, which represented 90% of the USA production (Bledsoe et al., 2003). Wild salmon roes can be purchased at prices ranging from 80 to 200 euros per kilo (Monfort, 2002).

Worldwide, there is currently no “industrial” Atlantic salmon egg production aiming for human consumption. It seems, that the main reason would be the “aquaculture taste” of Atlantic salmon eggs, since the egg production is primarily aiming for the reproduction of the fish and based on farmed salmon (Bledsoe et al., 2003). However, with the worldwide increased interest in fish eggs on the market, some Atlantic salmon farmers began maintaining fish until sexual maturity to collect their eggs for sold, but this commercial activity was only hold on a small scale (Bledsoe et al., 2003). The situation is currently still the same, with the exception of one fish farm in Australia, which appear to produce Atlantic salmon roes, known as "Yarra Valley Caviar" (Yarra Valley Caviar, 2014). Many other fish eggs are also produced and consumed as described in Table 1.

The Japanese population is the first consumer of fish eggs. Japanese are appreciating almost all roe including roe from salmon, pollock (*Pollachius virens*), herring (*Clupea harengus*), mullet (*Mugil cephalus*), flying fish (*Exocoetidae*) and smelt (*Osmeridae*) (Cordain, 2015).

All fish eggs found on the market are salted and pasteurised. The salting process represents the common method of preservation of fish eggs, by its inhibiting action towards microbial growth and enzyme activity. Moreover, a salting cure allows obtaining the desired viscosity, elasticity, volume and firmness of the eggs (Sternin & Dore, 1998). The quality of the salt used is very important. The salt used should be a non-iodised, non-chlorinated salt, without additives and any impurities such as micro-organisms. Many other factors can influence the duration of the salting process and its efficacy, such as the maturity, size and freshness of the eggs, the saturation and temperature of the brine, the fish species and the global brewing of the solution (Sternin & Dore, 1998). At the industry level, keeping the salt level at a constant rate is not easy and a margin of error of 0.2% is tolerated. The general salt level appreciated by consumers is around 3.5-4%. Such a level is not sufficient to ensure a good conservation of the product. Indeed, in order to ensure a good conservation, the salt level of a product should be around 10% (Sternin & Dore, 1998). The pasteurisation process is therefore essential. Considering salmonid eggs, the maximum temperature for pasteurisation is about 65-69°C for 60 to 120 minutes (Sternin & Dore, 1998; Bledsoe et al., 2003). With this range of temperature, the fish eggs keep their colour and their constituent integrity (Bledsoe et al., 2003).

Roe are sold and served in many different forms throughout the world. They can be processed into cured individual eggs, these can be called caviar, or as a whole egg product. They are used in various dishes as accompaniment or garnish in appetizers, seafood salads, sushi or pasta dishes (Bledsoe et al., 2003).

Table 1. Commonly consumed fish and aquatic animal roe (Cordain, 2015)

COMMON NAME	GENUS SPECIES
ANCHOVY	<i>Engraulis mordax</i>
CAPELIN (ATLANTIC)	<i>Mallotus villosus</i>
CARP	<i>Cyprinus carpio</i>
CATFISH/CLARIUS SP.	<i>Clarius species</i>
CATFISH (SHOVEL NOSE)	<i>Hemisorubim platyrhynchos</i>
CATFISH CHANNEL	<i>Ictalurus punctatus</i>
CROAKER (LARGE YELLOW)	<i>Pseudosciaena crocea</i>
FLOUNDER	suborder: <i>Pleuronectoidei</i>
FLOUNDER (SLIME)	<i>Microstomus achne</i>
FLYING FISH	<i>Cheilopogon furcatus</i>
HAKE (SOUTHWEST ATLANTIC)	<i>Merluccius hubbsi</i>
HERRING (PACIFIC)	<i>Clupea pallasii</i>
LUMPFISH	<i>Cyclopterus lumpus</i>
MACKEREL (ATKA)	<i>Pleurogrammus azonus</i>
MULLET	<i>Mugil cephalus</i>
ORANGE ROUGHY	<i>Hoplostethus atlanticus</i>
PADDLEFISH	<i>Polyodon spathula</i>
PIKE	<i>Esox species</i>
POLLOCK	<i>Theragra chatcogramma</i>
SALMON	
SALMON (CHUM)	<i>Oncorhynchus keta</i>
SALMON (ATLANTIC)	<i>Salmo salar</i>
SALMON (COHO)	<i>Oncorhynchus kisutch</i>
SALMON (PINK)	<i>Oncorhynchus gorbuscha</i>
SALMON (SOCKEYE)	<i>Oncorhynchus nerka</i>
SALMON (CHINOOK)	<i>Oncorhynchus tshawytscha</i>
SALMON (PACIFIC MASU)	<i>Oncorhynchus masou masou</i>
TROUT (RAINBOW)	<i>Oncorhynchus mykiss</i>
STEELHEAD TROUT	<i>Oncorhynchus mykiss</i>
TROUT	<i>Salmo trutta</i>
SAKHALINE TAIMEN	<i>Hucho perryi</i>
ARCTIC CHAR	<i>Salvelinus alpinus</i>
CHAR (JAPANESE)	<i>Salvelinus leucomaenis leucomaenis</i>
WHITE FISH	<i>Coregonus albula</i>
SOLE (DUSKY)	<i>Pleuronectes mochigarei</i>
SMELT (SHISHAMO)	<i>Spirinchus lanceolatus</i>
STURGEON	
BELUGA CAVIAR	<i>Huso huso</i>
OSETRA CAVIAR	<i>Acipenser gueldenstaedti</i>
IMPERIAL CAVIAR	<i>Acipenser persicus</i>
WHITE STURGEON	<i>Acipenser transmontanus</i>
STERLET	<i>Acipenser ruthensis</i>
SEVRUGA	<i>Acipenser stellatus</i>
CHINESE KALUGA STURGEON	<i>Huso dauricus, A. dauricus, A. mantschuricus</i>
AMUR RIVER STURGEON	<i>Acipenser schrenki</i>
SHIP STURGEON	<i>Acipenser nudiiventris</i>
TUNA	
SKIPJACK	<i>Katsuwonus pelamis</i>
TONGUL	<i>Thunnus tonggol</i>
BONITO	<i>Euthynnus affinis</i>
NON-FISH ROE	
SQUID (PURPLE SQUID)	<i>Sthenoteuthis oualaniensis</i>
SEA URCHIN	phylum -- <i>Echinodermata</i>
SHRIMP	class -- <i>Malacostraca</i>
SCALLOPS	class -- <i>Malacostraca</i>
LOBSTER	class -- <i>Malacostraca</i>
<i>Polyodon spathula</i>	<i>Polyodon spudon silyodon spathula</i>

2. Salmonids

The term salmonid, refers to a family of fish *Salmonidae* which includes, salmon, trout, chars (*Salvelinus*), graylings (*Thymallus thymallus*) and whitefish (*Coregonus*) (Collins Dictionary, 2018) .

There are two groups of salmon in the world: the Atlantic salmon and the Pacific salmon. The first one corresponds to the “*Salmo salar*” species. The second one includes five different species: King salmon (chinook), Sockeye salmon (red), Coho salmon (silver), Pink salmon (humpback) and Chum salmon (dog) (Government of Canada, 2018). Concerning the trouts, there are also different species. Among them we can find the rainbow trout, *Oncorhynchus mykiss* and the sea trout, *Salmo trutta* (Government of Canada, 2018).

2.1. Atlantic salmon (*Salmo salar*)

The Atlantic salmon is an amphidromous anadromous fish species originating from every rivers flowing into the North Atlantic Ocean and Baltic Sea. More specifically, on the western side, from the Hudson river to the north of the Ungava Bay at Quebec, and on the eastern side, in the area ranging from northern Portugal to the Kara sea in the north of Siberia, including the rivers draining into the Baltic sea (Figure 1) (Laird & Needham, 1988). Nowadays, the distribution of wild Atlantic salmon remains similar to the historical range. “However, the number of rivers supporting spawning runs in each country as well as the estimated population densities are much lower than those recorded historically” (Government of Canada, 2013). Indeed, nowadays Atlantic salmon disappeared from most of these rivers due to the industrial pollution and the construction of dams, which constituted barriers to migration. A third major cause of their disappearance is the overfishing, as they represent a high value food fish. The fishing in today strongly controlled (Laird & Needham, 1988).

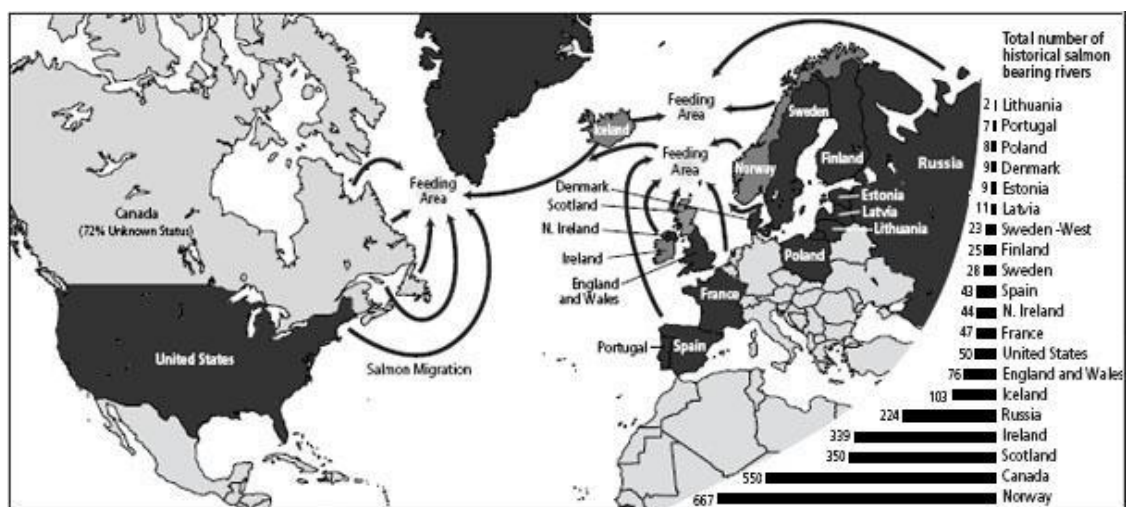


Figure 1. Migration patterns of wild Atlantic salmon and total number of historical salmon-bearing rivers worldwide (Government of Canada, 2013). Atlantic salmon historically occurred in every rivers flowing into the North Atlantic Ocean and Baltic Sea. Ranging, eastern, from the Kara Sea to northern Portugal and, western from the Hudson River in USA to the Ungava Bay in Quebec. Those fish can travel thousands of miles to their North Atlantic feeding grounds, usually near western Greenland or north of Scotland. Migrating patterns are indicated by the arrows (Government of Canada, 2013).

a) Life cycle

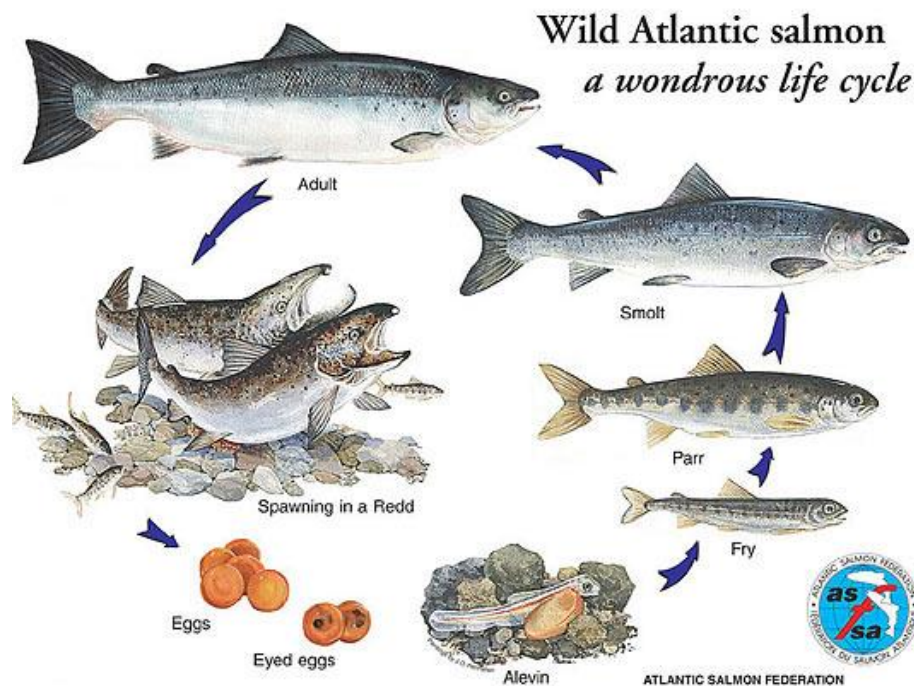


Figure 2. Wild Atlantic salmon life cycle (Atlantic Salmon Federation, 2017). Atlantic salmon spawn from November to December in their native river. The eggs hatch in April and May, to become alevins and then fry after three to four weeks. Fry begin feeding on tiny organisms. The young salmon, called parrs, spend one to three years in the stream where they were born, hunting for food. As they grow, they are called smolts and are ready to live in saltwater. They will then, migrate to the ocean, to their feeding grounds, to become mature (Atlantic Salmon Federation, 2017).

Wild Atlantic salmon have a complex life cycle (Figure 2). They are called anadromous fish, which means that they are born in freshwater, then migrate out to the ocean to find better feeding grounds and mature into adults. After one to three years, they swim back to freshwater, seeking for a better protection for their eggs and juveniles, to spawn. This ability of homing, strongly developed in Atlantic salmon, has permitted the emergence of local races (Atlantic Salmon Federation, 2017).

Adult female salmon bury their eggs at a depth of around 40 cm in the river gravel in autumn in special nests called redd. The eggs hatch the following early spring into alevins, which remain hidden in the gravel, feeding from their attached yolk sac. They reach at that point a length of two cm. Three to six weeks after hatching, alevins turn into fry and emerge from the gravel to start feeding on tiny water organisms. At this stage, they are mainly carnivorous and have a diet mostly based on aquatic insect larvae (Descroix et al. 2009; Atlantic Salmon Federation, 2017).

When fry are growing, we can progressively called them parr. Parrs present vertical markings and have a length of five to eight cm. These immature fish stay in their native river for two to six years depending on food availability and water temperatures (Atlantic Salmon Federation, 2017). During this stage some tiny male fish become prematurely mature (jack) and are able to fertilise eggs of adult female salmon. They are of interest in

enhancing the genetic diversity and ensuring fecundation of the eggs if only a few males return from sea (Rollin, 2017).

The smoltification is the next step, during which the becoming smolts develop a silver color and undergo a complex transformation of the body to survive in saltwater. They migrate in April, May or June to the ocean, according to the latitude, and during the downstream journey, they will imprint and memorise the odors of their native river in order to come back later (Atlantic Salmon Federation, 2017).

Adult wild salmon can travel long distances at sea to reach waters off Greenland or waters surrounding the Faroe Islands at north of Scotland (Figure 1). During the migration and at the end destination, they will actively feed mostly on crustaceans and smaller fish and grow at a high growth rate to become mature (Atlantic Salmon Federation, 2017).

After one to three winters spent in the ocean, adult salmon will begin returning in spring to their native river. Once in freshwater, they stop feeding before spawning. The female may lay 1,500 eggs or more per kg of body weight, and most weigh between 3 and 8 kg at maturity, completing the life cycle (Laird & Needham, 1988).

In the wild, most of the adult salmon will die from exhaustion after spawning. Less than 5% will survive, with a higher chance of survival among females than males (Laird & Needham, 1988). Survivors, called scavengers or kelts, can return to the ocean the following spring to feed and then return to their natal rivers to spawn again.

Losses of individuals are important during each step of the life cycle, even in an equilibrated population. If we begin with 7500 eggs per female, only 625 alevins will then emerge, to then give 200 parrs. At these young stages, fish are exposed to pollution or predators such as piscivorous birds or the brown trout and only a few will survive, to give 50 smolts migrating to sea. Four will reach maturity and return to the home river where only two adults will reproduce efficiently (Rollin, 2017).

b) Atlantic salmon in Belgium – The “Salmon Meuse” project

In the past, Belgian rivers were populated by numerous wild Atlantic salmons. In 1885, Atlantic salmon started to disappear from our rivers due to the increasing industrialization, which strongly degraded the water quality of our rivers, but also due to overfishing and finally, to dam construction, blocking the passage of fish. The Belgian and Dutch public authorities announced some modifications of the laws about fishing in 1883: Atlantic salmon fishery was forbidden during the reproductive period (from mid-October to mid-February) and a minimal fish size of 25 cm was required. In 1908, the law was again modified, the fishery was not allowed during the spawning period (from mid-October to mid-March) and the fish had to reach a minimal size of 40 cm (Secretin, 2003). Despite these measures, these factors did not allow the stock to rebuild and the species disappeared from the Meuse river system around 1935 (Malbrouck, 2007).

In 1983, a hydrobiological study was undertaken to assess the possibility of restoring Atlantic salmon in the Belgian River Meuse (Philippart, 1994). After the discovery of sea trout in the Berwinne (Meuse), near Liège, the “Salmon Meuse” programme emerged in

1987 as a contribution of Wallonia to the European year of environment. This discovery indicated that the water quality of the rivers had improved and that a suitable habitat for salmonid remained in some tributaries of the river Meuse (Prignon et al., 1999). A reintroduction of an Atlantic salmon strain was thus conceivable, managing some modifications of the dams obstructing the fish migration in the River Meuse of Belgium and The Netherlands. The passage of those dams with branch rivers and fish ways to allow the free circulation of the fish was the priority of the project (Rollin, 2017). Besides, the Atlantic salmon fishery was prohibited in Wallonia on 11 March 1993, and still is (Maison wallonne de la pêche ASBL, 2012).



Figure 3. River Meuse hydrographic basin and its tributaries. This map shows the main tributaries of the Meuse river, where Atlantic salmon are restocked, in Belgium (Prignon et al., 1999)

The second important aspect of the “Salmon Meuse” programme, was to restore the potential of smolt production in tributaries of the River Meuse (Figure 3), as well as the complete life cycle of Atlantic salmon and sea trout. Therefore, the construction of a modern hatchery was undertaken by the Walloon Region, at Erezée, and made available to the ‘Service de la Pêche’ (Nature and Forest Department, DGO3, Public Service of Wallonia). This hatchery has released in the wild reared salmon parrs and smolts obtained from foreign eggs from Scotland, Ireland and France (Malbrouck et al., 2007). In the first instance, eyed eggs were also released but their yield in alevins was too low. By the end of 1992, over 220,000 young salmon were released, especially in the basin of the river Ourthe (Prignon et al., 1999). Since 2010, between 250,000 and 500,000 parrs are released in the Mosan basin each year, with a record number registered in 2017, of an average of one million of emerging alevins and parrs released (Wallonie service public SPW, 2017;

Croufer (rtbf), 2017). Performances of the various strains were compared and studied in order to optimise the rehabilitation process and select the one showing the best survival rate and growth rate of juveniles, the best downstream migration of smolts and finally, the best returning rate of adults. In most cases, the released parr showed a good adaptation to the conditions of the tributary rivers but presented various biological responses according to water temperature (higher growth rate along with higher water temperature), initial density of the population released and physical characteristics of their new habitat, such as current velocity, water depth and substrate grading (Malbrouck et al., 2007). From 2016 onwards, the only strain still restocked in the Meuse river system (in Belgium, The Netherlands and Germany) is the Loire-Allier strain originating from the F2 eggs produced in Chanteuges (France, see below). Indeed, this strain showed by far the best returning rate of adults in the Meuse conditions in the early 21st century (Dierckx et al., 2017; Ovidio et al., 2016).

The first returning adult salmon were recorded in 1994 in the Meuse in The Netherlands and in 2002 in Belgium (Lixhe near Visé). These semi-wild adult salmon were artificially reproduced with the aim of creating a captive fresh water brood stock for the production of parrs and smolts of a "new Meuse strain" (Philippart et al., 2005). Between 2014 and 2017, the restocking enables the capture of 200 returning adult salmon in the Mosan basin (Wallonie service public SPW, 2017).

The salmonid farm CoSMos (Service de la Pêche – DNF) in Erezée, is the largest Belgian fish farm working on the project aiming at producing salmon juveniles (alevins, parrs and smolts), in order to amplify the efficient rehabilitation of the species in the Meuse river system and to reach 50,000 smolts released per year in the Meuse river as well as a minimum of 200,000 parrs in its principal tributaries (Ourthe, Amblève, Vesdre, Lesse, ect.) (Malbrouck et al., 2007).

c) Atlantic salmon in France – “Plan Loire Grandeur Nature”

A similar programme emerged in France in 2001. The “Conservatoire national du saumon sauvage” (CNSS, Chanteuges, France), regarded as the largest salmon farming of Europe, artificially reproduces Atlantic salmon of the Loire strain to restock the Allier basin and the rivers of the Loire-Allier axis. Twenty years ago, the number of Atlantic salmon returning from sea was just over a hundred each year. Nowadays, this number has risen to reach 800 and 1000 individuals each year, according to years, but this remains insufficient compared with the 50,000 individuals that were found at the same place in 1800 (Crozet, 2014; Conservatoire National du Saumon Sauvage, 2018). Moreover, the numbers identified in 2000 and 2010 were lower than those identified in the late 1970s. Despite this low number, the wild Atlantic salmon population persists with 30,000 salmon migrating to sea after the spawning of adults, each year (Hydrauxois, 2016).

d) Atlantic salmon farming

In the past, the Atlantic salmon fish activity was based on wild fish. However, as said above, these wild fish disappeared from our rivers in the 1930s. Therefore, the need for a culture of Atlantic salmon has emerged. Thanks to the development of salmon

farming, companies were able to propose fresh salmon throughout the year to consumers. Today, salmon farming is widespread. The main producers are Norway, Chile, Scotland, Canada and the Faroe Islands (Asche et al., 2013). It performs artificial reproduction and produce salmon for sale as food but also to restock rivers (Laird & Needham, 1988).

If in the future an Atlantic salmon egg production must be launched, it will be based on farmed fish, because most of the wild Atlantic salmon populations are depleted. The rearing of Atlantic salmon is possible, and their techniques well known. This fish can indeed accommodate to the conditions of life in fish farms (Asche et al., 2013). The only restraining factor of such a production in Wallonia, is the poor availability of sufficient amount of good quality water during summer. However, the possible use of farmed Atlantic salmon dedicated to egg production for human consumption is still an open question.

Existing fish farms have set up a controlled production of fish, focussed on the maximisation of their profit as well as the flesh quality of the salmon. They defined their optimum conditions according to the required final product, which implies: (1) the maximisation of the conversion of feed to edible fish flesh, (2) the production of appropriate fish size and quality demanded by the market and (3) the production at the appropriate time (Laird & Needham, 1988). These targets strongly impact the feeding regime of the fish. Expectations are different in the case of egg production for a dietary purpose. Some farms are already privileging the egg quality in their production, instead of the flesh quality, in case their goal is to restock rivers or produce self-sustaining populations. High quality eggs will probably lead to a better survival rate once the fry are transferred in rivers. The difference between flesh or egg production is major. Indeed, as soon as a female puts all her energy to produce good quality eggs, the flesh is no more valuable for human consumption.

Salmonid farming began in the 19th century for restocking purposes and has grown at an astonishing rate since (European Commission, 2018). “In 1985 out of a total world market of Atlantic salmon of 40,500 tonnes, 30,700 tonnes came from farming and 9,800 tonnes from wild fish” (Laird & Needham, 1988). In 2016, the total production of farmed salmonids reached 2 million tonnes, whereas the total catch volume of wild salmonids was about one third of the farmed production (Marine harvest, 2017).

We can distinguish two types of salmonid culture: the total and the partial culture. In the first type, fish are kept in captivity during their entire life. This is mostly the case when they are grown for their flesh or when they are produced as broodstock. The second type corresponds to salmon or trout that are first kept in captivity before being released into the wild to enhance wild stocks or to create a ‘put and take’ fishery (Laird & Needham, 1988). In both cases, the implementation of a fish farm is not easy and has to comply with certain criteria and standards.

Notation of age is specific in fish farming. If it is not possible to consider a fish to be one year older, a “+” should be assigned to their age (Baglinière et al., 2001). As an

example, salmon 1+ are salmon between the age of one and two years (Malbrouck et al., 2007).

For a fish to be suitable for farming, it has to possess several characteristics such as: a good adaptation to climate, a high growth rate, a feeding regime at the basis of the ecological pyramid, an aptitude to artificial reproduction with a high fertility rate, an aptitude to artificial feeding and the ability to valorise cheap ingredients, a high flesh value, if the final aim is for human consumption, a tolerance toward high stocking density and a special resistance to diseases, transport and manipulation (Rollin, 2017).

Among parameters, the water temperature and the oxygen content are of utmost importance in a fish farm and must be tightly controlled. The quality of the salmon feed is almost as important, since it represents the best resource for intensification and is central in the economic and environmental record of the farm and in the quality of the final product. Feed quality includes sanitary aspects, nutritional composition and sensory dimension (Rollin, 2017).

All farmers want to grow their fish as cheaply and quickly as possible and obtain a good quality of their product. As stated above, this can mostly be achieved through the feed that the fish receive. Their diet must be of the right type, given at the right moment and in the right quantities, in order to positively influence their growth rate, feed conversion, flesh/egg quality, maturation rate and, in most cases, the purity level of the water discharged (Laird & Needham, 1988).

e) Farmed salmon feeding

In the past, the major part of fish feeding was based on fish meal issued from dried and milled fish and fish oil obtained from the pressing of cooked fish (FAO, 2016). These feeds were composed of approximately 40 % of proteins that are balanced in indispensable amino acids in the proportion that fish need (Laird & Needham, 1988). Fish oil also constitutes the richest existing source of n-3 LC-PUFA, essential for fish development and contributing to many human health benefits. Prices of the fish-based feeds were low and their nutrient contents matched with the nutritional requirements of fish (Rollin, 2017). In the 1960s and even after, 33% of the total fish production was not designated for human consumption but was typically reduced to fish meal and fish oil (FAO, 2016).

Nowadays, the context has changed, aquaculture is expanding and reached a production of 76.6 million of tons in 2015 (FAO, 2017). Simultaneously, fish meal and fish oil demands have raised but not their productions from wild fisheries, so prices have increased. Besides, the use of fish meal and fish oil as feed for fish has been widely criticised for ethical and ecological reasons. Indeed, these fish meals are based on finite fishery resources, therefore the long-term ecological sustainability is not assured (Turchini et al., 2009). Based on such statements, it became urgent to find feed alternatives, which would be as appropriate in terms of protein and lipid supplies, especially in terms of n-3 LC-PUFA supply, to maintain their admitted benefits on fish development and on fish consumers health, along with an improvement of the sustainability and economic benefits of the fish farm industry (Turchini et al., 2009).

Feed producers turn themselves toward plant-derived oils (soybean oil, crude palm oil, corn oil, etc.) (Megdal et al., 2009). In consequence, the share of the fish total production transformed into fish meal decreased. In 2015, only 12% of the world fishery production was not designated for human consumption and transformed into fish meal, compared with one third in the 1960s (FAO, 2017). The plant-derived oils appear to be catabolised by fish as an energy source in a similar way as fish oil. They are also attractive because of their relatively low price, sustainable production and large availability. In addition, some of them, such as corn or soybean oils, are rich in linoleic acid (18:2 n-6, LA). Unfortunately, they are a poor source of n-3 fatty acids, which are essential for the growth and health of fish, and do not contain n-3 LC-PUFA (Megdal et al., 2009).

The nutritional requirements of farmed salmon can be, at least partially, covered through the substitution by plant derived oils and proteins. Plant-based feeds have been shown to be well tolerated to support normal growth rates and to provide fish with acceptable flesh flavour (Megdal et al., 2009). Nevertheless, fishmeal and fish oil still represent the most nutritious and digestible feed for farmed fish and are always used, but more selectively at lower concentrations, for critical steps during the production (Turchini et al., 2009).

Algae could also represent an alternative fish feed to fish oil. Algae are at the basis of the aquatic food chain and thus are the food resources that fish are adapted to. With high protein and arachidonic acid (20:4n-6, AA), eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA) contents (Towers, 2013). The potential use of DHA-rich oils derived from single-cell microalgae has been successful, precisely on Atlantic salmon, in 2009. However, these studies were kept at a small scale according to algae difficult and unpredictable supply and high production costs (Turchini et al., 2009). With recent technologies, more studies have been conducted and concluded that microalgae meal could replace fish oil as feed supplement for different aquaculture animals (Kousoulaki, 2014). In 2016, a study showed that whole cell microalgae *Phaeodactylum tricornutum* could replace 6 % of the fish meal of Atlantic salmon without adverse effects on digestibility and growth (Sorensen et al., 2016). However, this value remains low. More recent studies, discovered that the micro-algae, could not be the only marine organisms capable of synthesising the LC-PUFA. So far, micro-algae were considered as the primary producers of PUFA, which could then be modified by bioconversions as they pass up the marine food chain, by trophic upgrading. However, it seemed that organisms in higher trophic levels, including various marine invertebrates, possess the metabolic pathways that enable the organism to synthesise LC-PUFA *de novo*, in addition to the process of trophic upgrading (Kabeya et al., 2018). These marine invertebrates could also represent an alternative in the farmed fish diet, to provide the n-3 LC-PUFA.

2.2. Other salmonids

a) Sea trout (*Salmo trutta*) (Laird & Needham, 1988)

The sea trout is the anadromous ecological form of the brown trout, the latter exclusively living in freshwater. The native distribution of the sea trout expands from Iceland, through Europe and the Mediterranean countries, to Asia.

Like Atlantic salmon, sea trout smolts migrate to sea during the spring and early summer. However, once there, they remain closer to the coasts and most of them return to fresh water only a few months later. Unlike salmon, they may spawn many more times in their life than the Atlantic salmon and produce on average 1,800 eggs per kg of body weight (smaller eggs). Moreover, it seems that the majority of smolts and returning adults are female. As a consequence, these returning adult sea trouts may spawn with male trouts of the fresh water form.

The farming of sea trout also began in the 19th century and is now present in almost every region of the world. This expansion is only limited by water temperature since the species cannot tolerate a temperature higher than 21°C. Sea trout are farmed for stock enhancement or released in nature to create 'put and take' fisheries. They are rarely farmed for a human consumption, for which the rainbow trout is largely preferred.

b) Rainbow trout (*Oncorhynchus mykiss*)

The native range of the rainbow trout extends from the north of Mexico to the Bering Sea in Alaska. The species is now introduced all over the world.

The culture of rainbow trout in Europe was first developed in Denmark in the 1890s. One century later, in 1985, over 189,000 tonnes of rainbow trout were produced in Europe, 95% of the production being performed in fresh water (Laird & Needham, 1988). Nowadays, nearly all the rainbow trout fish found on the market come from aquaculture activity. In 2009, the main producers of farmed rainbow trout were the EU (Italy, France, Denmark, Germany and Spain), Chile, Turkey and Iran (European commission, 2012).

The rainbow trout is the most common trout species used for the fresh water farming meant for human consumption, because of its tolerance to relatively low oxygen levels and high-water temperature, its higher resistance to disease and its faster growth rate (Laird & Needham, 1988).

c) Pacific salmon (*Oncorhynchus spp*)

Several species of Pacific salmon exist. Six of them can be found on the western side of the Pacific Ocean and five on the North American side. The commercial interest for Pacific salmon began in 1814 with the first salmon canned but it truly developed along the Pacific coast 50 years later. With the high level of exploitation that followed, the stock levels considerably dropped, inducing the need for enhancing them (Laird & Needham, 1988).

3. Fatty acids

One of the reasons for the spectacular growth of fish farming and for the growing interest of consumers for fish deals with its richness in n-3 LC-PUFA, which are well known to be favourable for human health.

3.1. Definition

Lipids are a range of different compounds that are soluble in organic solvents and insoluble in water. This group includes several types of molecules varying in their structure such as fatty acids, waxes, sterols, fat-soluble vitamins, glycerides (mono-, di-, tri-) and phospholipids. They have various functions in the body involving energy storage, signalling pathways and structural composition of the cell membranes (Tocher, 2003).

The most important group of lipids is the fatty acid group. Fatty acids are organic compounds composed by an aliphatic chain and an acid function. They can be found in the form of (1) free fatty acids, (2) included is triacylglycerols (three fatty acids esterified to the hydroxyl groups in sn1, sn2 and sn3 positions of a glycerol), (3) included in phosphoglycerides (two fatty acids esterified to the hydroxyl groups in sn1 and sn2 positions of a glycerol linked to a polar head group) or (4) included in sphingolipids (single fatty acid linked to a sphingosine through an amide link) (Tocher, 2003). According to their structure, fatty acids can have different functions. Indeed, phospholipids are mainly involved in the structure and function of the cellular membranes, whereas triglycerides have mainly an energy storage role. Fatty acids can be classified according to (1) the fatty acid chain length, from 4 to 36 carbon atoms, (2) the unsaturation degree of the chain (number of double/ethylenic bonds): saturated (zero double bond), mono-unsaturated (one double bond), poly-unsaturated (several double bonds) and (3) the position of their ethylenic bonds (Tocher, 2003). These characteristics will influence the physicochemical properties of the fatty acid, particularly their sensitivity to temperature changes. As an example, saturated fatty acids (SFA) have a higher melting point than the unsaturated fatty acids (Cockbain et al., 2011). Besides their chemical name, established by the International Union of Pure and Applied Chemists (IUPAC) and their trivial name, fatty acids can be written X:Yn-Z, where X is the number of carbon atoms of the aliphatic chain, Y is the unsaturation degree and n-Z is the position of the first double bond from the methyl end of the carbon chain.

3.2. Polyunsaturated fatty acids and omega-3 long chain polyunsaturated fatty acids

PUFA can be divided in three groups, omega-3 (n-3), omega-6 (n-6) and omega-9 (n-9) PUFA, according to the position of the first unsaturated bond at 3, 6 or 9 carbon atoms from the methyl terminal group of the fatty acid (Cockbain et al., 2011).

The most important PUFA, which are playing a role in essential metabolic and physiological processes, are the long chain poly-unsaturated fatty acids (LC-PUFA). They have an aliphatic chain of 20 carbons or more. The predominant LC-PUFA are AA, from the n-6 family and EPA and DHA, from the n-3 family (Figure 4). Since bioconversion

between fatty acids is only possible within the same omega family, no interconversions between n-3 and n-6 fatty acids are possible.

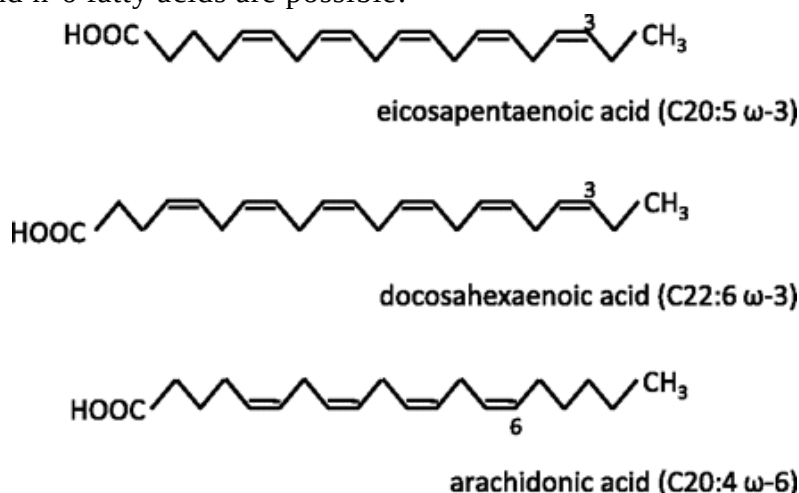


Figure 4. Structure and nomenclature of poly-unsaturated fatty acids (Cockbain et al.,2011).

Alpha-linolenic acid (18:3n-3, ALA) and LA are primordial as precursors for the respective n-3 LC-PUFA and n-6 LC-PUFA, but do not have specific metabolic roles. Some species have a low conversion capacity of these precursors into their corresponding LC-PUFA and have then a dietary requirement for the LC-PUFA instead of the C-18 ones (Cockbain et al., 2011). Figure 5 summarizes the anabolic pathways starting from LA and ALA in salmonids.

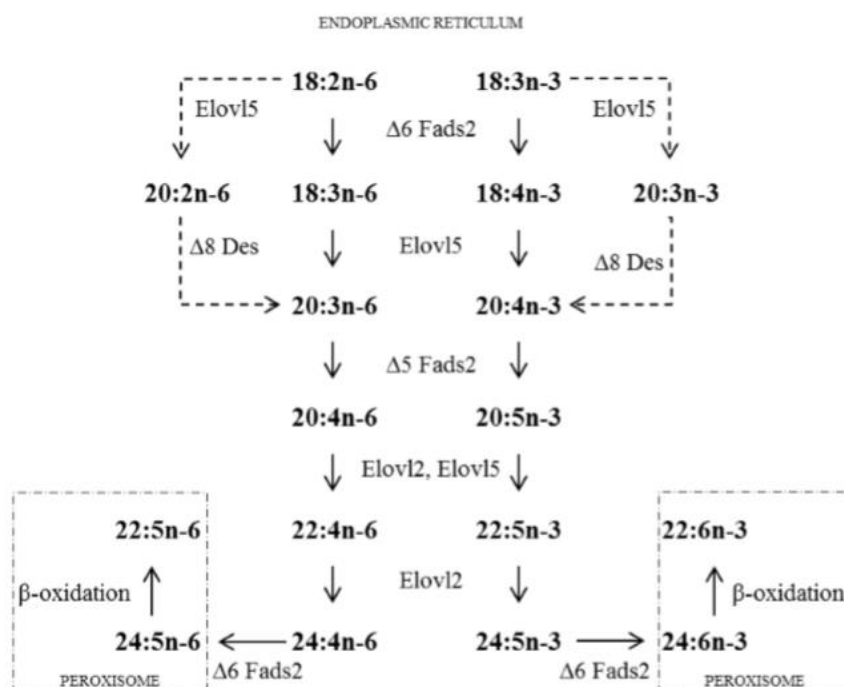


Figure 5. Synthesis of n-3 and n-6 long chain polyunsaturated fatty acids in salmonids. Δ5 Fads2, Δ5 fatty acid desaturase; Δ6 Fads2, Δ6 fatty acid desaturase; Δ8 Des, Δ8 desaturase; Elov12, very long fatty acid elongase 2; Elov15, very long fatty acid elongase 5; 18:2n-6, linoleic acid; 18:3n-6, γ-linolenic acid; 20:2n-6, eicosadienoic acid; 20:3n-6, dihomo-γ-linolenic acid; 20:4n-6, arachidonic acid; 22:4n-6, docosatetraenoic acid; 24:4n-6, tetracosatetraenoic acid; 24:5n-6, n-6 tetracosapentaenoic acid; 22:5n-6, n-6 docosapentaenoic acid; 18:3n-3, α-linolenic acid; 18:4n-3, stearidonic acid; 20:3n-3, eicosatrienoic acid; 20:4n-3, eicosatetraenoic acid; 20:5n-3, eicosapentaenoic acid; 22:5n-3, n-3 docosapentaenoic acid; 24:5n-3, n-3 tetracosapentaenoic acid; 24:6n-3, tetracosahexaenoic acid; 22:6n-3, docosahexaenoic acid (Mellery, 2017).

3.3. Poly-unsaturated fatty acids importance and dietary requirements

PUFA are biologically important, for both fish and humans, as they are involved in the phospholipid membrane structure and function, in the cellular signalling and the lipid metabolism. Furthermore, n-6 and n-3 fatty acids are important nutrients as they play a role in the growth, development and physiological functions of the body and are associated with many health benefits (Swanson et al., 2012).

For fish, n-3 PUFA and more precisely DHA are of first importance to maintain membrane fluidity and normal cell functions at low temperatures, because of the low melting points of HUFA compared with other membrane lipids (Brett & Muller-Navarra, 1997). In contrast, SFA ensure membrane rigidity thanks to its saturated chain. The fatty acid DHA plays also an important role in the development of the nervous system. PUFA are also important for the developing fish embryo and during further development steps as they are precursors of eicosanoids (Pickova et al., 1999). Indeed, eicosanoids, including prostaglandins, thromboxanes and leukotrienes, are produced mostly thanks to the presence of AA and EPA and play major roles in physiological processes, as the regulation of the egg-production, egg-laying, spawning and hatching of fish (Brett & Muller-Navarra, 1997).

For humans, the LC-PUFA are notably involved in the control of the inflammatory response, through the production of eicosanoids and other intercellular messengers. Among the n-3 LC-PUFA, DHA should be pointed out for its essential role in foetal development and optimal functioning of the brain and the retina. Next, the n-3 LC-PUFA seem to be important health factors, among others, against cholesterol excess and to prevent cardiovascular and Alzheimer's disease (Swanson et al., 2012).

Generally speaking, all vertebrate species, including human and fish, have an absolute dietary requirement in n-6 and n-3 PUFA (Turchini et al., 2009). For fish, dietary requirements appeared to vary between species (Brooks et al., 1997). Historically, all fish have the ability to convert the two LA and ALA precursors into their n-6 and n-3 LC-PUFA, but some species have lost this skill after adapting to a HUFA-rich environment, and thus have direct dietary requirements of these LC-PUFA, such as AA, EPA and DHA (Turchini et al., 2009). For humans, DHA is considered, in France, as an essential fatty acid, because our body needs are not met by our metabolism which is unable to synthesize it in sufficient amount. The capacity of humans to synthesize EPA is much better, and so this fatty acid is often called semi-essential. The minimal physiological requirement in DHA for a human adult has been estimated in France to be of 250 mg/day (AFSSA, 2010). And the recommended dietary allowance for EPA+DHA is 500 mg per day for an adult. It is important to have a high n-3 LC-PUFA blood content as much as a good n-6/n-3 LC-PUFA ratio. The recommended ratio range goes from one to four (Bresson, 2009; Peltomaa et al., 2017). Overall, the dietary intakes of n-3 LC-PUFA fatty acids are insufficient for most people. Indeed, in 2014, the Belgian population had an average intake of 190 mg of EPA + DHA per day (De Ridder et al., 2016). An increase in the consumption of foods rich in n-3 LC-PUFA fatty acids is thus required.

3.4. Human sources of n-3 polyunsaturated fatty acids

The current diet across the world has changed during the last decades. It is becoming higher in SFA and lower in n-3 LC-PUFA due to new eating habits centred on food items rich in SFA and poor in n-3 LC-PUFA. We thus need to better balance our diet and increase the intake of food items containing the precursors of LC-PUFA or the LC-PUFA themselves, to reach the appropriate intake of EPA and DHA.

Some terrestrial plants, such as colza, walnut and especially flax, have the ability to produce the first 18-C member of the n-3 PUFA family, namely ALA, which is the precursor of EPA and DHA, but does not produce the same health benefits as those two. Although our body is able to convert ALA obtained from our diet into EPA and DHA by elongase and desaturase enzymes this conversion does not seem to be sufficient. Only two to ten % of the dietary ALA is converted to EPA or DHA in human bodies (Swanson et al., 2012). As our body is incapable of synthesizing enough of the n-3 LC-PUFA from shorter ones, the only way is to get them directly through our diet, especially via marine animals, or with supplements (Swanson et al., 2012).

Fish, and more precisely, cold water fish are a good n-3 LC-PUFA dietary source for humans. Marine ecosystems, including micro-algae, which constitute the basis of the whole marine food chain, were until recently the only living organisms capable of synthesizing n-3 LC-PUFA, along with heterotrophic protists and bacteria (Kabeya et al., 2018). New studies concentrating on the organisms between the primary producers and the fish, in the marine trophic chain, and more precisely on marine invertebrates, revealed that they also contained high levels of EPA and DHA and the enzymatic components necessary for their *de novo* synthesis (Monroig et al., 2013). Fish, which are relatively high in the trophic chain of the marine ecosystem, can accumulate EPA and DHA and thus significantly participate to the trophic upgrading. This storage capacity is higher among cold-water fish, due to a physiological adaptation to the cold-water temperatures. This capacity of maintaining the integrity and fluidity of the fish cell membranes, thanks to the presence of such lipids, enable those fish to maintain activity even at low temperatures (Pickova et al., 1999; Brett & Muller-Navarra, 1997). In farmed fish, this high essential fatty acid level can be reached thanks to their commercial feed. In that respect, fish oils are recognised to be the best dietary lipid source in commercial fish feeds. A comparison between salmon flesh fatty acid content and their dietary fatty acid intake indicated that some fatty acids, such as EPA, were totally recovered in the animal body unlike others, like LA and oleic acid (18:1 n-9), found each in much smaller quantities in flesh (Megdal et al., 2009).

Fish eggs might be an alternative source of n-3 LC-PUFA for humans. The benefits of eating fish eggs are amazing for our health. These are indeed extremely rich in micronutrients and n-3 LC-PUFA, considering that the first objective of the egg is to give all the elements (vitamins, trace elements, proteins and other substances) necessary for the good development of the embryo (Brooks et al., 1997). Unfortunately, high levels of SFA and sodium are regularly present together with the n-3 LC-PUFA in eggs. Some egg

types are rich in n-3 LC-PUFA while being poorer in SFA and sodium. This is the case for the European hake eggs, and for the Atlantic salmon eggs (Claire, 2017).

4. Atlantic salmon eggs

4.1. Fish egg composition

The chemical composition of fish eggs varies “from species to species, according to the condition and maturity of the eggs, within and between runs or harvested areas and from year to year” (Bledsoe et al., 2003). Variations can even be recorded between eggs of a single female, as the eggs are becoming more mature as we progress from the tail to the head of the fish (Bledsoe et al., 2003).

Fish eggs have a high crude protein content, which range from 16 to 30 %, and a high crude lipid content, which can vary from 5 to 20 % (Bledsoe et al., 2003). Lipids are the most important constituents of fish eggs as they represent the main source of metabolic energy during embryonic development. The longer the time interval between spawning and egg hatching or first feeding of the larvae the higher the lipid reserve of the egg (Bromage & Roberts, 1995). As salmon are freshwater spawners, their eggs will have higher lipid levels as compared with marine fish (Bromage & Roberts, 1995). For salmon eggs, the average value of crude lipid content is around 10% (Bledsoe et al., 2003). The lipid composition of eggs is mostly influenced by the diet composition of the fish but also by its lipid metabolism (Pickova et al., 1999). In addition, the fatty acid composition of eggs and the fatty acid composition during the development of the fish will vary between individuals, depending on duration of development and environmental conditions (Murzina et al., 2011).

A study in 1985, revealed that the Atlantic salmon eggs contain approximately 30% of their dry weight as lipids, which comprise 48% of triacylglycerols, 44% of phospholipids and 8% of sterols and sterols esters (Cowey et al., 1985). In fertilised Atlantic salmon eggs, triacylglycerols mainly contain 16:0 and 18:1 (n-9) with the proportions of 11.4% and 30.9%, respectively. Concerning the n-3 fatty acids, EPA and DHA are present at 6.0% and 11.4%, respectively, in the total triacylglycerols. In contrast, the fatty acids of the phospholipids contain 46% of n-3 LC-PUFA with a ratio of DHA to EPA of 2:1 (Bromage & Roberts, 1995). A study on unfertilised eggs of freshwater Atlantic salmon, reported on 21.2% of SFA, 29.1% of MUFA and 49.0% of PUFA in total lipids. For the PUFA, 37.5% were of the n-3 family and 11.3% of the n-6 family (Murzina et al., 2011). During embryonic development, the fatty acid content remains globally stable, except at the stage of pre-larvae hatching where a significant decrease in LA, AA and n-3 PUFA are recorded (Murzina et al., 2011).

Apart from lipids and proteins, fish eggs also contain other nutrients conferring health benefits for humans. They first contain quite high levels of zinc that strengthens the immune system, but also calcium, iron, magnesium, manganese, phosphorous, potassium and copper. We also find vitamins A, D and K in fish egg. Fish eggs also contain cholesterol, generally in a higher level than for their corresponding muscle tissue, ranging from 300 to 500 mg/100 g (Bledsoe et al., 2003).

4.2. Fish egg quality to improve the survival rate

In a perspective of survival rate of the individuals, the two following definitions of fish egg quality may be considered.

- “Fish egg quality can be defined as the ability of the egg to be fertilised and subsequently develop into a normal embryo” (Bobe & Labbé, 2010).
- “In the fish farming industry, good-quality eggs have been defined as those exhibiting low mortalities at fertilization, eyeing, hatching and first feeding” (Brooks et al., 1997).

Several studies have pointed out various intrinsic egg characteristics or factors of the environment in which eggs are fertilised and subsequently incubated, that could directly influence egg quality, and in turn, their survival rate once released in nature or maintained under aquaculture conditions (Brooks et al., 1997). First, egg size could have an impact on the survival rate of the derived fry, as it has been demonstrated with eggs of brown trout. An individual female tends to produce eggs uniform in size. In contrast, different females of the same length, weight and stock, may produce eggs which vary in number and size. It has been demonstrated that large brown trout eggs produce larger fry that would grow at a higher rate than those from small eggs. Besides, these could survive longer without any food supply (Bagenal, 1969). With herring, large eggs have been shown to confer to larvae both a larger size and a longer survival time before feeding on exogenous food (Bagenal, 1969). However, other authors do not agree on those arguments and indicate that larger eggs would be more noticeable by predators and that, as larger eggs often hatch slower than small eggs, they would be longer exposed to predators and adverse abiotic conditions (Brooks et al., 1997). A second factor potentially affecting the survival rate of eggs, may be the initial density population. In that respect, a larger initial population density would not necessarily lead to a better survival rate (Brooks et al., 1997). A third factor might be the age of the fish, which might impact on the quality of the eggs. Notably, female rainbow trouts have been shown to produce better quality eggs in their second spawning season than in their first one (Brooks et al., 1997). This could be related to the size of the eggs produced, as more mature fish, with a larger body size, tend to produce larger eggs (Brooks et al., 1997), and we saw earlier that larger eggs could have a better survival rate.

The fatty acid egg composition appears to be an important factor affecting egg quality in terms of survival rate. This may be related to the fundamental roles that fatty acids, notably LC-PUFA, play first as structural components of storage and membrane lipids but also as major source of energy in metabolic processes of fish during their growth and development (Murzina et al., 2011). However, a high PUFA content in eggs may increase the risk of formation of free radicals that can damage tissues. This risk seems to be even worst with the high content of dissolved oxygen encountered in cold water. The antioxidant capacity is therefore determinant in the egg quality (Pickova et al., 1999). Fish feed, especially its lipid content, is an important factor susceptible to affect egg quality as it affects the egg composition. Fish diet composition has been shown to impact on

fecundity and some nutrients, such as fatty acids, vitamins, pigments, appear to be essential to ensure a good development of the embryo (Bobe & Labbé, 2010).

Environmental factors including temperature, photoperiod and water salinity could also have an influence on egg quality. Low as well as high water temperature could have a negative impact depending on the species (Bobe & Labbé, 2010). In the case of Atlantic salmon, a suboptimal temperature during the vitellogenesis stage can have an impact on the quality of the eggs (King et al., 2003). Concerning salinity, if a female Atlantic salmon is in sea water during the reproduction period, ovulation could be blocked (Haffray et al., 1995).

Egg quality seems to be higher in wild fish than captive ones. Indeed, eggs from wild Atlantic salmon showed a higher fertilization and hatching success than eggs from captive counterparts, due to a greater size and survival of embryos and alevins (Brooks et al., 1997). This seems logical in view of what was presented above with the influence of the diet of adult salmon and the physicochemical conditions of the water. In addition, the quality of the farming procedures (level of stress, fertilization procedures adopted, etc.) may be questioned (Brooks et al., 1997).

Many other factors are susceptible to affect egg quality, such as egg handling, endocrine status of the female during the growth of the oocyte in the ovary, exposure to xenobiotics or pollutants, etc. (Bobe & Labbé, 2010).

In Canada, a program aiming at optimising the egg quality of yellow walleye (*Sander Vitreus*) in captivity emerged in 2011. From their perspective, the relevant factors that could influence egg quality and the first embryonic development steps of the eggs are the age of the progenitors (the youngest, the best), the spawning period and the nutritional requirements in terms of fatty acids, amino acids and vitamin A (McPhee et al., 2015).

Despite the increasing attention given to this subject, the control of egg quality remains an issue in the aquaculture industry. Some of the factors identified as the cause of the variability of the egg quality are still poorly understood and others are largely unknown such as the genetic influence (Bobe & Labbé, 2010; Brooks et al., 1997).

5. Sensory analysis

To the best of our knowledge, Atlantic salmon eggs are not commercialised as a food item. One objective of the present study will thus be to evaluate if Atlantic salmon eggs could be appreciated as a food product by the Belgian consumers. Considering that no instrument can replace the human senses, a sensory evaluation seemed essential to be set up.

A sensory analysis is defined as “a multidisciplinary science that uses human panellists and their senses of sight, smell, taste, touch and hearing to measure the sensory characteristics and acceptability of food products, as well as many other materials” (Watts et al., 1989). In order to collect reliable and valid measurements, a sensory test has to be performed under controlled conditions defined by the “International Organisation for Standardisation” (ISO).

Sensory tests can be classified as consumer-oriented or product-oriented, according to the purpose of the test. The consumer-oriented test is used to evaluate the preference and acceptance for food products. In a preference test, consumers have to express a choice of preference between samples. The most common one is the paired-preference test but category scales or ranking test can also be used. A second consumer-oriented test is the hedonic test, used to test indirectly the preference or acceptance of a product, through the measurement of degree of liking by consumers, with a category scale ranging from dislike extremely to like extremely (Watts et al., 1989). In contrast, the product-oriented test, is used to measure sensory characteristics or to assess differences between products. More precisely, three types of tests can be implemented in the case of the product-oriented test. The first one is a test for difference to determine whether two samples are distinguishable from each other by sensory analysis. Secondly, a ranking for intensity test can be performed. In this type of test, the purpose is to order samples according to the perceived intensity of a sensory characteristic in order to obtain preliminary information on differences between products. The last one, the scoring for intensity test, requires scoring samples, on line scales or category scales, for the perceived intensity of a sensory characteristic. This allows measuring the level of difference between samples and to order them by increasing or decreasing intensity of a characteristic. Such tests gather 30 to 50 untrained tasters from personnel within the organization itself or others who would have a similar profile as the target population of consumers who will use the product (Watts et al., 1989).

Concerning facilities, basic requirements are as follows: a food preparation area, a panel area where distractions, noise and odours are kept as low as possible, a desk area and supplies for sensory testing. A particular attention has to be given to the sampling and the preparation of the food. The food presented must first be safe to eat and the sample be representative of the whole pack. Then, samples for sensory comparisons must be prepared following a standardised method to minimise the potential preparation effects. The presentation of each samples should also be standardised. Food should be served at the temperature the food is usually consumed and can be accompanied by a carrier. However, this could interfere with the evaluation of the main food product. Room

temperature water must be provided, so that the tasters can rinse their mouth between samples (Watts et al., 1989). Many other factors can influence the conduct of a sensory test and are accurately described in the Thesis written by Boutrolle I. and entitled “Mesure de l’appréciation des aliments par les consommateurs: état des pratiques et propositions méthodologiques” (Boutrolle, 2007).

III. OBJECTIVES AND EXPERIMENTAL STRATEGY

Atlantic salmon is beneficial for the regional biodiversity and for our health. Indeed, the reintroduction of the natural life cycle of Atlantic salmon in the Mosan basin, thanks to the release by the CoSMos centre, has a high ecological value. Furthermore, Atlantic salmon filet and Atlantic salmon eggs have a high nutritional value as they provide us with health-promoting n-3 LC-PUFA (EPA and DHA). These issues are the basis of the two main objectives of this master thesis.

The first objective aims at optimising the Atlantic salmon reintroduction in our region. This will be conducted by assessing to what extent the environment, the fish species, the fish life style or the fish feed may have an impact on the egg survival rate, considering the release of fry for restocking. For that purpose, the proximate composition and fatty acid profile of fish eggs have been determined. More precisely, the achievement of this objective was carried out on eggs from fish presenting different characteristics, including environment (Belgium and France), species (Atlantic salmon and sea trout) and lifestyle (semi-wild and captive).

The second objective is to determine the nutritional interest of an Atlantic salmon egg production for human consumption. The egg consumption could represent an additional way to include Atlantic salmon in our diet and also a food product from a local fish farming in contrast with current commercial Pacific salmon eggs. Firstly, comparisons between various fish egg proximate compositions and fatty acid profiles will help to point out differences of nutritional quality and evaluate the impact of the salting treatment on the eggs. More precisely, the achievement of this objective was carried out on eggs from fish presenting different characteristics, including species (Atlantic salmon, Pacific salmon, rainbow trout and sea trout) and lifestyle (semi-wild and captive). In addition, salted and pasteurised eggs of four of these conditions were also analysed. Commercial eggs and artificial fish feeds were also evaluated. Secondly, a sensory test was conducted in order to evaluate the Atlantic salmon egg appreciation by potential consumers.

IV. ATLANTIC SALMON EGGS FOR A BETTER SPAWNING SUCCESS

1. Material and methods

1.1. Egg and feed sampling

For this section of the work, egg samples have been selected with the aim to enhance the reintroduction of the Meuse Atlantic salmon strain in the rivers of the Meuse basin. Eggs have been mainly collected at CoSMos, during the artificial reproduction period from November 2017 to December 2017. Precisely, the egg sampling at CoSMos consisted in eggs from (1) two Atlantic salmon types: 3+ captive type and semi-wild type in which fish spent two years at sea and (2) two sea trout types: 2+ captive type and semi-wild type in which fish spent one year at sea. The expression "semi-wild" was chosen by CoSMos to describe the fish that were born in the fish farm, reared for a couple of months and then released in nature for the rest of their life cycle. Moreover, the CNSS (Chanteuges, France) provided us with eggs from semi-wild Atlantic salmon, which spent two years at sea.

The feed of the captive fish has been collected. Precisely, two feeds have been analysed: (1) "Repro floating" feed (Coppens, Helmond, The Netherlands) (See composition ANNEX 2) used to feed the captive sea trout of CoSMos and (2) "n-3 PUFA-rich Repro floating" feed prepared by CoSMos, from the "Repro floating" feed with the addition of cod liver oil (type A, ABC Chemicals, Braine-le-Château, Belgium) and "Carophyll Red" (Red animals, Ere, Belgium), and used to feed the captive Atlantic salmon of CoSMos.

Considering Atlantic salmon eggs from CoSMos, semi-wild females, which spent two years at sea, and 3+ captive females have been chosen, in order to have relevant comparisons. Indeed, semi-wild females, which spent two years at sea, represented the largest number of returning salmon, in comparison with semi-wild females, which spent one or three years at sea, and these were born in 2014, migrated to sea in 2015 and returned in river in 2017. Therefore, to use female captive Atlantic salmon with a similar life cycle, these have to be 3+. The same has been applied for sea trout females, in which the semi-wild sea trouts, which spent one year at sea, were compared with 2+ captive sea trouts.

The sampling dedicated to the better spawning success of the Atlantic salmon in the Mosan basin is resumed in Table 2. The complete egg and feed sampling is presented in ANNEX 1.

In order to control the variability of the egg composition existing within a condition, egg samples, from CoSMos as well as from CNSS, were collected from three females for each condition. About 50 g of eggs per female were harvested for analysis. The detailed description of the sampled females is provided in ANNEXES 4-7.

Table 2. List of the feed and egg samples analysed for the section aiming at a better spawning success of salmonid in Belgium.

of salmonids in Belgium.

FISH FEED					
Fish feed name		Origin (Brand, city, country)		Fish species feed	
Repro floating		Coppens, Helmond, The Netherlands		Captive sea trout (CoSMos)	
Repro floating improved ¹		Coppens, Helmond, The Netherlands		Captive Atlantic salmon (CoSMos)	
FISH EGGS					
Type	Species	Origin	Description	Years spent at sea	Treatment
Raw eggs	Atlantic salmon	CoSMos	Captive 3+ (first spawn)	0	Raw
	Atlantic salmon	CoSMos	Semi-wild (spring run, first spawn)	2	Raw
	Sea tout	CoSMos	Captive 2+ (first spawn)	0	Raw
	Sea tout	CoSMos	Semi-wild (spring run, first spawn)	1	Raw
	Rainbow trout	Du Ry Des Pres	Captive	0	Raw
	Atlantic salmon	CNSS	Semi-wild	2	Raw

¹Repro floating feed improved with cod liver oil (type A, ABC Chemicals, Braine-le-Château, Belgium) and “Carophyll Red” (Red animals, Ere, Belgium).

1.2. Analytical methods

After sampling, eggs and feeds were directly frozen after their arrival at the laboratory, and freeze-dried within 48h, in order to preserve the samples until further analyses. The feed and egg samples have been then homogenised following the 950.02 (1mm) protocol of the Association of Official Analytical Chemists (A.O.A.C., 1995). After the homogenisation process, each feed sample has been separated into three hermetic containers in order to obtain three replicates.

The chemical analyses consisted in the evaluation of (1) the proximate composition including the determination of the dry matter, crude ash, crude protein and crude lipid contents following the AOAC analytical methods (A.O.A.C., 1995) and (2) the fatty acid composition following the Bligh & Dyer extraction method followed by fatty acid methylation, separation and quantification by gaz chromatography and flame ionization detection. These methods are described in ANNEX 10.

1.3. Statistical methods

All the data are presented as mean $\pm$ standard error of the mean (SEM) of three biological replicates (n=3), for the fish egg samples. Concerning the feed sampling, three replicates were made up from one bag. Overall, the results of proximate composition and fatty acid profile were compared using a one-way analysis of variance (ANOVA), followed by Tukey’s (parametric with $\alpha = 0.05\%$) *post hoc* test in order to determine significant differences between conditions. Prior to statistical analyses, assumptions for

statistical methods were verified: (1) normal distribution of residuals with the Shapiro-Wilk's test and (2) equality of variances with the Levene's test. In case these assumptions were not verified, results were analysed by the Wilcoxon's test, followed by the Steel-Dwass's (nonparametric version of Tuckey's method, with $\alpha = 0.05\%$) *post hoc* test. Besides, fatty acid profiles (% of total identified fatty acids (TFA)) of eggs from different treatments or of eggs and feeds from the same treatment, were compared thanks to a linear regression. Statistical analyses were performed using the JMP software (JMP Pro 13, SAS, Cary, NC, USA).

2. Results

Concerning the project aiming at a better survival rate, several relevant comparisons between the proximate composition and fatty acid profile of fish eggs were made. A first one intended to evaluate the *effect of the fish wild environment* on the eggs fatty acids composition by comparing eggs from Atlantic salmon of the same Loire-Allier genetic strain, which spent two years at sea, caught either in the river Meuse in Lixhe and coming from CoSMos (Erezée, Belgium) or in the river Allier in Vichy (France) and coming from CNSS (Chanteuges, France). The second comparison focussed on semi-wild fish eggs of different salmonid species, either from the Atlantic salmon of Loire-Allier strain, which spent two years at sea, or from the sea trouts, which spent one year at sea, both caught in the river Meuse in Lixhe, to evaluate the *effect of the species* on the eggs fatty acid composition. Another interesting comparison was made between the eggs of the captive and semi-wild fish from both the Atlantic salmon and the sea trout species, in order to evaluate the *influence of their life-style* (wild *versus* culture). Finally, comparing the captive fish eggs with the artificial fish feeds that the adult fish received could give information about the fish *feed impact*.

a) Environment impact

In order to evaluate the environmental impact, we will compare the composition of the eggs of Atlantic salmon of the Loire-Allier origin, which spent two years at sea, but one group, caught in its own Allier system in Vichy (France) provided by CNSS (Chanteuges, France) and the other group caught in the Meuse river system in Lixhe provided by CoSMos (Erezée, Belgium).

Table 3. Proximate composition (g/100 g) of eggs from Atlantic salmon adult females of Loire-Allier strain, which spent two years at sea, caught in 2017 either in the river Allier in Vichy (France) or in the river Meuse in Lixhe (Belgium).

Proximate composition	Atlantic salmon eggs (Allier)	Atlantic salmon eggs (Meuse)
Dry matter	39.9 ± 0.8	39.3 ± 0.3
Crude ash	4.6 ± 0.3	4.3 ± 0.6
Crude lipids (DM ¹)	21.8 ± 0.8	24.4 ± 0.4
Crude proteins (DM)	70.3 ± 0.8	69.1 ± 0.2
Crude lipids (FM ¹)	8.7 ± 0.3	9.6 ± 0.2
Crude proteins (FM)	28 ± 0.4	27.2 ± 0.2

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05).

¹DM, Dry Matter ; FM, Fresh Matter.

No impact of the environmental conditions was observed on the egg proximate composition, as observed in Table 3. However, a tendency for a higher lipid content in the eggs from the salmon females caught in the river Meuse was observed, as compared with the river Allier female eggs (P=0.0726 (DM), P=0.0576 (FM)).

Globally, there was a good correlation between the fatty acid profiles (% TFA) of both egg conditions: Semi-wild Atlantic salmon eggs fatty acid level (Allier, CNSS) = - 0.10724 + 1.0284*Semi-wild Atlantic salmon eggs fatty acid level (Meuse, CoSMos),

$R^2=0.9876$ ($P<0.0001$). When expressed in mg/g of FM, some significant differences of fatty acid composition were observed in the eggs from the salmon females of the same Loire-Allier genetic strain, caught either in the Allier river system in Vichy or in the Meuse river in Lixhe (Table 4). However, most of these differences disappeared when expressed in % TFA (Table 4). When expressed in these units, the various environmental conditions induced no significant difference of content for most fatty acids between the eggs from salmon caught in the Allier and those caught in the Belgian Meuse, except for a significantly higher 20:1n-9 content and a slightly lower SFA level ($P<0.05$), mainly due to 14:0, in the Allier eggs, as compared with the Meuse eggs (Table 4). Both fish eggs presented low levels of Σ C18 n-6 PUFA and Σ C18 n-3 PUFA and high levels of Σ n-3 HUFA, mainly due to higher DHA content and to a lesser extent to EPA and 22:5n-3.

Table 4. Fatty acid profile (mg/g of fresh matter and % of total identified fatty acids) of eggs from Atlantic salmon adult females of Loire-Allier strain, which spent two years at sea, caught in 2017 either in the river Allier in Vichy (France) or in the river Meuse in Lixhe (Belgium).

Expressed as	mg/g of fresh matter		% of total identified fatty acids	
Fatty acid	Atlantic salmon eggs (Allier)	Atlantic salmon eggs (Meuse)	Atlantic salmon eggs (Allier)	Atlantic salmon eggs (Meuse)
12:0	0.02±0.01	0.04±0	0.03±0.02	0.05±0
14:0	1.1±0.05 ^b	1.6±0.13 ^a	1.57±0.05 ^b	2.02±0.12 ^a
16:0	9.39±0.16 ^b	10.67±0.35 ^a	13.28±0.04	13.41±0.18
18:0	3.84±0.15 ^b	4.55±0.09 ^a	5.4±0.14	5.69±0.05
20:0	0.03±0.03	0.02±0.02	0.04±0.04	0.03±0.03
22:0	0±0	0±0	0±0	0±0
24:0	0±0	0±0	0±0	0±0
14:1n-5	0.01±0.01	0.03±0	0.01±0.01	0.03±0.01
16:1n-7	3.48±0.2	6.01±1.38	4.92±0.24	7.49±1.54
18:1n-7	2.68±0.12	3.45±0.45	3.78±0.13	4.29±0.47
18:1n-9	15.22±0.46 ^b	17.01±0.38 ^a	21.42±0.57	21.31±0.74
20:1n-9	2.12±0.14 ^a	1.4±0.12 ^b	2.98±0.24 ^a	1.74±0.17 ^b
22:1n-9	0.07±0.01	0.07±0	0.1±0.01	0.08±0.01
24:1n-9	0±0	0±0	0±0	0±0
18:2n-6	0.92±0.03	1.15±0.12	1.29±0.05	1.43±0.11
18:3n-6	0.02±0.02	0.02±0.02	0.03±0.03	0.02±0.02
20:2n-6	0.17±0.01	0.15±0.01	0.24±0.01 ^a	0.19±0.01 ^b
20:3n-6	0.18±0.01	0.23±0.06	0.25±0.01	0.28±0.06
20:4n-6	0.43±0.05	0.45±0.04	0.61±0.08	0.56±0.06
22:4n-6	0.02±0.01	0±0	0.03±0.02	0±0
22:5n-6	0.1±0	0.1±0.01	0.14±0.01	0.12±0
18:3n-3	0.35±0.01	0.41±0.05	0.49±0.02	0.51±0.07
18:4n-3	0.28±0.02	0.27±0.03	0.39±0.04	0.34±0.05
20:3n-3	0.1±0	0.06±0.03	0.14±0	0.07±0.04
20:4n-3	1.3±0.01	1.46±0.23	1.82±0.01	1.83±0.31
20:5n-3	7.36±0.1 ^b	8.39±0.13 ^a	10.32±0	10.48±0.33
22:5n-3	4.68±0.31	5.32±0.36	6.53±0.39	6.6±0.3
24:5n-3	0±0	0.02±0.01	0±0	0.02±0.01

24:6n-3	0.02±0.01	0.01±0.01	0.03±0.02	0.02±0.02
22:6n-3	17.01±0.8	17.15±0.62	23.78±1.13	21.37±1.23
Σ SFA ¹	14.39±0.31 ^b	16.88±0.55 ^a	20.32±0.17 ^b	21.19±0.26 ^a
Σ MUFA ²	23.86±0.67	27.95±1.64	33.21±0.79	34.94±1.27
Σ C18 n-6 PUFA ³	0.94±0.04	1.16±0.13	1.33±0.07	1.45±0.13
Σ n-6 HUFA ⁴	0.91±0.07	0.93±0.09	1.28±0.11	1.16±0.09
Σ C18 n-3 PUFA ⁵	0.62±0.02	0.68±0.08	0.88±0.04	0.86±0.11
Σ n-3 HUFA ⁶	30.46±0.66	32.41±0.52	42.62±0.76	40.39±1.52
n-3/n-6 ⁷	16.97±1.41	16.15±1.81	16.9±1.4	16.09±1.8
n-3 HUFA/n-6 HUFA ⁸	34.05±3.46	35.46±3.79	34±3.46	35.4±3.79
Total fatty acids	71.18±0.97 ^b	80.02±1.79 ^a	99.62±0.13	100±0

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row, per mode of expression, with different superscript letters are significantly different.

¹ Sum of saturated fatty acids, includes 12:0, 14:0, 16:0, 18:0, 20:0, 22:0 and 24:0.

² Sum of monounsaturated fatty acids, includes 14:1n-5, 16:1n-7, 18:1n-7, 18:1n-9, 20:1n-9, 22:1n-9 and 24:1n-9.

³ Sum of omega-6 polyunsaturated fatty acids with 18 C.

⁴ Sum of omega-6 long chain polyunsaturated fatty acids with 20 C and 22 C.

⁵ Sum of omega-3 polyunsaturated fatty acids with 18C.

⁶ Sum of omega-3 long chain polyunsaturated fatty acids with 20 C, 22 C and 24 C.

⁷ Ratio of total omega-3 polyunsaturated fatty acids to total omega-6 polyunsaturated fatty acids.

⁸ Ratio of omega-3 long chain polyunsaturated fatty acids to omega-6 long chain polyunsaturated fatty acids.

b) Species impact

The comparison of the egg composition between the semi-wild sea trout, which spent one year at sea, and the semi-wild Atlantic salmon of the Loire-Allier strain, which spent two years at sea, was meant to provide information about the species impact as well as a possible difference of feeding-growth area of the two species in the wild at sea or a difference of time spent at sea. Both species were caught in the Meuse river system in Lixhe. The number of years spent at sea, different for the two species in this case, was chosen on purpose to take into account the migration habits of these two species, including their respective average time spent at sea (See "2.2.Other salmonids a) Sea trout" p.19).

Table 5. Proximate composition (g/100 g) of eggs, either from semi-wild sea trouts, which spent one year at sea, or from semi-wild Atlantic salmon, which spent two years at sea, both caught in 2017 the Belgian river Meuse.

Proximate composition	Sea trout eggs	Atlantic salmon eggs
Dry matter	38.4 ± 0.8	39.3 ± 0.3
Crude ash	4.3 ± 0.6	4.3 ± 0.6
Crude lipids (DM ¹)	21.9 ± 0.7 ^b	24.4 ± 0.4 ^a
Crude proteins (DM)	71.4 ± 0.9	69.1 ± 0.2
Crude lipids (FM ¹)	8.4 ± 0.4	9.6 ± 0.2
Crude proteins (FM)	27.4 ± 0.3	27.2 ± 0.2

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row with different superscript letters are significantly different.

¹DM, Dry Matter; FM, Fresh Matter.

No impact of the species was observed on the egg proximate composition, with the exception of a reduced crude lipid content, on a dry matter basis, in the eggs from the sea

trouts, as compared with the eggs from the Atlantic salmon (Table 5). The same trend was observed regarding the crude lipid content on a fresh matter basis ($P=0.0714$).

Table 6. Fatty acid profile (mg/g of fresh matter and % of total identified fatty acids) of eggs, either from semi-wild sea trouts, which spent one year at sea, or from semi-wild Atlantic salmon, which spent two years at sea, both caught in 2017 the Belgian river Meuse.

Expressed as	mg/g of fresh matter		% of total identified fatty acids	
Fatty acid	Sea trout eggs	Atlantic salmon eggs	Sea trout eggs	Atlantic salmon eggs
12:0	0.03±0	0.04±0	0.04±0.01	0.05±0
14:0	1.02±0.15 ^b	1.6±0.13 ^a	1.48±0.18	2.02±0.12
16:0	9.99±0.64	10.67±0.35	14.46±0.63	13.41±0.18
18:0	3.84±0.33	4.55±0.09	5.52±0.31	5.69±0.05
20:0	0.02±0.02	0.02±0.02	0.03±0.03	0.03±0.03
22:0	0±0	0±0	0±0	0±0
24:0	0±0	0±0	0±0	0±0
14:1n-5	0.02±0.01	0.03±0	0.03±0.01	0.03±0.01
16:1n-7	3.83±0.16	6.01±1.38	5.54±0.06	7.49±1.54
18:1n-7	3.9±0.43	3.45±0.45	5.61±0.52	4.29±0.47
18:1n-9	13.41±0.88 ^b	17.01±0.38 ^a	19.43±1.78	21.31±0.74
20:1n-9	0.75±0.07 ^b	1.4±0.12 ^a	1.08±0.11 ^b	1.74±0.17 ^a
22:1n-9	0.01±0.01 ^b	0.07±0 ^a	0.01±0.01 ^b	0.08±0.01 ^a
24:1n-9	0±0	0±0	0±0	0±0
18:2n-6	0.61±0.23	1.15±0.12	0.88±0.34	1.43±0.11
18:3n-6	0±0	0.02±0.02	0±0	0.02±0.02
20:2n-6	0.16±0.04	0.15±0.01	0.23±0.06	0.19±0.01
20:3n-6	0.16±0.06	0.23±0.06	0.24±0.08	0.28±0.06
20:4n-6	1.44±0.5	0.45±0.04	2.08±0.73	0.56±0.06
22:4n-6	0.4±0.17	0±0	0.58±0.25	0±0
22:5n-6	0.33±0.18	0.1±0.01	0.48±0.26	0.12±0
18:3n-3	0.5±0.1	0.41±0.05	0.72±0.15	0.51±0.07
18:4n-3	0.11±0.02 ^b	0.27±0.03 ^a	0.15±0.02 ^b	0.34±0.05 ^a
20:3n-3	0.13±0.02	0.06±0.03	0.19±0.04	0.07±0.04
20:4n-3	0.71±0.17	1.46±0.23	1.02±0.23	1.83±0.31
20:5n-3	5.82±0.83	8.39±0.13	8.31±0.93	10.48±0.33
22:5n-3	5.78±0.67	5.32±0.36	8.24±0.68	6.6±0.3
24:5n-3	0.06±0.01	0.02±0.01	0.09±0.02 ^a	0.02±0.01 ^b
24:6n-3	0.06±0.01 ^a	0.01±0.01 ^b	0.08±0.01 ^a	0.02±0.02 ^b
22:6n-3	16.15±0.17	17.15±0.62	23.17±0.63	21.37±1.23
Σ SFA ¹	14.9±1.01	16.88±0.55	21.53±0.83	21.19±0.26
Σ MUFA	22.13±0.28 ^b	27.95±1.64 ^a	31.71±1.29	34.94±1.27
Σ C18 n-6 PUFA	0.61±0.23	1.16±0.13	0.88±0.34	1.45±0.13
Σ n-6 HUFA	2.51±0.94	0.93±0.09	3.62±1.38	1.16±0.09
Σ C18 n-3 PUFA	0.6±0.09	0.68±0.08	0.87±0.14	0.86±0.11
Σ n-3 HUFA	28.71±1.75	32.41±0.52	41.09±1.31	40.39±1.52
n-3/n-6	12.06±3.64	16.15±1.81	12.03±3.63	16.09±1.8

n-3 HUFA/n-6 HUFA	14.66±4.37 ^b	35.46±3.79 ^a	14.64±4.37 ^b	35.4±3.79 ^a
Total fatty acids	69.45±2.24 ^b	80.02±1.79 ^a	99.7±0.12	100±0

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row, per mode of expression, with different superscript letters are significantly different.

¹See definition of abbreviations in Table 4.

Globally, there was a good correlation between the fatty acid profiles (% TFA) of both egg conditions: Semi-wild sea trout eggs fatty acid level = $0.02778 + 0.9887 \times \text{Semi-wild Atlantic salmon eggs fatty acid level (CoSMos)}$, $R^2=0.975$ ($P<0.0001$). Overall, the fatty acid levels (%TFA), were similar in the Atlantic salmon eggs and in the sea trout eggs, except for significantly higher 20:1n-9 and 22:1n-9 contents, higher LA/ALA and n-3 HUFA/n-6 HUFA ratios and lower AA level in the Atlantic salmon eggs, as compared with the sea trout eggs (Table 6). Both fish eggs presented low levels of Σ C18 n-6 PUFA and Σ C18 n-3 PUFA and high levels of Σ n-3 HUFA, mainly due to DHA and to a lesser extent to EPA and 22:5n-3.

c) Life style impact

By comparing the eggs of captive and semi-wild species, including both Atlantic salmons and sea trouts, we may have a better insight in the fish “life style” (wild *versus* captive) influence on the egg composition.

c.1. Atlantic salmon

Table 7. Proximate composition (g/100 g) of eggs from Atlantic salmons, either captive or semi-wild which spent two years at sea (CoSMos¹).

Proximate composition	Captive Atlantic salmon eggs	Semi-wild Atlantic salmon eggs
Dry matter	37.8 ± 0.4 ^b	39.3 ± 0.3 ^a
Crude ash	5.7 ± 1.4	4.3 ± 0.6
Crude lipids (DM ¹)	24.4 ± 0.9	24.4 ± 0.4
Crude proteins (DM)	67.6 ± 0.5 ^b	69.1 ± 0.2 ^a
Crude lipids (FM ¹)	9.2 ± 0.4	9.6 ± 0.2
Crude proteins (FM)	25.5 ± 0.1 ^b	27.2 ± 0.2 ^a

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row with different superscript letters are significantly different.

¹DM, Dry Matter; FM, Fresh Matter; CoSMos, “Conservatoire du Saumon Mosan”.

Lower dry matter and crude protein (DM and FM) contents were observed in the eggs from the captive Atlantic salmons, as compared with those of the semi-wild Atlantic salmons, which spent two years at sea. In contrast, no impact of the life type was observed on the crude lipid content (DM and FM) (Table 7).

Globally, there was a good correlation between the fatty acid profiles (% TFA) of both egg conditions: Captive Atlantic salmon eggs fatty acid level = $0.0232 + 0.9918 \times \text{Semi-wild Atlantic salmon eggs fatty acid level (CoSMos)}$, $R^2=0.8051$ ($P<0.0001$). Furthermore, as can be seen in Table 8, no significant impact of the Atlantic salmon life type was observed on the Σ MUFA egg content, except for higher 18:1n-9 and lower 20:1n-9 contents in the captive Atlantic salmon eggs, as compared with the semi-wild ones ($P<0.05$). Higher levels of Σ C18 n-6 PUFA and Σ n-6 HUFA were observed in

the captive fish eggs, as compared with the semi-wild fish eggs ($P<0.05$), mostly due to a huge increase in LA content, as well as higher 20:3n-6 and AA contents ($P<0.05$). The same was observed concerning the Σ C18 n-3 PUFA content, mainly represented by ALA, higher in the captive fish eggs ($P<0.05$). In contrast, the Σ SFA and Σ n-3 HUFA levels and the n-3 HUFA/n-6 HUFA ratio were significantly higher in the eggs from the semi-wild Atlantic salmons, which spent two years at sea, as compared with the captive ones. The higher Σ n-3 HUFA level was mainly related to the significantly higher levels of 20:4n-3, EPA and 22:5n-3 in the semi-wild Atlantic salmon eggs, as compared with the captive ones. By contrast, no significant difference in the DHA level was observed between the two egg conditions.

Table 8. Fatty acid profile (mg/g of fresh matter and % of total identified fatty acids) of eggs from Atlantic salmons, either captive or semi-wild which spent two years at sea (CoSMos²).

Expressed as	mg/g of fresh matter		% of total identified fatty acids	
Fatty acid	Captive Atlantic salmon eggs	Semi-wild Atlantic salmon eggs	Captive Atlantic salmon eggs	Semi-wild Atlantic salmon eggs
12:0	0.04±0	0.04±0	0.05±0	0.05±0
14:0	1.02±0.06 ^b	1.6±0.13 ^a	1.18±0.03 ^b	2.02±0.12 ^a
16:0	10.92±0.28	10.67±0.35	12.52±0.06 ^b	13.41±0.18 ^a
18:0	4.29±0.08	4.55±0.09	4.9±0.13 ^b	5.69±0.05 ^a
20:0	0±0	0.02±0.02	0±0	0.03±0.03
22:0	0±0	0±0	0±0	0±0
24:0	0±0	0±0	0±0	0±0
14:1n-5	0.01±0.01	0.03±0	0.01±0.01	0.03±0.01
16:1n-7	3.55±0.2	6.01±1.38	4.07±0.12	7.49±1.54
18:1n-7	2.68±0.14	3.45±0.45	3.06±0.07	4.29±0.47
18:1n-9	26.53±1.05 ^a	17.01±0.38 ^b	30.24±0.33 ^a	21.31±0.74 ^b
20:1n-9	0.65±0.01 ^b	1.4±0.12 ^a	0.73±0.01 ^b	1.74±0.17 ^a
22:1n-9	0.06±0.05	0.07±0	0.07±0.06	0.08±0.01
24:1n-9	0±0	0±0	0±0	0±0
18:2n-6	9.05±0.25 ^a	1.15±0.12 ^b	10.33±0.08 ^a	1.43±0.11 ^b
18:3n-6	0.43±0.07 ^a	0.02±0.02 ^b	0.49±0.08 ^a	0.02±0.02 ^b
20:2n-6	0.71±0.01 ^a	0.15±0.01 ^b	0.81±0.01 ^a	0.19±0.01 ^b
20:3n-6	1.6±0.1 ^a	0.23±0.06 ^b	1.82±0.09 ^a	0.28±0.06 ^b
20:4n-6	1.22±0.09 ^a	0.45±0.04 ^b	1.39±0.14 ^a	0.56±0.06 ^b
22:4n-6	0.08±0	0±0	0.09±0.01	0±0
22:5n-6	0.13±0 ^a	0.1±0.01 ^b	0.14±0.01 ^a	0.12±0 ^b
18:3n-3	1.52±0.15 ^a	0.41±0.05 ^b	1.73±0.13 ^a	0.51±0.07 ^b
18:4n-3	0.38±0.03	0.27±0.03	0.44±0.03	0.34±0.05
20:3n-3	0.13±0.07	0.06±0.03	0.15±0.08	0.07±0.04
20:4n-3	0.65±0.08 ^b	1.46±0.23 ^a	0.73±0.07 ^b	1.83±0.31 ^a
20:5n-3	4.33±0.03 ^b	8.39±0.13 ^a	4.93±0.18 ^b	10.48±0.33 ^a
22:5n-3	1.72±0.11 ^b	5.32±0.36 ^a	1.94±0.07 ^b	6.6±0.3 ^a
24:5n-3	0±0	0.02±0.01	0±0	0.02±0.01

24-6n-3	0.04±0	0.01±0.01	0.05±0	0.02±0.02
22:6n-3	15.88±0.34	17.15±0.62	18±0.34	21.37±1.23
Σ SFA ¹	16.28±0.39	16.88±0.55	18.65±0.17 ^b	21.19±0.26 ^a
Σ MUFA	33.59±1.4	27.95±1.64	38.18±0.52	34.94±1.27
Σ C18 n-6 PUFA	9.49±0.29 ^a	1.16±0.13 ^b	10.82±0.16 ^a	1.45±0.13 ^b
Σ n-6 HUFA	3.73±0.13 ^a	0.93±0.09 ^b	4.25±0.19 ^a	1.16±0.09 ^b
Σ C18 n-3 PUFA	1.91±0.16 ^a	0.68±0.08 ^b	2.17±0.12 ^a	0.86±0.11 ^b
Σ n-3 HUFA	22.75±0.43 ^b	32.41±0.52 ^a	25.81±0.39 ^b	40.39±1.52 ^a
n-3/n-6	1.87±0.05	16.15±1.81	1.86±0.05	16.09±1.8
n-3 HUFA/n-6 HUFA	6.11±0.28 ^b	35.46±3.79 ^a	6.1±0.28 ^b	35.4±3.79 ^a
Total fatty acids	87.75±2.58	80.02±1.79	99.88±0.06	100±0

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row, per mode of expression, with different superscript letters are significantly different.

¹See definition of abbreviations in Table 4.

² CoSMos, “Conservatoire du Saumon Mosan”.

c.2. Sea trout

Table 9. Proximate composition (g/100 g) of eggs from sea trouts, either captive (CoSMos¹) or semi-wild which spent one year at sea.

Proximate composition	Captive sea trout eggs	Semi-wild sea trout eggs
Dry matter	38.5 ± 0.4	38.4 ± 0.8
Crude ash	4.5 ± 0.7	4.3 ± 0.6
Crude lipids (DM ¹)	21.5 ± 0.7	21.9 ± 0.7
Crude proteins (DM)	71 ± 1.1	71.4 ± 0.9
Crude lipids (FM ¹)	8.3 ± 0.3	8.4 ± 0.4
Crude proteins (FM)	27.3 ± 0.6	27.4 ± 0.3

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05).

¹DM, Dry Matter; FM, Fresh Matter; CoSMos, “Conservatoire du Saumon Mosan”.

No significant impact of the life style of the sea trout species was observed on the egg proximate composition (Table 9).

Table 10. Fatty acid profile (mg/g of fresh matter and % of total identified fatty acids) of eggs from sea trouts, either captive (CoSMos²) or semi-wild which spent one year at sea.

Expressed as	mg/g of fresh matter		% of total identified fatty acids	
Fatty acid	Captive sea trout eggs	Semi-wild sea trout eggs	Captive sea trout eggs	Semi-wild sea trout eggs
12:0	0.04±0	0.03±0	0.06±0	0.04±0.01
14:0	0.82±0.02	1.02±0.15	1.19±0.03	1.48±0.18
16:0	8.84±0.14	9.99±0.64	12.73±0.4	14.46±0.63
18:0	2.85±0.1 ^b	3.84±0.33 ^a	4.08±0.06	5.52±0.31
20:0	0.02±0.02	0.02±0.02	0.02±0.02	0.03±0.03
22:0	0.01±0.01	0±0	0.02±0.02	0±0
24:0	0±0	0±0	0±0	0±0
14:1n-5	0±0	0.02±0.01	0±0	0.03±0.01
16:1n-7	2.23±0.09 ^b	3.83±0.16 ^a	3.2±0.1 ^b	5.54±0.06 ^a
18:1n-7	2.83±0.02	3.9±0.43	4.05±0.05 ^b	5.61±0.52 ^a

18:1n-9	18.03±0.96 ^a	13.41±0.88 ^b	25.78±0.9 ^a	19.43±1.78 ^b
20:1n-9	1.54±0.09 ^a	0.75±0.07 ^b	2.19±0.13 ^a	1.08±0.11 ^b
22:1n-9	0.09±0.07	0.01±0.01	0.13±0.11	0.01±0.01
24:1n-9	0±0	0±0	0±0	0±0
18:2n-6	6.1±0.33 ^a	0.61±0.23 ^b	8.72±0.33 ^a	0.88±0.34 ^b
18:3n-6	0.08±0.01 ^a	0±0 ^b	0.11±0.01 ^a	0±0 ^b
20:2n-6	1.44±0.03 ^a	0.16±0.04 ^b	2.05±0.05 ^a	0.23±0.06 ^b
20:3n-6	1.19±0.03 ^a	0.16±0.06 ^b	1.7±0.01	0.24±0.08
20:4n-6	0.98±0.09	1.44±0.5	1.39±0.11	2.08±0.73
22:4n-6	0.15±0.02	0.4±0.17	0.21±0.02	0.58±0.25
22:5n-6	0.23±0.02	0.33±0.18	0.32±0.03	0.48±0.26
18:3n-3	1.1±0.06 ^a	0.5±0.1 ^b	1.58±0.07 ^a	0.72±0.15 ^b
18:4n-3	0.11±0	0.11±0.02	0.16±0	0.15±0.02
20:3n-3	0.18±0.09	0.13±0.02	0.26±0.13	0.19±0.04
20:4n-3	0.38±0.04	0.71±0.17	0.55±0.06	1.02±0.23
20:5n-3	2.43±0.16	5.82±0.83	3.47±0.16	8.31±0.93
22:5n-3	1.62±0.26 ^b	5.78±0.67 ^a	2.29±0.32 ^b	8.24±0.68 ^a
24:5n-3	0.04±0.01	0.06±0.01	0.06±0.01	0.09±0.02
24:6n-3	0.12±0 ^a	0.06±0.01 ^b	0.17±0.01 ^a	0.08±0.01 ^b
22:6n-3	16.4±0.73	16.15±0.17	23.37±1.42	23.17±0.63
Σ SFA ¹	12.58±0.08	14.9±1.01	18.1±0.37 ^b	21.53±0.83 ^a
Σ MUFA	24.8±1.07	22.13±0.28	35.36±0.85	31.71±1.29
Σ C18 n-6 PUFA	6.17±0.34 ^a	0.61±0.23 ^b	8.83±0.34 ^a	0.88±0.34 ^b
Σ n-6 HUFA	3.99±0.17	2.51±0.94	5.68±0.17	3.62±1.38
Σ C18 n-3 PUFA	1.22±0.06 ^a	0.6±0.09 ^b	1.74±0.07 ^a	0.87±0.14 ^b
Σ n-3 HUFA	21.17±0.37	28.71±1.75	30.15±0.95 ^b	41.09±1.31 ^a
n-3/n-6	2.21±0.12	12.06±3.64	2.2±0.12	12.03±3.63
n-3 HUFA/n-6 HUFA	5.33±0.25	14.66±4.37	5.32±0.24	14.64±4.37
Total fatty acids	69.94±1.3	69.45±2.24	99.87±0.06	99.7±0.12

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row, per mode of expression, with different superscript letters are significantly different.

¹See definition of abbreviations in Table 4.

Globally, there was a good correlation between the fatty acid profiles (% TFA) of both egg conditions: captive sea trout eggs fatty acid level = 0.0371 + 0.9906*semi-wild sea trout eggs fatty acid level, $R^2=0.8478$ ($P<0.0001$). No significant impact of the sea trout life type could be observed, in Table 10, in terms of Σ MUFA egg content. In contrast, higher 18:1n-9 and 20:1n-9 contents were noticed in the captive sea trout eggs, as compared with the semi-wild ones ($P<0.05$). A significantly higher level of Σ C18 n-6 PUFA was observed, mostly due to the LA content, in the captive sea trout eggs, as compared with the semi-wild ones. The same was observed concerning the Σ C18 n-3 PUFA content, mainly represented by ALA, higher in the captive fish eggs ($P<0.05$). In contrast, the Σ n-3 HUFA level was higher in the eggs from the sea trouts, which spent one year at sea, as compared with the captive sea trout eggs, mainly due to a significantly higher 22:5n-3 content and a higher EPA content. By contrast, no significant difference

in the DHA level was observed between the two egg conditions. Finally, the Σ SFA content and the n-3 HUFA/n-6 HUFA ratio recorded were higher in the semi-wild sea trout eggs, as compared with the captive ones ($P<0.05$).

d) Fish feed impact

In order to study the potential influence of the artificial feed given to the captive fish on their egg composition, a comparison has been made in terms of composition trends between the artificial fish feeds of CoSMos, used either to feed the captive Atlantic salmon or to feed the captive sea trouts and the respective eggs of these two fish catches, having been fed on these artificial feeds.

Regarding the proximate composition, observed in Table 11, no similar trends were observed between the fish feed and the fish egg compositions, except regarding the crude protein content (FM), for which a higher level was obtained for both the captive sea trout feed and the eggs produced, as compared with the captive Atlantic salmon feed and eggs.

Table 11. Proximate composition (g/100 g) of artificial feeds, used to feed either captive sea trouts or captive Atlantic salmon, and of eggs produced by these fish.

Proximate composition	Feed		Eggs	
	Sea trout	Atlantic salmon	Sea trout	Atlantic salmon
Dry matter	90.1 $\pm$ 0.6 ^b	93.7 $\pm$ 0.3 ^a	38.5 $\pm$ 0.4	37.8 $\pm$ 0.4
Crude ash	8.2 $\pm$ 0.3 ^b	9.6 $\pm$ 0.3 ^a	4.5 $\pm$ 0.7	5.7 $\pm$ 1.4
Crude lipids (DM ¹)	15.8 $\pm$ 0.9	19.1 $\pm$ 0.3	21.5 $\pm$ 0.7	24.4 $\pm$ 0.9
Crude proteins(DM)	52.2 $\pm$ 0.3 ^a	48.3 $\pm$ 0.1 ^b	71 $\pm$ 1.1	67.6 $\pm$ 0.5
Crude lipids (FM ¹)	14.2 $\pm$ 0.7 ^b	17.9 $\pm$ 0.3 ^a	8.3 $\pm$ 0.3	9.2 $\pm$ 0.4
Crude proteins(FM)	47.1 $\pm$ 0.3 ^a	45.2 $\pm$ 0 ^b	27.3 $\pm$ 0.6 ^a	25.5 $\pm$ 0.1 ^b

For each condition, three samples were analysed and results are expressed as mean values $\pm$ SEM ($p<0.05$). Results within a row, and for one sample type (feed or egg), with different superscript letters are significantly different. ¹DM, Dry Matter; FM, Fresh Matter.

Concerning the fatty acid profiles, there was a moderate to good correlation between the fatty acid levels (% TFA) in the eggs and feed of either sea trouts or Atlantic salmon. For the sea trout eggs and feed, this gives: Captive sea trout eggs fatty acid level = $0.8467 + 0.7459 \times \text{Sea trout feed fatty acid level}$, $R^2=0.6709$ ($P<0.0001$). For the Atlantic salmon eggs and feed, this gives: Captive Atlantic salmon eggs fatty acid level = $0.1797 + 0.9472 \times \text{Atlantic salmon feed fatty acid level}$, $R^2=0.8616$ ($P<0.0001$). Table 12 shows several significant differences between the feed and the fish egg fatty acid compositions (mg/g FM), as the contents in Σ MUFA, Σ C18 n-3 PUFA and Σ n-3 HUFA, mostly represented by EPA, which were significantly higher, both in the feed and in the eggs, of the captive Atlantic salmon, as compared with the feed and eggs of the captive sea trouts. Regarding the results of the fatty acid profile expressed in % TFA in Table 12, the Σ SFA, Σ MUFA, Σ C18 n-6 PUFA, mainly due to LA, Σ C18 n-3 PUFA, mainly due to ALA, levels were higher in the feeds, as compared with the captive fish eggs. By contrast, the Σ n-6 HUHA and Σ n-3 HUFA contents were higher in the eggs, as compared with the feeds, mostly due to significant higher AA and DHA contents in the eggs. The EPA content was similar in the feed and in the eggs.

Table 12. Fatty acid profile (mg/g of fresh matter and % of total identified fatty acids) of artificial feeds, used to feed either captive sea trouts or captive Atlantic salmon, and of eggs produced by these fish.

Expressed as	mg/g of fresh matter				% of total identified fatty acids			
Fatty acid	Feed		Eggs		Captive sea trout		Captive Atlantic salmon	
	Sea trout	Atlantic salmon	Sea trout	Atlantic salmon	Feed	Eggs	Feed	Eggs
12:0	0.56±0.01	0.14±0	0.04±0	0.04±0	0.47±0 ^a	0.06±0 ^b	0.11±0 ^a	0.05±0 ^b
14:0	3.22±0.02 ^b	3.87±0.03 ^a	0.82±0.02	1.02±0.06	2.66±0.02 ^a	1.19±0.03 ^b	2.85±0.01 ^a	1.18±0.03 ^b
16:0	17.57±0.17 ^b	18.47±0.14 ^a	8.84±0.14 ^b	10.92±0.28 ^a	14.45±0.05 ^a	12.73±0.4 ^b	13.53±0.04 ^a	12.52±0.06 ^b
18:0	4.04±0.02	4.25±0.04	2.85±0.1 ^b	4.29±0.08 ^a	3.31±0.02 ^b	4.08±0.06 ^a	3.1±0.02 ^b	4.9±0.13 ^a
20:0	0.37±0	0.42±0.02	0.02±0.02	0±0	0.3±0	0.02±0.02	0.3±0.02	0±0
22:0	0.23±0.02	0.2±0.01	0.01±0.01	0±0	0.19±0.02 ^a	0.02±0.02 ^b	0.15±0.01	0±0
24:0	0±0	0.11±0.08	0±0	0±0	0±0	0±0	0.08±0.06	0±0
14:1n-5	0.1±0.01	0.08±0.01	0±0	0.01±0.01	0.08±0.01 ^a	0±0 ^b	0.06±0.01 ^a	0.01±0.01 ^b
16:1n-7	4.13±0.03 ^b	6.54±0.07 ^a	2.23±0.09 ^b	3.55±0.2 ^a	3.4±0.01	3.2±0.1	4.79±0.01 ^a	4.07±0.12 ^b
18:1n-7	3.43±0.02 ^b	4.75±0.03 ^a	2.83±0.02	2.68±0.14	2.81±0.01	4.05±0.05	3.46±0.02 ^a	3.06±0.07 ^b
18:1n-9	41.56±0.29	42.55±0.41	18.03±0.96 ^b	26.53±1.05 ^a	34.02±0.06 ^a	25.78±0.9 ^b	31.03±0.03	30.24±0.33
20:1n-9	3.4±0.03 ^b	5.5±0.09 ^a	1.54±0.09 ^a	0.65±0.01 ^b	2.77±0.01 ^a	2.19±0.13 ^b	4±0.04 ^a	0.73±0.01 ^b
22:1n-9	0.47±0.03	0.53±0.04	0.09±0.07	0.06±0.05	0.38±0.02	0.13±0.11	0.38±0.03 ^a	0.07±0.06 ^b
24:1n-9	0.44±0.02	0.42±0.02	0±0	0±0	0.36±0.02 ^a	0±0 ^b	0.31±0.01 ^a	0±0 ^b
18:2n-6	21.14±0.26	20.83±0.33	6.1±0.33 ^b	9.05±0.25 ^a	17.31±0.09 ^a	8.72±0.33 ^b	15.19±0.11 ^a	10.33±0.08 ^b
18:3n-6	0.13±0 ^b	0.16±0 ^a	0.08±0.01 ^b	0.43±0.07 ^a	0.11±0	0.11±0.01	0.12±0 ^b	0.49±0.08 ^a
20:2n-6	0.73±0	0.52±0.01	1.44±0.03	0.71±0.01	0.6±0	2.05±0.05	0.37±0.01 ^b	0.81±0.01 ^a
20:3n-6	0.21±0 ^a	0.18±0 ^b	1.19±0.03	1.6±0.1	0.17±0 ^b	1.7±0.01 ^a	0.13±0	1.82±0.09
20:4n-6	0.64±0.01 ^b	0.77±0.01 ^a	0.98±0.09	1.22±0.09	0.52±0.01 ^b	1.39±0.11 ^a	0.56±0 ^b	1.39±0.14 ^a
22:4n-6	0.14±0	0.05±0.05	0.15±0.02	0.08±0	0.12±0	0.21±0.02	0.03±0.03	0.09±0.01
22:5n-6	0.19±0 ^b	0.26±0 ^a	0.23±0.02 ^a	0.13±0 ^b	0.16±0 ^b	0.32±0.03 ^a	0.19±0 ^a	0.14±0.01 ^b
18:3n-3	5.14±0.05	5.01±0.11	1.1±0.06 ^b	1.52±0.15 ^a	4.21±0.02 ^a	1.58±0.07 ^b	3.66±0.06 ^a	1.73±0.13 ^b
18:4n-3	0.96±0.01 ^b	1.58±0 ^a	0.11±0	0.38±0.03	0.79±0.01 ^a	0.16±0 ^b	1.16±0.01 ^a	0.44±0.03 ^b

20:3n-3	0.38±0.01	0.26±0	0.18±0.09	0.13±0.07	0.31±0.01	0.26±0.13	0.19±0	0.15±0.08
20:4n-3	0.5±0.01	0.51±0.01	0.38±0.04 ^b	0.65±0.08 ^a	0.41±0	0.55±0.06	0.37±0 ^b	0.73±0.07 ^a
20:5n-3	4.34±0.04 ^b	7.8±0.08 ^a	2.43±0.16 ^b	4.33±0.03 ^a	3.54±0.01	3.47±0.16	5.67±0	4.93±0.18
22:5n-3	1.1±0.01 ^b	1.39±0.02 ^a	1.62±0.26	1.72±0.11	0.89±0.01	2.29±0.32	1.01±0.01 ^b	1.94±0.07 ^a
24:5n-3	0.11±0 ^b	0.15±0.01 ^a	0.04±0.01	0±0	0.09±0 ^a	0.06±0.01 ^b	0.11±0	0±0
24:6n-3	0.01±0.01	0.04±0.02	0.12±0 ^a	0.04±0 ^b	0.01±0.01 ^b	0.17±0.01 ^a	0.03±0.02	0.05±0
22:6n-3	6.67±0.08 ^b	9.41±0.07 ^a	16.4±0.73	15.88±0.34	5.42±0.03 ^b	23.37±1.42 ^a	6.82±0.02 ^b	18±0.34 ^a
Σ SFA ¹	25.99±0.18 ^b	27.46±0.28 ^a	12.58±0.08	16.28±0.39	21.38±0.05 ^a	18.1±0.37 ^b	20.12±0.05 ^a	18.65±0.17 ^b
Σ MUFA	53.7±0.33 ^b	60.72±0.61 ^a	24.8±1.07 ^b	33.59±1.4 ^a	43.82±0.03 ^a	35.36±0.85 ^b	44.03±0.03	38.18±0.52
Σ C18 n-6 PUFA	21.27±0.27	20.99±0.33	6.17±0.34 ^b	9.49±0.29 ^a	17.41±0.09 ^a	8.83±0.34 ^b	15.31±0.11 ^a	10.82±0.16 ^b
Σ n-6 HUFA	1.92±0.02 ^a	1.77±0.04 ^b	3.99±0.17	3.73±0.13	1.56±0.01	5.68±0.17	1.28±0.02	4.25±0.19
Σ C18 n-3 PUFA	6.1±0.05 ^b	6.59±0.11 ^a	1.22±0.06 ^b	1.91±0.16 ^a	5±0	1.74±0.07	4.81±0.06 ^a	2.17±0.12 ^b
Σ n-3 HUFA	13.1±0.12 ^b	19.57±0.19 ^a	21.17±0.37 ^b	22.75±0.43 ^a	10.67±0.03 ^b	30.15±0.95 ^a	14.2±0.01 ^b	25.81±0.39 ^a
n-3/n-6	0.83±0 ^b	1.15±0.01 ^a	2.21±0.12	1.87±0.05	0.83±0	2.2±0.12	1.15±0.01 ^b	1.86±0.05 ^a
n-3 HUFA/n-6 HUFA	6.82±0.01	11.08±0.21	5.33±0.25	6.11±0.28	6.82±0.01	5.32±0.24	11.07±0.21 ^a	6.1±0.28 ^b
Total fatty acids	122.08±0.94 ^b	137.1±1.44 ^a	69.94±1.3 ^b	87.75±2.58 ^a	99.84±0.08	99.87±0.06	99.75±0.13	99.88±0.06

For each condition, three samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row, per mode of expression and for one sample type (feed or eggs and sea trout or Atlantic salmon), with different superscript letters are significantly different.

¹See definition of abbreviations in Table 4.

3. Discussion

This section of the project was aiming to study the egg composition of the Atlantic salmon and sea trouts of CoSMos in order to improve the survival rate of these fish eggs and enhance the reintroduction of the species in the Meuse river. By comparing the proximate composition and fatty acid profile of eggs from fish presenting different characteristics, we could have a better insight into the environment, species and life style impacts of the fish on the derived eggs. The artificial fish feeds were also analysed, in order to assess their influence on the derived captive fish eggs.

The egg fatty acid composition might be an important factor affecting egg quality in terms of fertilisation rate, hatching, survival and growth rate of fish larvae (Parma et al., 2014). This could be related to the fundamental roles that fatty acids, notably LC-PUFA, play first as structural components of storage and membrane lipids but also as major source of energy in metabolic processes of fish during their growth and development. (Murzina et al., 2011). The LA, ALA, AA, EPA and DHA, are the five principal PUFA identified as providing beneficial impacts on the animal growth, reproduction, immunity and product quality of most aquaculture species (Brett, 2009). Nevertheless, the optimal dietary intake and the ideal proportions between the fatty acid classes (n-3 and n-6) and chain lengths (18C, 20C or 22C) vary according to the fish species (Brett, 2009). In some fish species, a deficiency in n-3 HUFA showed to have a negative impact on fecundity, fertilisation rate and hatching rate. Furthermore, high EPA content, compared with DHA content, could affect normal growth and survival of larvae due to an imbalance in the structural composition of the phospholipids (Rainuzzo et al., 1997). A study on the relationships between the egg fatty acid compositions of the domesticated common sole (*Solea solea*) and its egg and larval viability, revealed that the SFA and MUFA contents of the eggs were positively correlated with the hatching rate of the larvae and that the MUFA, EPA and n-6/n-3 egg contents were positively correlated with the survival rate of the larvae (Parma et al., 2014). However, these relationships, effective for the common sole, could not be observed for other fish species.

The fish eggs generally have a high crude protein content, which range from 16 to 30 %, and a high crude lipid content, which can vary from 5 to 20 % (Bledsoe et al., 2003). Indeed, the proximate composition determination of the fish eggs revealed that all the eggs, tested for this section of the work, had a protein content ranging from 25.5 to 30.1% (FM) and a crude lipid content ranging from 7.3 to 10.3 % (FM) (Tables 3, 5, 7 and 9). These overall similar protein and lipid compositions between the eggs of different fish, point out the involvement of these proteins and lipids in basic functions, common to all the fish species (Tocher & Sargent, 1984).

Results are expressed, for each comparison, as mg/g of FM and as % of TFA. Overall, these two modes of expression showed the same differences between the sample conditions compared, except for some fatty acid levels which were considered as different in the two sample conditions when expressed in mg/g of fresh matter, as compared when they are expressed in % of TFA. However, the results expressed in FM are influenced by

the different total fatty acid contents registered in the sample conditions compared, which could make some comparisons more difficult.

In the present work, the studied semi-wild Atlantic salmon were of the same genetic strain (Loire-Allier) but were caught in 2017 in two different hydrosystems: the Allier river in Vichy (France) or the Meuse river in Lixhe (Belgium). This is because of salmon disappearing in the Meuse system around 1935 and the occurrence of a reintroduction programme (“Salmon Meuse”) since 1988 in Belgium. This reintroduction programme used mostly these last years the Loire-Allier strain for restocking of small parrs and smolts in the principal Meuse tributaries in Wallonia (Ourthe, Lesse, etc.). Those juveniles are produced in CoSMos (Erezée) from imported eggs of the Loire-Allier hydrosystem (Dierckx et al., 2017; Ovidio et al., 2016). Thus we are facing an interesting situation where a same genetic strain of Atlantic salmon begins its life cycle in two different freshwater environment, the river Allier (a tributary of the Loire, France), where it evolved naturally during many millennia, or the river Ourthe (a tributary of the Meuse, Belgium), where it was artificially introduced. After smoltification, smolts went to sea but arrived at two very different points: the estuary of the Loire in the Atlantic Ocean in Nantes (France) or the estuary of the Meuse in North Sea in Haringvliet (The Netherlands). Therefore, the two smolts stocks arrive at two different sides of the English Channel. From that moment, the behaviour of the two smolts stocks are poorly known, in particular their precise northern migration trajectories as well as their “final” feeding grounds (Holst, 2012). In the present study, the eggs of Atlantic salmon of the Loire-Allier origin, which were coming from females returners after two years spent at sea, caught either in the Allier in Vichy or in the Meuse in Lixhe revealed no significant impact of these two different environment origins on the egg fatty acid composition (Tables 3 and 4). Both fatty acid profiles were extremely well correlated ($R^2=0.998$). This suggests that these two Atlantic salmon stocks shared an identical feeding ground at sea. Recent investigations (Holst, 2012) suggested that both stocks may use different trajectories to reach similar feeding grounds in the northern part of the Norwegian Sea (Figure 6). Our results are in line with this study. However, the significantly higher level of 20:1n-9 in the eggs of semi-wild (or wild) Allier returners, compared to their Meuse counterparts, may reveal a more northern feeding ground for the first compared to the latter. Indeed, northern origin marine-oils are known to contain high levels of 20:1n-9 (and 22:1n-11) (Linko et al., 1992; Peng et al., 2003) and this level is possibly related to latitude. However, this statement is based on the hypothesis that the 20:1n-9 egg level is mainly influenced by the fish 20:1n-9 diet content at sea, but we cannot exclude the possibility of the implication of bioconversion processes. The two main sources of 20:1n-9 in salmonid fish are either from a diet based on marine-oils rich in this fatty acid or from bioconversion processes of the n-9 precursor, the 18:1n-9. However, since the 18:1n-9 content of both eggs conditions is similar, this hypothesis of a differential elongation of the 18:1n-9 for explaining the higher 20:1n-9 content in the eggs of salmon caught in the Allier is uncertain. The difference of 20:1n-9 is thus probably of dietary origin. Pickova et al. (1998) reported that the DHA content of the phospholipid fraction of the Atlantic salmon eggs from three Swedish anadromous river stocks correlated inversely with the

mean river temperature. We did not analyse fatty acids in the phospholipids fraction of our eggs in the present study, but a somewhat higher (but not significant) DHA level (in % TFA) was noticed in the eggs of Allier returners, as compared with the Meuse returners (Table 6). Further studies will be needed to clarify these questions.

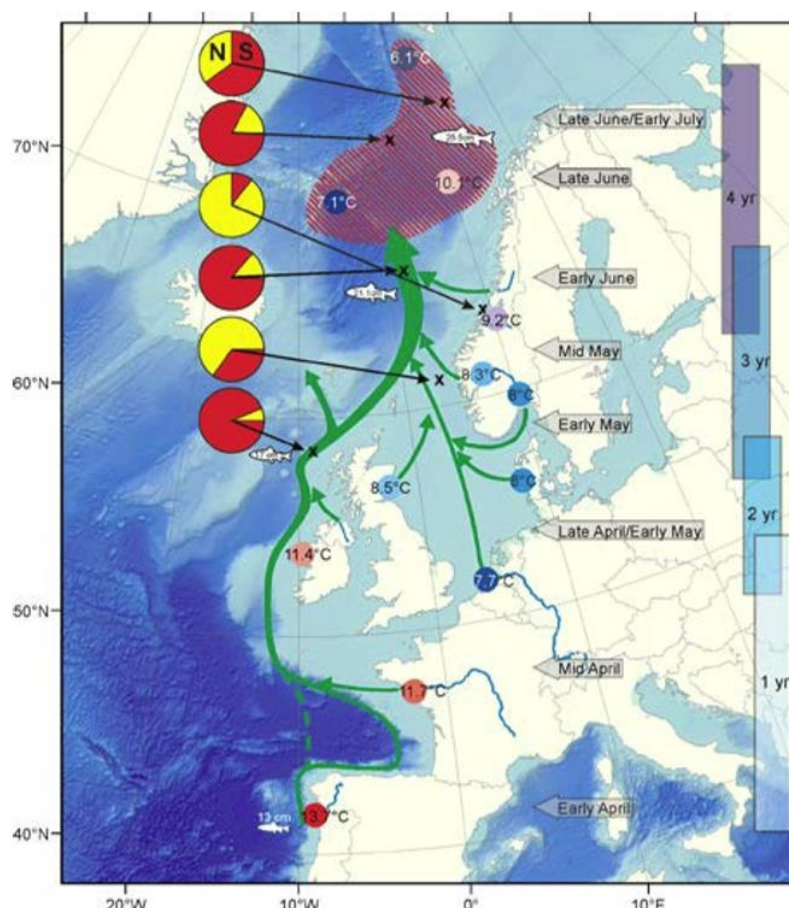


Figure 6. Migration routes for European salmon stocks (Holst, 2012). The ambient temperature, the fish body length, the migration dates, the smolts ages and the ratios between distribution of southern and northern stocks, at the start of the sea migrations and at the feeding area, can also be observed on the figure (Holst, 2012).

The comparison of egg composition between the semi-wild sea trouts, which spent one year at sea, and the semi-wild Atlantic salmons of the Loire-Allier strain, which spent two years at sea, both caught in the Meuse river in Lixhe (Belgium), was meant to provide information about the species impact as well as a possible difference of feeding-growth grounds of the two species in the wild at sea or a difference of time spent at sea. The proximate composition results showed that the crude lipid content (DM) was higher in the Atlantic salmon eggs, as compared with the sea trout eggs. However, the fatty acid levels (% TFA) were overall similar between the two egg species, both fatty acids profiles being extremely well correlated ($R^2=0.975$), except for significantly higher 20:1n-9 and 22:1n-9 levels and n-3 HUFA/n-6 HUFA or LA/ALA ratios in the Atlantic salmon eggs, as compared with the sea trout eggs (Table 6). This higher n-3 HUFA/n-6 HUFA ratio registered in the Atlantic salmon eggs was mostly due to the higher EPA content and lower AA content. All these differences suggest a more “marine-type” pattern in the Atlantic salmon eggs, compared to sea trout eggs. For instance, the 20:1n-9 and 22:1n-9 fatty acids

are found in high levels in northern origin marine oils and in very low proportions in freshwater fish (Linko et al., 1992; Peng et al., 2003). A similar trend was observed for the n-3 HUFA, including EPA. On a contrary, wild fish in freshwater accumulate AA from invertebrates (Ackman & Takeuchi, 1986; Peng et al., 2003) and higher AA levels in the eggs of the sea trouts may reveal a higher dependence of this species to freshwater ecosystems for feeding. Sea trout are known to migrate to sea during shorter periods than Atlantic salmon and on much shorter distances, preferring coastal or estuarine areas to pelagic environment (Laird & Needham, 1988; Jonsson & Jonsson, 2011). Our observed differences of fatty acid contents are thus in line with these differences of life cycle and ecological preferences between the two species. Besides, the higher number of years spent at sea of the Atlantic salmons, as compared with the sea trouts, could explain the higher crude lipid content recorded in the Atlantic salmon eggs. It has been reported that a fish tending to migrate further distances may produce eggs with higher lipid content (Bledsoe et al., 2003). This is confirmed by our results. Little information is available about the migration routes of sea trout, in particular in the Meuse hydrosystem. Apparently, sea trout would remain closer to the coasts after their migration at sea, as compared with Atlantic salmon (Laird & Needham, 1988), and could even go no further than the river estuary. It has to be noticed that the natural estuary of the Meuse was artificially transformed by the construction of the big dams of the Delta Plan (Department of waterways and public works, 2018). Therefore, the estuary is actually transformed to a great freshwater reservoir (Haringvliet, The Netherlands) upstream of the dam. Therefore, it cannot be excluded that the so-called sea trouts caught in the Meuse in Belgium are in fact big trouts not going at sea and living in the Haringvliet. However, the eggs of our semi-wild sea trouts contained high levels of marine-type fatty acids as n-3 HUFA, suggesting that these Meuse trouts are effectively going at sea for their feeding and growing. Studies revealed that cold-water fish developed a high storage capacity of the n-3 HUFA, which present a low melting point, in order to maintain the integrity and fluidity of their cell membranes, even at low temperatures (Pickova et al., 1999). As the semi-wild Atlantic salmons probably travelled more to the north, to cold water, during their migration, as compared with the semi-wild sea trouts, then would be able to store more n-3 HUFA, including EPA. This could explain the higher EPA level observed in the Atlantic salmon eggs, as compared with the sea trout eggs. Besides, the slightly, but well significantly higher 20:1n-9 level recorded in the eggs of the semi-wild Atlantic salmons, as compared with the semi-wild sea trout eggs, could confirm the more northern feeding grounds of the Atlantic salmons, as northern origin marine-oils are known to contain high levels of 20:1n-9 (Peng et al., 2003). Again, this statement is based on the assumption that the 20:1n-9 egg level is mainly influenced by the fish diet at sea. Nevertheless, it is impossible to determine if either the species or the number of years spent at sea or both are responsible for one or another effects observed in the fish eggs.

The comparison of the captive and semi-wild fish egg species was made in order to study the life style influence. For both fish species tested, the Atlantic salmons and the sea trouts, higher Σ C18 n-6 PUFA levels, mainly due to a really great increase of LA content (related to soybean meal), as well as increased Σ n-6 HUFA and Σ C18 n-3 PUFA

contents, were observed in the captive egg species, as compared with the semi-wild egg species (Tables 8 and 10). By contrast, significantly higher levels of Σ n-3 HUFA, mostly due to higher EPA content, were recorded in the semi-wild Atlantic salmon eggs, as compared with the captive Atlantic salmon eggs (Table 8), except for the DHA content which was similar in both egg conditions. The same trend was observed in the sea trout species, semi-wild as compared with captive (Table 10). At sea, wild fish might have a diet based on n-3 HUFA-rich organisms, thanks to a trophic chain starting with microalgae and comprising marine invertebrates, which contain high levels of EPA and DHA (Pickova et al., 1999; Monroig et al., 2013). Furthermore, the storage capacity of the n-3 HUFA might be higher among cold-water fish due to a physiological adaptation to specific environmental conditions (Pickova et al., 1999). Cold-water fish developed this higher capacity of storage in order to maintain the integrity and fluidity of their cell membranes, when evolving in cold-water temperature, thanks to the low melting point of the HUFA (Pickova et al., 1999). As the semi-wild Atlantic salmon travelled long distances at sea to reach their feeding grounds, near Greenland or along the Faroe Islands, they evolved in cold water during a major part of their life cycle (Figure 6), on the contrary of the captive fish, and could then be able to store more n-3 HUFA (Atlantic Salmon Federation, 2017). This was confirmed by previous studies that showed that total n-3 HUFA content was significantly higher in the phospholipid fraction of wild anadromous Atlantic salmon parrs whole body, as well as in eggs, as compared with freshwater landlocked counterparts (Pickova et al., 1999; Peng et al., 2003; Rollin et al., 2003). Therefore, the semi-wild fish diet at sea and the cold-water temperatures to which they are subjected, could explain the higher Σ n-3 HUFA and EPA content registered in the semi-wild fish eggs, as compared with the captive ones. However, we can again not exclude the possible implication of the fatty acid bioconversion capacity of the fish on the n-3 HUFA content of the eggs. A way to verify this hypothesis was to study if the captive fish egg composition and the feed given to the respective adult fish were related.

Studies on Atlantic salmon already proved that the fatty acid profiles of the whole body of the fish reflected, to a large extent, those of the fish diet (Rollin et al., 2003). Next, the lipid composition of fish eggs would also be mainly influenced by the diet composition of the fish, but also by its lipid metabolism (Pickova et al., 1999). Regarding the results of the fatty acid profile (% TFA) in Table 12, the Σ SFA, Σ MUFA, Σ C18 n-6 PUFA, Σ C18 n-3 PUFA, levels were higher in the feeds, as compared with the captive fish eggs, which could reveal a link between these fatty acid levels in the feed and in the eggs. By contrast, the Σ n-6 HUFA and Σ n-3 HUFA contents were higher in the eggs, as compared with the feeds, mostly due to significant higher AA and DHA contents in the eggs (EPA content was similar between the feed and the eggs). The AA and DHA egg contents would then be less influenced by the fish feed composition in AA and DHA and more by the fish metabolism. It has already been demonstrated that the DHA level, and partially the AA level, in salmon eggs were under genetic control (Peng et al., 2003; Rollin et al., 2003). Indeed, regarding the AA and DHA contents of all the egg conditions compared, in the present section of the study, they were overall similar, with the exception of the AA content between the captive and semi-wild Atlantic salmon eggs. However, the

captive fish egg EPA content, seemed to be effectively link to the captive fish feed. Besides, as the EPA level is mainly responsible for the higher n-3 HUFA level recorded in the semi-wild Atlantic salmon eggs, as compared with the captive counterparts, the EPA level in the semi-wild fish eggs could be a marker of the marine diet of the fish at sea. Moreover, in this case, the higher 20:1n-9 level recorded in the semi-wild Atlantic salmon eggs, as compared with the captive counterparts, cannot be explain by the marine source of this fatty acid. Indeed, as explained above, the 20:1n-9 content in fish eggs can also result from the bioconversion of the dietary 18:1n-9 (oleic acid). In Table 12, we can observe that the 18:1n-9 content of the sea trout feed was higher, as compared with the Atlantic salmon feed, resulting in a higher 20:1n-9 content in the captive sea trout eggs, as compared with the captive Atlantic salmon eggs. This confirm the implication of the fish metabolism in the resulting 20:1n-9 egg content of captive fish. However, we still don't know the relative influence of both, the wild-fish diet at sea and the conversion capacity of wild-fish on the 20:1n-9 contents of wild-fish eggs. The environment influence could be greater at sea, and mask the bioconversion process influence. We thus cannot completely exclude 20:1n-9 as a marine marker of the feeding grounds of the wild fish.

A study in 1997, noticed that the egg quality seemed to be higher with wild fish than captive ones. The wild Atlantic salmon eggs had showed a greater size and survival rate of embryos and alevins, along with a higher fertilization and hatching success, as compared with the captive counterparts (Brooks et al., 1997). Indeed, at CoSMos, in 2014, the egg survival rate, until hatching, was much higher among the semi-wild Atlantic salmons, as compared with the captive ones. The same trend was observed among the sea trout fish, but less pronounced. The n-3 HUFA egg content, and more precisely, the EPA content, could thus be related to the better egg survival rate registered in the semi-wild fish, as compared with the captive fish. Besides, the comparison between the mean egg size of the semi-wild and captive Atlantic salmons and sea trouts, revealed no effect of this factor on the egg quality, as the egg sizes were similar between the captive and semi-wild fish of each species (ANNEXES 4-7).

In this work, the two feeds analysed (Tables 11 and 12), were of the same origin, except that the Atlantic salmon feed has been improved with cod liver oil. Cod liver oil is rich in n-3 HUFA, and more precisely, is highly concentrated in EPA and DHA. Indeed, regarding the results obtained of the fatty acid profile (mg/g FM), the EPA and DHA levels were significantly higher in the Atlantic salmon feed, as compared with the sea trout feed. Nevertheless, regarding egg quality, a study in 1993, revealed that the Nile tilapia (*Oreochromis niloticus*) fed with a diet enriched with cod liver oil, showed a high weight gain but poor reproductive performance. Whereas a higher fry production was observed with the same fish species fed with a diet supplemented with soybean oil, rich in n-6 fatty acids (Santiago & Reyes, 1993). As the two feeds analysed in this study are intended to two different fish species, it is difficult to compare their relative influence on fish growth and reproductive success, and therefore assess the impact of the cod liver oil addition into the feed. Overall, the trends of the increased Σ C18 n-3 PUFA and Σ n-3 HUFA (mainly due to EPA) contents in the Atlantic salmon feed, compared with the sea trout feed, were found in the respective eggs, as observed in Table 12 (mg/g FM).

However, this is not observed for the rest of the fatty acid content. We have to keep in mind, that we are comparing two different fish species which are physiologically different and could be characterised by variable digestive capacity of the artificial feed received, as well as variable dietary PUFA conversion capacity, which could have a great impact on the fatty acid contents of the eggs (Holt, 2011; Peng 2003). A study of the impact of variable n-3 HUFA concentration feeds on the eggs issued from one fish species, namely the gilthead seabream (*Sparus aurata* L.), showed that the n-3 HUFA content in the fish eggs was positively correlated with the dietary n-3 HUFA content, and mainly to the dietary EPA content (Fernandez-Palacios et al., 1995). Moreover, higher levels of n-3 HUFA in fish diet were linked to improved spawning quality in terms of fecundity, hatching and larval survival of the gilthead seabream. Nevertheless, a too high n-3 HUFA diet content led to a decreased of the fecundity of the fish (Fernandez-Palacios et al., 1995).

The analysis of the egg quality and the possible enhancement of the survival rate of salmonid eggs have been studied, in this work, through the fatty acid determination of eggs, assuming that fatty acids were the major factors influencing the egg quality. Indeed, the n-3 HUFA egg content appeared to play a major role in the fish egg quality and seemed to be linked to the fish dietary content (Fernandez-Palacios et al., 1995). We could thus try to control the n-3 HUFA egg content through the fatty acid diet content of the captive fish, with previous knowledge about the fish fatty acid conversion capacity, as well as the fish fatty acid dietary requirements, in order to improve the quality of the fish eggs. However, other nutrients in fish eggs could also play a role. Some nutrients, such as fatty acids, vitamins, pigments, appeared to be essential to ensure a good development of the fish embryo (Bobe & Labbé, 2010). It has been proved that for some species, a vitamin E deficiency could affect the reproductive performance, inducing a reduced hatching rate and survival of the offspring. The protein component could also play an important role (Izquierdo et al., 2001). Another study on Atlantic salmon, also suggested that astaxanthin content of the eggs was important and that the need for a minimum dietary concentration of 5.1 mg/kg was needed to reach maximum growth and survival during the first feeding moments of the fish (Christiansen et al., 1995). The presence of pollutants in the fish eggs might also influenced its quality, by reducing the hatching success of the eggs (Ochokwu et al., 2015). Besides, environmental factors including temperature, photoperiod and water salinity could also have an influence on the egg quality. Indeed, low as well as high water temperatures could have a negative impact depending on the species (Bobe & Labbé, 2010). In the case of Atlantic salmon, a suboptimal temperature during the vitellogenesis stage can have an impact on the quality of the eggs (King et al., 2003).

V. ATLANTIC SALMON EGGS FOR HUMAN CONSUMPTION

1. Material and methods

1.1. Egg and feed sampling

Samples were collected from November 2017 to January 2018. We harvested eggs from Atlantic salmon and sea trouts at CoSMos, during the artificial reproduction period. Precisely, samplings consisted in eggs from (1) two Atlantic salmon types: 3+ captive type and semi-wild type in which fish spent two years at sea and (2) two sea trout types: 2+ captive type and semi-wild type in which fish spent one year at sea. Next, the fish farm “Du Ry Des Pres” (Philippeville, Belgium) provided captive rainbow trout eggs. Among those, four egg types were selected for the sensory test, namely eggs from: captive rainbow trouts (“Du Ry Des Pres” farm), captive sea trouts (CoSMos), captive Atlantic salmon (CoSMos) and Atlantic salmon, which spent two years at sea (CoSMos). These four egg types selected for the sensory test were salted (see “1.3. Sensory test”, p.54).

Samples of the feeds dedicated to the captive fish were also collected. Precisely, these feeds corresponded to (1) the “Repro floating” feed (Coppens, Helmond, The Netherlands) used to feed the captive sea trouts of CoSMos, (2) the “n-3 PUFA-rich Repro floating” feed prepared by CoSMos and used to feed the captive Atlantic salmon and (3) the “T-3P Optiline” feed (Skretting, Fontaine les Verviers, France) (See composition ANNEX 3) used to feed the captive rainbow trouts of the “Du Ry Des Pres” farm. Eggs currently found on the market were also analysed, including the Pacific salmon eggs from the following brands: Carrefour (*O. keta*), Kaviari (*O. keta*), Gabriel (*O. keta*) and Petrossian (*O. gorbusha*). Two trout egg brands were also found on the market: Carrefour (unknown species) and Gabriel (rainbow trout). Each of them has been bought at three different periods of the year (November 2017, December 2017 and January 2018) (ANNEX 9).

The sampling dedicated to the evaluation of an Atlantic salmon egg production for human consumption is resumed in Table 13. The complete egg and feed sampling is presented in ANNEX 1.

As described in the first section of the work, eggs of three females were harvested for each condition, in order to evaluate the variability of the egg compositions within the same condition. The analyses were made on the same 50 g of eggs that have been sampled, per female, for the first part of the work. Moreover, 110 g of eggs from five females by condition were pooled, before being salted and dedicated to the sensory test and further analyses. The detailed description of the sampled females is provided in ANNEXES 4-8.

Table 13. List of the feed and egg samples analysed for the section aiming at an Atlantic salmon egg production in Belgium, for human consumption.

Production in Belgium, for human consumption.					
FISH FEED					
Fish feed name	Origin (Brand, city, country)		Fish feed species		
Repro floating	Coppens, Helmond, The Netherlands		Captive sea trout (CoSMos)		
Repro floating improved ¹	Coppens, Helmond, The Netherlands		Captive Atlantic salmon (CoSMos)		
T-3P Optiline	Skretting, Fontaine les Verviers, France		Captive rainbow trout (Du Ry Des Pres farm)		
FISH EGGS					
Type	Species	Origin	Description	Years spent at sea	Treatment
Raw eggs	Atlantic salmon	CoSMos	Captive 3+ (first spawn)	0	Raw
	Atlantic salmon	CoSMos	Semi-wild (spring run)	2	Raw
	Sea tout	CoSMos	Captive 2+ (first spawn)	0	Raw
	Sea tout	CoSMos	Semi-wild (spring run, first spawn)	1	Raw
	Rainbow trout	Du Ry Des Pres	Captive	0	Raw
Salted eggs for the sensory test	Atlantic salmon	CoSMos	Captive 3+ (first spawn)	0	Salted
	Atlantic salmon	CoSMos	Semi-wild (spring run, first spawn)	2	Salted
	Sea tout	CoSMos	Captive 2+ (first spawn)	0	Salted
	Rainbow trout	Du Ry Des Pres	Captive	0	Salted
Market eggs	Pacific salmon (<i>O. keta</i>)	Delhaize	Gabriel	/	Tansformed
	Pacific salmon (<i>O. kisutch</i>)	Bio c' Bon	Kaviari	/	Tansformed
	Pacific salmon (<i>O. keta</i>)	Carrefour	Carrefour	/	Tansformed
	Pacific salmon (<i>O. gorbuscha</i>)	Petrossian	Petrossian	/	Tansformed
	Trout	Carrefour	Carrefour	/	Tansformed
	Rainbow trout	Delhaize	Gabriel	/	Tansformed

¹ Repro floating feed improved with cod liver oil (type A, ABC Chemicals, Braine-le-Château, Belgium) and “Carophyll Red” (Red animals, Ere, Belgium).

1.2. Analytical methods

As explained in the first section of this work, eggs and feeds were directly frozen after their arrival at the laboratory and were next freeze-dried within 48h after sampling. The same was applied to the salted eggs at the end of the sensory test (12/12/2017). The feed and egg samples have then been homogenised following the 950.02 (1mm) protocol of the AOAC (A.O.A.C., 1995). After the homogenisation process, the salted fish eggs and the three feeds, have been separated into three hermetic containers in order to obtain three replicates.

The chemical analyses of the samples are similar to those described in the first section of the work and are detailed in ANNEX 10. They consisted in the evaluation of the proximate composition, namely dry matter, crude ash, crude protein and crude lipid contents, as well as in the determination of the fatty acid composition.

1.3. Sensory test

In order to evaluate the potential of the Atlantic salmon eggs as a consumption product, their appreciation by potential customers has been determined through a sensory test.

The sensory test has been designed on the basis of (1) a previous master thesis of our laboratory in which similar tests have been performed on another food item (Sébastien Delandmeter, 2016) and (2) the book “Basic sensory methods for food evaluation” (Watts, 1989).

The eggs selected for the sensory test had to meet various criteria. Firstly, five females by condition were required to prepare a representative batch in which the inter-individual variability was minimised. Moreover, these had to reach maturity on the same day, in order to preserve the freshness of the eggs. Fortunately, both fish farms provided us with eggs, of a considered condition, from five females reaching maturity on the same day. Since the sensory test had to take place in December 2017, we had to exclude the fish species, which came to maturity after this month, such as the semi-wild sea trouts, which spent one year at sea. Indeed, these animals reached maturity in January 2018. The eggs collected in the fish farms, and salted by ourselves, have not been compared with those found on the market. Indeed, the pronounced organoleptic differences between the market eggs and those salted by hand, would have probably hidden the less pronounced differences existing among our salted eggs. Moreover, we would not have been able to apply exactly the same treatment than the one used for the marketed eggs. Considering the above, four types of fish eggs have been selected for the sensory test, namely eggs from (1) captive rainbow trouts (“Du Ry Des Pres” farm), (2) captive sea trouts (CoSMos), (3) captive Atlantic salmons (CoSMos) and (4) Atlantic salmons, which spent two years at sea (CoSMos).

In order to be as close as possible to the marketed eggs, the sampled raw eggs used in the sensory test had to be processed in a similar manner as the commercial ones. After some investigations, we noticed that the Pacific salmon eggs on the market were only salted (3 to 4%) and pasteurised. We decided to process our eggs on a similar way. For

the salting process, we used a non-iodised salt (the most natural) “sel de guérande”, demineralised water and the salt meter “LAQUATwin B-721” (Horiba, Japan), capable of measuring the sodium ion concentration, to determine the salinity level of the product. After some preliminary tests to reach approximately the same salinity level as that of the eggs from the market, namely around 4%, we defined the conditions of the process. These are to put the raw fish eggs in a 360g/l brine solution for a defined time depending on the egg size and the egg membrane permeability. The captive rainbow trout eggs have been put for 35 minutes in the brine to reach the salt concentration of 4 %. In contrast, the captive sea trout eggs required 60 minutes to reach the same concentration, the captive Atlantic salmon eggs 75 minutes and the semi-wild Atlantic salmon eggs 90 minutes. The salting process was performed directly after the egg-laying, with a maximum delay of one hour, in order to preserve the integrity of the eggs and to enhance the conservation. The eggs were then rinsed and put in 350 ml glass containers hermetically sealed. The second step consisted in a pasteurisation step performed by incubating the hermetically closed containers, containing the eggs, in a water-bath at a constant temperature of 69°C, for one hour (Sternin & Dore, 1998). The eggs were finally stored in the fridge (2-4°C) in darkness until the sensory test.

The sensory test took place on 12 December 2017, with 58 participants. The target population should not have any specific nutritional knowledge or previous information about the project (Sternin & Dore, 1998). The only information that the participants received about the products was that they will test fish eggs. The survey consisted in four questionnaires, one for the general information about the participant, one for the visual test, one for the tasting test and finally one to evaluate general opinion about such products. The survey was anonymous.

Each participant was isolated and had access to a bottle of still water, a glass and two slices of white bread. Four cups with 40 g of each egg sample were presented to the participants, with four little spoons. The participants were asked to follow a specific order to evaluate the eggs. That order was however randomly determined and changed between the participants in order to avoid any systematic effect of one sample on the next one in the mouth. The questionnaires of the sensory test are available in ANNEX 11.

1.4. Statistical methods

All the data are presented as mean $\pm$ SEM of three biological replicates (n=3) for the fish egg samples. Concerning the salted eggs and the feed sampling, three replicates were made up from one bag. For the salted eggs, the original bag was composed of the eggs of five females of the condition concerned. Overall, the results of the proximate composition and fatty acid profile of the egg and feed samples were compared using a one-way analysis of variance (ANOVA), followed by Tukey's (parametric with $\alpha = 0.05\%$) *post hoc* test in order to determine significant differences between conditions. Prior to statistical analyses, assumptions for statistical methods were verified: (1) Normal distribution of residuals with the Shapiro-Wilk's test and (2) equality of variances with the Levene's test. In case these assumptions were not verified, results were analysed by the Wilcoxon's test, followed by the Steel-Dwass's (nonparametric version of Tuckey's method, with $\alpha =$

0.05%) *post hoc* test. Statistical analyses were performed using the JMP software (JMP Pro, SAS, Cary, NC, USA).

Concerning the results of the sensory test, all the data are presented as mean $\pm$ SEM of the participants, which represent the replicates (n= max 58). The number of replicate was lower than 58 for some tests, because several participants did not systematically answered to all the questions. The data were statistically analysed by one-way analysis of variance (ANOVA) in a mixed model, involving fixed effects of the four products and random effects of the participants, followed by Tukey's (parametric with $\alpha = 0.05\%$) *post hoc* test in order to determine significant differences between the characteristics studied. Prior to statistical analyses, assumptions for statistical methods were verified: (1) Normal distribution of residuals with the Shapiro-Wilk's test and (2) equality of variances with the Levene's test. Statistical analyses were performed using the JMP software (JMP Pro, SAS, Cary, NC, USA).

2. Results

In order to evaluate the interest of an Atlantic salmon egg production for human consumption in Belgium, a first comparison concentrated on the raw and salted fish eggs, either from the Atlantic salmons, captive or semi-wild, or from the captive rainbow trouts or from the captive sea trouts, in order to evaluate the *salting process impact*. A second one intended to compare the four salted eggs, in order to determine their *nutritional quality*. A third one was the comparison of the salted eggs, either from the semi-wild Atlantic salmons or from the captive rainbow trouts, with the respective Pacific salmons and rainbow trouts commercial eggs, in order to evaluate their *nutritional benefits*. At last, the comparison between the artificial fish feeds and the eggs from the adult fish fed on this diet, will tell us more about the captive *fish feed impact*. Furthermore, a sensory test was conducted with four salted egg samples selected between the fish conditions, namely the eggs from the captive sea trouts, the captive rainbow trouts and the Atlantic salmons, both captive and semi-wild, in order to *assess the appreciation* by potential consumers, of the products.

2.1. Chemical analyses

a) Salting process impact

In order to evaluate if there is a negative impact of the salting process, performed on the four raw eggs used during the sensory test, we will compare the composition of the four salted eggs with their respective raw counterparts. The four egg samples used for this comparison are the eggs from the Atlantic salmons, captive and semi-wild, from the captive rainbow trouts and from the captive sea trouts.

Regarding the proximate composition of the eggs, the dry matter, crude ash and crude protein (FM) contents were significantly lower in the raw eggs, as compared with the salted eggs of the four fish conditions, as observed in Table 14, with the exception of the sea trout eggs, for which no significant difference was observed concerning the crude protein content, and the captive Atlantic salmon eggs, for which no significant difference was observed regarding the crude ash content. Concerning the crude lipid (FM) content of all the egg conditions, no impact of the salting process was observed. The same observation was made regarding the crude lipid content (DM), except for the sea trout and rainbow trout eggs, where higher contents were observed in the raw eggs, as compared with the salted eggs (Table 14).

As shown in Table 15, there was, overall, no difference between the fatty acid composition of the raw and salted eggs, except, regarding the rainbow trout eggs for which, an increased Σ n-3 HUFA content was recorded in the salted eggs, mostly due to the increased DHA content, as compared with the raw eggs. Concerning the captive Atlantic salmon eggs, slightly higher contents in 18:0 and 20:1n-9 were observed in the salted eggs.

Table 14. Proximate composition (g/100 g) of raw and salted eggs, from captive rainbow trouts or from captive Atlantic salmons or from Atlantic salmons, which spent two years at sea (CoSMos¹) or from captive sea trouts.

Proximate composition	Raw	Salted
Captive Rainbow trout eggs		
Dry matter	38.9 ± 0.8 ^b	45.2 ± 0.3 ^a
Crude ash	4.8 ± 1.1 ^b	9.3 ± 0.3 ^a
Crude lipids (DM ¹)	22.9 ± 0.3 ^a	18.8 ± 0.9 ^b
Crude proteins (DM)	70.9 ± 0.9 ^a	66.7 ± 0.6 ^b
Crude lipids (FM ¹)	8.9 ± 0.3	8.5 ± 0.4
Crude proteins (FM)	27.6 ± 0.3 ^b	30.1 ± 0.1 ^a
Captive Atlantic salmon eggs		
Dry matter	37.8 ± 0.4 ^b	46 ± 0.3 ^a
Crude ash	5.7 ± 1.4	10.1 ± 0.2
Crude lipids (DM)	24.4 ± 0.9	22.4 ± 0.2
Crude proteins (DM)	67.6 ± 0.5 ^a	62.5 ± 0.8 ^b
Crude lipids (FM)	9.2 ± 0.4	10.3 ± 0.1
Crude proteins (FM)	25.5 ± 0.1 ^b	28.7 ± 0.2 ^a
Semi-wild Atlantic salmon eggs (CoSMos)		
Dry matter	39.3 ± 0.3 ^b	46.4 ± 0.2 ^a
Crude ash	4.3 ± 0.6 ^b	9.6 ± 0.1 ^a
Crude lipids (DM)	24.4 ± 0.4	21 ± 0.1
Crude proteins (DM)	69.1 ± 0.2 ^a	64.7 ± 0.4 ^b
Crude lipids (FM)	9.6 ± 0.2	9.8 ± 0.1
Crude proteins (FM)	27.2 ± 0.2 ^b	30 ± 0.1 ^a
Captive sea trout eggs		
Dry matter	38.5 ± 0.4 ^b	43.3 ± 0.4 ^a
Crude ash	4.5 ± 0.7 ^b	11.2 ± 0.5 ^a
Crude lipids (DM)	21.5 ± 0.7 ^a	17.9 ± 0.4 ^b
Crude proteins (DM)	71 ± 1.1 ^a	65.7 ± 0.7 ^b
Crude lipids (FM)	8.3 ± 0.3	7.8 ± 0.2
Crude proteins (FM)	27.3 ± 0.6	28.4 ± 0.1

For each condition, three samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row with different superscript letters are significantly different.

¹DM, Dry Matter; FM, Fresh Matter; CoSMos, “Conservatoire du Saumon Mosan”.

Table 15. Fatty acid profile (mg/g of fresh matter) of raw and salted eggs, from captive rainbow trouts or from captive Atlantic salmon or from Atlantic salmon, which spent two years at sea (CoSMos²) or from captive sea trouts.

Fatty acid	Captive rainbow trout eggs		Captive Atlantic salmon eggs		Semi-wild Atlantic salmon eggs		Captive sea trout eggs	
	Raw	Salted	Raw	Salted	Raw	Salted	Raw	Salted
12:0	0.05±0.01	0.07±0	0.04±0	0.06±0.01	0.04±0	0.02±0.01	0.04±0	0.04±0
14:0	0.67±0.08	0.68±0.02	1.02±0.06	1.1±0.04	1.6±0.13	1.38±0.05	0.82±0.02	0.84±0.03
16:0	9.76±0.43	10.23±0.1	10.92±0.28	11.65±0.24	10.67±0.35	10.97±0.24	8.84±0.14	9.26±0.3
18:0	3.48±0.12	3.69±0.04	4.29±0.08 ^b	4.61±0.03 ^a	4.55±0.09	4.67±0.04	2.85±0.1	2.92±0.07
20:0	0.05±0.03	0.05±0.02	0±0	0±0	0.02±0.02	0.03±0.03	0.02±0.02	0.04±0.02
22:0	0.04±0.02	0.06±0.03	0±0	0±0	0±0	0±0	0.01±0.01	0±0
24:0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
14:1n-5	0±0	0±0	0.01±0.01	0±0	0.03±0	0.01±0.01	0±0	0±0
16:1n-7	2.06±0.13	2.05±0.04	3.55±0.2	3.87±0.14	6.01±1.38	5.34±0.21	2.23±0.09	2.21±0.08
18:1n-7	2.4±0.14	2.49±0.02	2.68±0.14	2.93±0.08	3.45±0.45	3.38±0.08	2.83±0.02	2.83±0.08
18:1n-9	20.61±0.99	20.14±0.2	26.53±1.05	27.96±0.56	17.01±0.38	17.65±0.54	18.03±0.96	17.77±0.5
20:1n-9	1.54±0.37	1.72±0.01	0.65±0.01 ^b	0.73±0.02 ^a	1.4±0.12	1.61±0.04	1.54±0.09	1.64±0.05
22:1n-9	0.05±0	0.03±0.02	0.06±0.05	0±0	0.07±0	0.08±0	0.09±0.07	0.05±0
24:1n-9	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
18:2n-6	7.38±0.27	7.24±0.1	9.05±0.25	9.29±0.24	1.15±0.12	1.11±0.04	6.1±0.33	6±0.19
18:3n-6	0.53±0.09	0.5±0.01	0.43±0.07	0.31±0.15	0.02±0.02	0.01±0.01	0.08±0.01	0.08±0
20:2n-6	1.28±0.24	1.39±0.01	0.71±0.01	0.55±0.2	0.15±0.01	0.17±0	1.44±0.03	1.47±0.04
20:3n-6	2.05±0.3	2.1±0.02	1.6±0.1	1.86±0.04	0.23±0.06	0.22±0	1.19±0.03	1.29±0.04
20:4n-6	2.65±0.14	2.93±0.06	1.22±0.09	1.33±0.02	0.45±0.04 ^b	0.61±0.03 ^a	0.98±0.09	1.07±0.01
22:4n-6	0.28±0.03	0.3±0	0.08±0	0.05±0.03	0±0	0.03±0.02	0.15±0.02	0.15±0
22:5n-6	0.29±0.03	0.33±0	0.13±0	0.14±0.01	0.1±0.01 ^b	0.13±0 ^a	0.23±0.02	0.24±0.01
18:3n-3	1.12±0.04	1.09±0.03	1.52±0.15	1.53±0.05	0.41±0.05	0.4±0.02	1.1±0.06	1.05±0.04
18:4n-3	0.42±0.06	0.4±0.01	0.38±0.03	0.3±0.11	0.27±0.03	0.25±0.01	0.11±0	0.11±0.01
20:3n-3	0.16±0.02	0.15±0	0.13±0.07	0.22±0	0.06±0.03	0.09±0	0.18±0.09	0.25±0.01
20:4n-3	0.25±0.04	0.25±0	0.65±0.08	0.72±0.02	1.46±0.23	1.22±0.04	0.38±0.04	0.38±0.01
20:5n-3	2.92±0.12	3.03±0.04	4.33±0.03	4.51±0.12	8.39±0.13	8.26±0.22	2.43±0.16	2.4±0.09

22:5n-3	1.16±0.2	1.15±0.01	1.72±0.11	1.84±0.02	5.32±0.36	5.08±0.09	1.62±0.26	1.44±0.04
24:5n-3	0±0	0±0	0±0	0±0	0.02±0.01	0±0	0.04±0.01 ^a	0±0 ^b
24:6n-3	0.1±0.02	0.11±0	0.04±0	0.03±0.02	0.01±0.01	0±0	0.12±0	0.12±0
22:6n-3	12.8±0.35 ^b	13.89±0.14 ^a	15.88±0.34	17.13±0.39	17.15±0.62	18.24±0.42	16.4±0.73	17.37±0.53
Σ SFA ¹	14.07±0.64	14.78±0.13	16.28±0.39	17.42±0.31	16.88±0.55	17.07±0.29	12.58±0.08	13.1±0.4
Σ MUFA	26.71±1.6	26.54±0.23	33.59±1.4	35.62±0.77	27.95±1.64	28.18±0.8	24.8±1.07	24.63±0.72
Σ C18 n-6 PUFA	7.91±0.19	7.74±0.11	9.49±0.29	9.6±0.21	1.16±0.13	1.12±0.04	6.17±0.34	6.08±0.19
Σ n-6 HUFA	6.56±0.47	7.04±0.06	3.73±0.13	3.93±0.2	0.93±0.09	1.16±0.03	3.99±0.17	4.22±0.09
Σ C18 n-3 PUFA	1.54±0.04	1.49±0.04	1.91±0.16	1.84±0.09	0.68±0.08	0.65±0.03	1.22±0.06	1.16±0.04
Σ n-3 HUFA	17.39±0.16 ^b	18.57±0.18 ^a	22.75±0.43	24.46±0.52	32.41±0.52	32.9±0.76	21.17±0.37	21.96±0.67
n-3/n-6	1.31±0.06	1.36±0.01	1.87±0.05	1.95±0.06	16.15±1.81	14.75±0.09	2.21±0.12	2.24±0.01
n-3 HUFA/n-6 HUFA	2.68±0.19	2.64±0.04	6.11±0.28	6.26±0.45	35.46±3.79	28.42±0.39	5.33±0.25	5.2±0.05
Total fatty acids	74.19±2.91	76.17±0.64	87.75±2.58	92.87±1.65	80.02±1.79	81.07±1.95	69.94±1.3	71.15±2.11

For each condition, three samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row, for the same fish condition, with different superscript letters are significantly different.

¹See definition of abbreviations in Table 4.

²CoSMos, “Conservatoire du Saumon Mosan”.

b) Nutritional quality of the salted eggs

In order to evaluate which fish egg sample, used during the sensory test, has the best nutritional value for human consumption, we compared the composition of our four salted eggs. In addition, these data allowed us to evaluate, as in the first section of this work, the species impact, by comparing the three salted captive fish eggs, as well as the life style influence, by comparing the salted eggs from captive and semi-wild Atlantic salmons.

Table 16. Proximate composition (g/100 g) of salted eggs, from captive rainbow trouts or from captive Atlantic salmons or from Atlantic salmons, which spent two years at sea (CoSMos¹), or from captive sea trouts.

Proximate composition	Captive rainbow trout eggs	Captive Atlantic salmon eggs	Semi-wild Atlantic salmon eggs	Captive sea trout eggs
Dry matter	45.2 ± 0.3 ^a	46 ± 0.3 ^a	46.4 ± 0.2 ^a	43.3 ± 0.4 ^b
Crude ash	9.3 ± 0.3	10.1 ± 0.2	9.6 ± 0.1	11.2 ± 0.5
Crude lipids (DM ¹)	18.8 ± 0.9 ^{bc}	22.4 ± 0.2 ^a	21 ± 0.1 ^{ab}	17.9 ± 0.4 ^c
Crude protein (DM)	66.7 ± 0.6 ^a	62.5 ± 0.8 ^b	64.7 ± 0.4 ^{ab}	65.7 ± 0.7 ^a
Crude lipids (FM ¹)	8.5 ± 0.4 ^b	10.3 ± 0.1 ^a	9.8 ± 0.1 ^a	7.8 ± 0.2 ^b
Crude proteins(FM)	30.1 ± 0.1 ^a	28.7 ± 0.2 ^b	30 ± 0.1 ^a	28.4 ± 0.1 ^b

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row with no common superscript letter are significantly different.

¹DM, Dry Matter; FM, Fresh Matter; CoSMos, “Conservatoire du Saumon Mosan”.

We can observe, in Table 16, that the proximate composition of the salted eggs presents some differences related to the origin of the eggs, with the exception of the crude ash content, which was similar for all eggs. The dry matter content of the four salted eggs was similar, except for the sea trout eggs, in which a lower dry matter content was recorded. The Atlantic salmon eggs, both captive and semi-wild, had a higher crude lipid (FM) content, as compared with the rainbow trout and sea trout eggs.

Table 17. Fatty acid profile (mg/g of fresh matter) of salted eggs, from captive rainbow trouts or from captive Atlantic salmons or from Atlantic salmons, which spent two years at sea (CoSMos²), or from captive sea trouts.

Fatty acid	Captive rainbow trout eggs	Captive Atlantic salmon eggs	Semi-wild Atlantic salmon eggs	Captive sea trout eggs
12:0	0.07±0 ^a	0.06±0.01 ^a	0.02±0.01 ^b	0.04±0 ^{ab}
14:0	0.68±0.02 ^d	1.1±0.04 ^b	1.38±0.05 ^a	0.84±0.03 ^c
16:0	10.23±0.1 ^{bc}	11.65±0.24 ^a	10.97±0.24 ^{ab}	9.26±0.3 ^c
18:0	3.69±0.04 ^b	4.61±0.03 ^a	4.67±0.04 ^a	2.92±0.07 ^c
20:0	0.05±0.02	0±0	0.03±0.03	0.04±0.02
22:0	0.06±0.03	0±0	0±0	0±0
24:0	0±0	0±0	0±0	0±0
14:1n-5	0±0	0±0	0.01±0.01	0±0
16:1n-7	2.05±0.04 ^c	3.87±0.14 ^b	5.34±0.21 ^a	2.21±0.08 ^c
18:1n-7	2.49±0.02 ^c	2.93±0.08 ^b	3.38±0.08 ^a	2.83±0.08 ^b
18:1n-9	20.14±0.2 ^b	27.96±0.56 ^a	17.65±0.54 ^c	17.77±0.5 ^c
20:1n-9	1.72±0.01 ^a	0.73±0.02 ^b	1.61±0.04 ^a	1.64±0.05 ^a
22:1n-9	0.03±0.02	0±0	0.08±0	0.05±0
24:1n-9	0±0	0±0	0±0	0±0

18:2n-6	7.24±0.1 ^b	9.29±0.24 ^a	1.11±0.04 ^d	6±0.19 ^c
18:3n-6	0.5±0.01	0.31±0.15	0.01±0.01	0.08±0
20:2n-6	1.39±0.01	0.55±0.2	0.17±0	1.47±0.04
20:3n-6	2.1±0.02 ^a	1.86±0.04 ^b	0.22±0 ^d	1.29±0.04 ^c
20:4n-6	2.93±0.06	1.33±0.02	0.61±0.03	1.07±0.01
22:4n-6	0.3±0	0.05±0.03	0.03±0.02	0.15±0
22:5n-6	0.33±0 ^a	0.14±0.01 ^c	0.13±0 ^c	0.24±0.01 ^b
18:3n-3	1.09±0.03 ^b	1.53±0.05 ^a	0.4±0.02 ^c	1.05±0.04 ^b
18:4n-3	0.4±0.01	0.3±0.11	0.25±0.01	0.11±0.01
20:3n-3	0.15±0 ^c	0.22±0 ^b	0.09±0 ^d	0.25±0.01 ^a
20:4n-3	0.25±0	0.72±0.02	1.22±0.04	0.38±0.01
20:5n-3	3.03±0.04 ^c	4.51±0.12 ^b	8.26±0.22 ^a	2.4±0.09 ^d
22:5n-3	1.15±0.01 ^d	1.84±0.02 ^b	5.08±0.09 ^a	1.44±0.04 ^c
24:5n-3	0±0	0±0	0±0	0±0
24:6n-3	0.11±0	0.03±0.02	0±0	0.12±0
22:6n-3	13.89±0.14 ^b	17.13±0.39 ^a	18.24±0.42 ^a	17.37±0.53 ^a
Σ SFA ¹	14.78±0.13 ^b	17.42±0.31 ^a	17.07±0.29 ^a	13.1±0.4 ^c
Σ MUFA	26.54±0.23 ^{bc}	35.62±0.77 ^a	28.18±0.8 ^b	24.63±0.72 ^c
Σ C18 n-6 PUFA	7.74±0.11 ^b	9.6±0.21 ^a	1.12±0.04 ^d	6.08±0.19 ^c
Σ n-6 HUFA	7.04±0.06 ^a	3.93±0.2 ^b	1.16±0.03 ^c	4.22±0.09 ^b
Σ C18 n-3 PUFA	1.49±0.04 ^b	1.84±0.09 ^a	0.65±0.03 ^d	1.16±0.04 ^c
Σ n-3 HUFA	18.57±0.18 ^c	24.46±0.52 ^b	32.9±0.76 ^a	21.96±0.67 ^b
n-3/n-6	1.36±0.01	1.95±0.06	14.75±0.09	2.24±0.01
n-3 HUFA/n-6 HUFA	2.64±0.04	6.26±0.45	28.42±0.39	5.2±0.05
Total fatty acids	76.17±0.64 ^{bc}	92.87±1.65 ^a	81.07±1.95 ^b	71.15±2.11 ^c

For each condition, three samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row with no common superscript letter are significantly different.

¹See definition of abbreviations in Table 4.

²CoSMos, “Conservatoire du Saumon Mosan”.

Significantly higher levels of Σ SFA were recorded in the Atlantic salmon eggs, captive and semi-wild, mainly due to the higher 16:0 and 18:0 contents, as compared with the two other fish egg conditions (Table 17). Significantly higher Σ MUFA, Σ C18 n-6 PUFA and Σ C18 n-3 PUFA levels were observed in the captive Atlantic salmon eggs, as compared with the salted eggs of the three other conditions, due to the increased 18:1n-9, LA and ALA contents, respectively. In contrast, Σ MUFA, Σ C18 n-6 PUFA and Σ C18 n-3 PUFA levels were significantly lower in the semi-wild Atlantic salmon eggs, as compared with the three other egg conditions. Conversely, the Σ n-3 HUFA level was significantly higher in the semi-wild Atlantic salmon eggs, mostly due to the significantly higher contents in EPA and 22:5n-3, as compared with the other egg conditions. Finally, the Σ n-6 HUFA level was significantly higher in the captive rainbow trout eggs, as compared to the three other egg conditions.

Regarding the captive species, the salted Atlantic salmon eggs contained significantly higher levels of Σ SFA, Σ MUFA, Σ C18 n-6 PUFA and Σ C18 n-3 PUFA, compared with the rainbow trout and sea trout eggs. The Σ n-3 HUFA contents were higher in the Atlantic salmon and sea trout eggs, mostly due to the DHA content in both species, as compared with the rainbow trout eggs. In contrast, the Atlantic salmon eggs contained the lowest level of Σ n-6 HUFA, as compared with the sea trout eggs and the rainbow trout

eggs, the difference being particularly important in the latter case. Concerning the salted Atlantic salmon eggs, there was no impact of the life type on the Σ SFA (16:0 and 18:0) and DHA contents. Higher levels of Σ MUFA (18:1n-9), Σ C18 n-6 PUFA (LA), Σ n-6 HUFA and Σ C18 n-3 PUFA (ALA) were recorded in the captive fish, as compared with the semi-wild fish. In contrast, the Σ n-3 HUFA levels were higher in the semi-wild fish, mostly due to the EPA and 22:5n-3 contents, as compared with the captive fish.

c) Nutritional quality compared with the commercial eggs

A comparison between the salted eggs, produced in the present work, with similar eggs already available on the market has been performed to give a better insight into the nutritional quality of our salted eggs. To that end, the composition of the salted eggs from the Atlantic salmons, which spent two years at sea, has been compared with the commercial Pacific salmon eggs from four different brands (Carrefour, Gabriel, Kaviari and Petrossian). Similarly, the salted rainbow trout eggs have been compared with the commercial rainbow trout eggs of two different brands (Carrefour and Gabriel).

c.1. Salmon species

Regarding the dry matter and crude ash contents, no differences were observed within the four commercial Pacific salmon eggs, as seen in Table 18. In contrast, the crude lipid (DM and FM) and crude protein (DM and FM) contents of the four commercial Pacific salmon eggs were not similar.

By comparing the four commercial eggs with the salted semi-wild Atlantic salmon eggs, no significant differences in the dry matter and crude protein (DM) contents were observed. However, this was not the case for the crude ash content, which was significantly higher in the salted Atlantic salmon eggs, as compared with the commercial eggs. The crude lipid content (DM and FM) of the salted Atlantic salmon eggs was similar to the one of the "Kaviari" and "Petrossian" brands and lower, compared with the two other brands. The crude protein content (FM) of the Atlantic salmon eggs, was lower than the one of the "Carrefour" brand, but similar when compared to the other brands (Table 18).

In terms of fatty acid profiles, the data are provided in Table 19. Comparing the Pacific salmon egg brands with each other, higher levels of Σ n-6 HUFA and Σ C18 n-3 PUFA can be noticed in the "Carrefour", "Gabriel" and "Petrossian" brands, compared with the "Kaviari" brand. The same goes for the Σ n-3 HUFA, mainly due to the increase of EPA and DHA contents.

Overall, there was no significant difference in fatty acid profile and content between the salted semi-wild Atlantic salmon eggs and the four commercial Pacific salmon egg brands, except regarding the systematically lower 14:1n-5 and EPA contents and the systematically higher 20:1n-9 and 20:3 n-6 contents in the salted Atlantic salmon eggs, as compared with the commercial ones. Overall, the salted semi-wild Atlantic salmon eggs were closer to the Pacific salmon eggs of the "Kaviari" brand, regarding their fatty acid profiles.

Table 18. Proximate composition (g/100 g) of commercial Pacific salmon eggs, of four different brands: Carrefour, Gabriel, Kaviari and Petrossian, or of salted eggs from Atlantic salmon, which spent two years at sea (CoSMos¹).

Proximate composition	Pacific salmon eggs (Carrefour)	Pacific salmon eggs (Gabriel)	Pacific salmon eggs (Kaviari)	Pacific salmon eggs (Petrossian)	Salted semi-wild Atlantic salmon eggs
Dry matter	51.7±0.2	48±0.4	45.4±0.1	45.7±0.1	46.4±0.2
Crude ash	6.1±0.4 ^b	6 ±0.5 ^b	7.6±0.5 ^b	7.7±0.2 ^b	9.6±0.1 ^a
Crude lipids (DM ¹)	24.9±0.8 ^a	24.4±0.4 ^{ab}	21.7±0.5 ^c	22.6±0.2 ^{bc}	21±0.1 ^c
Crude proteins (DM)	63.4±0.8 ^b	64.7±0.5 ^{ab}	66.5±0.4 ^a	63.6±0.6 ^b	64.7±0.4 ^{ab}
Crude lipids (FM ¹)	12.9±0.3 ^a	11.7±0.2 ^b	9.8±0.3 ^c	10.3±0.1 ^c	9.8±0.1 ^c
Crude proteins (FM)	32.8±0.5 ^a	31.1±0.4 ^b	30.2±0.1 ^{bc}	29±0.2 ^c	30±0.1 ^{bc}

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row with no common superscript letter are significantly different.

¹DM, Dry Matter; FM, Fresh Matter; CoSMos, “Conservatoire du Saumon Mosan”.

Table 19. Fatty acid profile (mg/g of fresh matter) of commercial Pacific salmon eggs of four different brands: Carrefour, Gabriel, Kaviari and Petrossian, and of salted eggs from Atlantic salmon, which spent two years at sea (CoSMos²).

Fatty acid	Pacific salmon eggs (Carrefour)	Pacific salmon eggs (Gabriel)	Pacific salmon eggs (Kaviari)	Pacific salmon eggs (Petrossian)	Salted semi-wild Atlantic salmon eggs
12:0	0.11±0.01 ^a	0.14±0.01 ^a	0.04±0 ^b	0.06±0 ^b	0.02±0.01 ^b
14:0	3.76±0.28	4.08±0.17	1.37±0.1	2.1±0.01	1.38±0.05
16:0	12.81±0.62 ^a	11.13±0.26 ^{ab}	10.64±0.46 ^b	10.02±0.04 ^b	10.97±0.24 ^b
18:0	5.17±0.18	4.66±0.12	4.67±0.17	4.11±0.03	4.67±0.04
20:0	0.13±0.04	0±0	0±0	0.03±0.03	0.03±0.03
22:0	0±0	0±0	0±0	0±0	0±0
24:0	0±0	0±0	0±0	0±0	0±0
14:1n-5	0.05±0 ^a	0.05±0 ^a	0.04±0 ^a	0.06±0 ^a	0.01±0.01 ^b
16:1n-7	5.56±0.4	4.33±0.45	5.4±0.25	4.35±0.06	5.34±0.21
18:1n-7	3.66±0.22 ^a	2.5±0.24 ^c	3.51±0.16 ^{ab}	2.81±0.03 ^{bc}	3.38±0.08 ^{ab}
18:1n-9	19.83±1.17 ^a	17.2±1.02 ^{abc}	13.97±0.51 ^c	14.8±0.18 ^{bc}	17.65±0.54 ^{ab}
20:1n-9	0.75±0.04 ^c	0.95±0.03 ^b	0.87±0.04 ^{bc}	0.82±0.01 ^{bc}	1.61±0.04 ^a
22:1n-9	0.17±0.02	0.23±0.03	0.03±0.03	0.07±0.07	0.08±0

24:1n-9	0±0	0±0	0±0	0±0	0±0
18:2n-6	1.35±0.1	1.28±0.04	0.75±0.04	1.26±0.01	1.11±0.04
18:3n-6	0±0	0±0	0±0	0±0	0.01±0.01
20:2n-6	0.36±0.02	0.35±0.01	0.16±0.01	0.33±0	0.17±0
20:3n-6	0.16±0.01 ^b	0.14±0.01 ^{bc}	0.12±0.01 ^c	0.16±0 ^b	0.22±0 ^a
20:4n-6	1.31±0.16 ^a	1.02±0.05 ^a	1.13±0.08 ^a	1.05±0.05 ^a	0.61±0.03 ^b
22:4n-6	0.08±0.04	0.08±0.01	0.1±0	0.08±0	0.03±0.02
22:5n-6	0.41±0.03	0.39±0.06	0.15±0.01	0.26±0	0.13±0
18:3n-3	1.33±0.11 ^a	1.25±0.09 ^a	0.5±0.03 ^b	1.06±0.02 ^a	0.4±0.02 ^b
18:4n-3	0.86±0.08 ^a	0.93±0.05 ^a	0.32±0.03 ^b	0.99±0.01 ^a	0.25±0.01 ^b
20:3n-3	0.3±0.03	0.22±0	0.1±0.05	0.15±0.08	0.09±0
20:4n-3	1.93±0.15	1.93±0.1	0.57±0.03	1.91±0.02	1.22±0.04
20:5n-3	17.34±1.15 ^a	14.38±0.56 ^{ab}	11.75±0.68 ^b	16.43±0.08 ^a	8.26±0.22 ^c
22:5n-3	6.8±0.45	5.2±0.43	6.69±0.25	5.72±0.01	5.08±0.09
24:5n-3	0±0	0±0	0.04±0	0±0	0±0
24:6n-3	0±0	0±0	0±0	0±0	0±0
22:6n-3	25.38±1.15 ^a	24.4±1.06 ^a	20.21±0.81 ^{bc}	22.73±0.07 ^{ab}	18.24±0.42 ^c
Σ SFA ¹	21.99±1.05	20±0.54	16.72±0.73	16.31±0.06	17.07±0.29
Σ MUFA	30.24±1.77 ^a	25.46±0.79 ^{abc}	24.06±0.95 ^{bc}	23.05±0.14 ^c	28.18±0.8 ^{ab}
Σ C18 n-6 PUFA	1.35±0.1	1.28±0.04	0.75±0.04	1.26±0.01	1.12±0.04
Σ n-6 HUFA	2.3±0.23 ^a	1.97±0.12 ^{ab}	1.64±0.1 ^{bc}	1.87±0.05 ^{ab}	1.16±0.03 ^c
Σ C18 n-3 PUFA	2.2±0.19 ^a	2.18±0.14 ^a	0.82±0.06 ^b	2.05±0.02 ^a	0.65±0.03 ^b
Σ n-3 HUFA	51.76±2.84 ^a	46.13±1.26 ^{ab}	39.36±1.79 ^{bc}	46.94±0.11 ^{ab}	32.9±0.76 ^c
n-3/n-6	14.85±0.44 ^c	14.87±0.45 ^c	16.83±0.27 ^a	15.67±0.33 ^{ab}	14.75±0.09 ^c
n-3 HUFA/n-6 HUFA	22.69±1.07 ^b	23.53±1.16 ^b	23.98±0.44 ^b	25.09±0.73 ^{ab}	28.42±0.39 ^a
Total fatty acids	109.83±6.09 ^a	97.03±2.78 ^{ab}	83.36±3.67 ^b	91.49±0.29 ^b	81.07±1.95 ^b

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row with no common superscript letter are significantly different.

¹See definition of abbreviations in Table 4.

² CoSMos, “Conservatoire du Saumon Mosan

c.2. Rainbow trout species

Table 20. Proximate composition (g/100 g) of commercial rainbow trout eggs of two different brands: Carrefour and Gabriel, and of salted eggs from captive rainbow trouts.

Proximate composition	Rainbow trout eggs (Carrefour)	Rainbow trout eggs (Gabriel)	Salted captive rainbow trout eggs
Dry matter	36.1 ± 0.4	43 ± 1.1	45.2 ± 0.3
Crude ash	6.9 ± 1	9.2 ± 0.5	9.3 ± 0.3
Crude lipids (DM ¹)	24.2 ± 0.9 ^a	21.4 ± 0.7 ^{ab}	18.8 ± 0.9 ^b
Crude proteins (DM)	62.3 ± 0.2 ^b	64.8 ± 0.4 ^a	66.7 ± 0.6 ^a
Crude lipids (FM ¹)	8.7 ± 0.4	9.2 ± 0.4	8.5 ± 0.4
Crude proteins (FM)	22.5 ± 0.2	27.9 ± 0.8	30.1 ± 0.1

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row with no common superscript letter are significantly different.

¹DM, Dry Matter; FM, Fresh Matter.

The two commercial eggs were similar in their proximate composition, except for their crude lipid (DM) content, higher in the "Carrefour" brand, as compared with the "Gabriel" brand (Table 20). Overall, the proximate composition of the salted eggs, prepared in house, was similar to that of the commercial rainbow trout eggs of the "Gabriel" brand. It was also very similar to that of the rainbow trout eggs of the "Carrefour" brand, except for the crude lipid and crude protein contents in FM.

Regarding the fatty acid profiles, the comparison of the two commercial eggs indicated higher levels of Σ SFA (16:0), Σ n-6 HUFA and Σ n-3 HUFA (DHA) were recorded in the "Gabriel" brand, compared to the "Carrefour" brand, whereas increased level of Σ MUFA (18:1n-9) was recorded in the "Carrefour" brand, compared to the "Gabriel" one (Table 21). The salted rainbow trout eggs, prepared in the present work, had a fatty acid composition quite similar to those of the commercial rainbow trout eggs, with the exception of significantly higher AA and Σ n-6 HUFA levels, and significantly lower Σ n-3 HUFA level, mainly due to lower EPA content and lower ALA or DHA content, in the salted rainbow trout eggs, compared with the two commercial eggs.

Table 21. Fatty acid profile (mg/g of fresh matter) of commercial rainbow trout eggs of two different brands: Carrefour and Gabriel, and of salted eggs from captive rainbow trouts.

Fatty acid	Rainbow trout eggs (Carrefour)	Rainbow trout eggs (Gabriel)	Salted captive rainbow trout eggs
12:0	0.03±0 ^c	0.05±0 ^b	0.07±0 ^a
14:0	1.13±0.05 ^a	1.15±0.03 ^a	0.68±0.02 ^b
16:0	8.89±0.24 ^b	10.91±0.43 ^a	10.23±0.1 ^a
18:0	3.03±0.06 ^c	4.52±0.06 ^a	3.69±0.04 ^b
20:0	0.1±0.01	0.09±0.01	0.05±0.02
22:0	0±0	0±0	0.06±0.03
24:0	0±0	0±0	0±0
14:1n-5	0±0	0±0	0±0
16:1n-7	2.09±0.09 ^b	2.51±0.12 ^a	2.05±0.04 ^b
18:1n-7	2.56±0.08	2.31±0.15	2.49±0.02
18:1n-9	20.94±0.86 ^a	15.14±0.42 ^b	20.14±0.2 ^a

20:1n-9	1.87±0.04	1.5±0.18	1.72±0.01
22:1n-9	0.16±0.1	0.02±0.01	0.03±0.02
24:1n-9	0.03±0.01	0±0	0±0
18:2n-6	8.17±0.41	9.74±2.04	7.24±0.1
18:3n-6	0.1±0	0.16±0.06	0.5±0.01
20:2n-6	1.58±0.04 ^b	1.95±0.04 ^a	1.39±0.01 ^c
20:3n-6	0.61±0.02	0.92±0.11	2.1±0.02
20:4n-6	0.77±0.06 ^c	1.33±0.12 ^b	2.93±0.06 ^a
22:4n-6	0.08±0 ^c	0.16±0.01 ^b	0.3±0 ^a
22:5n-6	0.18±0	0.23±0.11	0.33±0
18:3n-3	2.68±0.14 ^a	1.67±0.2 ^b	1.09±0.03 ^b
18:4n-3	0.31±0.02	0.29±0.09	0.4±0.01
20:3n-3	0.32±0.16	0.38±0.05	0.15±0
20:4n-3	0.56±0.02 ^a	0.39±0.03 ^b	0.25±0 ^c
20:5n-3	4.7±0.14 ^a	4.42±0.26 ^a	3.03±0.04 ^b
22:5n-3	1.71±0.05 ^a	1.68±0.03 ^a	1.15±0.01 ^b
24:5n-3	0.07±0	0.08±0	0±0
24:6n-3	0.12±0	0.12±0.01	0.11±0
22:6n-3	15.65±0.34 ^b	18.86±0.6 ^a	13.89±0.14 ^b
Σ SFA ¹	13.18±0.34 ^c	16.72±0.42 ^a	14.78±0.13 ^b
Σ MUFA	27.67±0.99 ^a	21.56±0.91 ^b	26.54±0.23 ^a
Σ C18 n-6 PUFA	8.27±0.41	9.9±2.1	7.74±0.11
Σ n-6 HUFA	3.22±0.12 ^c	4.59±0.16 ^b	7.04±0.06 ^a
Σ C18 n-3 PUFA	2.99±0.16	1.95±0.29	1.49±0.04
Σ n-3 HUFA	23.13±0.68 ^b	25.94±0.36 ^a	18.57±0.18 ^c
n-3/n-6	2.28±0.03	2.01±0.28	1.36±0.01
n-3 HUFA/n-6 HUFA	7.18±0.08	5.67±0.26	2.64±0.04
Total fatty acids	78.46±2.69	80.67±1.74	76.17±0.64

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row with different superscript letters are significantly different.

¹See definition of abbreviations in Table 4.

d) Fish feed impact

In order to study if there is a possible influence of the artificial feed given to the captive fish on their egg composition, we observed the composition trends between the artificial fish feeds, used to feed the captive Atlantic salmons, or the captive sea trouts or the captive rainbow trouts, and the respective eggs of these three fish species that received these artificial feeds.

We can observe in Tables 22 and 23, that there was no specific link between the feed and the fish eggs, in terms of proximate composition and fatty acid profile for the three fish species under investigation.

Table 22. Proximate composition (g/100 g) of artificial feeds, used to feed captive sea trouts or captive Atlantic salmons or captive rainbow trouts, as well as of the eggs produced by these animals.

Proximate composition	Feed			Eggs		
	Sea trout	Atlantic salmon	Rainbow trout	Sea trout	Atlantic salmon	Rainbow trout
Dry matter	90.1±0.6 ^b	93.7±0.3 ^a	91.6±0.4 ^b	38.5±0.4	37.8±0.4	38.9±0.8
Crude ash	8.2±0.3 ^b	9.6±0.3 ^a	7±0.2 ^b	4.5±0.7	5.7±1.4	4.8±1.1
Crude lipids (DM ¹)	15.8±0.9	19.1±0.3	19.2±0.1	21.5±0.7	24.4±0.9	22.9±0.3
Crude proteins(DM)	52.2±0.3 ^a	48.3±0.1 ^b	45±0.4 ^c	71±1.1	67.6±0.5	70.9±0.9
Crude lipids (FM ¹)	14.2±0.7 ^b	17.9±0.3 ^a	17.6±0.2 ^a	8.3±0.3	9.2±0.4	8.9±0.3
Crude proteins(FM)	47.1±0.3 ^a	45.2±0 ^b	41.2±0.2 ^c	27.3±0.6 ^a	25.5±0.1 ^b	27.6±0.3 ^a

For each condition, three samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row, and for one sample type (feed or egg), bearing different superscript letters are significantly different.

¹DM, Dry Matter; FM, Fresh Matter.

Table 23. Fatty acid profile (mg/g of fresh matter) of artificial feeds, used to feed captive sea trouts or captive Atlantic salmons or captive rainbow trouts, as well as of the eggs produced by these animals.

Fatty acid	Feed			Eggs		
	Sea trout	Atlantic salmon	Rainbow trout	Sea trout	Atlantic salmon	Rainbow trout
12:0	0.56±0.01	0.14±0	0.7±0.02	0.04±0	0.04±0	0.05±0.01
14:0	3.22±0.02 ^b	3.87±0.03 ^a	3±0.02 ^c	0.82±0.02 ^{ab}	1.02±0.06 ^a	0.67±0.08 ^b
16:0	17.57±0.17 ^c	18.47±0.14 ^b	24.53±0.12 ^a	8.84±0.14 ^b	10.92±0.28 ^a	9.76±0.43 ^{ab}
18:0	4.04±0.02 ^b	4.25±0.04 ^b	7.54±0.08 ^a	2.85±0.1 ^c	4.29±0.08 ^a	3.48±0.12 ^b
20:0	0.37±0 ^b	0.42±0.02 ^b	0.78±0.01 ^a	0.02±0.02	0±0	0.05±0.03
22:0	0.23±0.02 ^b	0.2±0.01 ^b	1.3±0.03 ^a	0.01±0.01	0±0	0.04±0.02
24:0	0±0	0.11±0.08	0.07±0.07	0±0	0±0	0±0
14:1n-5	0.1±0.01 ^b	0.08±0.01 ^b	0.15±0 ^a	0±0	0.01±0.01	0±0
16:1n-7	4.13±0.03 ^c	6.54±0.07 ^a	5.51±0.04 ^b	2.23±0.09 ^b	3.55±0.2 ^a	2.06±0.13 ^b
18:1n-7	3.43±0.02 ^c	4.75±0.03 ^a	4.04±0.03 ^b	2.83±0.02	2.68±0.14	2.4±0.14
18:1n-9	41.56±0.29 ^b	42.55±0.41 ^b	61.73±0.15 ^a	18.03±0.96 ^b	26.53±1.05 ^a	20.61±0.99 ^b
20:1n-9	3.4±0.03 ^b	5.5±0.09 ^a	1.33±0.03 ^c	1.54±0.09 ^a	0.65±0.01 ^b	1.54±0.37 ^a
22:1n-9	0.47±0.03 ^a	0.53±0.04 ^a	0.28±0.01 ^b	0.09±0.07	0.06±0.05	0.05±0

24:1n-9	0.44±0.02 ^a	0.42±0.02 ^a	0.24±0.01 ^b	0±0	0±0	0±0
18:2n-6	21.14±0.26 ^b	20.83±0.33 ^b	29.77±0.2 ^a	6.1±0.33 ^c	9.05±0.25 ^a	7.38±0.27 ^b
18:3n-6	0.13±0 ^b	0.16±0 ^a	0.17±0.01 ^a	0.08±0.01 ^b	0.43±0.07 ^a	0.53±0.09 ^a
20:2n-6	0.73±0	0.52±0.01	0.22±0	1.44±0.03	0.71±0.01	1.28±0.24
20:3n-6	0.21±0 ^a	0.18±0 ^b	0.16±0.01 ^c	1.19±0.03	1.6±0.1	2.05±0.3
20:4n-6	0.64±0.01 ^b	0.77±0.01 ^a	0.81±0.03 ^a	0.98±0.09 ^b	1.22±0.09 ^b	2.65±0.14 ^a
22:4n-6	0.14±0	0.05±0.05	0.18±0	0.15±0.02 ^b	0.08±0 ^b	0.28±0.03 ^a
22:5n-6	0.19±0 ^b	0.26±0 ^a	0.14±0.01 ^c	0.23±0.02 ^a	0.13±0 ^b	0.29±0.03 ^a
18:3n-3	5.14±0.05 ^b	5.01±0.11 ^b	7.66±0.11 ^a	1.1±0.06 ^b	1.52±0.15 ^a	1.12±0.04 ^{ab}
18:4n-3	0.96±0.01 ^b	1.58±0 ^a	0.64±0.01 ^c	0.11±0	0.38±0.03	0.42±0.06
20:3n-3	0.38±0.01	0.26±0	0±0	0.18±0.09	0.13±0.07	0.16±0.02
20:4n-3	0.5±0.01 ^a	0.51±0.01 ^a	0.17±0.01 ^b	0.38±0.04 ^b	0.65±0.08 ^a	0.25±0.04 ^b
20:5n-3	4.34±0.04 ^c	7.8±0.08 ^a	4.78±0.07 ^b	2.43±0.16 ^b	4.33±0.03 ^a	2.92±0.12 ^b
22:5n-3	1.1±0.01 ^b	1.39±0.02 ^a	0.68±0.01 ^c	1.62±0.26	1.72±0.11	1.16±0.2
24:5n-3	0.11±0 ^b	0.15±0.01 ^a	0±0 ^c	0.04±0.01	0±0	0±0
24:6n-3	0.01±0.01	0.04±0.02	0±0	0.12±0 ^a	0.04±0 ^b	0.1±0.02 ^a
22:6n-3	6.67±0.08 ^b	9.41±0.07 ^a	2.97±0.03 ^c	16.4±0.73 ^a	15.88±0.34 ^a	12.8±0.35 ^b
Σ SFA ¹	25.99±0.18 ^c	27.46±0.28 ^b	37.91±0.23 ^a	12.58±0.08	16.28±0.39	14.07±0.64
Σ MUFA	53.7±0.33 ^c	60.72±0.61 ^b	73.72±0.38 ^a	24.8±1.07 ^b	33.59±1.4 ^a	26.71±1.6 ^b
Σ C18 n-6 PUFA	21.27±0.27 ^b	20.99±0.33 ^b	29.94±0.2 ^a	6.17±0.34 ^c	9.49±0.29 ^a	7.91±0.19 ^b
Σ n-6 HUFA	1.92±0.02 ^a	1.77±0.04 ^b	1.51±0.04 ^c	3.99±0.17 ^b	3.73±0.13 ^b	6.56±0.47 ^a
Σ C18 n-3 PUFA	6.1±0.05 ^c	6.59±0.11 ^b	8.3±0.11 ^a	1.22±0.06 ^b	1.91±0.16 ^a	1.54±0.04 ^{ab}
Σ n-3 HUFA	13.1±0.12 ^b	19.57±0.19 ^a	8.6±0.07 ^c	21.17±0.37 ^b	22.75±0.43 ^a	17.39±0.16 ^c
n-3/n-6	0.83±0 ^b	1.15±0.01 ^a	0.54±0.01 ^c	2.21±0.12 ^a	1.87±0.05 ^a	1.31±0.06 ^b
n-3 HUFA/n-6 HUFA	6.82±0.01	11.08±0.21	5.71±0.12	5.33±0.25 ^a	6.11±0.28 ^a	2.68±0.19 ^b
Total fatty acids	122.08±0.94 ^c	137.1±1.44 ^b	159.98±0.79 ^a	69.94±1.3 ^b	87.75±2.58 ^a	74.19±2.91 ^b

For each condition, three independent samples were analysed and results are expressed as mean values ± SEM (p<0.05). Results within a row, and for one sample type (feed or egg), bearing no common superscript letter are significantly different.

¹See definition of abbreviations in Table 4.

2.2. Sensory test

The sensory test took place the 12 December 2017 with 58 participants. These participants followed a visual and tasting test with four salted egg samples, i.e. from (1) captive Atlantic salmons, (2) Atlantic salmons, which spent two years at sea, (3) captive sea trouts and (4) captive rainbow trouts.

a) Participant population

In order to ensure representative results, a diverse population of participants was needed. Regarding sex we roughly obtained the same ratio of men and women, 55% and 45% respectively. Concerning the average age, the participant population was quite young, with more than half of the participants between 19 and 29 years old (Figure 7).

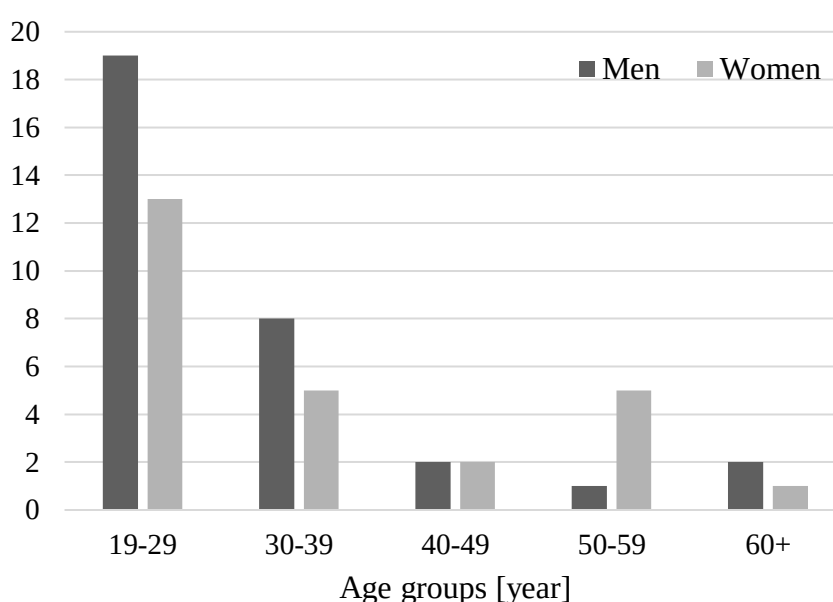


Figure 7. Age distribution of the men and women who participated to the sensory test on 12 December 2017.

In total, almost half of the participants were workers (55%) and the other half were students (45%). The majority of the students who participated were from the Faculty of Bioscience engineering (92% of the total student number), who are familiar with nutrition issues. However, these students were by no means linked to the laboratory or to the project.

A large proportion of the participants had already tasted fish eggs. Indeed, 91% had already experienced the tasting of lump, salmon and trout eggs, and for some sturgeon eggs, and this mostly during festive days or in restaurants, on toasts as starters. Only 9%, which represent 6 participants on 58, had never tasted fish eggs.

b) Visual test

The participants had first to follow a visual test and evaluate eggs on their stickiness, colour and brightness. They had to score each feature for each egg sample on a scale of one (less appreciated) to five (more appreciated). The mean and SEM of the results of the

participant appreciations obtained for each feature per sample are presented in Table 24 and Figure 8.

Table 24. Mean $\pm$ SEM of the scores attributed by each participant, on a scale of one (less appreciated) to five (more appreciated), to four different egg samples, in terms of the visual aspect of the sensory test.

Feature	Captive Atlantic salmon eggs	Semi-wild Atlantic salmon eggs	Captive sea trout eggs	Captive Rainbow trout eggs
Global appreciation	3.3 $\pm$ 0.1 ^b	3.6 $\pm$ 0.1 ^b	4.2 $\pm$ 0.1 ^a	3.5 $\pm$ 0.1 ^b
Colour	3.1 $\pm$ 0.1 ^c	3.7 $\pm$ 0.2 ^b	4.2 $\pm$ 0.1 ^a	3.5 $\pm$ 0.2 ^{bc}
Brightness	3.6 $\pm$ 0.1 ^b	3.5 $\pm$ 0.1 ^b	4.2 $\pm$ 0.1 ^a	3.7 $\pm$ 0.1 ^b
Stickiness	3.5 $\pm$ 0.1	3.5 $\pm$ 0.1	3.7 $\pm$ 0.1	3.5 $\pm$ 0.1

Results are expressed as mean values $\pm$ SEM ($p < 0.05$, $n = 53$). Results within a row with different superscript letters are significantly different.

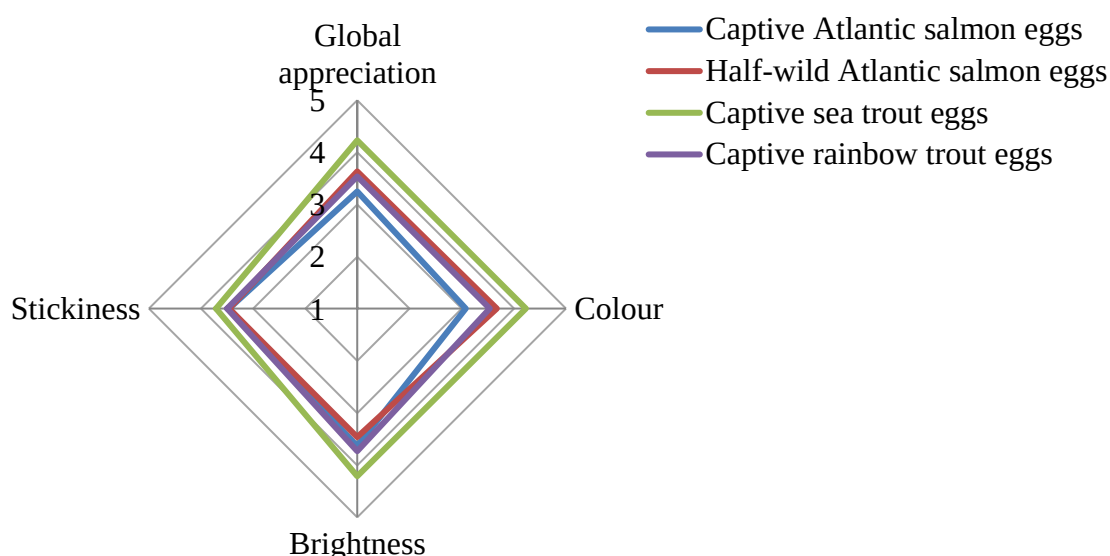


Figure 8. Means of the scores attributed by each participant, on a scale of one (less appreciated) to five (more appreciated), to four different egg samples, in terms of the visual aspect of the sensory test.

We can observe that overall, all eggs had a good visual appreciation, but the captive sea trout eggs were significantly more appreciated regarding all the features, as compared with the other egg samples, except regarding the stickiness (Table 24). The salted eggs from the captive Atlantic salmons seemed to be less appreciated mostly because of their colour. Furthermore, we can observe that the colour was the most relevant aspect allowing to differentiate the four egg samples (Figure 8).

The participants were asked to give their order of preference of the four egg samples, from less appreciated to most appreciated (for example: "...<Sample A < Sample B<...", with equality accepted, "...Sample A = Sample B.."), for the global visual aspect. A number, from one to four, has then been associated to the samples following the ranking attributed to the eggs (from one for the less appreciated, until four for the most appreciated). The results are presented in Table 25.

Table 25. Mean $\pm$ SEM of the preference order attributed by each participant to four different egg samples, in terms the visual aspect of the sensory test.

Preference evaluation	Captive Atlantic salmon eggs	Semi-wild Atlantic salmon eggs	Captive sea trout eggs	Captive Rainbow trout eggs
Order of Preference	1.71 $\pm$ 0.11 ^b	2.15 $\pm$ 0.14 ^b	2.86 $\pm$ 0.12 ^a	2.09 $\pm$ 0.14 ^b

Results are expressed as mean values $\pm$ SEM ($p < 0.05$, $n = 58$). Results with different superscript letters are significantly different.

Overall, the captive sea trout eggs were visually preferred, as compared with the three other egg conditions.

c) Taste test

After having evaluated the eggs on their visual aspect, the participants performed a taste test in order to assess the taste, aftertaste, crunchiness and saltiness of the four egg samples. As for the visual test, they had to score each feature for each egg sample on a scale from one (less appreciated) to five (most appreciated). The mean and SEM of the results of the participant appreciations obtained for each feature per sample are presented in Table 26 and Figure 9.

Table 26. Mean $\pm$ SEM of the scores attributed by each participant, on a scale of one (less appreciated) to five (more appreciated), to four different egg samples, in terms of the tasting aspect of the sensory test.

Feature	Captive Atlantic salmon eggs	Semi-wild Atlantic salmon eggs	Captive sea trout eggs	Captive rainbow trout eggs
Global appreciation	3.3 $\pm$ 0.1	3.4 $\pm$ 0.2	3.5 $\pm$ 0.2	3.1 $\pm$ 0.2
Taste	3.5 $\pm$ 0.1	3.4 $\pm$ 0.2	3.7 $\pm$ 0.2	3.2 $\pm$ 0.2
Crunchiness	3.6 $\pm$ 0.2	3.4 $\pm$ 0.2	3.8 $\pm$ 0.2	3.5 $\pm$ 0.2
Saltiness	2.9 $\pm$ 0.2 ^b	3.5 $\pm$ 0.2 ^a	3 $\pm$ 0.2 ^{ab}	3.4 $\pm$ 0.2 ^{ab}
Aftertaste	3.6 $\pm$ 0.1 ^{ab}	3.7 $\pm$ 0.2 ^a	3.7 $\pm$ 0.2 ^{ab}	3.1 $\pm$ 0.2 ^b

Results are expressed as mean values $\pm$ SEM ($p < 0.05$, $n = 46$). Results within a row with no common superscript letter are significantly different.

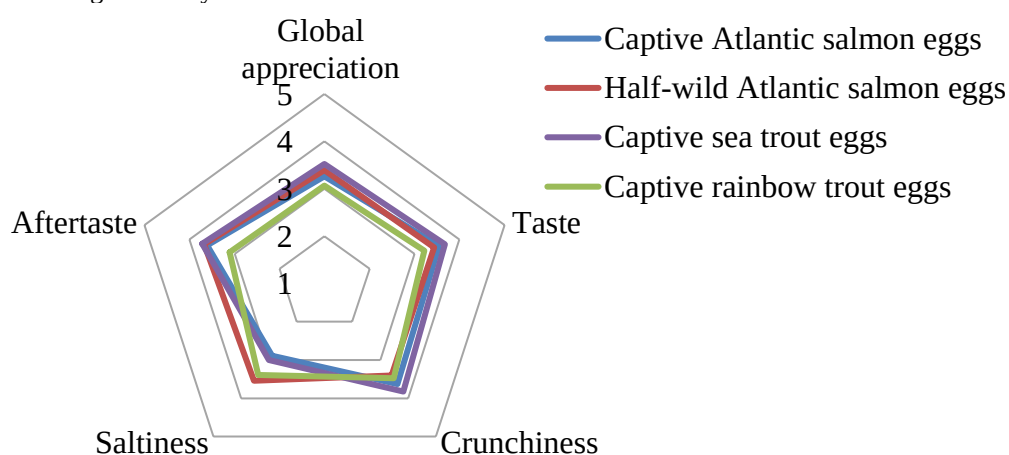


Figure 9. Means of the scores attributed by each participant, on a scale of one (less appreciated) to five (more appreciated), to four different egg samples, in terms of the tasting aspect of the sensory test.

As visualized on Figure 9, that overall, all egg samples showed a good appreciation. Regarding the features separately, the aftertaste and saltiness seemed to be the more variable features between the four egg samples. The captive rainbow trout eggs seemed to be less appreciated, mostly due to their aftertaste, the difference being significant when compared to the semi-wild Atlantic salmon eggs. The semi-wild Atlantic salmon eggs were more appreciated, regarding their saltiness, compared with the captive Atlantic salmon eggs (Table 26).

As for the visual test, the participants were asked to give their order of preference for the tasting aspect of the four egg samples. A number from one to four has then been associated following the ranking attributed to the samples. Results of the Mean and SEM of the preference order attributed by the participants for the four egg samples are presented in Table 27.

Table 27. Mean $\pm$ SEM of the preference order attributed by each participant to four different egg samples, in terms of the tasting aspect of the sensory test.

Preference evaluation	Captive Atlantic salmon eggs	Semi-wild Atlantic salmon eggs	Captive sea trout eggs	Captive Rainbow trout eggs
Order of preference	2.15 $\pm$ 0.12	2.33 $\pm$ 0.14	2.46 $\pm$ 0.13	2.02 $\pm$ 0.15

Results are expressed as mean values $\pm$ SEM ($p < 0.05$, $n = 58$).

No significant difference between the preference attributed by the participants to the four egg samples was observed, regarding the tasting aspect of the sensory test (Table 27).

d) Consumption criteria

After the tasting, 82% of the participants said they did like the products and 18% not. The participants were also asked if they would buy such a product. Fortunately, 81% answered yes and 19% no, because they did not like it, mostly due to the too high saltiness of the eggs.

The participants had furthermore, to score, from one to five, the importance, in their opinion, on some consumption criteria. The results are presented in Figure 10. This indicated that the taste was an important criterion for most participants, followed by the price, the sustainability, the local production, and the visual aspect. At last we had the high nutritional quality and the n-3 PUFA content of the product (Figure 10). However, according to the participants, if one criterion had to be retained as publicity on the product, the local production aspect and the tastiness of the product were more important, followed by the higher nutritional quality, the sustainability and the omega-3 contents. At last, we had the price and finally the visual aspect of the product.

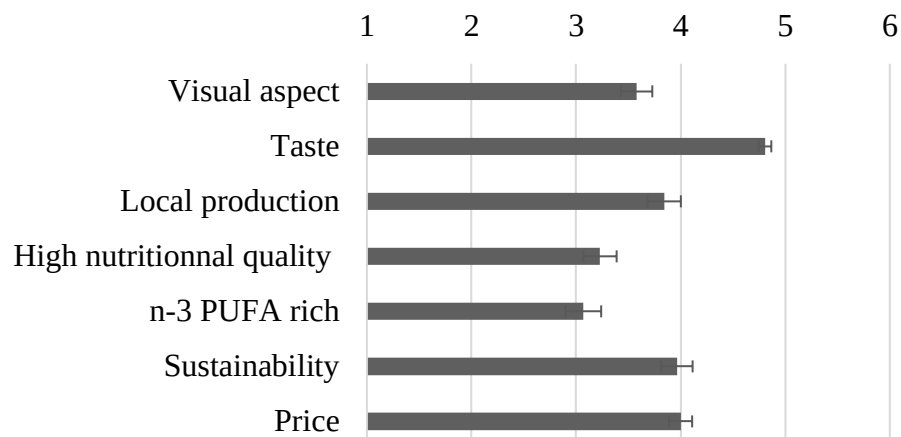


Figure 10. Mean $\pm$ SEM of the scoring of consumption criteria by the participants of the sensory test.

e) Age of the participant influence

Considering the eating habits, generally different between young (19-29 years old) and adult and elderly people (30-58+ years old), the results of the global appreciation for the visual aspect and the tasting aspect of the sensory test were observed separately for these two groups. However, no important difference in the appreciation trends were seen between the two different age groups, compared with the situation where these two groups are gathered, except for a generally slightly higher score attributed by the young participants in the tasting appreciation of the four fish egg products, as compared with the second group.

3. Discussion

The aim of this section of the work was to evaluate the possible development of a Walloon sustainable Atlantic salmon egg production for human consumption. To reach this objective, chemical analyses and more precisely, proximate composition and fatty acid profile of eggs were determined, aiming at evaluating their quality as food item. Second, a sensory test with four salted salmonid egg samples has been performed, to assess the appreciation of the products by potential consumers.

3.1. Chemical analyses

The n-6 and n-3 PUFA play major roles in the growth, development and physiological functions of the human body and are associated with many health benefits. The LC-PUFA are notably involved in the control of the inflammatory response, through the production of eicosanoids and other intercellular messengers. Among the n-3 LC-PUFA, DHA should be pointed out for its essential role in foetal development and optimal functioning of the brain and the retina (Swanson et al., 2012). Humans have an absolute dietary requirement in these two fatty acid families, because the body is unable to synthesise them *de novo* and also unable to bioprocess pre-existing n-3 PUFA into DHA with a sufficient efficacy (Turchini et al., 2009). However, the dietary intakes of n-3 LC-PUFA are, currently, insufficient for most people (De Ridder et al., 2016). Indeed, the minimal physiological requirement in DHA for a human adult has been estimated in France to be of 250 mg/day (AFSSA, 2010). Yet, in 2014, the Belgian population had an average intake of EPA + DHA of 190 mg per day (De Ridder et al., 2016). An increase in the consumption of food items rich in n-3 LC-PUFA is thus required. Atlantic salmon eggs might contain high levels of n-3 LC-PUFA and could then be used in human diet. Moreover, this could represent an additional way to valorise the Atlantic salmon, as these animals are starting again to be present in our Belgian rivers. This could also improve the commercial sustainability of the Walloon Atlantic salmon production, through a diversification of its production.

Firstly, as the fish eggs already commercially available are salted, we applied the same treatment on four raw salmonid eggs. It was then important to test the impact of the salting process on our salmonid raw egg samples. The data, presented in Table 15, revealed that there was no negative impact of the salting process, performed on the raw eggs, on their fatty acid profile. Higher dry matter, crude ash and crude protein (FM) contents were recorded in the proximate composition of the salted eggs, as compared with their respective raw eggs (Table 14). The same trend was observed with the crude lipid (FM) content of the salted eggs. These higher contents observed in the salted eggs are due to the removal of water of the raw eggs, as a consequence of the salting process (Bledsoe et al., 2003). Therefore, the moisture of the salted eggs was lower, compared with the raw eggs, and induced higher values for the crude protein (FM) and crude lipid (FM) contents of the salted eggs, compared with the raw counterparts.

To evaluate which salted eggs might have the best nutritional quality for human consumption, the composition of the four salted eggs was compared (Tables 16 and 17).

The data showed that the semi-wild Atlantic salmon eggs had the best nutritional quality regarding their n-3 LC-PUFA content, which was significantly higher, compared with the three other egg conditions, mostly due to higher EPA and 22:5 n-3 contents. As discussed in the first section of the present work, this higher Σ n-3 HUFA egg content could be explained by the migration habit of the semi-wild Atlantic salmon to their feeding grounds, to cold water, during their life cycle, as well as by their diet at sea, mainly based on a trophic chain starting with micro-algae and marine invertebrates, which contain high levels of EPA and DHA (Pickova et al., 1999; Monroig et al., 2013). Thus, a fish migrating to sea during its life cycle could contain higher levels of n-3 LC-PUFA, as compared with a captive fish, and this could then be reflected in the fish egg composition. Surprisingly, the DHA content was at the same level in the Atlantic salmon eggs, either captive or semi-wild, and in the sea trout eggs. Regarding the captive Atlantic salmon eggs, this could be partly explained thanks to the diet, enriched in EPA and DHA, that the Atlantic salmon received. However, the sea trout feed had significantly lower levels of DHA, as compared with the Atlantic salmon feed (Table 17), providing no obvious explanation to the elevated DHA level recorded in the sea trout eggs. In another perspective, the high DHA level found in all egg samples might reflect a specific transfer of that fatty acid to allow a better development of the future new born. In contrast with the high Σ n-3 HUFA level, the semi-wild Atlantic salmon eggs, presented significantly lower levels of Σ n-6 FA and Σ C18 n-3 PUFA, as compared with the three other egg conditions. Next, the salted eggs of the captive Atlantic salmon had a more diverse content, thanks to the higher Σ C18 n-6 PUFA and Σ C18 n-3 PUFA levels recorded, as compared with the other salted eggs, and a high Σ n-3 HUFA content, mostly due to DHA, which is lower as compared with the semi-wild Atlantic salmon eggs, but higher as compared with the sea trout and rainbow trout eggs. However, the Atlantic salmon eggs, both captive and semi-wild, presented low levels of Σ n-6 HUFA, which was higher in the rainbow trout eggs, mainly due to the high LA and AA contents (Table 17). In brief, regarding the nutritional aspect and as humans have an absolute dietary requirement in n-3 LC-PUFA, the semi-wild Atlantic salmon eggs, followed by the captive Atlantic salmon eggs, are of greater interest for humans, as compared with the rainbow trout and sea trout eggs.

In order to have a better picture of the nutritional quality of the semi-wild Atlantic salmon eggs, which had the highest Σ n-3 HUFA content, as compared with the three other salted egg conditions, in the human diet, a small calculation can be made. The semi-wild Atlantic salmon eggs contained, on average, 8.3 mg of EPA per g of fresh egg and 18.2 mg of DHA per g of fresh egg (Table 17). Considering an average egg weight of 0.093 g for the five semi-wild Atlantic salmon, from which we sampled the eggs, (ANNEX 5), a person will eat 0.77 mg of EPA and 1.68 mg of DHA per egg ingested. To meet the dietary requirements of 250 mg of DHA per day (AFSSA, 2010), this same individual should eat 148 of these eggs per day, which corresponds, on average, to one third of a commercial egg pot of 50 g. For the captive rainbow trout eggs for which the eggs are smaller, with an average egg weight of 0.068 g (ANNEX 8) and the EPA and DHA levels are of 0.205 mg and 0.952 mg per egg, respectively (Table 17), 262 eggs should be eaten to reach the human daily dietary requirement in DHA, which corresponds to an egg weight of 17.82

g. Such quantities can be plausible ingested. However, fish eggs are rather considered as a food item used in starters or as decoration on dishes and are thus consumed in small quantities. It remains that their good n-3 LC-PUFA content may contribute to an improvement of DHA intake. Salmonid eggs will most probably not be used as unique food item to reach the human n-3 LC-PUFA requirements.

Regarding the commercial eggs, for each brand, eggs from containers sold at three different periods of the year were collected. However, regardless of this precaution taken to have a representative sample of the whole brand, the three egg containers purchased per brand, were generally coming from the same lot number (ANNEX 9). In order to have a better representation of the brand diversity, we should have collected eggs of different lot numbers and therefore collect them on a larger period of time, but this was not feasible due to the limited period of time available to carry out the analyses. Variations of the egg composition between different commercial brands of two Pacific salmon species (*O. keta* and *O. gorbusha*) and within a brand, were recorded. These variations between the Pacific salmon egg brands were mostly observed regarding the Σ n-3 HUFA content of the "Carrefour (*O. keta*)", "Gabriel (*O. keta*)" and "Kaviari (*O. keta*)" brands (Table 19). Yet, we could have assumed that the commercial eggs would be more uniform in their composition, between brands of the same product or within the same brand, as their production is at the industrial level. Nevertheless, regarding the limited control possible on all the factors susceptible to influence the composition of a product of biological origin, it is expected to observe a variability in the composition of such products. First, the primary product quality highly influences the quality of the final product, but this could be mainly controlled by appropriate handling practices of the fishermen. Second, slight variations in the processing techniques to obtain the final product might also influence the quality and composition of the fish eggs (Tsuyuki et al., 1976). Furthermore, as observed in Tables 18-21, composition variations between the Pacific salmon egg brands, from wild fish, were not larger than the composition variations observed between rainbow trout egg brands, from captive fish. The same was observed with the non-marketed eggs. This showed that the composition variability of the eggs from wild fish of the same condition, is not necessarily bigger than the composition variability of the eggs from captive fish of the same condition, on which we assumed to have a greater control on the composition, through the artificial diet.

Overall, the commercial rainbow trout eggs had a poorer nutritional value for humans, compared with the commercial Pacific salmon eggs, due to decreased levels of n-3 HUFA, mostly due to the reduced EPA content. Indeed, the n-3 HUFA content of the commercial Pacific salmon eggs ranged from 39 to 51 mg/g of fresh eggs, whereas it ranged from 23 to 25 mg of n-3 HUFA per g of fresh egg for the commercial rainbow trout eggs (Tables 18 and 20). Regarding the commercial Pacific salmon egg composition, the "Carrefour" brand seemed to have the best nutritional quality with the higher lipid content, and higher Σ n-3 HUFA content (Table 19), as compared with the other brands. However, due to the broad variability in the egg compositions between the different brands, it is difficult to stand out one brand, which presents a higher nutritional quality than the others. The comparison between our Atlantic salmon eggs and the commercial

Pacific salmon eggs was considered as the most appropriate, considering the absence of Atlantic salmon eggs on the market. The salted semi-wild Atlantic salmon eggs had a similar nutritional quality, compared with the commercial Pacific salmon eggs, except concerning the AA and EPA levels, which were significantly lower in the Atlantic salmon eggs, compared with the four Pacific salmon eggs available on the market. The same trend was observed regarding the DHA content (Table 19). The Σ n-6 HUFA, Σ C18 n-3 PUFA and Σ n-3 HUFA contents were also significantly lower in the Atlantic salmon eggs, as compared with the commercial eggs, except for the "Kaviari" brand (Table 18). Similar trends were observed with the rainbow trout eggs, for which a significantly lower level of Σ n-3 HUFA was recorded in the salted rainbow trout eggs from "Du Ry Des Pres" farm, as compared with the two commercial ones. Conversely, the Σ n-6 HUFA level was the highest in the salted rainbow trout eggs, as compared with the two commercial ones (Table 21).

Slight variations of the dry matter content were observed between the hand salted eggs and the respective commercial eggs, and also within the commercial eggs (Tables 18 and 20). Such differences could be induced by the various treatments that the eggs received, that could influence the texture of the grain, and in consequence the ability of the salt to penetrate the membrane (Sternin & Dore, 1998). Yet, this slight variation in dry matter did not induce different results in the fatty acid compositions, expressed in DM, of the various eggs, as compared with the results expressed on a FM basis. The results of the fatty acid profiles in DM of all the samples are available in ANNEXES 13-16.

At last, we wanted to assess if the artificial feeds used with the captive fish could explain some variations observed in the derived egg composition. No real trends, in Tables 22 and 23, were observed between the feed and the fish egg composition of the captive Atlantic salmon, sea trout and rainbow trout. However, this statement is based on the comparison of three different fish species, which received three different feeds presenting various properties. The physiological differences between these fish species might influence the feed digestion and induce different composition profiles in the eggs (Holt, 2011). The feed characteristics could also have an impact and induce differences between fish egg compositions. As an example, the captive rainbow trout feed is described as a high energy diet aiming at obtaining optimal growth rates and low feed conversion ratios (Skretting company, 2018). Therefore, its assimilation by fish could be different, as compared to other fish feeds.

3.2. Sensory test

Many factors might influence the fish egg quality and impact the consumers sensory and visual perceptions. Fish eggs must present several characteristics to ensure a good appreciation of potential consumers. According to a study, the most important factors of the fish eggs appeared to be: "a proper colour and glossiness, texture, a desirable mouth feel, and their characteristic flavour with limited fishy, bitter or oxidized flavour notes" (Bledsoe et al., 2003). Other studies stated that the major characteristics impacting the quality of the product and the consumer appreciations were the colour, the salt content, the elasticity, the shape and the appearance (Tsuyuki et al., 1976). Appreciation by

consumers of several of these factors were tested on four salmonid egg samples, during a sensory test implemented in December 2017. The results of the sensory test revealed that, among the parameters tested, the colour and the brightness, for the visual aspect (Table 24), and the saltiness and aftertaste, for the tasting aspect (Table 26), played important roles in the appreciation of the products by the participants, and were indeed, more variable between the four egg samples tested. The stickiness, taste and crunchiness were the less significant factors, influencing the appreciation of the participants to the sensory test.

Results of the technical report of the “Fish and marine services” of Canada in 1976 reported that, “colour is the particularly important as it is the first factor that draws the attention of the consumer’s sensory perception”. The same report noticed that consumers had a preference for products with a bright and “deep orange-red” colour (Tsuyuki et al., 1976). Accordingly, the results of the visual test in Table 24 and Figure 8 indicate that the colour of the egg samples seemed to be the feature, which generated the most reactions among the participants, as compared with the other features tested. In turn, it allowed to best differentiate the four egg samples. Overall, the eggs with a great orange colour (captive sea trout eggs) received a better score, compared with eggs, which presented a paler colour (rainbow trout eggs and captive Atlantic salmon eggs) or a too “orange-red”/“fluo” colour (semi-wild Atlantic salmon eggs), as described by the participants of the sensory test. In brief, the captive sea trout eggs received better scores for the visual test, due to a good colour and brightness of the eggs (Tables 24 and 25). In contrast, the captive Atlantic salmon eggs were visually less appreciated, mainly due to their “yellow - pale” colour, as described by participants (Table 24). Besides, these results indicated that there was a very low or no impact of the artificial feeds that the captive Atlantic salmon and the captive sea trouts received, on the derived egg pigmentations. Indeed, the same feed “repro floating” was given to these two captive fish species, with the exception that the captive Atlantic salmon feed, was enriched, compared with the sea trout feed, notably, with the carotenoid-rich “Carophyll red”, which is usually used as feed additive to improve the appearance of fish flesh (DSM company, 2018). Nevertheless, as said above, the captive Atlantic salmon eggs were less appreciated by consumers, mostly due to their pale colour, whereas the captive sea trout eggs presented a more orange “warmer” colour, which was more attractive, according to the participants. However, the pasteurisation of the product could also have had an influence on the egg colours. Indeed, salmon eggs might become slightly paler due to high pasteurisation temperatures. To minimise this effect, the pasteurisation temperature must remain below 70°C (Bledsoe et al., 2003). The salted eggs all underwent the same pasteurisation treatment, at a constant temperature of 69°C, but eggs from some fish species might be more sensitive to heat than others. It would be interesting to find a way to influence the colour of the fish eggs, as it is an important factor impacting the consumer appreciations. However, it seems that each fish species has its own egg colour standard, and that egg colour can vary even within a fish species and within the same lot (Bledsoe et al., 2003). Thus, the strategy found to influence a specific egg sample colour should be adapted for each egg sample.

Regarding the tasting results, the captive sea trout eggs were slightly preferred and the captive rainbow trout eggs slightly less appreciated by the participants of the sensory test, compared with others. However, these preferences were not significant. The semi-wild Atlantic salmon eggs received a better appreciation regarding saltiness, compared with the captive Atlantic salmon eggs (Table 26), which were described by participants as too salty. The captive Atlantic salmon eggs had a higher mean salt level, of 4.45% (after 75 min in the brine solution) (ANNEX 4), compared with the semi-wild Atlantic salmon eggs, which had a mean salt level of 4.25% (after 90 min in the brine solution) (ANNEX 5). The salt level was thus slightly higher in the captive Atlantic salmon eggs, as compared with the semi-wild ones, but the better saltiness appreciation of the participants of the semi-wild Atlantic salmon eggs, could also be due to the higher "fish-aftertaste" noticed in those ones, which could have partially masked the saltiness of the product. Indeed, the semi-wild Atlantic salmon eggs received a better appreciation regarding their aftertaste feature, as compared with the captive rainbow trout eggs (Table 26). The aftertaste of the semi-wild fish eggs was characterised of "fishy/sea aftertaste", "stronger, pleasant aftertaste", whereas the rainbow trout eggs had more a bad "soil/sand" aftertaste, as described by participants. This could be explained by the time spent at sea by the semi-wild Atlantic salmons, as compared with the captive rainbow trouts, which lived in outdoor fish ponds. Therefore, the environment and life type of adult fish seem to have an impact on the derived egg taste, and mainly on the egg aftertaste. It could be interesting to see if the "sea/fish" taste of the eggs could be due to the higher n-3 LC-PUFA content recorded in the semi-wild fish eggs, as compared with the captive fish (Martin et al., 2010).

Testing the taste preferences is not an easy task, considering the enormous variety of taste existing worldwide. Furthermore, the eating habits, such as eating salty or not, were not considered in the implementation of this sensory test, and yet have great influences on the taste appreciation of a product. We also did not took into account if the participants were smokers or not, which might also impact the taste in the mouth. Besides, the participant population, with a high proportion of young participants mostly made of students in bioscience engineering, was not representative of the whole population. Moreover, as fish eggs are a bit considered as a luxury product, not affordable for everyone, we should have selected a population with good financial resources, which is generally not the case of students, which represented the half of the sensory test participants (Figure 7).

VI. GENERAL DISCUSSION AND PERSPECTIVES

Atlantic salmon is beneficial for both the regional biodiversity and our health. The reintroduction of the natural life cycle of Atlantic salmon in the Mosan basin, thanks to the release by the CoSMos centre, has a high ecological value. Furthermore, Atlantic salmon eggs have a high nutritional value as they provide us with health-promoting n-3 LC-PUFA (EPA and DHA). The objectives of this master thesis were to assess the quality of Atlantic salmon eggs in order to (1) ensure a better spawning success aiming at enhancing the efficacy of the reintroduction of the Atlantic salmon in the Mosan basin and (2) determine the nutritional and sensory interest of a sustainable and Walloon Atlantic salmon egg production for human consumption.

The fatty acid egg composition and the n-3 LC-PUFA seemed to be an important factor affecting egg quality in terms of survival rate and nutritional benefit. This may be related to the fundamental roles that fatty acids, notably LC-PUFA, play as structural components of storage and membrane lipids but also as major source of energy and regulatory elements in metabolic processes of fish during their growth and development, as well as in humans (Murzina et al., 2011). The proximate composition and the fatty acid profile of all the eggs sampled, as well as the feeds, were determined. For the first objective, we assessed to what extent the environment, the species, and the life style of the fish, associated to specific fatty acid profiles, may impact the egg composition of the fish. For the second objective, we compared the nutritional quality of various fish eggs, notably with commercial eggs, and assessed the possible impact of the salting process on the raw fish eggs. Next, a sensory test was conducted in order to evaluate the Atlantic salmon egg appreciation by potential consumers.

In the first part of the present study, the assessment of the environment impact by the comparison of the egg compositions of Atlantic salmon of the Loire-Allier origin, which were coming from females returners after two years spent at sea, caught in the Allier in Vichy or the Meuse in Lixhe, suggested that these two Atlantic salmon stocks shared an identical feeding ground at sea. However, the significantly higher level of 20:1n-9, probably of dietary origin, in the eggs of semi-wild (or wild) Allier returners, compared to their Meuse counterparts, may reveal a more northern feeding ground for the first compared to the latter. The study of the species impact by the comparison of the egg composition of the semi-wild sea trout, which spent one year at sea, and the semi-wild Atlantic salmon of Loire-Allier strain, which spent two years at sea, both caught in the Meuse river in Lixhe (Belgium), showed an overall similar fatty acid content between these two fish eggs, with the exception of higher 20:1, 22:1 and EPA levels and a lower AA level in the Atlantic salmon eggs, as compared with the sea trout eggs. These differences suggest a more “marine-type” pattern for the Atlantic salmon, as well as a higher dependence of the sea trouts to freshwater ecosystems for feeding. However, the high levels of n-3 HUFA marine-type fatty acids in the eggs of our semi-wild sea trouts, suggests that these Meuse trouts are effectively going at sea for feeding and growing. For further research, the impact of the number of years spent at sea should be studied with two semi-wild fish of the same species, which spent a different number of years at sea, in

order to be able to take relevant conclusion about the number of sea year influence on the fish migrating distances, and thus on the fish feeding grounds. However, the comparison of such conditions was not feasible in this case, due to their unavailability during the sampling period, in November-December 2018, at the fish farm CoSMos. Next, to confirm the hypothesis of a more northern feeding ground for some fish species, further studies in relation with a possible higher DHA content in the phospholipids fraction of our eggs, inversely correlated with the mean river temperature, will be needed. Moreover, the 22:1n-11 fatty acid could also confirm this hypothesis, as it is an important marker of the marine environment of the fish (Linko et al., 1992; Peng et al., 2003) and as its level could be possibly related to latitude. However, this fatty acid has not been quantified in this study, and should be considered for further research.

It seemed that we had a clear impact of the life style of the fish on the egg fatty acid composition. Indeed, higher Σ n-3 HUFA, mostly due to EPA, content and lower Σ C18 n-6 PUFA, Σ n-6 HUFA and Σ C18 n-3 PUFA contents were quantified in the eggs of the semi-wild fish species, as compared to the captive counterparts (Tables 8 and 10). This higher Σ n-3 HUFA content in the migrating fish eggs, could be due to (1) the physiological adaptation of the wild fish to the specific cold environmental conditions, in order to maintain their body activity and membrane fluidity (Pickova et al., 1999; Peng et al., 2003), as well as to (2) the diet of the wild fish at sea, which would be mainly based on a trophic chain with micro-algae and marine invertebrates, which contain high levels of EPA and DHA (Monroig et al., 2013). The egg survival rate, until hatching, was higher among the semi-wild Atlantic salmons, as compared with the captive ones, at CoSMos in 2014. The same was noticed in studies on Atlantic salmons in 1997, where the wild Atlantic salmons showed a greater survival rate of embryos and alevins, along with a higher hatching success, as compared with the captive counterparts (Brooks et al., 1997). The n-3 LC-PUFA, and more precisely EPA, egg content, could thus be related to the better egg survival rate registered in the semi-wild fish, as compared with the captive fish.

The observations made above, are assuming that the fatty acid content of the fish egg is entirely reflecting the fish diet fatty acid content. However, we cannot exclude the possibility that the adult fish could adjust the fatty acid composition of the eggs, by fatty acid bioconversion processes and specific fatty acid transfer, to fill some fatty acid deficiencies of the eggs. We should have studied the flesh fatty acid composition of the fish, along with its eggs, in order to determine the impact of the fish metabolism on the egg composition. Besides, we assumed in this work that lipids were the major factor influencing the egg survival rate, as studies revealed that the n-3 LC-PUFA egg content appeared to play a major role in the fish egg quality (Fernandez-Palacios et al., 1995). However, other nutrients present in the fish eggs could be involved. In order to determine all the factors involved in the better quality of fish eggs, a complete egg composition analysis should be performed, including determination of amino acid content, vitamins, pigments and possible contaminants. The egg fatty acid composition could also be determined by separating the three fatty acid fractions (free fatty acids, triacylglycerols and phospholipids) in order to assess if the fatty acid repartition between these fractions has an influence on the egg survival rate.

Better insights in the feasibility of an Atlantic salmon egg production in Belgium for human consumption was brought through the study of the second aspect of the project. The raw salmonid eggs obtained from a farmed fish can go through a salting and a pasteurisation process without causing any negative effect on the proximate composition and the fatty acid profile of the eggs (Tables 14 and 15). If a sustainable Atlantic salmon egg production for human consumption has to be launched, it will be based on captive Atlantic salmon. The captive Atlantic salmon eggs presented high levels of n-6 PUFA and C18 n-3 PUFA and a relatively high Σ n-3 HUFA level, but still lower than the Σ n-3 HUFA level in the semi-wild Atlantic salmon eggs (Table 17). Moreover, the Σ n-3 HUFA content, mainly due to EPA and DHA, recorded in the semi-wild Atlantic salmon eggs were still significantly lower, as compared with the commercial Pacific salmon eggs (Table 19). As the dietary intake of n-3 LC-PUFA are, currently, insufficient for most people (De Ridder et al., 2016), we could try to improve the artificial fish feed to enhance the EPA and DHA contents of the captive Atlantic salmon eggs, in order to increase the nutritional benefits for humans and compete with the actual commercial eggs from Pacific salmon.

For both aspects of the present work, the n-3 LC-PUFA egg content seemed to play a major role in the fish egg quality, from an ecological and a nutritional point of view. Higher n-3 LC-PUFA levels in the captive Atlantic salmon eggs, would enhance their survival rate at the fish farm, as well as their nutritional benefit for human. We could next try to define the appropriate n-3 LC-PUFA fish diet content in order to improve the quality of the captive fish eggs, through the previous knowledge of the fish fatty acid conversion capacity. Indeed, the egg composition could be mainly impacted by the fish feed, but we cannot exclude the possible implication of bioconversion processes of dietary fatty acids by the fish. In addition, specific fatty acid transfers are more than likely. For further research, we should compare the egg composition of fish of the same species fed with the same diet, varying in fatty acid concentration, notably with its n-3 LC-PUFA, in order to remove the influence of the physiological differences between species.

For further research, the chemical analyses of fish eggs should be carried out on eggs of more than three females per condition ($n > 3$) in order to have a better statistical power. Indeed, for some data the statistical hypotheses were not verified, and a non-parametric test has then been applied to these data, the latter having however a much lower statistical power.

The results of the sensory test revealed that the colour and the brightness, for the visual aspect, and the saltiness and the aftertaste, for the tasting aspect, were the most important factors, among those tested, for the appreciation of the products by potential consumers (Tables 24 and 26). It would be interesting to find a way to influence the colour of the captive Atlantic salmon eggs, to transform their pale orange colour in a brighter colour, as this has a major influence on consumer appreciation. The comparison of fish egg compositions from fish of the same species, which received the same artificial feed but with variable pigment quantities in it, could be tested in order to determine the optimal feed pigment concentration to obtain the right egg colour. Astaxanthin has been identified

as the main carotenoid pigment involved in the orange-red colour of fish eggs (Tsuyuki et al., 1976). Regarding the taste results, the four salted egg samples showed a similar appreciation (Table 27). The aftertaste of the product had a great influence on participant appreciations, and it would thereby be interesting to try to develop the appreciated "sea-aftertaste" of the semi-wild Atlantic salmon eggs, in the captive fish eggs. If this sea aftertaste could be due to the higher n-3 LC-PUFA level quantified in the semi-wild fish eggs is remaining a question. We should keep in mind, that, as discussed earlier, the appreciation test did not use consumers who are representative of the whole population. The sensory test implemented was a first test to obtain preliminary and global information about the appreciation of our salted fish eggs by potential consumers. Indeed, we saw in the sensory test results that the taste was an important consumption criterion, according to participants (Table 17). Overall, we can conclude that our product, the Atlantic salmon eggs, was appreciated, regarding the visual and tasting aspects. This conclusion motivates to implement a new sensory test at a larger scale.

Fish eggs are overall consumed in small quantities and are thus likely to have only a limited effect on the nutrition of an individual. Besides, analysis of the other egg compounds, like cholesterol or potential pollutants should be carried out, as this can have a serious impact on human health. Indeed, results of a study on the cholesterol content of Pacific salmon roe indicated a level of 716 mg/g in the egg, which corresponded to 200 mg of cholesterol per egg (Urena & Rywkin, 2010). Considering that the cholesterol nutritional recommendation for a Belgian adult is still set at a maximum of 300 mg per day, cholesterol appears to be an issue. In addition, the daily salt intake of an adult should stay below 5 to 6 g (Conseil Supérieur de la santé, 2016). Consumers with cardiovascular diseases are therefore advised to avoid consumption of fish egg products already available on the market, due to their high salt and cholesterol contents (Johannesson, 2006). Nevertheless, an Atlantic salmon egg production for human consumption, could represent an additional way to give an added value to Atlantic salmon in human nutrition and improve the commercial sustainability of the Walloon Atlantic salmon production, through this diversification of its production.

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Abstract Atlantic salmon disappeared from our rivers in the 1930s due to pollution, dam construction and overfishing. Therefore, the reintroduction of the natural life cycle of this species in the Meuse system has a high ecological value. Furthermore, salmonid and salmonid egg consumption are of nutritional interest as they provide us with health-promoting omega-3 highly unsaturated fatty acids (n-3 HUFA) (EPA and DHA).

The present work was designed to evaluate the way to (1) optimise the Atlantic salmon reintroduction in our region and (2) introduce their eggs in our diet. Regarding the first objective, we worked in association with the CoSMos centre where salmonids are artificially reproduced to restock the rivers of the Meuse basin. For this purpose, we assessed the egg quality to ensure a better spawning success and hence a higher survival rate of salmonids. The second objective was to assess the egg nutritional quality in order to evaluate the interest for a Walloon Atlantic salmon egg production. In addition, a sensory test gave us a better insight into the way potential consumers could appreciate the eggs.

The egg n-3 HUFA content could have a direct impact on their survival rate. Survival rate of salmonids registered at CoSMos were higher among semi-wild fish, as compared with captive counterparts. The life type difference between captive and semi-wild salmonids appeared to have a direct impact on the n-3 HUFA content of the eggs, which was higher in the migrating semi-wild fish eggs. Besides, the results of the environment impact between the eggs of salmonids, which spent two years at sea, either caught in the Meuse system or in their own Loire-Allier system, suggested that they both shared an identical feeding ground at sea. The Allier returners, compared with their Meuse counterparts, may however migrate to a more northern feeding ground. The study of the species impact between the eggs of the semi-wild sea trouts, which spent one year at sea, and the semi-wild Atlantic salmonids of Loire-Allier strain, which spent two years at sea, both caught in the Meuse river in Lixhe (Belgium), suggested a more “marine-type” pattern for the Atlantic salmonids, compared with the sea trouts. However, the results showed that our semi-wild sea trouts were effectively going at sea for feeding and growing. If the n-3 HUFA egg content could be related to the latitude of the fish feeding area, with higher n-3 HUFA content in fish travelling to more northern feeding areas, needs further studies to be clarified.

Atlantic salmon eggs could be a good additional source of n-3 HUFA in human nutrition. Their n-3 HUFA content was lower but in the same range as the commercial Pacific salmon eggs. Moreover, the sensory test revealed that the salmonid egg samples were globally well appreciated. The colour, brightness, saltiness and aftertaste were the more variable features between the four egg samples tested. Atlantic salmon eggs may further be improved in nutritional and sensory terms, but could definitively become a valid opportunity to give an added value to the Atlantic salmon farming.

More extensive research needs to be performed in order to find out the relative implication of the fish metabolism and diet in the derived egg composition, as well as the possible impact of other compounds, present in the fish eggs, on the egg quality.

