Heinrich-Heine-Universität Düsseldorf
in corporation with aquaFUTURE e.K.

Physical and Biological Parameters Influencing Egg Hatching Success in the Atlantic salmon (Salmo salar)

Master thesis in the course of studies biology
Institute of metabolic physiology

Presented by: Julien Kocabiyik
Matriculation number: 2501895

Supervisor:

1. apl. Prof. Dr. Christopher Robert Bridges, Institute of metabolic physiology
2. Prof. Dr. Eckhard Lammert, Institute of metabolic physiology

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Abstract

The objective of this theses was to detect biological and physical parameters which influence the egg hatching success in the Atlantic Salmon (*Salmo salar*). Nine physical different female and male parental salmons were used for 27 crossbreeds (three one-year old, three two-years old and three three-years old). All parental fish experienced no special treatment and had the same environmental conditions before the experiment started. The eggs of every crossbreed were treated the same way in terms of water parameters, light incidence, measurements and the fertilization process. The degree days until hatching (C°), total egg production (g), total sperm volume (ml), spermatocrit (%), mean individual egg- and alevin weight (mg) and size/ yolk sac length (mm) at different developmental stages, sinking rate (cm/sec) and mortality rate (%) was recorded for/ within 90 days.

The total egg production of longer salmons is higher than the one of small salmons, regarding fully grown salmons. Furthermore, the eggs from older salmons needed more degree days until hatching than younger salmons, while the cumulative egg mortality rate after 90 days was lower for faster hatching eggs.

Longer and older salmons produced bigger egg, regarding fully grown salmons.

The alevin yolk sacs are generally bigger than the eggs were right after spawning. The eggs reached their maximum weight and size right after fertilization and swelling in water for one hour. After that, the weight and size decreased until the alevin hatched. The decrease speed was different for eggs from different female parental salmons. A slower increase in egg weight (%) resulted in a lower cumulative mortality rate after 90 days.

The total production of eggs, the spermatocrit and the egg size and its increase (%) did not influence the cumulative mortality rate after 90 days. The carotenoid content and other genetic influences by the parental salmons play a key role for the mortality rate and so for the hatching success.

In general, the maternal influence on the next generation is highly stronger than the paternal influence. Already by observation with the eye, some tendencies for the hatching success can be identified.

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1. Introduction

1.1. Aquaculture

Aquaculture is a very diverse and unexplored subject until now and this won't change soon. There are many reasons contributing to a need for a better understanding in marine ecology and Aquaculture as a part of it, but what is Aquaculture?

Aquaculture is the rearing of cultivated marine life (aquatic animals or plants). In this study, we are only focusing on one specific aquatic animal, the Atlantic salmon (*Salmo salar*). Aquaculture is very important and needed, to remain most of the aquatic animals, which are not able to keep a consistent population without human intervention, although also human interventions led to that state. Unfortunately, many reasons are contributing to dropping aquatic populations, like the pollution of our marine systems around the world with e.g., antibiotics (Han et al, 2020) or plastic (Xanthos, Walker, 2017), the chemistry changes because of the increasing carbon dioxide emission (Kapsenberg, Cyronak, 2019) and especially the overfishing of the oceans (FAO, 2020). Anitibotics released in the wastewater can be observed in aquatic environmental (sediment, water, organisms and feed samples). They can bioaccumulate in fish tissue, exposing it to a high toxicity, which can cause genotoxicity and developmental disorders (Yang, Song, Lim, 2020).
Plastic in the marine environmental is a global issue and there is still no end to it. Even if innovations and policies try to reduce the plastic use, it is already too late to completely avoid connected problems for aquatic life, considering the current amount of plastic inside the oceans. Plastic persists a long time period in the environment and because of the nearly indestructible morphology and the toxins it contains, it becomes a serious problem to ecosystems (Hammer, Kraak, Parsons, 2012)
The carbon dioxide exposure can cause different gene expression as usual, which can for instance prevent growth of Atlantic salmon gills (Mota et al, 2019).
Overfished stocks are considered biologically unsustainable. In 2017, 34.2% of the fish stocks of the world's marine fisheries were classified as overfished. Since the current successes accomplished in some countries and regions are not sufficient to reverse the global declining trend of overfished stocks, nothing will change much, but the increasing importance of aquaculture (FAO, 2020).
Also, fish consumption accounted for 17% of the global population's intake of animal proteins, and 7% of all proteins consumed. Globally, fish provided more than 3.3 billion people with 20% of their average per capita intake of animal proteins (FAO, 2020).

In 2018 global capture fisheries production reached a record of 96.4 million tonnes. World aquaculture fish production reached a record of of 114.5 million tonnes (32.4 million tonnes of aquatic algae and 26 000 tonnes of ornamental seashells and pearls, excluded in Fig. 1). Global catches in inland waters accounted for 12.5% of total capture fisheries production, but inland water catches are more concentrated than marine catches, both geographically and by country (Fig. 1).

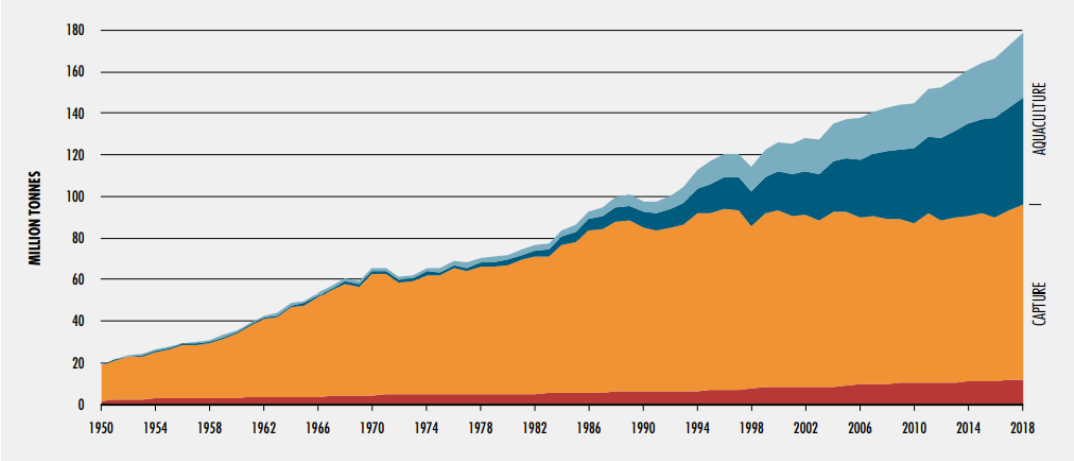

Fig. 1. World capture fisheries and aquaculture production. (**Red**) Capture – inland waters. (**Orange**) Capture – marine areas. (**Blue)** Aquaculture – inland waters. (**Light blue**) Aquaculture – marine areas. Aquatic mammals, crocodiles, alligators and caimans, seaweeds and other aquatic plants are excluded (FAO 2020).

The contribution of world aquaculture to global fish production is increasing in most parts of the world. Europe increased its total fish production through aquacultures to 17.0%, close behind Africa with 17,9%, but still more than America with an increase of up to 15.7%. Oceania even decreased its contribution within the past 20 years to “only” 12.7%.
In comparison to this, other countries already acknowledged the huge potential of aquacultures. Asian fish production (excluding China) reached 42.0% in 2018, up from 19.3% in 2000. Fish farming is actually dominated by Asia (including China), which has produced 89% of the global total in volume terms in the last 20 years (Fig. 2).

The oceans cover roughly up to 71% water of the planet earth. A good part of these 71% should be used as aquacultures. The rapid increasing human population creates many different and also different valued problems, but one, which is definitely worth to emphasise, is the food problem (Crist, Mora, Engelman, 2017). In 2012 the world population of more than 7 billion was estimated to approximately 9 billion by 2030 and to 10 billion by 2050 (Gerland et al, 2020). Although the actual pandemic (Covid) might change this prediction, the population will recover and grow for sure. In 2018, over 156 million tonnes of the 179 million

tonnes of total fish production were utilized for direct human consumption (Fig. 3), while the remaining 22 million tonnes were used for non-food purposes (e.g., fish food production, as bait, in pharmaceutical uses, for pet food, etc.).

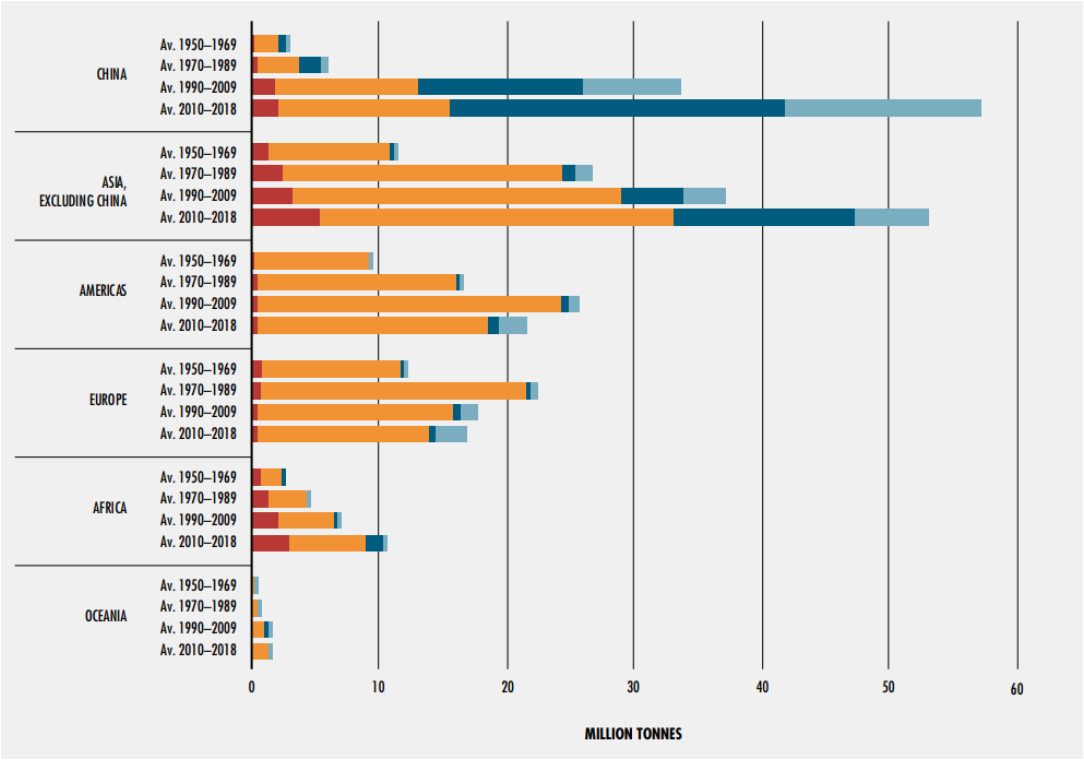

Fig. 2. Regional contribution to world fisheries and aquaculture production from 1950 to 2018. (**Red**) Capture – inland waters. (**Orange**) Capture – marine areas. (**Blue)** Aquaculture – inland waters. (**Light blue**) Aquaculture – marine areas. Aquatic mammals, crocodiles, alligators and caimans, seaweeds and other aquatic plants are excluded (FAO 2020).

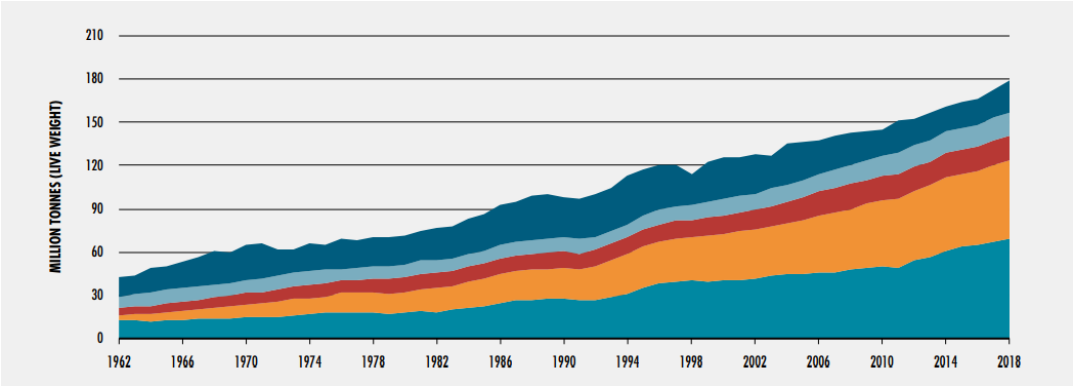

Fig. 3. Utilization of world fisheries and aquaculture production, 1962–2018. (**Blue**) Live fresh or chilled. (**Orange**) Frozen. (**Red**) Prepared or preserved. (**Light blue**) Cured. (**Dark blue)** Non-food purposes. (FAO 2020).

Several sources can be used to gain more food, but using the water as a basis for food production (Seafood and fish) offers many opportunities to use other strong restricted resources and spaces for other projects.

A great benefit of food like fish is the Freezing. While fresh or chilled fish represents the largest share of utilization for consumption with 44%, freezing still represents the main method of preserving fish for food, accounting for 62% of all processed fish for human consumption. The fish consumption of frozen (35%), prepared and preserved (11%) and cured (10%) fish, by humans differ significantly across continents (FAO, 2020).

As already mentioned, aquaculture is the rearing of cultivated marine life, but this must not mean that aquaculture is the rearing of cultivated marine life in terms of food. Aquacultures such as “aquaFuture e.k.” produce fish for restocking surrounding water systems. The aim behind this process is to create and establish a local brood stock. Aquacultures should be supporters to world fisheries, which are based on natural reproduction instead of human-made reproduction. Another interesting benefit of aquacultures is, that a specific fish can be produced. Depending on the desire/ need of a specific species more, or less aquacultures can focus on this species, while fisheries cannot guarantee a specific fish species forever.

At the same time aquacultures do not contribute to the mentioned ocean pollution, while fisheries produce a lot of microplastic (Xue et al, 2020). Microplastic has a negative effect on many organisms e.g., the Atlantic salmon. The consumption of natural prey when microplastics were present was decreased. Negative effects on growth, reproduction and even survival are also evident (Granby et al, 2017).
It’s a fact, that overfishing will not stop and might even increase, but rearing high quality fish as supporters could help. Capture fishes have unknown genetics and might be really good in terms of reproducing, but this will also remain unknown. In comparison to that (usually), fishes from aquacultures are not only trackable by their genetics, they have a high vitality and survival rate, a high reproduction rate and even more factors, which make them suitable for the oceans. The quality of aquaculture fishes is depending on different factors like the water quality or the fish food used (Kong et al, 2020).
In anyway, the aquaculture fish production must continuously increase within the next decades if the world keeps on changing the way it does right now. Already 52% of the fish available for human consumption originate from aquaculture production (FAO, 2020).
An increase in food production from sustainable capture fisheries is simply unlikely (Garcia, Grainger, 2005).

To come back to the Atlantic salmon, in addition to the demand as food, it is also important for the environment and ecosystems. For example: Salmon runs function as enormous pumps that push vast amounts of marine nutrients from the ocean to the headwaters of otherwise low productivity rivers (Rahr, 2016).

1.2. The Atlantic salmon (*Salmo salar*)

Every fish species is unique. Different feed compositions and sizes, different water parameters, different handling, vaccination and many other aspects are required to raise a perfectly conditioned fish. One also has to differ between the farmed fish and the wildlife fish. Since this study deals with the Atlantic salmon (*Salmo salar*), I am going to mainly focus on all of these aspects in terms of farmed salmons.

The salmon life cycle (Fig. 4) starts as an egg. After fertilization, the eggs typically need 200-300 degree days to reach the eyed stage and in total 480- 520 degree days until hatching. The time of egg development is measured in degree days. Degree days (DD) are defined as water temperature (°C) multiplied by number of days. The time of the hatching process itself is also depending on the water temperature. They require a sudden temperature increase to hatch. For example, if the eggs incubated for 520degree days and the temperature always stays around a low value, the eggs will not hatch. If the incubated eggs, then experience a temperature increase of 3 degrees from one day to the next, most likely all of these eggs will hatch. The dissolved oxygen (DO) concentrations always need to be above 80% saturation. In addition to that, the pH value may not become less than six or higher than seven. Another important factor, which will influence the salmon welfare in every life cycle stage is the stocking density, but already as an egg, its strongly depending on the facility and equipment. In general, they can be stocked from 10.000 to 80.000 per m^2 surface area. Aside from that, the eggs should be kept in darkness to avoid light, which causes stress (Benchmark Genetics, 2019). Fish eggs are very sensitive to vibrations. They can become injured very fast and the embryo will not develop any further. Thus, making it difficult to handle and sort them correctly. While hatching, many damaged eggs start to fungus and build spores. These eggs need to be removed, otherwise they will infect neighbour eggs and interrupt their development as well. This state of sensitivity ends after the egg reach the eyed stage. Then, they become more resistant to vibration because most of the first developmental progression is done.

The eggs hatch into the alevin stage. The small alevins (<2.5cm) have a yolk sac, which they feed on for two to four weeks. In this time, no additional food is required. Newly hatched alevins are also very sensitive to light and covering their tank minimises stress. Their temperature optimum is equal to the one of eggs (2°C- 8°C), but for the first feeding a

temperature above 10°C is even better. The first feeding starts when approximately 90% of the yolk sac is absorbed. In comparison to eggs, the water exchange must now be increased and the stocking density can vary from 10.000 to “only” 20.000 per m^2 surface area. Furthermore, pathogens including bacteria, viruses and parasites can affect the salmon from now on, what makes health management and monitoring important. The importance of monitoring remains (with changes) the whole life of a farmed salmon (or until it gets released to water systems). Dissolved oxygen and pH are still as important as before but the limits do not change for now.

After alevins have absorbed their yolk sac and start to eat, they enter the fry stage (2.5-6.5cm). This is considered to be the rapid growing stage. Many factors are key players when it comes to growth. The light impact can increase the grow rate, the size they reach within this time and also gene expression levels of myogenic regulatory factors (Churova et al. 2020). At this point an aquaculture needs to decide whether the fish should rapidly grow for food production purpose or slowly grow and learn to hide in shadows for a better survival in future wildlife. The fish feed composition affects the health and growth of cultured salmons as well. The content of phospholipids in the feeds is one aspect which needs to be taken into account, as they are key components of cell membranes and indispensable for normal growth and development (Betancor et al, 2014) and also for the modulation of immune defence against pathogens (Binder, Papac-Milicevic, Witztum, 2016). The content of protein in the fish feed as well as which type of protein must be taken into account. Protein from fish meal leads to a better growth than plant-based proteins (Egerton et al, 2020). Apart from that, the total amount of fish feed and the frequency of feeding is very important for the growth and survival rate up to a limit, which is variable depending on the temperature and fish size (Storebakken, Austreng, 1987). It is important to remember, that fishes also produce more wastes when they feed more. So, with increasing fish feed use and reaching healthy limits, more cleaning has to be done for good water parameters. A good rule of thumb is, that 1kg of fish feed produces 0.03kg ammonia (apart from the extra oxygen needed for the fish when feeding and resulting organic carbon, nitrate and CO_2). Also, the carotenoid astaxanthin in fish feed benefits the growth and survival rate. A minimum of 5.1 mg per kg astaxanthin concentration is contributing to a maximum growth and survival during the start-feeding period (Christiansen, Lie, Torrisson, 1995).

As now clearly pointed out the fish feed composition plays a key role for good conditioned salmons with way more factors than mentioned. This will last the salmon for its lifetime, but with different developmental stages, different feed compositions and sizes are required (Wańkowski, Thrope, 1979), because the elemental composition of salmon changes within the life cycle and the fish size (Shearer et al, 1994).

The growing fry reach the next developmental stage, the parr stage. The Parr (6.5- 12cm) is the last freshwater stage of a wildlife salmon life cycle. They can weight approximately 30-40g and develop camouflaging vertical strips. They start swimming with the current instead of against the current. The smoltification (also called parr-smolt transformation) begins as a parr. It prepares the salmon for downstream migration and transition to the marine life (Stefansson et al, 2008) The salmon undergoes biochemistry, physiology, morphology, and behaviour changes for life in seawater (McCormick, 2012). A parr is very sensitive to pathogens, because the changes due to smoltification possess a high energetic cost for the salmon and correlate with decreased defences related to the immune system (Pontigo et al, 2016). As they move into seawater, the osmotic gradient is reversed and as some compensatory mechanisms, they drink seawater, reduce their urine production, and actively secrete salts across the gill's epithelium through specialised cells called ion- ocytes, mitochondria-rich (MR) cells, or chloride cells (Clarke et al, 1996). Smoltification can be influenced by several factors e.g., temperature (Handeland et al, 2014) and many other environmental factors (Wedemeyer, Saunders, Clarke, 1980).

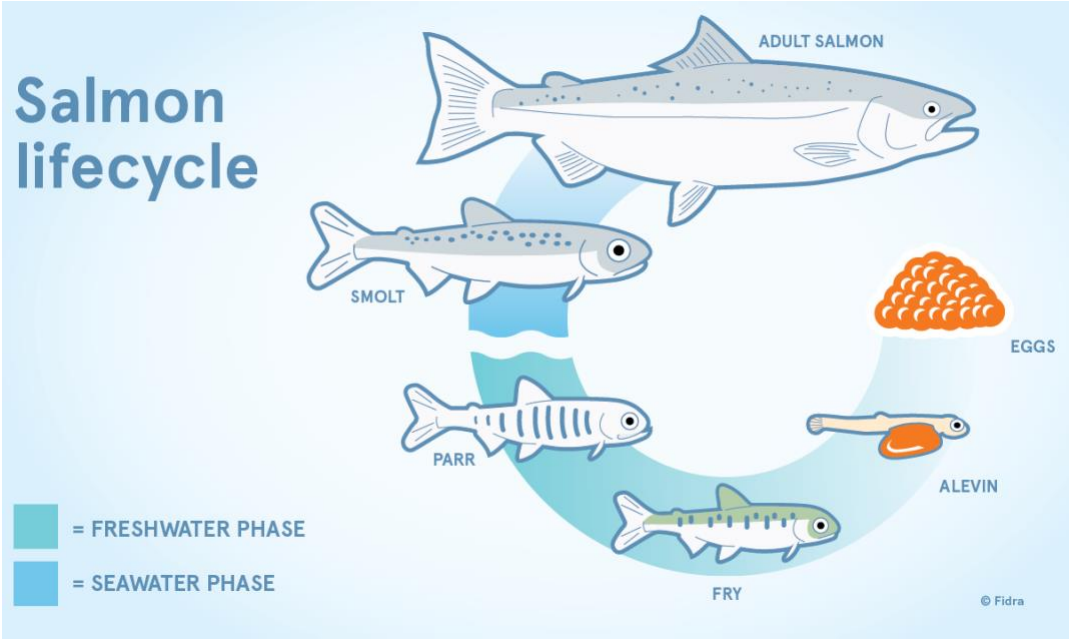

Fig. 4. The salmon lifecycle split into freshwater- / and seawater- phase. (Salmon Life cycle - Best Fishes)

After smoltification the parr becomes a smolt. Smolts (12-15cm, 50-80g) are only adapted to sea water if they experience a migration time in brackish water, where their body chemistry becomes accustomed to osmoregulation (McCormick 2012), otherwise they can keep on living in fresh water. Some physiological adjustments in the function of osmoregulatory

organs such as the gills, which leads to large increases in their ability to secrete salt, are still slightly occurring, but this can be compensated through the fish feed composition and other ways to increase the salt concentration inside water. The main characteristic of smolts apart from the mentioned adjustments, is the silvery colouring with scales that easily rub off. In addition to that, male maturation starts within smoltification, while female maturation starts after completion of smoltification (Fjelldal et al, 2018).

Finally, at approximately 100g wet weight, the smolt becomes an adult salmon. The adult salmon reaches its full size within 12-14 months. They can already spawn eggs before reaching its full size (average length: 71-76cm, average weight 3.6-5.4kg) in farms. In the wildlife, the salmons return to their natal river within 24-36 months to spawn. They can grow up to a known maximum of 13.6kg, depending on the exact species (Salmon Life Cycle, 17 April 2021). After that, the life cycle of a new generation begins again, as eggs.

In summary we can say, that some factors are important for the quality of the salmon, other factors are important for its survival and some factors are even important for the migration into sea water.

1.3. The Hatching

Hatching can be compared to the human birth. It is one, if not the most crucial day of an Atlantic salmon (*Salmo salar*) life. Different intrinsic (e.g., parental) and extrinsic (e.g., environmental) factors influence the hatching process and can either improve or worsen the hatching success.

The most important extrinsic factors are water parameters. Salmon eggs require even more dissolved oxygen when the hatching starts (in comparison to the incubation with > 80% DO). This has to be acknowledged because hypoxia can cause a huge decrease in the metabolic rate of embryos. Furthermore, it also decreases growth and development, and delays the hatching (Wood et al, 2020). Incubation temperature is a key factor affecting phenology of hatch, with warmer incubation temperatures resulting in faster physiological development and shorter incubation periods (Jeuthe, Brännäs, Nilsson, 2016). Obviously, water chemistry, especially alkalinity, nitrates and phosphates, are important regulators of production, as well as ammoniac and nitrite for alevins (Gibson, 1993). All extrinsic factors are usually on a good basis level in aquaculture systems. In aquacultures, monitoring systems and water purification systems are working all day every day. A salmon in wildlife could theoretically spawn its eggs in water with inappropriate parameters, which is just one, but for sure the less important factor resulting in no natural reproduction within many water systems. The most

important factors leading to this, which I will not discuss here, are the many biological predators (cormorant (*Phalacrocoracidae*) & wels catfish (*Silurus glanis*)) and the lack of expansion of water routes, that are used for migration.

Some of many important recorded intrinsic factors are inherited traits by the maternal salmon. Maternal effects play a large role in shaping early life phenotype in salmonids (Thorn, Morbey, 2018). Especially in small tanks, specified for hatching many eggs, the density is important as well as already mentioned. A high density can result in decreased growth in juvenile salmons (Rich et al, 2009).

Aside from water parameters, also stress has a huge impact on hatching in the early life stage. On the one hand stress exposure can modify the expression of thousands of genes, rendering many important functions in developmental processes, but on the other hand it can also reduce sensitivity to stress later in the juvenile fish, therefore having long-lasting effects on an organism's ability to adapt to environmental perturbations (Auperin, Geslin, 2008; Moghadam, 2017). In addition to this, also the hatching strategy is an interesting parameter. In natural systems salmons spawn their eggs in either nests or redds by making a shallow depression in the stream bottom. This slightly protects the eggs from the stream and predators. In aquacultures, tanks for only hatching are used, with every egg and/or alevin directly next to each other.

1.4. Objective of the presented thesis

Over the past years, methods for implementing efficient breeding and hatching programs were constantly optimized and improved. Based on the findings from human studies as well as a few model organisms, it is known that at least some environmental exposures, particularly those that are encountered during early life stages, can trigger developmental trajectories with lifetime impacts on the health, metabolism or behaviour of the animal (Szyf, 2013) Same will be for the natural and farmed Atlantic salmon (*Salmo salar*).
For the production and management of farmed species, every knowledge will become increasingly more important, as it will provide a basis for a better farming success. One day the humanity might depend on these farmed species. The production efficiency needs to improve.
The Atlantic salmon, is a species of great social, cultural, environmental, evolutionary and economic importance and is the most produced of all cultured marine cold-water fish in Europe (FEAP, 2019). Salmons spawn only once per year. The hatching success should be as high as possible.

Based on the mentioned aspects in the introduction this study deals with physical and biological parameters influencing the egg hatching success.

The hypothesis of this study is, that the egg hatching success, will differ significantly between different parental crossbreeds of one brood stock. Differences were measured by mortality rate, sinking rate, egg wet weight, egg size, parental salmon traits (e.g., body size, optical traits, condition, total sperm/eggs, age) and time until hatching.
A significant trackable difference in the hatching success could help an aquaculture to prefer specific salmons, due to their hatching success or the hatching success of the parental fish. It could also help to sort the eggs from salmon groups, depending on the expected outcome. Furthermore, a ranking inside the brood stock could be done.
Hence, the tracked crossbreeds can be used for future analysis e.g., growth, survival and more. Genetical material was taken as well, which is highly interesting and important for possible future analysis.

2. Materials and methods

2.1. Test facility and structure

“AquaFUTURE e.k.” is located in Ennepetal (Germany, North Rhine Westphalia) next to the “Hasper Talsperre”, which is a dam and grants the water supply. The water can be used for the facility because of an air pressure compensator. It mixes the water every day at 5pm to generate an equal distribution of all water parameters e.g., oxygen and temperature. In comparison to a normal lake where the temperature is 4°C at the bottom, it is equal everywhere. The water is transferred through a control system inside the facility, where the stream is divided. The facility exists out of 4 great halls (Fig. 5). Three halls are working with a flow-through system, where most of the water is needed. In average about 7,5 l/s, depending on the season. These halls contain different developmental stages of fish, depending on the season (fry, parrs, smolts and adult Atlantic salmons (*Salmo salar*)). A flow-through system constantly transfers fresh water through the facility. This increases natural oxygen, cools the water inside the system and most importantly can also wash out parasites etc. But at the same time, it can bring parasites and is strongly depending on the water supply and the parameters of the used water. In case the water inside the “Hasper Talsperre” becomes polluted or has too high nitrogen-, nitrate- or ammonium-concentrations, the water inside the facility will as well. This is not dangerous for the adult fish or smolt, but for the eggs, alevin and fry. Therefore, a recirculating aquaculture system (RAS) is used in the last hall.

As the name says the system uses water more than just one time and has a smaller total exchange. Less water supply means a lower hydraulic retention time (HRT), which means that it takes way more time to change the whole water in the system, than in a flow-through system. There is a freshwater supply of only 0,2l/s. Inside the hall is a filtration system to provide water purification. The water which enters this part of the facility must fit the necessary parameters. The whole filtration system exists twice, for the case that one part is broken. The whole filtration system, pipes, pumps, tubes and tanks were cleaned before the start of the experiment. Maintenance and calibration of everything that needed these steps were carried out as well and as often as needed.

Fig. 5. "AquaFUTURE e.k." in front of the "Hasper Talsperre"
The 4 halls are supplied with deep water from the sea. The left hall has an own RAS and is only used for hatching, containing eggs and alevins. The 3 other halls work with a flow-through system and contain fry, parrs, smolts and adult Atlantic salmons (*Salmo salar*).

The filtration system starts with 2 slow sand filters (Fig. 6. A). This filtration is based on granular loose media depth filters. By simply flowing through the filter, it removes fine solids (<100mm) and >90% of pathogens from the water. The sand filters were cleaned by the own washing function every 2 days, which is more than enough at the given filtration capability. They were working continuously.

After the water passes them, it flows through a drum filter (Fig. 6. C). This filtration is based on the mechanical trapping of particles via a filter screen. The control unit detects clogging on the filter mesh and activates the backwashing process to clean the filter mesh and wash the dirt particles into the waste water outlet, removing solids depending on the filter mesh (60µm stainless steel mesh in my case). This filter is not only used for new water entering the facility, it also works for the backwash. At this point recirculating water and fresh water out of the sand filters are mixed. Both flow at the same time inside the steel drum. The drum filter by "SENECT" has an own cleaning mechanic, which allowed me to ignore any maintenance after the calibration at the start of the experiment. One optical sensor for dissolved oxygen and one temperature sensor were placed inside the drum filter (next to the steel drum). Both of them are connected to the control unit of the drum filter, which usually displayed time until next wash, oxygen (mg/l and %) and temperature (°C). The washing process was set to

every 10 mins. This was the time until the water inside the drum reached a specific volume at the given flow rate.

The next filtration step was UV treatment, which is used to reduce bacterial numbers and control pathogens (Fig. 6. B). The water flows through a reactor where several UV lamps are placed inside specialised tubes (4 lamps/tubes in my case). UV light is damaging and denaturating the DNA of organisms. Most microorganisms (90-95% of known microorganisms) inside the water are killed this way. The exposure time needs to be between 240- 280nm. The UV-dosage is depending on the flowrate, UV transmittance of the water and the lamp intensity. Because of the low flowrate in this RAS, only 2/4 lamps were active. This also benefitted the water temperature. The rest of the settings were correctly set by “ULTRAQUA”, the reactor and lamp producer. The water disinfection via UV-light was continuous.

Fig. 6. Essential parts of the RAS for hatching. (**A**) A slow sand filter after the experiment, not in use. (**B**) The UV-reactor, which includes 4 tubes with UV-lamps. (**C**) The open drum filter with its control unit, not in use. (**D**) The cooling system after the experiment, not in use.

The cleaned water then flew through pipes into the tanks. Inside each of the 2 tanks were around 20 aquatic pond baskets. The baskets had 2mm wide holes on each side. The holes had the perfect size to prevent eggs from falling through them and to let the hatched alevin swim out of them. Since eggs need upstream water for oxygen, all 4 sites were taped off with cling film (Fig. 7.B). Water supply was then only possible via upstream. Each basket was signed with a waterproof pen to recognize the specific crossbreed inside of it. The buoyancy of the baskets and the eggs were enough to made it possible for the baskets to simply float inside the tank without sinking. To prevent too much movement of the baskets, which could harm the eggs, the density of baskets inside each tank was increased to a limit, where they could not move anymore (Fig. 7. A)

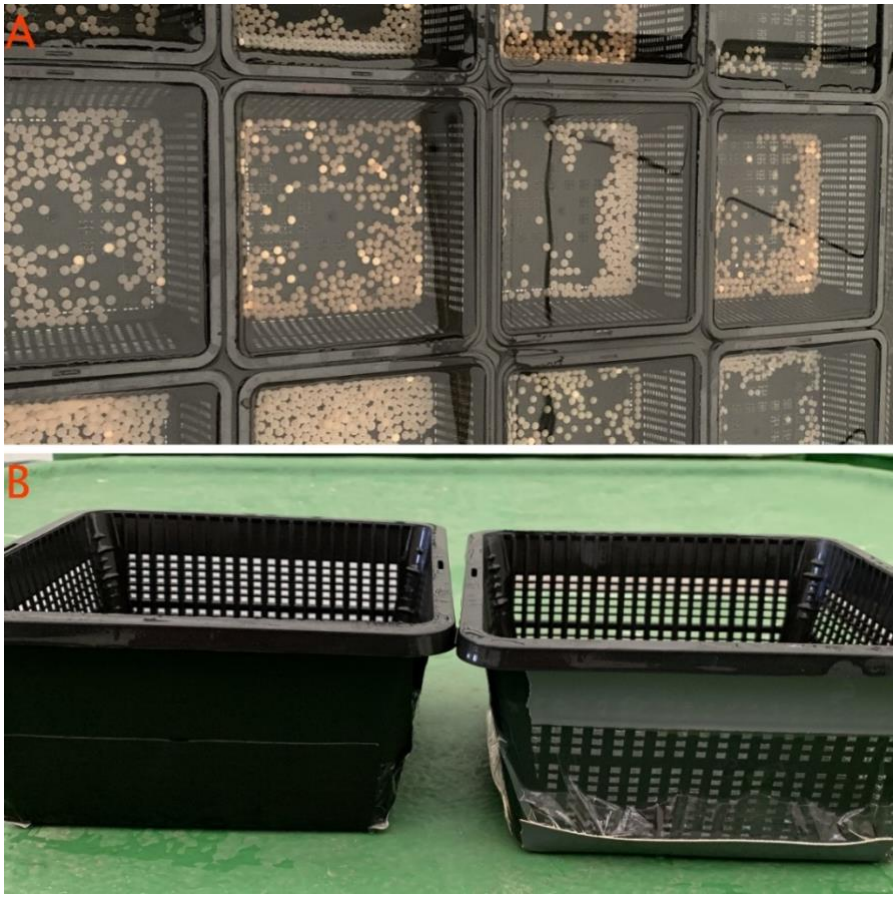

Fig. 7. Aquatic pond baskets in the tank. (**A**) The high density of baskets inside the water filled tank (94 x 94 x 19cm / water up to 13cm≈ 114l water) to prevent any movement. (**B**) Two examples of taped aquatic pond baskets (19 x 19 x 9mm) to guarantee up stream and oxygen supply.

The last part of the RAS was a cooling system. It mainly worked in the beginning of the experiment (during November/December), when the water temperature was still too high for healthy salmon growth. The cooling system also always displayed temperature and worked continuously when it was turned on (Fig. 6. D)
At this point the pipes led back to the drum filter.

2.2. Water parameters

The most important water parameters (temperature and oxygen) were measured continuously across the experiment. They were noted once per day, each day at 8:45am. As already mentioned, inside the drum filter were both optical sensors placed, one for oxygen (mg/L and %) and one for temperature (°C) measurement. The values were displayed on the control unit of the drum filter. Both sensors were calibrated before the experiment. To have guaranteed safe values, the measurements were double checked just before noting them with an optical sensor by "aquaFuture e.k.", which was calibrated once per week. The temperature was also measured and displayed a third time at the cooling system.
Another very important parameter is the pondus hydrogenii (pH). It was measured every day as well, but selectively and not continuously, because the change of the pH value is very slow in aquatic systems, if nothing dramatic happens to the system. It was measured using pH strips. Furthermore, the ammonium-content, nitrate-content and nitrite-content (mg/L) were measured every five days, because these values also change very slowly, and are less important in comparison to the pH. These measurements were made by using specific test-kits. The measurement range of these test kits were covering the average observed water parameters from the "Lachszentrum Hasper Talsperre". Fresh water from one tank was added to a test flask, using syringes (one for every single test).

The ammonium-content was measured using the "JBL ammonia test for fresh water and salt water". It indicates concentrations from <0,05 - 5 mg/l. Sodium hydroxide was added (0.4ml of one test kit liquid) to the fresh water, which reacted with the ammonium ions (NH_4^+), resulting in ammoniac (NH_3). Ammonia then reacts with the fresh water and produces ammonium ions and hydroxide ions. After shacking the flask for 30 seconds, a fluid universal indicator was added (0.4ml), which reacts with the resulting hydroxide ions. After 15 minutes the colour of the water inside the flask could be compared to a specific colour scale of the test kit (appendix) to receive a result.

$$NH_4Cl + NaOH \rightarrow NH_3 + H_2O + NaCl$$

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^-$$

The nitrate-content was measured using the "JBL nitrate test for fresh water and salt water". It indicates concentrations from 0-50 mg/l. The fresh water was mixed with a given amount of a powder containing zinc dust and glacial acetic acid. As a first result the nitrate ions were reduced to nitrite ions.

$$NO_3^- + Zn + 2H^+ \rightarrow NO_2^- + Zn^2 + H_2O$$

Then, the Lunges reagent was used (a mix of sulfanilic acid and 1-naphthylamine) to detect the nitrite ions. After adding the fluid Lunges reagent (0.6ml), the liquid had to be shaken for one minute. After 10 minutes of incubation the final result could be detected by comparing the colour change to a specific colour scale of the test kit (appendix).

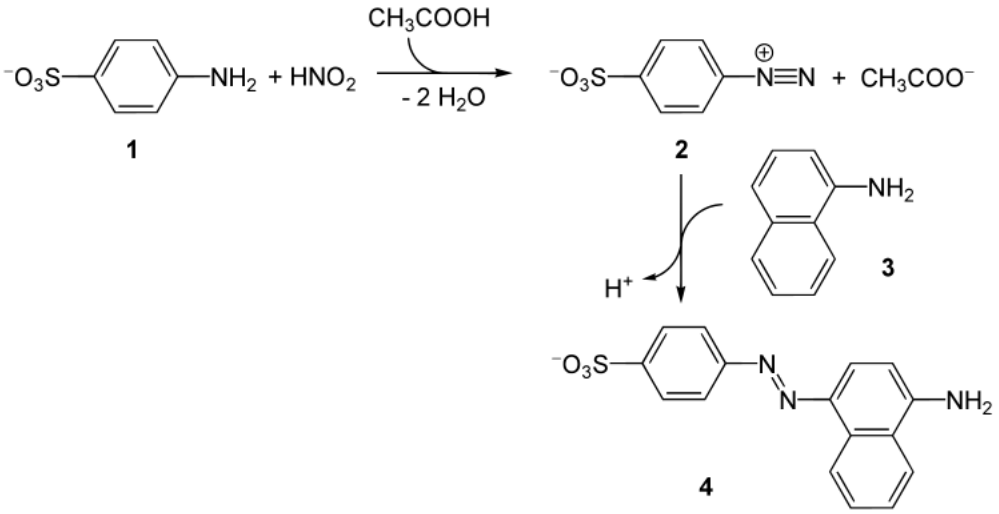

Fig. 8. Lunges reagent in water. Sulfanilic acid (1) turns into a diazonium salt (2), which reacts with 1-naptthylamine (3) to an azo dye (4), which turns the liquid red.

The nitrite content was measured using the "JBL nitrite test for fresh and salt water". It indicates concentrations from 0-0.2 mg/l. Only the fluid Lunges reagent (ml) was added to the fresh water (Fig. 8). After swinging the flask for 30 seconds and an incubation time of three minutes, a colour change could be observed and compared to a specific colour scale of the test kit (appendix).

2.3. Parental salmon treatment and striping

Before the striping and the experiment began, all used salmons (*Salmo salar*) had the same general treatment. They were in a good condition and not fed much, so the salmons could produce many eggs. The used salmons were chosen by different parameters. Three one-year old, three two-years old and three three-years old female salmons were used. Inside these groups the females had different colours and a different body size (if possible). All of the male salmons were between one- / and two-years old, because most of the male salmons only spawn once in their life and so for the first time. The males were also chosen by selecting different colours, patterns and body sizes (if possible). First of all, the female salmons were taken out of a huge tank and put inside a smaller one with just a little water. After that 10-20ml clove oil (depending on the water amount inside the smaller tank) was added to the water (1ml: 1l) and the salmons mixed everything by simply moving around. This was an absolute stress situation for the salmon and so their heart frequency increased. This made them exchange more water and air through the gills, where the clove oil showed its effect. The salmons were calming down by the numbing effect and became deeply relaxed within 2 minutes. After that it was possible to hold the salmon without it strongly moving and trying to get away. A ruler was fixed on the surface of the smaller tank to make pictures while placing the salmon next to it, for the body size measurements. A weight measurement was not possible, because all salmon become reconditioned after striping at “aquaFuture e.k.”. The Weighing of them would be too much stress and too risky in that state and consequently forbidden to me.

As a next step the salmons were washed inside fresh water from another tank, to get rid of any clove oil rest. Then a fin clip was taken. This was done by using a special device (Fig. 9. A) to precisely take out genetic material without harming the salmon (Fig. 9. D). The fin cures within some months.

Immediately after the fin clip, a swab from the gills was taken (Fig. 9. B). This was done by placing the swab between gills and turning it around five times, so enough blood could be gathered for possible later analysis.

Finally, the striping began. It is required to be very careful with the salmons to neither injure them nor burst the eggs inside them. Since it is better to do this with help one person hold the salmon and another person hold a dry bowl directly under the salmon. It is really important, that the bowl is dry, otherwise the eggs touching water start to swell, thus preventing fertilization. By gently striping with 2 fingers on each side of the stomach of the salmon, from cranial to caudal, the eggs literally flow out of the salmon in the dry bowl. After a female salmon spawned all of its eggs it was put into a third tank (as big as the first) to recover. The bowl with the eggs was instantly covered with a lid to prevent incidence of light.

Fig. 9. Body size measurement and devices for collecting genetic material. (**A**) The tool used to produce fin clips and instantly place the clipped part into ethanol for conservation the genetic material for possible later use. (**B**) A swab used for blood sampling between the gills. (**C**) The male parental Atlantic salmon (*Salmo salar*) M7 next to the ruler fixed in the small tank. (D) A fresh fin clip of an Atlantic salmon done with the shown tool.

Then we could continue with the male salmons (still two persons). The process before striping is similar to the process with female salmons (clove oil effect, photo next to ruler, wash, fin clip, gill swab). The bowl to collect the sperm had to be dry again, otherwise fresh water shortly extends sperm activity and the sperm becomes inactive after a minute. The sperm was then collected in a syringe (new ones for each male salmon). After the male spawned all of its sperm it was put into a fourth tank (as big as the first, but only for male salmons) to recover.

The process was repeated for three female salmons and three male salmons in one day, because other methods took a long time, which slowed everything down. The three female salmons were striped right after each other. The three male salmons had at least 1- 1,5h between the striping.

2.4. Egg and sperm parameters

It was required to make the analysis as fast as possible. Most of them were done between the striping of two male parental Atlantic salmons (*Salmo salar*). In a first step the sperm analysis was done. The total volume (ml) could be observed with the syringe scaling. After that the spermatocrit was detected, which works similar to haematocrit, just for semen cells and not for blood cells. For this process 10µl sperm was sucked in a capillary, by capillary forces. A centrifuge (Fig. 10. A.) by “Compur-Electronic GmbH” (COMPUR M1100) was used to detect the semen cell concentration (%) inside the sperm volume (ml). One minute of centrifugation was enough for each sample. Also, the sperm concentration (cell/ml) was detected by using a haemocytometer. A “Zeiss” brightfield microscope with a magnification of 400x was required to see any semen cells. The dilution factor was 1:10000.

After the sperm analysis, the egg analysis started. The total egg production (g) of each female salmon was measured, using an analytical balance (Fig. 10. B.) by “Kern®” (ABJ 220-4NM). The eggs were decanted in another bowl, which was already placed on the balance and used for calibration. While decantation nothing was removed. Neither overripe, nor damaged eggs. After measuring and noting the total egg production, ten eggs were taken away from the bowl and a next measurement was made to declare a new weight. This new weight was used to split the remaining eggs in three equal parts for three crossbreeds (three new labelled bowls). The ten single eggs were placed next to a ruler fixed on a wet paper (Fig. 10. C.) to make pictures for an as exact as possible determination of the egg size (mm) with ImageJ. The underground of the eggs had to be wet so they would not instantly lose weight or shrink because of losing water to any other surface. Right after that, the single eggs were weighed with the analytical balance (mg). Moreover, the sinking rate was observed. Each egg was dropped inside a measuring cylinder, containing a 25cm high water column. The time from the first moment when an egg touched the water until the time it reached the ground was measured.

These processes were repeated with all three female/ male salmons per day.

After fertilization, the eggs inside the bowls were put in the associated labelled baskets, which were already placed in water inside the tanks.

The egg weight and egg seize were measured several times after the first time. Directly after the fertilization, seven days after incubation in water, right after reaching the eyed stage and after hatching. For the last measurement of this kind, the parameters turned into alevin weight and alevin length. These were measured right after the first ten+ alevin spawned inside one basket. The eggs or alevins were taken out of each basket by using different specialised big syringes with a wide opening, so neither the alevin nor the eggs became damaged. Every egg and alevin ever measured was placed in a labelled Eppendorf tube and was frozen.

Labels were made in terms of crossbreed e.g., F1 x M2 -> female 1 fertilized by male 2.

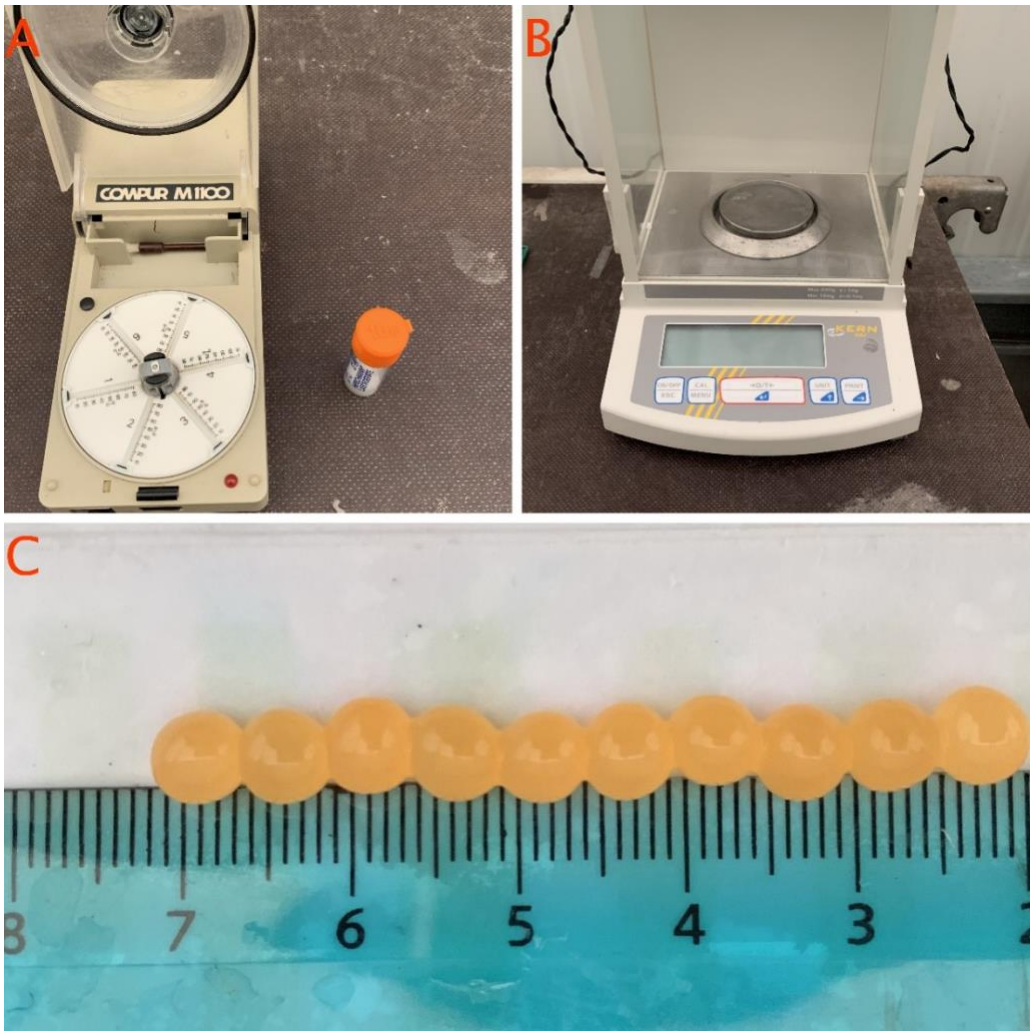

Fig. 10. Devices for measurements. (**A**) The centrifuge COMPUR M1100 for spermatocrit measurement and 10µl capillaries. (**B**) The analytical balance ABJ 220-4NM by Kern®. (**C**) The ruler with wet paper fixed to it as an underground for the eggs.

2.5. Fertilization process

It was required to do the fertilization process between the striping of each male Atlantic salmon (*Salmo salar*). Every bowl filled with eggs was covered until and directly after its own fertilization phase, to prevent incidence of light. When the fertilization began, exactly 9 bowls were there the first time. Starting with male M1, it was required to start with 3 bowls, filled with eggs from a different female salmon (F1xM1, F2xM1, F3xM1). Now, hand disinfection and hand washes were made twice to ensure egg safety. Then 0,7ml sperm was added to the first bowl and one was able to start gently mixing the eggs with the sperm using the bare hands. After exactly 40 seconds of mixing, fresh water was added into the bowl (until all eggs were covered) and the mixing continued for 20 more seconds. As a next step, it was required to wash the sperm out of the fresh water in the bowl. More fresh water was added with a

hose, which created an upswing of semen cells. The water was decanted while adding new fresh water with the hose. After most of the turbidity (semen cells) was gone, it was stopped adding water with the hose and instead one stirred the fresh water inside the bowl and decanted it for a last time. Two more washes were made in the same way. After that, the bowl was filled with as much fresh water as possible and the eggs remained there for 1:15h to swell. The eggs were then resistant to most vibrations etc. for the next 24h, after that already any medium vibration (e.g., touching an egg) was able to fatally damage the eggs. The whole procedure was done with the first three bowls filled with eggs from different female salmons. Afterwards, it was waited for the next striping of a male salmon. The next sperm was then used for the next three bowls (F1xM2, F2xM2, F3xM2) and so on.

2.6. Mortality rate

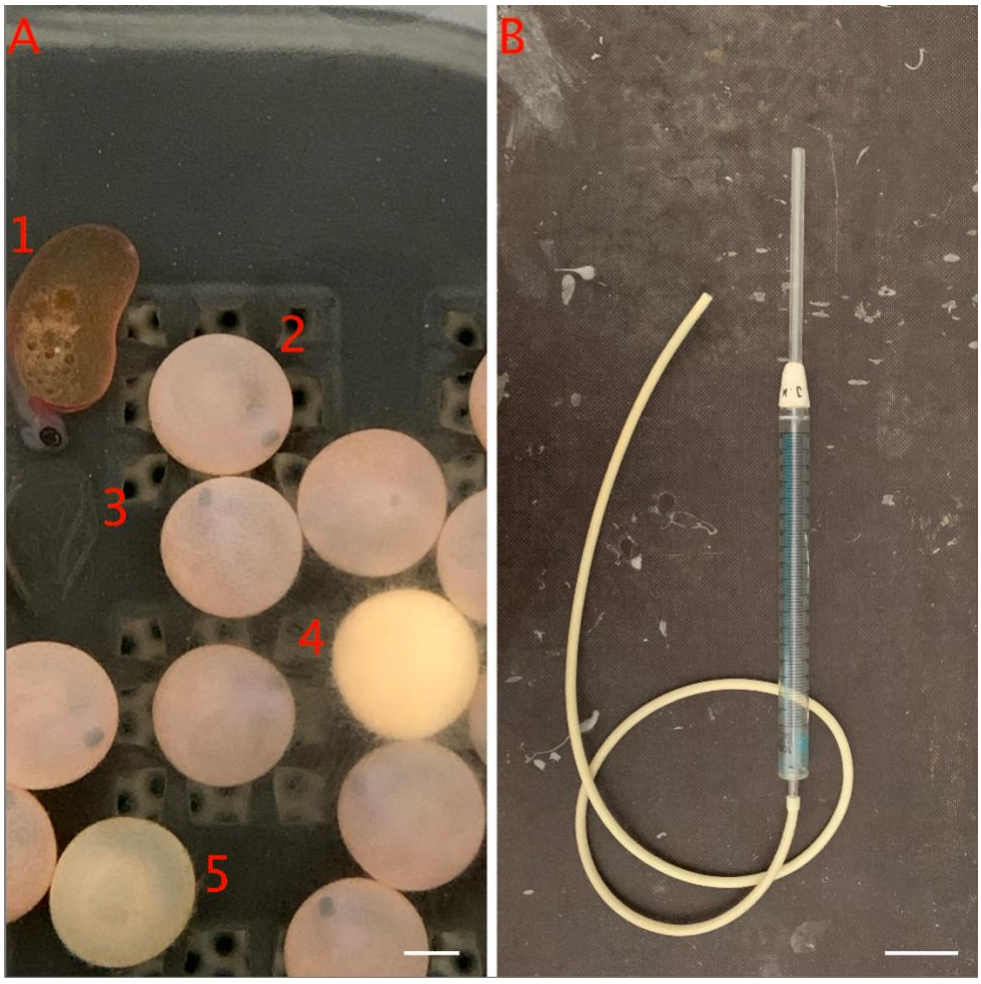

Fig. 11. The different possible observations inside a basket except overripe eggs, and the device to handle them cautiously. (**A**) 1) A newly hatched Atlantic salmon (*Salmo salar*) as an alevin. 2) An eyed stage egg. 3) An egg shell. 4) An egg with fungus and spore growth infecting its neighbour eggs. 5) A damaged/ dead egg, without any fungus. (**B**) A modified large syringe for handling eggs. Scale bar (A), 2mm. Scale bar (B), 5cm.

The cumulative egg mortality rate was measured by observing all baskets every two days. The number of dead or damaged eggs was noted, they could be recognized by white spots or blurring (Fig. 11. A, 5). Egg shells and overripe eggs were noted, but not counted to the mortality rate (Fig. 11. A, 3) Dead eggs were not taken out of the baskets until they fungus and spores grew (Fig. 11. A, 4) “Healthy” eggs which were infected with spores from another damaged egg, were not taken out of the baskets because of that. The measurement was made from 9:30am – 10:30am every time for 90 days (45 times). A large modified syringe with a wide opening (at least wide enough for salmon eggs) was used when eggs had to be taken out of the baskets because of fungus. The syringe had a usual barrel but instead of a syringe plunder, a thin hose was connected (Fig. 11. B). This made it possible to either smoothly pull the eggs inside the barrel or blow them away from each other to prevent collision while trying to select the damaged eggs. New dead eggs were always added to the count, while the eggs taken out were noted separately. The days, when the first eggs reached the eyed stage and the first eggs hatched were noted as well.

2.7. Statistical programs

The website “https://www.socscistatistics.com” was used for all statistical tests. The students t-test for independent means was used to determine whether there was statistical evidence that the independent means are significantly different to each other (https://www.socscistatistics.com/tests/studentttest/default2.aspx).

In addition to that, “Microsoft excel” was used for mean and standard deviation calculation. Diagrams and tables were also made that way.

Apart from that, “Fiji (ImageJ) was used for size measurements. The plugin “FigureJ” was used to create some figures.

3. Results

The nine female parental Atlantic salmons (*Salmo salar*) are called F1- F9. The nine male parental Atlantic salmons are called M1-M9. The Atlantic salmon group F1-F3 contains three-years old salmons, group F4-6 contains two-years old salmons and group F7-9 contains one-year old salmons. A salmon group solely means, that they are sorted by age. Egg and alevin weights are wet weights.

3.1. Water parameters and degree days

The water parameters varied between 1.3 and 7.9°C over 3 months of measurement (Fig. 12). Dissolved oxygen was never too low or too high. It was constantly between 11 and 13.8mg/l. The nitrate content was constantly at 30mg/l, the ammonium content was constantly at < 0.05mg/l and the nitrite content was constantly at 0.01mg/l. The pH was constantly at seven (appendix). All Values were always inside the limits for good conditions. A total count of 453,9-degree days were observed.

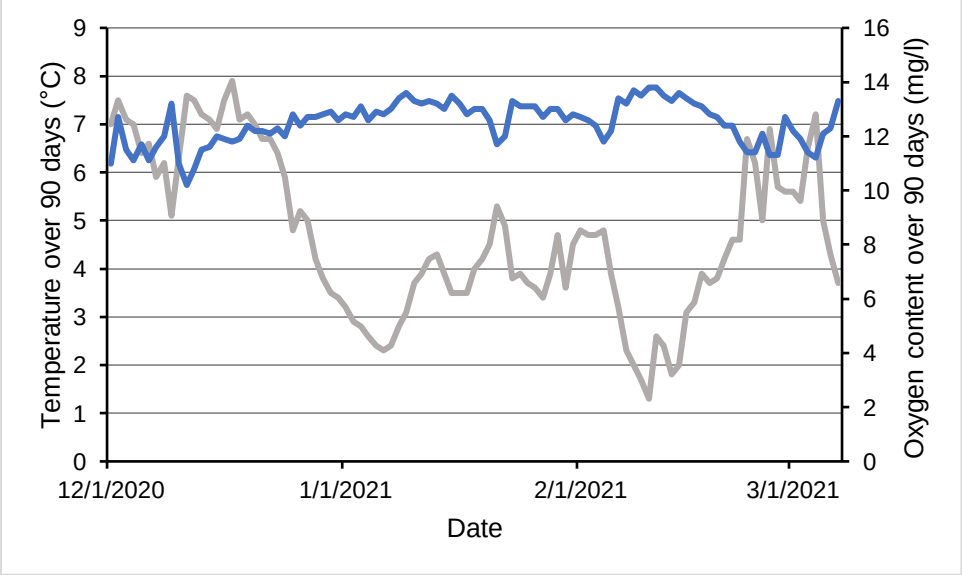

Fig. 12. Water temperature (°C, **grey**) and Oxygen content (mg/l, **blue**) over 90 days.

The degree days until the first alevin hatched do not display an average degree day for hatching. There is no significant age dependence on degree days until the salmon eggs hatch, but a strong correlation has been detected. In average salmon eggs from older parental female fish seem to take longer for hatching (Fig. 13).

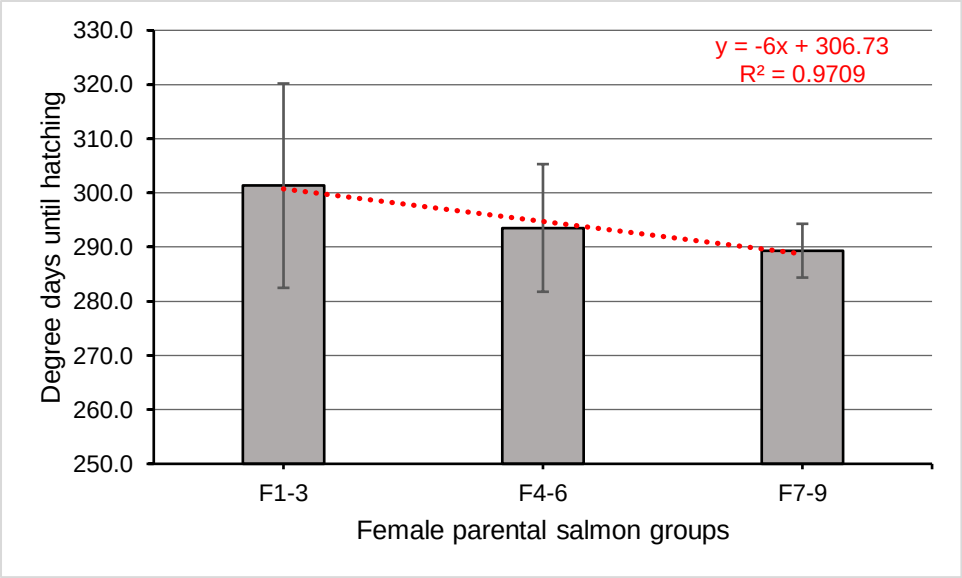

Fig. 13. Degree days until hatching of the female parental salmon groups sorted by age. Data are mean ± s.d. (*n*= 3 salmon per group).

The mean alevin weight (mg) after hatching from different salmon groups showed no correlation with the degree days until hatching (Fig. 14). The difference between the degree days until hatching was also not significant.

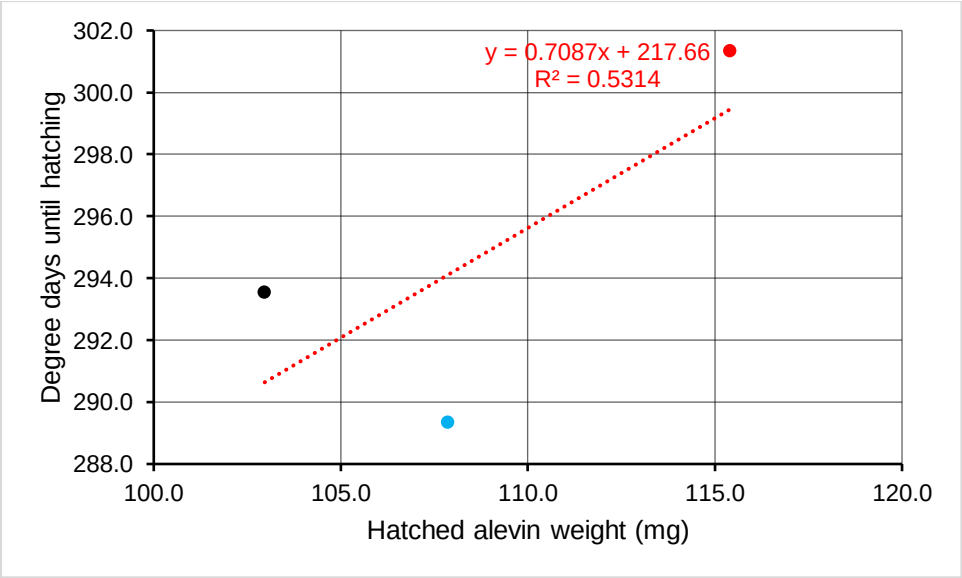

Fig. 14. Degree days until hatching in comparison to the hatched alevin weight (mg) after ten+ alevins hatched from female parental salmon groups sorted by age (*blue*= one-year old; *black*= two-years old; *red*= three-years old). Data are mean (*n*= 30 alevins per group).

The degree days until hatching showed no correlation to the cumulative egg mortality rate after 90 days (%) of different parental female salmons. Therefore, a little tendency shows, that eggs seem to have a lower cumulative egg mortality rate after 90 days if they hatch

faster (Fig. 15). An equal observation was made for the comparison between the degree days until hatching and the cumulative egg mortality rate after 90 days of different salmon groups. In this case a lower cumulative egg mortality rate occurred with faster hatching as well (Fig. 16).

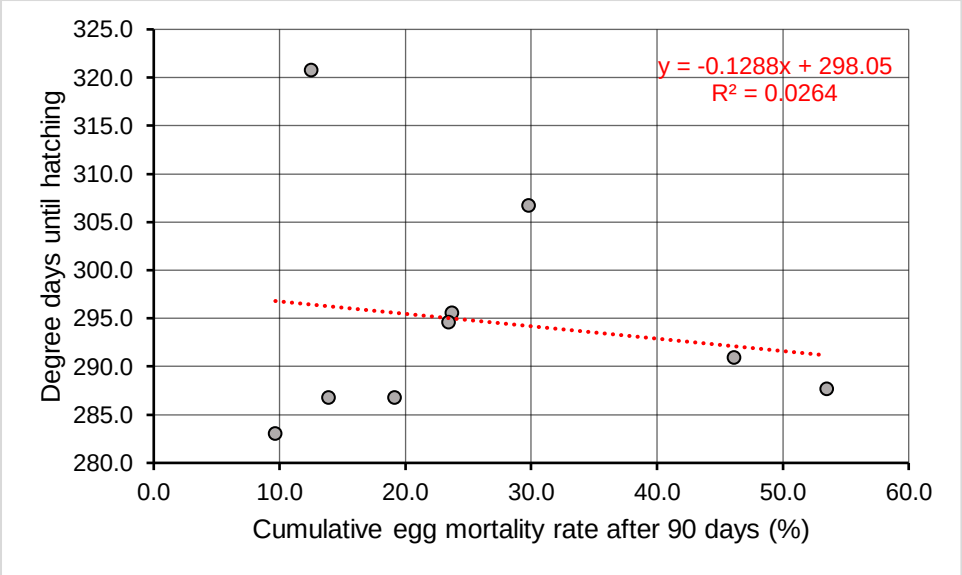

Fig. 15. Degree days until hatching in comparison to the cumulative egg mortality rate after 90 days (%) of female parental salmons. Data are mean.

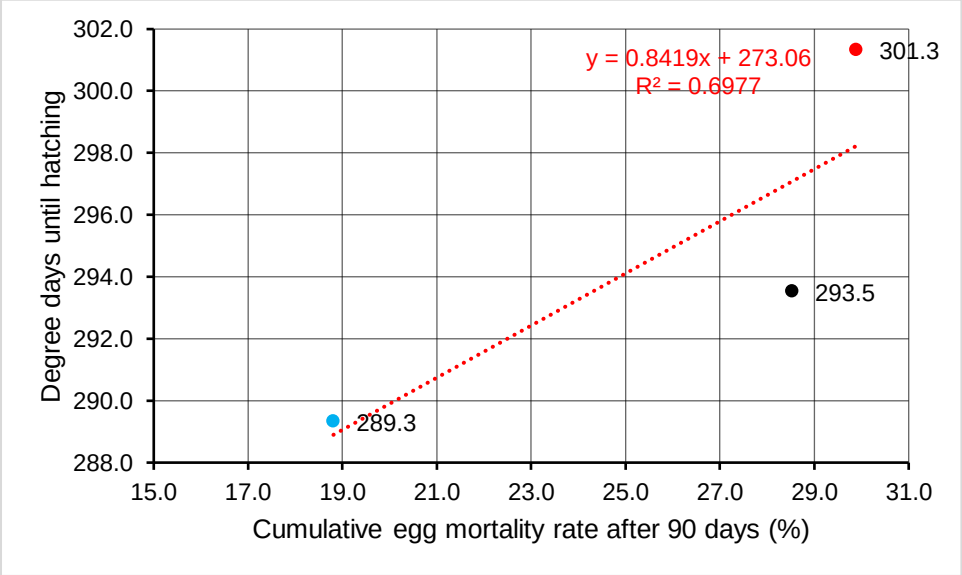

Fig. 16. Degree days until hatching in comparison to the cumulative egg mortality rate after 90 days (%) of female parental salmon groups sorted by age (*blue*= one-year old; *black*= two-years old; *red*= three-years old). Data are mean (*n*= 3 salmon per group).

3.2. Influence of biometric salmon data on eggs and sperm

The female parental salmon length is correlated to the total egg production (g) spawned after one completed striping process (Fig. 17). A longer salmon has a higher total egg production, which can be connected to the estimated number of eggs spawned by that salmon (Table 1). This applies more to old and fully grown salmons than to young salmons. Furthermore, a salmon must have a minimal size of approximately 40cm to produce eggs.

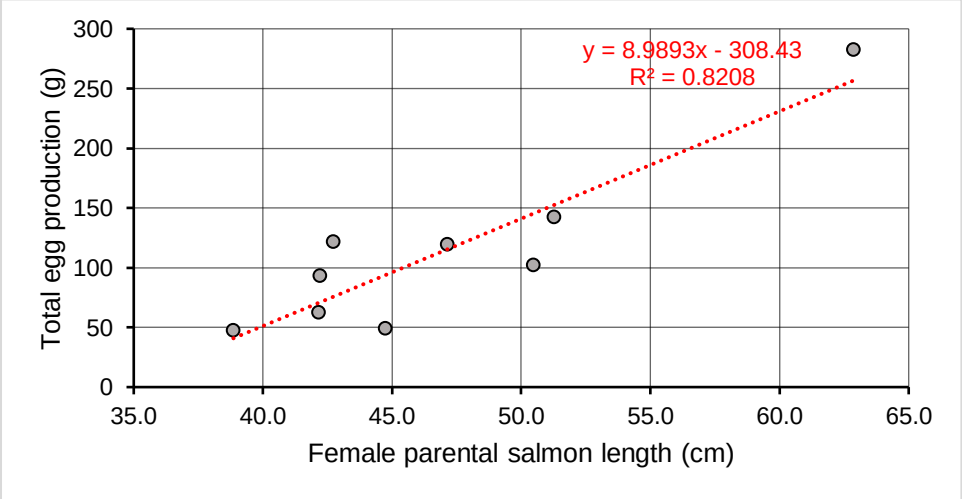

Fig. 17. Total egg production (g) spawned by a female parental salmon in comparison to its length (cm).

Table. 1. Parental female salmon length (cm) in connection with its total egg production (g) and the estimated total number of eggs spawned in one striping process.

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Parental length (cm)	62,9	51,3	50,5	42,2	47,1	42,7	44,7	38,9	42,2
Total egg production (g):	282,5	142,4	102,3	93,5	119,8	121,8	49,1	47,5	62,4
Estimated total eggs:	2123	1409	1043	1293	1175	1045	474	492	576

The male parental length is not correlated to the total amount of sperm (ml) given by one striping process (Fig. 18). Yet, a minimum size of approximately 30cm is required to produce any sperm. There is still a little tendency, that smaller salmons down to the limit of approximately 30cm produce more total sperm volume (ml). Spermatocrit (%) from 16% to 26% was measured. Total sperm volumes (ml) from 3,1ml to 7,8ml were measured (Fig. 19). There is no correlation between the spermatocrit and the total sperm volume produced after one striping process.

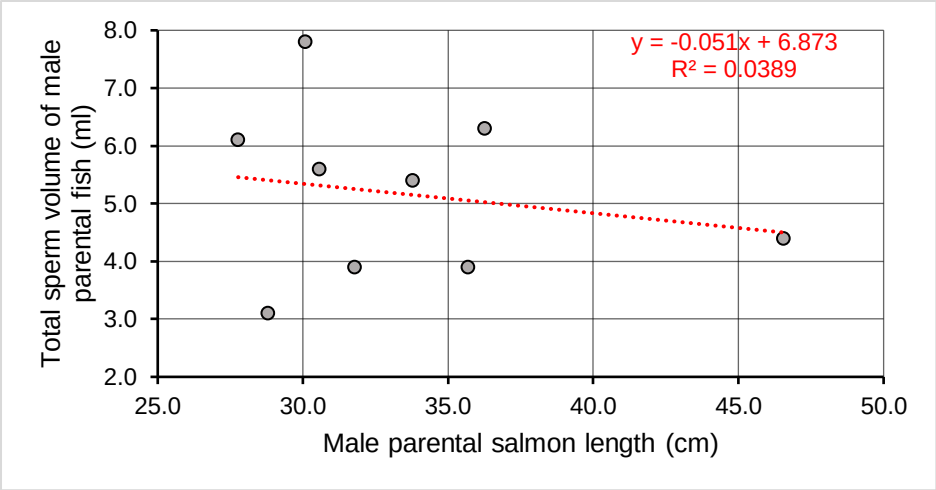

Fig. 18. Total sperm volume taken of a male parental salmon (ml) in one striping process in comparison to its length (cm).

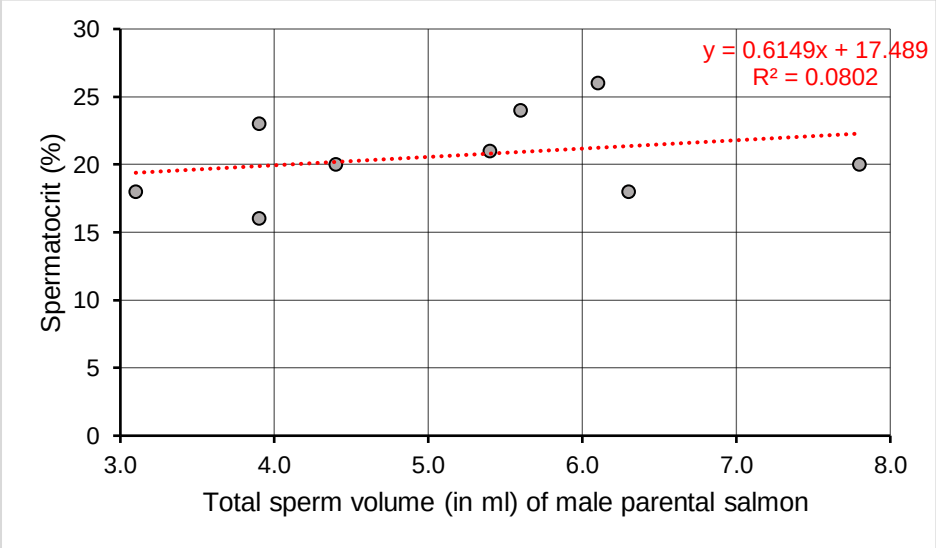

Fig. 19. The spermatocrit (%) inside 10µl sperm volume of male parental salmons in comparison to their total sperm volume (ml).

3.3. Differences in egg parameters

Egg weight

The mean individual egg weight (mg) of most female parental salmon was significantly different to each other at the beginning of the experiment (Fig. 20. Table 2). Salmon F4 spawned very light eggs compared to the others. Salmon F1 spawned the biggest eggs. Moreover, the mean individual weight of eggs from one-year old salmons was close to each other and to the mean for all eggs. The other salmon groups had highly divergent values within the group. In average three-years old salmons, which are most likely fully grown, have significantly heavier eggs than younger salmons (Fig. 21). There was no significant difference between two-years old and one-year old salmons.

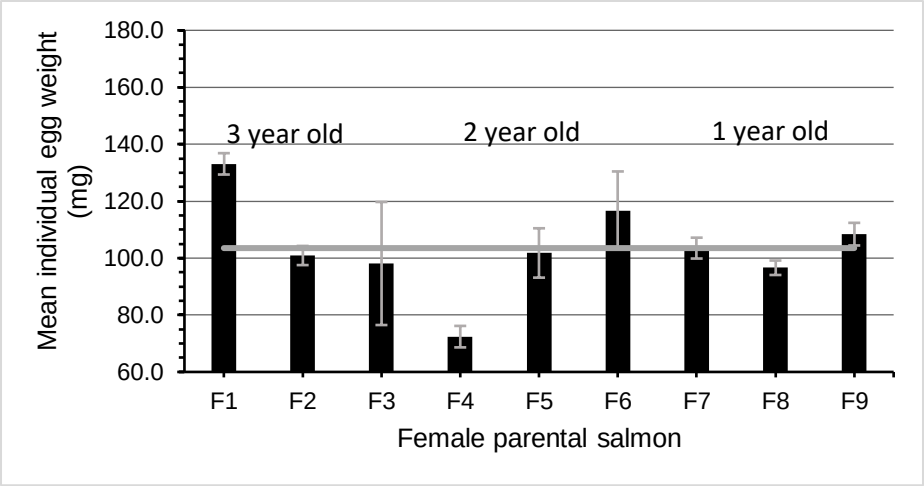

Fig. 20. Mean individual egg weight (mg) of female parental salmons right after spawning. The grey bar shows the mean value for all eggs. Data are mean ± s.d. (*n*= 10 eggs per salmon).

Table 2. T/- and p-values of the difference between the mean individual egg weights (mg) of different female parental salmons right after spawning (*red*= not significant; *blue*= significant).

Salmon	F1	F2	F3	F4	F5	F6	F7	F8
F1								
F2	19.2/ 0.001							
F3	4.8/ 0.001	0.4/ 0.352						
F4	34.2/ < 0.001	16.9/ < 0.001	3.5/ 0.001					
F5	9.9/ < 0.001	-0.3/ 0.387	-0.5/ 0.320	-9.3/ < 0.001				
F6	3.5/ 0.001	-3.4/ 0.001	-2.2/ 0.021	-9.3/ < 0.001	-2.8/ 0.007			
F7	16.9/ < 0.001	-1.6/ 0.067	-0.7/ 0.234	-17.7/ < 0.001	-0.5/ 0.297	2.8/ 0.006		
F8	24.2/ < 0.001	3.1/ 0.003	0.2/ 0.419	-16.0/ < 0.001	1.7/ 0.051	4.3/ 0.001	4.6/ 0.001	
F9	13.5/ < 0.001	-4.3/ 0.001	-1.4/ 0.088	-19.7/ < 0.001	-2.1/ 0.026	1.7/ 0.049	-2.7/ 0.007	-7.5/ < 0.001

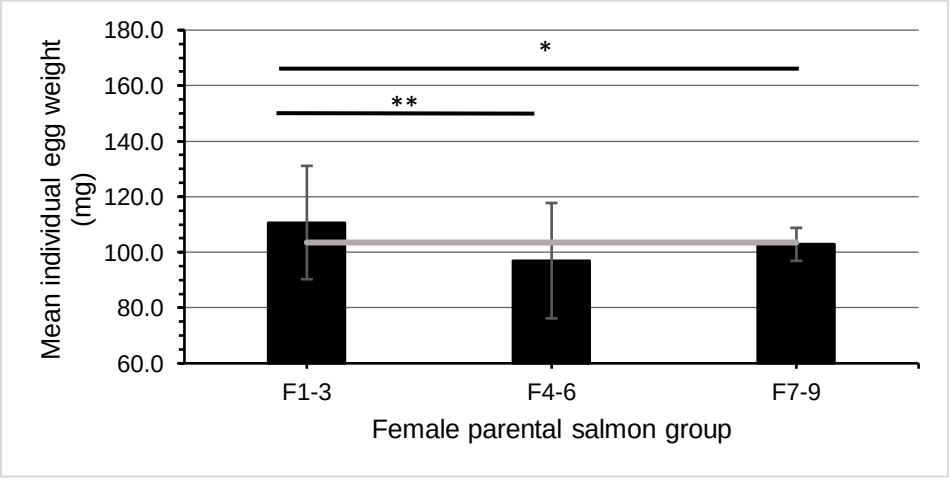

Fig. 21. Mean individual egg weight (mg) of female parental salmon groups sorted by age, right after spawning. The grey bar shows the mean value for all eggs. Data are mean ± s.d. (*n*= 3 salmon per group; 10 eggs per salmon, **P*= 0.025, ***P*= <0.001).

The mean individual alevin weights (mg) of different female parental salmons, after the first ten+ alevins hatched were significantly different to each other in most cases (Fig. 22. Table 3). The ranking is similar to the ranking of the mean individual egg weight right after spawning (Fig. 20), but for F3. The eggs /alevins of F3 increased their weight even more than the other eggs /alevins. Also, the mean individual alevin weight of one-year old salmons was close to each other, in comparison to the divergent values within the other salmon groups.

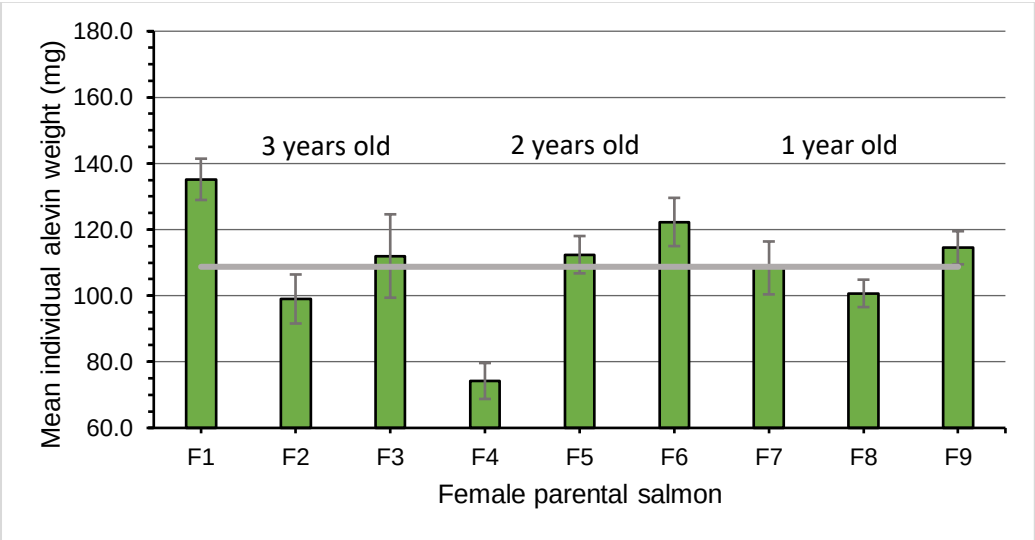

Fig. 22. Mean individual alevin weight (mg) of alevins from different female parental salmons, after the first ten+ alevins hatched. The grey bar shows the mean value for all alevins. Data are mean ± s.d. (*n*= 10 alevins per salmon).

Table 3. T/- and p-values of the difference between the mean individual alevin weights (mg) of different female parental salmons, after the first ten+ alevins hatched (*red*= not significant; *blue*= significant).

Salmon	F1	F2	F3	F4	F5	F6	F7	F8
F1								
F2	11.2/ < 0.001							
F3	4.9/ 0.001	-2.7/ 0.008						
F4	22.1/ < 0.001	8.1/ < 0.001	8.3/ < 0.001					
F5	8.1/ < 0.001	-4.3/ < 0.001	-0.1/ 0.466	-14.6/ < 0.001				
F6	4.0/ < 0.001	-6.7/ < 0.001	-2.1/ 0.024	-15.9/ < 0.001	-3.2/ 0.002			
F7	7.9/ < 0.001	-2.6/ 0.009	0.7/ 0.239	-10.6/ < 0.001	1.2/ 0.118	3.9/ < 0.001		
F8	13.8/ < 0.001	-0.6/ 0.278	2.6/ 0.01	-11.6/ < 0.001	5.0/ < 0.001	7.7/ < 0.001	2.6/ 0.01	
F9	7.8/ < 0.001	-5.2/ < 0.001	-0.6/ 0.294	-16.3/ < 0.001	-0.8/ 0.207	2.6/ 0.008	-1.9/ 0.034	-6.4/ < 0.001

In average, the eggs of the older salmons, which are most likely fully grown, significantly result in heavier individual alevins than younger salmons (Fig. 23). There was no significant difference in the mean individual alevin weight for two-years old and one-year old salmons.

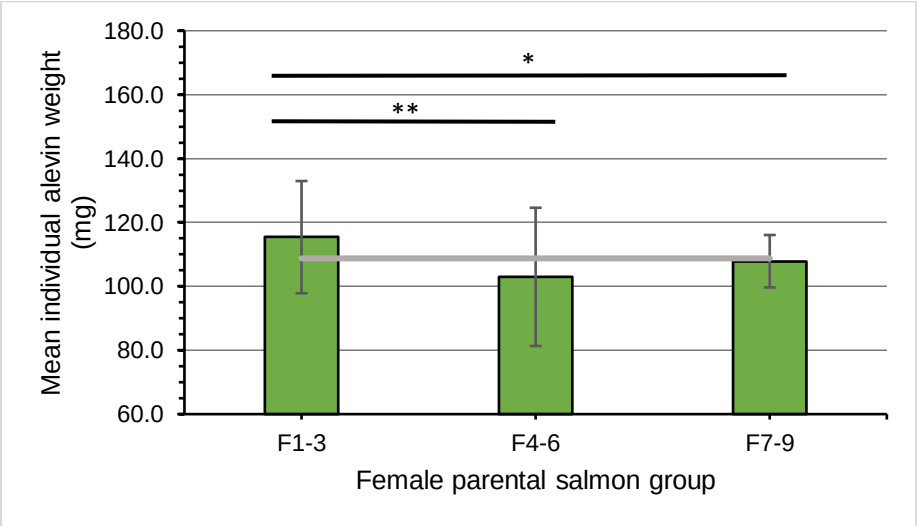

Fig. 23. Mean individual alevin weight (mg) of alevins from different female parental salmon groups sorted by age, right after spawning. The grey bar shows the mean value for all alevins. Data are mean ± s.d. (*n*= 3 salmon per group; 10 alevins per salmon, **P*= 0.02, ***P*= 0.01).

The mean individual egg weight strongly increased from spawning to after fertilization for all eggs (Fig. 24).

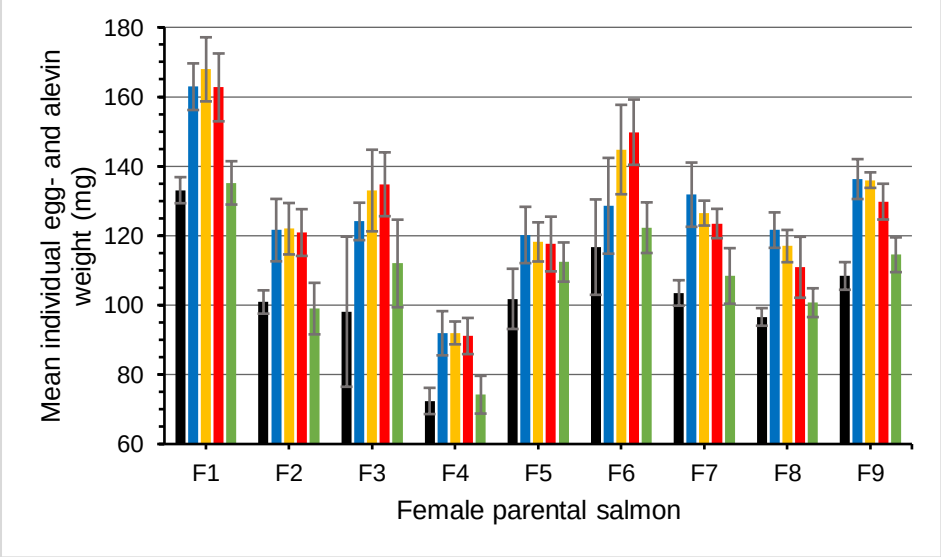

Fig. 24. Mean individual egg- and alevin weight (mg) of female parental salmons. (**Black**) egg weight right after spawning. (**Blue**) egg weight after fertilization and 60 min incubation in fresh water. (**Yellow**) egg weight after 7 days of incubation in fresh water. (**Red**) egg weight after the first ten+ eggs reached the eyed stage. (**Green**) alevin weight after the first ten+ eggs hatched. Data are mean ± s.d. (*n*= 10 eggs /alevins per salmon).

For some salmons eggs the mean weight then increased after seven days of incubation in water and when reaching the eyed stage (F3, F6). In other salmon eggs the individual mean weight increased after seven days of incubation in water and then decreased while reaching the eyed stage (F1, F2, F4). For most salmon eggs the individual mean weight continuously decreased after a maximum at fertilization (F5, F7. F8, F9). The individual mean alevin weight was lower for all salmons than their individual mean egg weight after fertilization (Fig. 19).

The progress from fertilization to the eyed stage of the individual mean egg weight was observed for the salmon groups (Fig. 25) The individual mean egg weight of the oldest tested salmons is higher than the ones of youngest tested salmons.

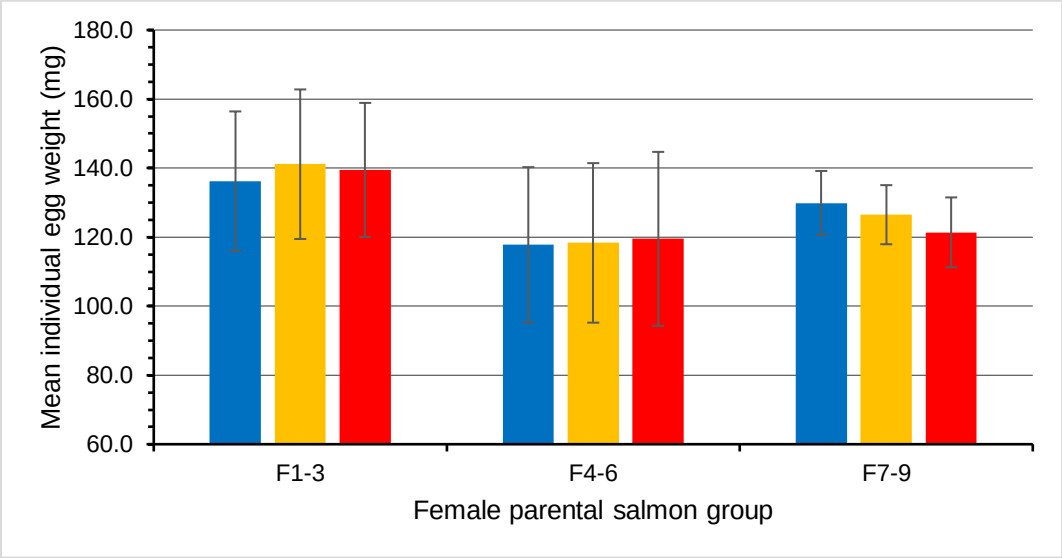

Fig. 25. Mean individual egg- and alevin weight (mg) of female parental salmon groups sorted by age. (**Blue**) egg weight after fertilization and 60 min incubation in fresh water. (**Yellow**) egg weight after 7 days of incubation in fresh water. (**Red**) egg weight after the first eggs reached the eyed stage. Data are mean ± s.d. (*n*= 10 eggs per salmon).

Egg size

The mean individual size (mm) of eggs from all (but two) parental female salmons showed no significant difference at the beginning of the experiment. There was a significant difference between the size of eggs from the three-years old salmon group and the two-years old salmon group.

The mean individual alevin yolk sac length (mm) was not significantly different for any comparison (Fig. 26. Table 4). There is always a big distribution within the salmon groups. There was also no significant difference in the salmon groups. Still, the alevin yolk sacs of alevins from the oldest salmons seem to be averagely longer than the ones of alevins from younger salmons (Fig. 27).

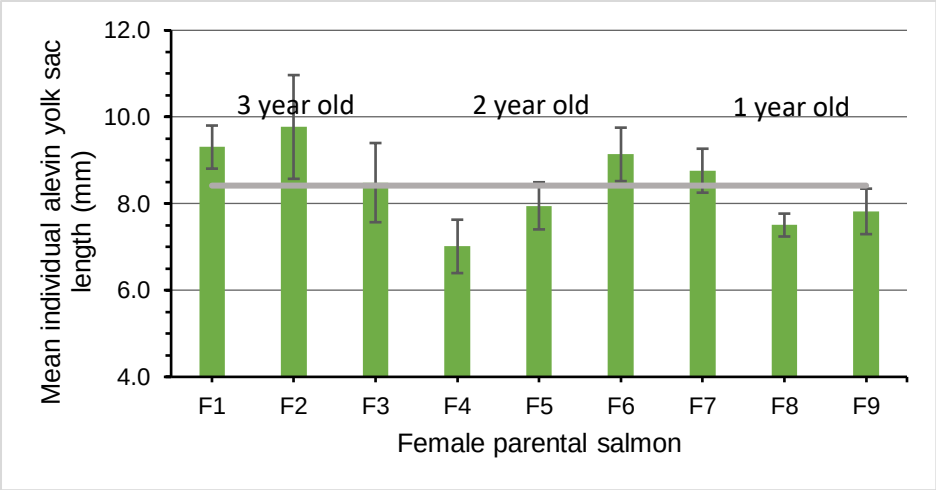

Fig. 26. Mean individual alevin yolk sac length (mm) of alevins from different female parental salmons after the first ten+ alevins hatched. The grey bar shows the mean value for all alevins. Data are mean ± s.d. (*n*= 10 alevins per salmon).

Table 4. T/- and p-values of the difference between mean individual alevin yolk sac lengths (mm) of alevins from different female parental salmon after the first ten+ alevins hatched (*red*= not significant).

Salmon	F1	F2	F3	F4	F5	F6	F7	F8
F1								
F2	0.2/ 0.433							
F3	0.1/ 0.459	-0.1/ 0.472						
F4	-0.02/ 0.490	-0.2/ 0.420	-0.1/ 0.448					
F5	0.3/ 0.396	0.1/ 0.455	0.2/ 0.430	0.3/ 0.382				
F6	-0.1/ 0.442	-0.3/ 0.37	-0.3/ 0.397	-0.1/ 0.45	-0.4/ 0.337			
F7	-0.2/ 0.403	-0.4/ 0.334	-0.4/ 0.36	-0.2/ 0.409	-0.1/ 0.305	-0.1/ 0.457		
F8	0.01/ 0.497	-0.2/ 0.433	-0.1/ 0.460	0.03/ 0.487	-0.3/ 0.394	0.2/0.437	0.3/ 0.397	
F9	-0.1/ 0.475	-0.2/ 0.405	-0.2/ 0.432	-0.04/ 0.483	-0.3/ 0.369	0.1/ 0.467	0.2/ 0.426	-0.1/ 0.471

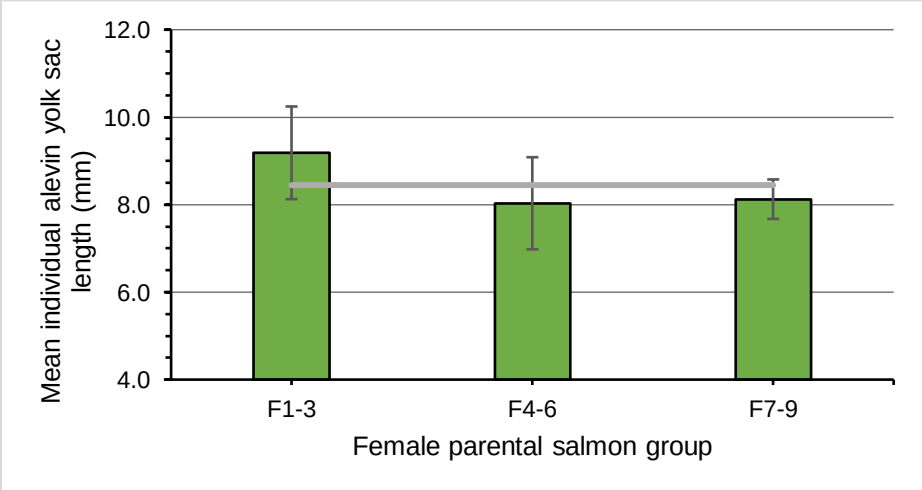

Fig. 27. Mean individual alevin yolk sac length (mm) of alevins from different female parental salmon groups sorted by age, after the first ten+ alevins hatched. The grey bar shows the mean value for all alevins. Data are mean ± s.d. (*n*= 3 salmon per group; 10 alevins per salmon).

The mean individual egg size of eggs from female parental salmons already showed different progress right after fertilization (Fig. 28). The eggs from most salmons grew significantly and directly after fertilization (F1, F2, F3, F7, F8, F9). After seven days of incubation in water the eggs of these salmons were smaller. After reaching the eyed stage different outcomes could be observed. Either the mean individual egg size kept on to decrease (F1, F7, F9), or it started to increase again (F2, F3, F8). The eggs from salmons of salmon group F4-6 seemed to shrink at fertilization. Therefore, all of the eggs from these salmons (F4, F5, F6) grew after fertilization until hatching. The mean individual alevin yolk sac length was always significantly higher than the mean individual egg size at any time. The biggest recorded mean individual egg size was not from the salmon with the biggest recorded mean individual alevin length. The mean individual egg size and alevin yolk sac length from the oldest salmons tends to be higher (Fig. 29).

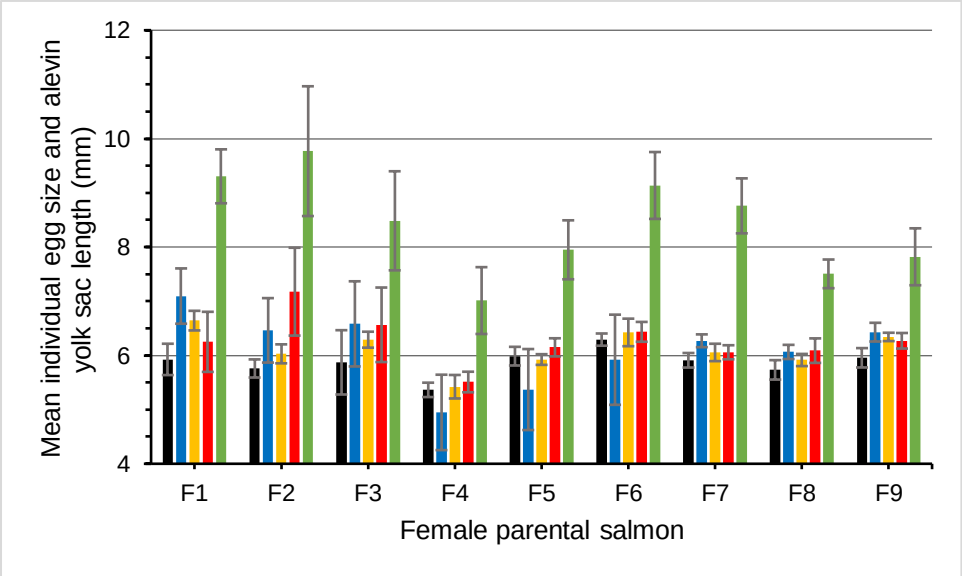

Fig. 28. Mean individual egg size and alevin yolk sac length (mm) of female parental salmons. (**Black**) egg size right after spawning. (**Blue**) egg size after fertilization and 60 min incubation in fresh water. (**Yellow**) egg size after 7 days of incubation in fresh water. (**Red**) egg size after the first ten+ eggs reached the eyed stage. (**Green**) alevin yolk sac length after the first ten+ eggs hatched. Data are mean ± s.d. (*n*= 10 eggs /alevins per salmon).

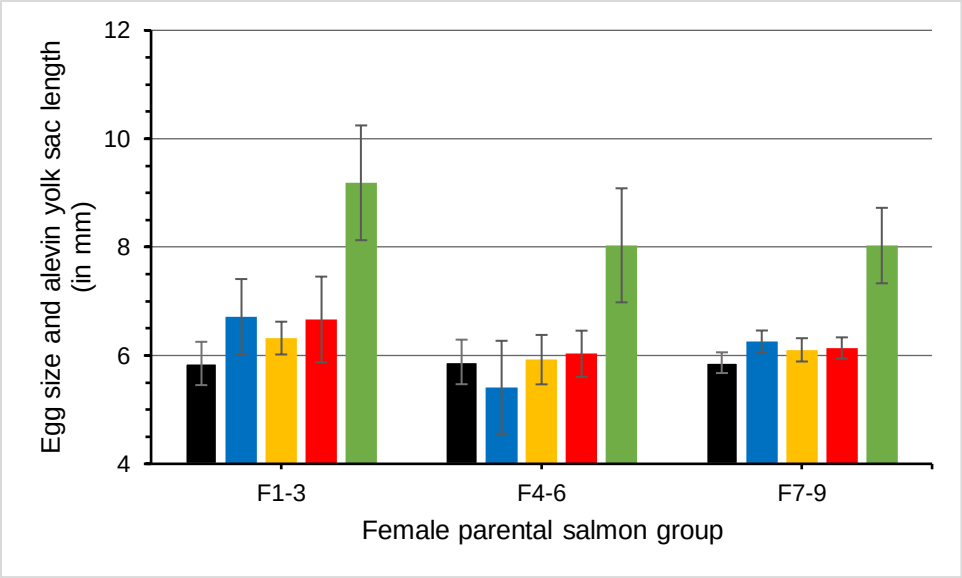

Fig. 29. Egg size and alevin weight (mm) of female parental salmon groups sorted by age. (**Black**) egg size right after spawning. (**Blue**) egg size after fertilization and 60 min incubation in fresh water. (**Yellow**) egg size after 7 days of incubation in fresh water. (**Red**) egg size after the first eggs reached the eyed stage. (**Green**) alevin yolk sac length after the first eggs hatched. Data are mean ± s.d. (*n*= 10 eggs /alevins per salmon).

Sinking rate

Eggs with a higher density (g/cm^3) tend to have a higher sinking rate (cm/sec), although there is no correlation (Fig. 30). Thus, the sinking rate is as expected mainly depending on weight and volume.

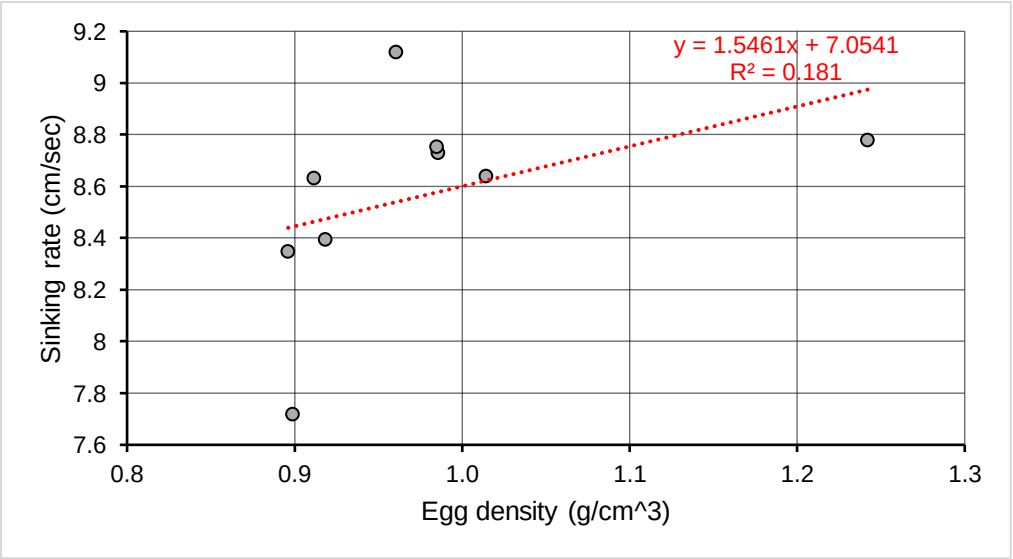

Fig. 30. The sinking rate (cm/sec) of eggs in comparison to the egg density (g/cm^3), both right after spawning. Data are mean. (*n*= 10 eggs salmon).

3.4. Mortality rate of fertilized eggs

The cumulative mortality rate after 90 days (%) of fertilized eggs significantly differed between crossbreeds, where different female parental salmons within the first salmon group were used. The same goes for the second salmon group. The male parental salmon M1 always contributed to the lowest cumulative mortality rate after 90 days within the three crossbreeds of one female parental salmon. The combined crossbreeds of the third (and youngest) salmon group showed the lowest cumulative mortality rate after 90 days (Fig. 31).

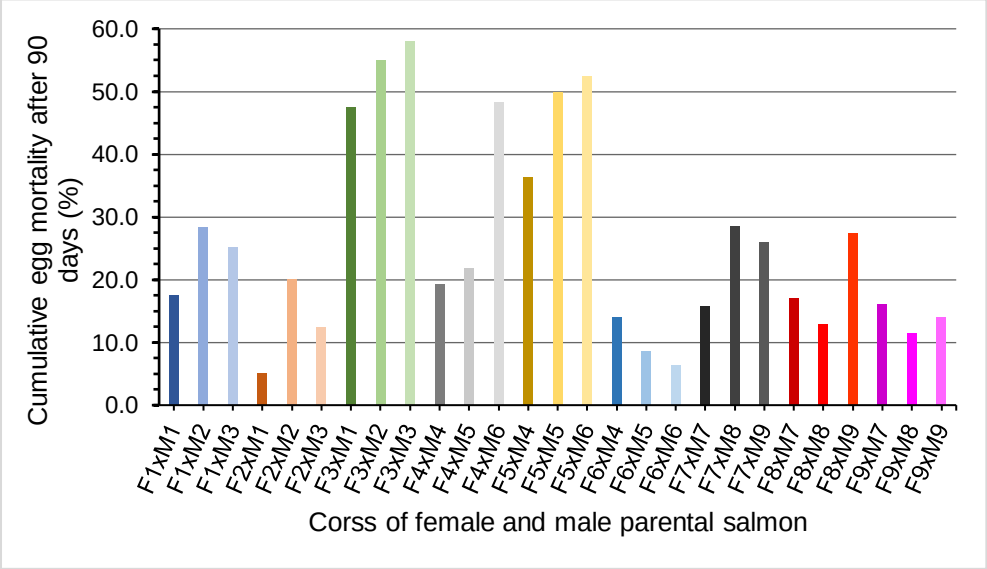

Fig. 31. Cumulative egg mortality (%) after 90 days for all intersections between the single male- (different colour contrast) and female (different colour) parental salmon.

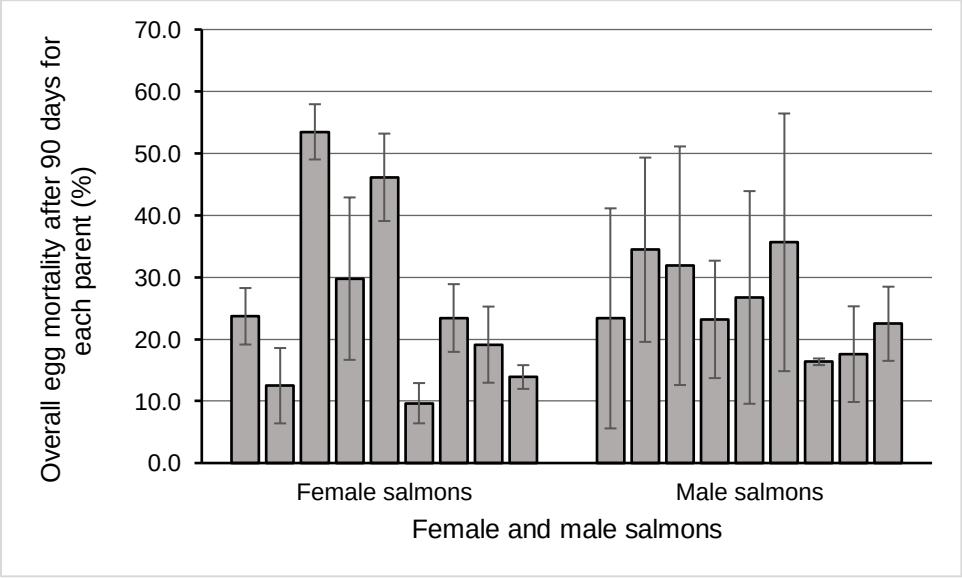

Fig. 32. Estimated overall egg mortality after 90 days for each parental salmon (%). Salmon (female and male) 1-9 from left to right. Data are mean ± s.d.

The lowest estimated overall egg mortality rate (%) from a single parental female salmon was overserved for F6 (9,7%). The highest estimated overall mortality rate of eggs from a single parental female salmon was observed for F3 (53,5%). The male values have very high standard deviations (Fig. 32). The lowest average estimated overall mortality rate within a salmon group was observed for the third (and youngest) salmon group (18.8%), which is much lower to the one of the other two salmon groups (F1-3: 29,9%, F4-6: 28,5%). It shows a trend, that eggs from younger salmons have lower mortality rates.
The estimated mortality rate from one crossbreed is depending more on the female parental salmon (Fig. 31, Fig. 32).

The female parental egg mortality rate is not correlated to the total increase in the egg weight (%). Nevertheless, the highest and lowest increase in egg weight were connected to the highest female parental egg mortality rate (Fig. 33). Those 2 values can be counted as outliers. Apart from them, a small tendency shows, that eggs, which increase their weight slower, have a lower cumulative egg mortality rate after 90 days.
The total egg production by one female parental salmon in one striping period is not correlated at all to its egg mortality rate (Fig. 34). The total egg production (g) a salmon spawns has no influence on the cumulate egg mortality rate after 90 days. Hence one can say, that the cumulative egg morality rate after 90 days is also not depending on the salmon length, since the total egg production is correlated to the salmon length.

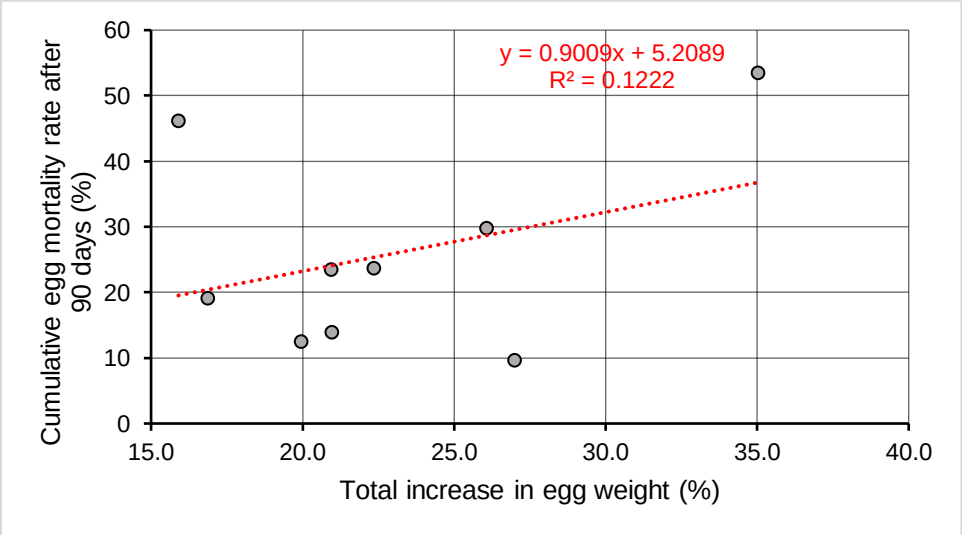

Fig. 33. Cumulative mortality rate after 90 days of eggs from different female parental salmon (%) in comparison to the total increase in the egg weight (%) until hatching. Data are mean (*n*= 30 eggs per salmon).

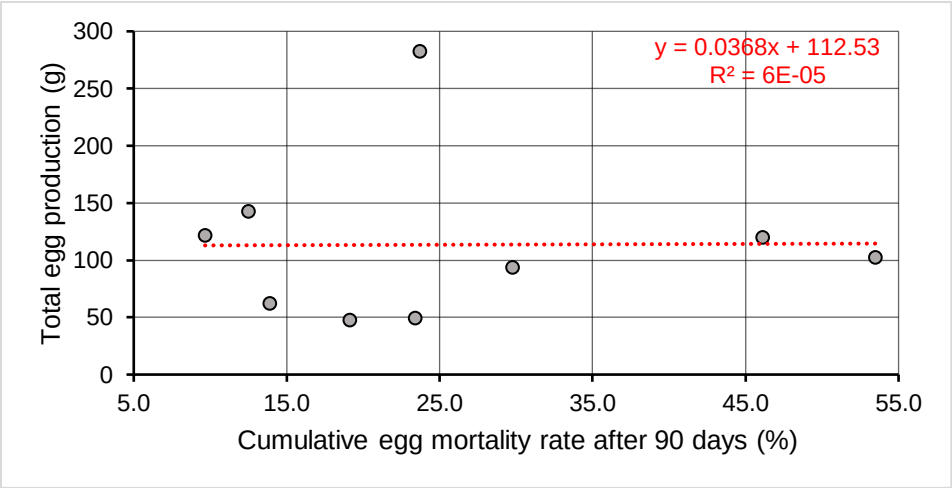

Fig. 34. Total egg production (g) spawned by a female parental salmon in comparison to its cumulative egg mortality rate after 90 days (%). Data are mean.

The spermatocrit (%) shows also no correlation to the cumulative egg mortality rate of eggs fertilized by the different male parental salmons (Fig. 35). Values were varying from 16% to 26% spermatocrit inside 10µl sperm volume.

A small correlation between the spermatocrit and the cumulative egg mortality rate after 90 days exists. A higher spermatocrit results in a lower cumulative egg mortality rate. This is very interesting. Aside from the fertilization success it also influences the cumulative egg mortality rate over 90 days. The Cumulative egg mortality rate after five days after fertilization (%) showed a reverse trend, where a higher spermatocrit results in a higher cumulative egg mortality rate (Fig. 36).

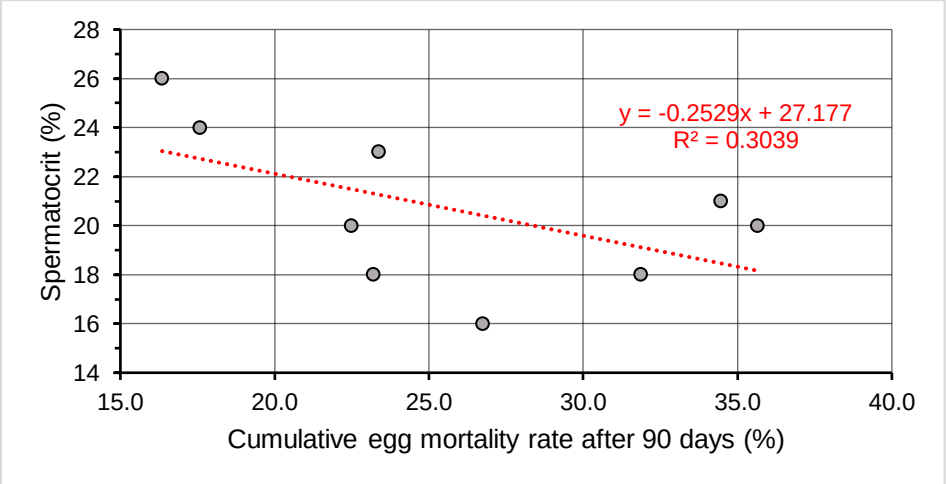

Fig. 35. Spermatocrit (%) inside 10 µl sperm volume of male parental salmons in comparison to the cumulative egg mortality rate after 90 days (%) of eggs fertilized by these male salmons.

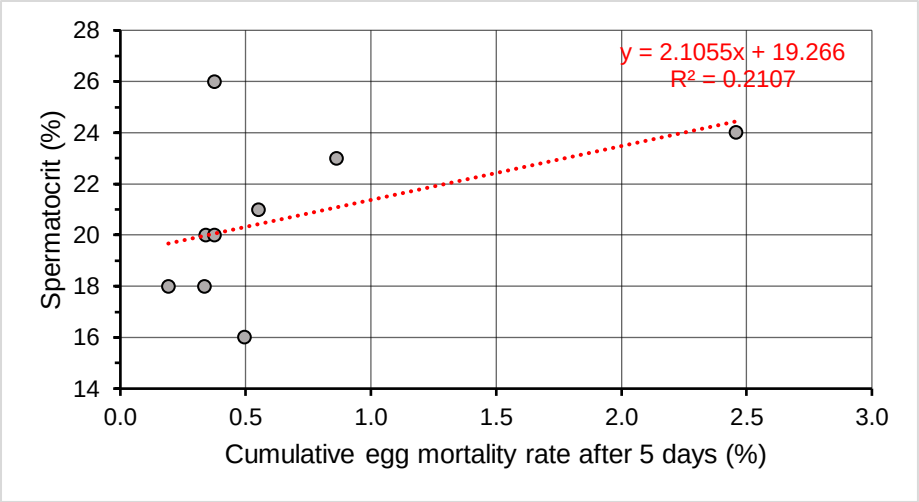

Fig. 35. Spermatocrit (%) inside 10µl sperm volume of male parental salmons in comparison to the cumulative egg mortality rate after five days (%) of eggs fertilized by these male salmons.

4. Discussion

4.1. Experimental procedure

Evidence showed, that the hatching success was influenced by another factor. The hatching strategy improved the total hatching success. In comparison to other methods, where all eggs after fertilization are laying inside the tanks next to each other (which means next to eggs from different females), this experiment could control contamination (Fig. 35).

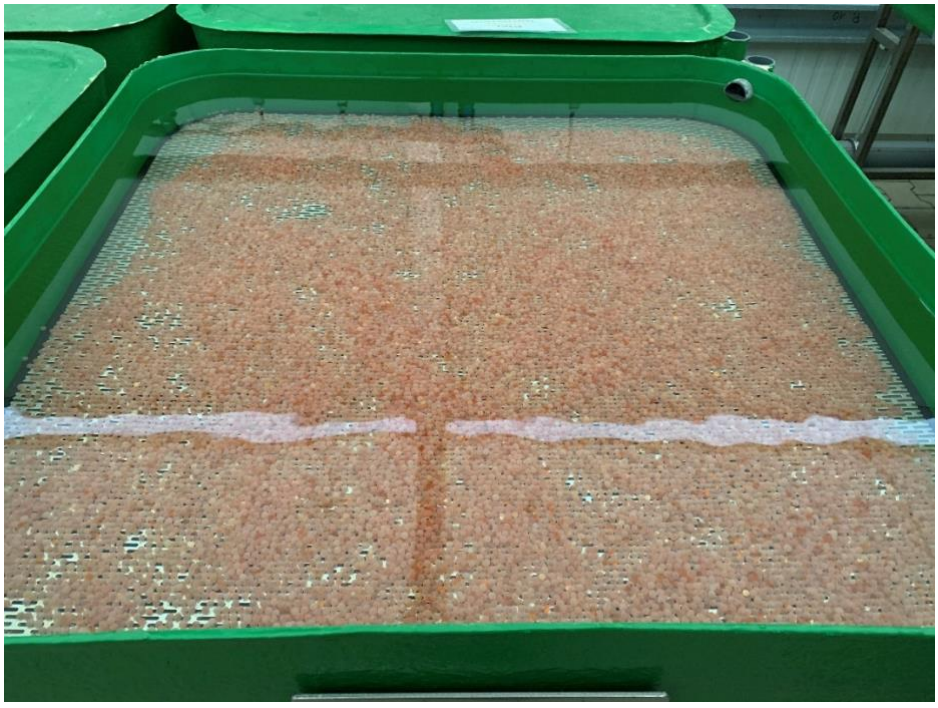

Fig. 35. Atlantic salmon (*Salmo* salar) egg incubation after fertilization inside a tank (94 x 94 x 19cm / water up to 13cm≈ 114l water) for breeding purpose only.

The eggs from different female parental salmons (*Salmo salar*) had different mortality rates. The worse observed one was from F3 and the best from F6 (Fig. 30). If for instance eggs from F3 are laying directly next to eggs from F6, the chance of wasting good eggs is increasing. The spores are usually moving in every direction. This means, that at a maximal density inside one tank, where every egg has at least 6 neighbour eggs, already one dead egg can infect 6 others. This way also fungus clusters can occur. Clusters can contain up to 30 eggs based on my own experience, just connected through spores of fungus. As an example, if more than 50% of eggs spawned by one salmon tend to fungus and build spores, many others eggs, depending on the total eggs from this salmon (total salmon eggs * 0.5 * 6), would be at risk. This can cause huge losses. By separating the eggs from different female parental salmons with baskets, exactly these losses could be prevented. Moreover, after hatching, most alevins escaped from the baskets inside the tank, which was perfect for them, because the salmon density was very adequate inside the tanks. Observations showed one more thing. The egg shells, which usually float inside the tanks too and polluting the water, by growing spores and fungus, stay inside the baskets. By coincidence, I also noticed, that conjoined twins, which have an extremely low survival chance and which are connected through the yolk sac (Fjelldal, 2016), cannot swim through the holes of the baskets. This prevents them from mixing with other casual alevins. Baskets are just one way, there are surely other good options depending on the equipment and the tanks in an aquaculture. Incidence of light was prevented the whole experiment, but when mortality observations were made. Low light intensities are better for the egg development (Peng et al, 2019). The tanks were also not touched by anyone else than me, because of that dead eggs due to vibrations are out of the question.

Apart from all egg parameters and only a few sperm parameters, more sperm parameters should be measured. The sperm was analysed under a brightfield microscope to create videos with a magnification of 400x.
These videos should be evaluated with the CASA (computer-assisted sperm analysis) plug in for ImageJ. All sperm samples were free from water and urine. This was important because water (or urine) acts as an extender for teleost fish sperm. Usually, it is immotile but motility is induced by contact with water or urine (Perchec-Poupard et al, 1998). This motility remains for less than 2 mins. In this time pictures can be taken for morphology analysis, otherwise the sperm flagellar will bend, making it impossible to measure precisely. Sperm cells should stay alive in 1-4°C. A dilution factor of 1:100 should be enough to see the cells under a brightfield microscope (Kime et al, 2001).

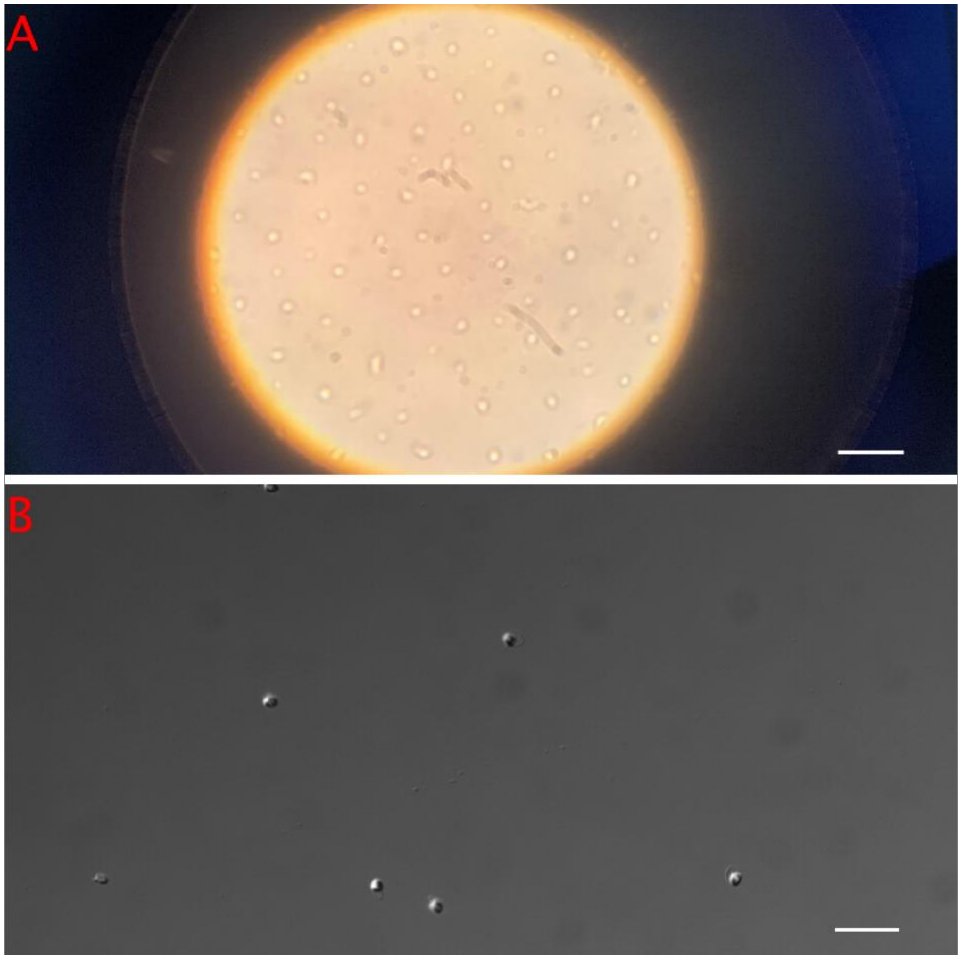

Fig. 36. Atlantic salmon (*Salmo* salar) sperm cells diluted in fresh water 1:1000. (**A**) Sperm cells under a brightfield microscope by Zeiss with a 400x magnification. (**B**) Sperm cells under a confocal microscope by Zeiss using DIC microscopy (differential interference contrast) with a magnification of 400x. Scale bars, 100µm.

In my experiment, I took the sperm sample and analysed it within 1min. No sperm cells were detectable. I tested the dilution factor of 1:100 but it was not enough, so I increased it to 1:1000, which seemed good (Fig. 36, A). I still could not see any flagellar. Then I tried to collect samples and analyse them as fast as possible under a confocal microscope (at the Heinrich-Heine University) using DIC microscopy for a better imaging of unstained samples (Fig. 36, B). There was again no motility, although fresh water was used as an extender again. The temperature was always under 4°C. Aside from the morphology, I wanted to test the straight-line velocity (VSL) and the trajectory of sperm. They are the most reliable indicators of fertility (Moore, Akhondi, 1996). From thousands of observed sperm cells only 2 were motile. Because of this I decided to not evaluate and insert this part of the experiment into the results. For fertilization of the eggs 0.7ml sperm was used. The spermatocrit (%) is so high, that also 0.1ml would be enough since one egg need exactly one semen cell to become fertilized. (Jamieson 1991). I wanted to prevent, that the fertilization rate itself will

occur as a problem, so the value 0.7 was chosen because the lowest total sperm amount given by the striping of one male was 3.1ml. I wanted to save 1ml for spermatocrit and to freeze the rest for later analysis. 2.1ml divided by three because of 3 bowls per parental male salmon made 0.7ml. Obviously, all bowls were fertilized with the exact same amount of sperm.
The experiment ended after the alevins hatched because after this they spread inside the tanks, which made it impossible to detect crossbreeds, expect with genetic analysis. Genetically analysis of blood samples and fin clips could not be analysed by now due to financial and time factors.
As already mentioned in the methods, all experimental steps were performed in a hurry. Therefore, I only had the limited devices we placed at the “Lachszentrum Hasper Talsperre” before the experiment started.

4.2. Water parameters and degree days

The water parameters were constantly within limits and around optimal values. The most important one while hatching is the temperature. Atlantic salmon (*Salmo* salar) eggs need to be held in over 0°C and under 8°C water temperature. In my case the lowest recorded temperature was 1.3°C and the highest was 7.9°C. Too high temperatures can increase the probability of skeletal deformations. The average degree days until hatching were different depending on the salmon age. Surprisingly, my values are totally different from literature degree days until hatching. The first hatched alevin was most likely around 300degree days, when salmon eggs should usually reach the eyed stage (Benchmark Genetics, 2019). The eyed stage was averagely reached after already 225degree days in my experiment. The difference could be explained, because I noted the date, when the first ten+ eggs reached the eyed stage or ten+ alevins hatched. After that sometimes no more eggs reached the eyed stage or no more alevins hatched. This could shift my degree day count to values + 100degree days. This is a little bit closer to the literature but still not as near as it should be. But that could be explained by a very interesting aspect. Also, within the same salmon species, different tribes exist, which show different characteristics (Crozier, 1993). The “Lachszentrum Hasper Talsperre” works with its own brood stock, but once per year wildlife returnees are collected (if possible) and taken into the brood stock too after quarantine. This can lead to genetically differences even within the tribe and brood stock, resulting in differences like this. One strong correlation I detected was the correlation between the degree days until hatching and the cumulative egg mortality rate after 90 days (%) of female parental salmon groups (Fig. 16). The eggs from older female parental salmons needed more degree days until hatching and had a higher estimated mortality rate. A possible reason for this is the spawning. In wildlife and under normal circumstances the salmons spawn only

once per life. Although the recondition in aquacultures is very good (95% of salmons survive the striping season), the salmon quality could decrease (Reid, Chaput, 2012). Furthermore, the maternal age can influence the mortality rate as well (Shelton et al, 2012).
Another reason for this difference could be a wrong temperature measurement, but since the temperature was measured three times each day with three different devices (all calibrated) and all of them displaying the same value, makes this reason kind of irrelevant. One last observation definitely needs to be emphasised. The hatching is not only depending on the degree days themselves. It requires a sudden temperature increase of 2-3°C for hatching. Just before the experiment ended the water temperature increased by 2°C+. Inside one basket (F2xM1) all of the eggs reached the eyed stage at the average time, but only a few eggs hatched. At this day, all of the eggs (100+) hatched (appendix).

The impact by all water parameters was equal on all baskets since they were inside 2 tanks next to each other with the exact same pipes transferring water to them. A RAS (recirculating aquaculture system) also supports the equal water parameters everywhere. Because of this an impact on any other measured parameter due to water parameters can be ignored.

4.3. Egg and sperm characteristics

In the beginning of the experiment close to all egg weights (mg) were significantly different from each other (Fig. 20). This significant difference between the egg weights and alevin weights (mg) was constant in most cases. Physiological parameters such as metabolic rate can influence the egg weight (Lagos, White, Marshall, 2017). Mainly after reaching the eyed stage and at least after hatching the egg-/ alevin- weight dropped in all eggs /alevins. The weight of eggs from different female parental salmons changes differently. The reason behind the different progress could be the metabolic rate. A higher metabolic rate could decrease the egg weight and lead to the alevin consuming its yolk sac even faster. Another association could be a diversification of the parental salmon's life history related to reproductive strategy (Yamamoto et al, 2020) For this analysation more genetically data would be required. In my experiment, the ranking of the egg weight was the same as that of the alevin weight (Fig. 20, Fig. 22). Usually larger eggs produce larger offspring, which can influence survival during this vulnerable life stage (Thorn, Morbey, 2018).
The cumulative egg mortality rate after 90 days (%) is not in correlation with the increase in egg weight (%), but the female salmon with the highest cumulative egg mortality rate after 90 days had the highest increase in egg weight as well (Fig. 33). The samples of 10 eggs for each measurement is enough. The use of more female parental salmons to gather more data would have been better.

The egg size (mm) was just very rarely significantly different between any of different female parental salmons from beginning of the experiment. In average, the egg size increased stronger in older salmons, while the egg weight increased randomly.
There was also no correlation between the length of a female parental salmon and the total increase in egg weight of its eggs, but a tendency showed, that the cumulative egg mortality rate after 90 days is lower for eggs which have a smaller increase in egg weight (Fig. 33). This also supports the fact, that the eggs from 1-year old female parental salmons have a lower cumulative mortality rate after 90 days, because their eggs increase the egg weight slower. But the main reason for the much lower cumulative egg mortality rate after 90 days will be the first spawning, instead of repeated spawning (Reid, Chaput, 2012).
Also, no correlation between the alevin yolk sac length (mm) and the female parental salmon length (cm) could be detected. I worked with the alevin length and not volume, because it is really difficult to make exact picture to measure all required data. Considering my limited tools at the “Lachszentrum Hasper Talsperre” this was the best possible way.
The second strong correlation I detected was between the total egg production (g) spawned by a single female parental salmon and its length (cm). Longer parental female salmons spawn more eggs (Fig. 27).

The total sperm volume after striping (ml) is not correlated to the parental male salmon length. Therefore, the result indicates the minimal length of approximately 30 a male salmon needs to produce a good amount of sperm (Fig.18). This minimal value could be even bigger, if precocious male salmons would be used for the experiment, which was not the case for me. The spermatocrit (%) is neither correlated to the sperm volume (Fig. 19), nor to the male salmon length. There is a little trend, showing that the spermatocrit is lower when the total sperm volume is lower as well. This could be accurate due to the fact, that more “total sperm volume” can contain more other liquids and pollute the sperm (e.g., water or urine). This directly results in a lower percentage of semen cells.
After yolk sac absorption and after the salmon reaches the fry stage the sperm could influence salmon growth (Garant et al, 2002), but this cannot be evaluated by my case, because the experiment ended before the majority of alevins absorbed their yolk sac.

4.4. Mortality of fertilized eggs

In the beginning of this part, I want to emphasise, that the impact of the parental male salmon is very low and random in this experiment. When looking at the standard deviation, which is a measure for the amount of dispersion, the cumulative egg mortality rate after 90 days (%) of eggs, which were fertilized by different parental male salmons are varying extremely strong in comparison to the values from parental female salmons (Fig. 32).

Therefore, I will mainly focus on the maternal impact on the cumulative mortality of fertilized eggs after 90 days

The eggs from different female parental salmons looked different, but apart from the size, the colour differed as well. Egg colour and pigmentation is influenced by carotenoids such as astaxanthin or canthaxanthin (Craik, 1985). A high astaxanthin content should increase egg production and larval growth, while it lowers egg incubation mortality (Hansen, Puvanendran, Bangera, 2014). In this experiment, the most pigmented and so very reddish eggs had the highest cumulative mortality rates after 90 days. There could be too much astaxanthin inside these eggs, which lead to a higher mortality instead of a lower. This could then only be reasoned because of the female parental salmon. The different female parental salmons got the same fish feed since they feed. The only reasoning left could be the genetically impact on astaxanthin content in the produced eggs from different salmons. On the other hand, eggs with very low pigmentation, which were quite bright, had a high cumulative mortality rate after 90 days as well, which emphasises the importance of an adequate carotenoid content (Fig. 37).

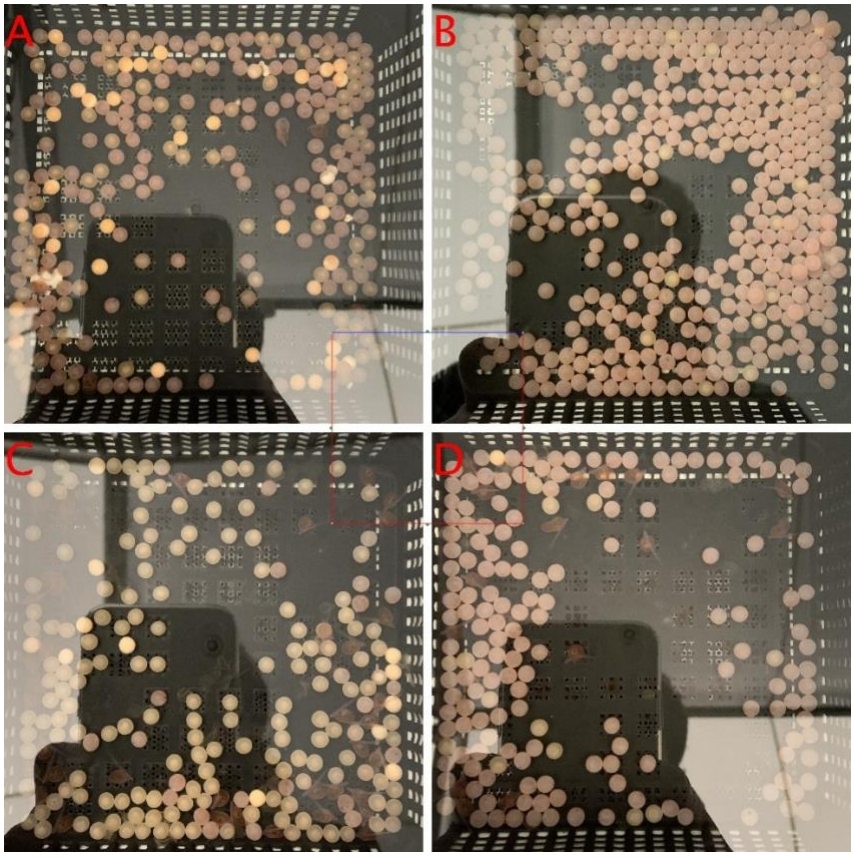

Fig. 37. The different pigmentation of Atlantic salmon (*Salmo salar*) eggs. (**A**) High pigmented eggs from F5 with a cumulative egg mortality rate after 90 days of 46,1%. (**B**) Well pigmented eggs from F2 with a cumulative egg mortality rate after 90 days of 12,5%. (**C**) Low pigmented eggs from F3 with the highest cumulative egg mortality rate after 90 days (53,5%). (**D**) Well pigmented eggs from F6 with the lowest cumulative mortality rate after 90 days (9,7%).

The colour contrast between eggs would be even greater outside of water. But I wanted to present all eggs in one basket and taking a basket out of the water would have changed the experimental outcome. The egg pigmentation is mainly maternal, because eggs of the same female parental salmon could be detected with the naked eye.

The sinking rate (cm/sec) improves the mortality for wildlife salmons. A higher sinking rate tends to indicate a higher egg density (g/cm^3). Usually a strong current exists in rivers where salmons breed. The redds made by the salmons are not directly under the salmon when they spawn the eggs, which can lead to a high distribution of redds and eggs (Chapman, 1986). The faster the eggs sink, the higher is the chance to be placed in a safe place (e.g., a prepared redd) chosen by the parental salmon.
The baskets covered a surface of 0,361m^2. This surface area was never fully covered by the eggs from any crossbreed. This means, that the egg density has been kept low in every basket and a contribution to the results by the egg number placed in a basket can be ignored.

The spermatocrit (%) influences the fertilization success without saying. Because of this, the cumulative egg mortality rate after five days (Fig. 35) was observed as well. Also, if the outlying value would be ignored, the tendency is the same with a lower correlation. The extremely small tendency could have been coincidence. According to this, the spermatocrit does not influence the early cumulative egg mortality rate after five days. Surprisingly, the spermatocrit seems to influence the cumulative egg mortality rate after 90 days (Fig. 35). The tendency shows, that a higher spermatocrit results in lower cumulative egg mortality rate after 90 days. This must be coincidence, because the sperm was carefully but surely washed out of the water after fertilization. The water inside the tanks was also free from sperm, so a long-term effect from the spermatocrit seems impossible to me.

As already mentioned above, the cumulative mean egg mortality rate after 90 days for three-years old salmons is higher, maybe because they are repeated spawners. They already spawned eggs for one or two times. Apart from the fact, that the salmons were selected randomly in terms of reproduction, the salmons have an individual influence on the cumulative egg mortality rate after 90 days. Genetic differences must be the main reason for the different hatching success, like the carotenoid content. Furthermore, the condition of a female parental salmon before spawning the eggs had an influence on the mortality as well. Salmon F6 with the lowest cumulative egg mortality rate after 90 days looked “perfect” for fish farmers, while salmon F3 with the highest cumulative egg mortality rate after 90 days already had fungus growth at its ventral and tail fin. In addition to that it was very thin and the

light brown colour should be bad in comparison to the brown of F6, which slightly fades from dorsal to ventral, what was claimed by the fish farmers (Fig. 38).

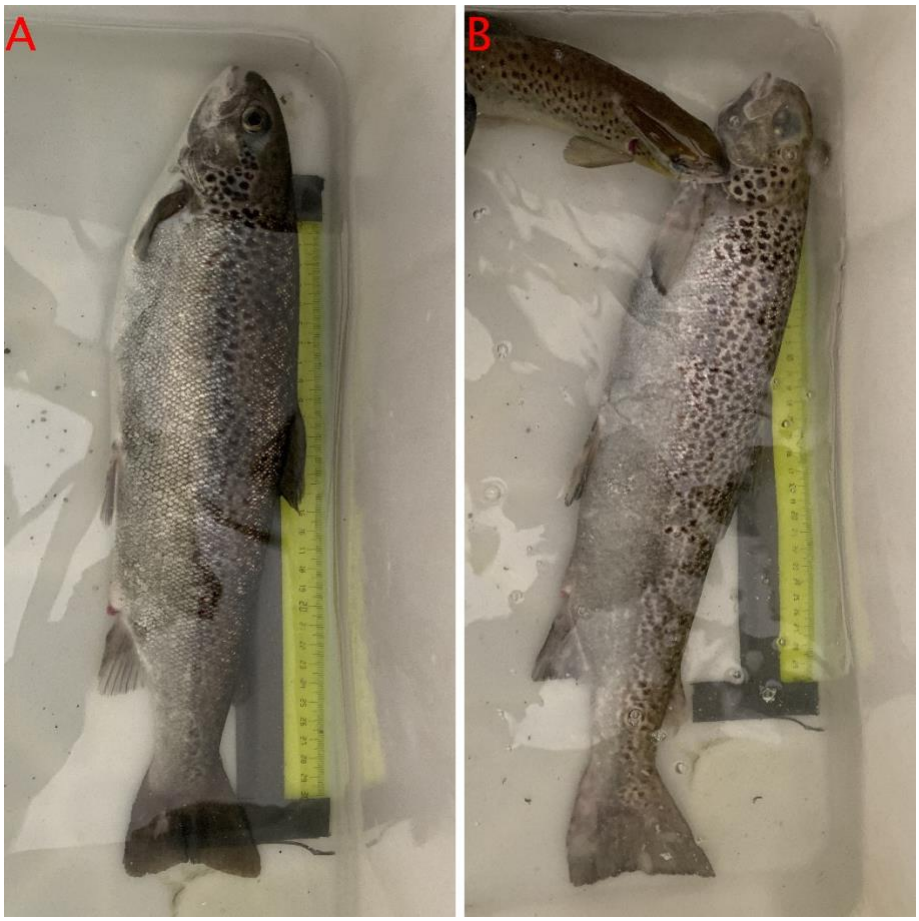

Fig. 38. Female parental Atlantic salmon (Salmo salar). (**A**). Salmon F6 with the lowest cumulative mortality rate (%) over 90 days. (**B**) Salmon F3 with the highest cumulative mortality rate after 90 days (%).

4.5. Conclusion

The hatching success describes nothing less than the survival rate of salmons between spawning as an egg and hatching into an alevin. In wildlife, the hatching success and everything that goes with it is influenced by parental Atlantic salmon (*Salmo* salar) genetics, physical appearance, fish feed and the water parameters. In aquacultures, it is influenced by all of these aspects as well as the care by fish farmers. Many comparisons can be detected better in fully grown salmons than in one- or two-years old salmons.

The egg size (mm) and its increase, the spermatocrit (%) and the total egg production (g) have no influence on the cumulative egg mortality rate after 90 days (%). This also means, these parameters will not worsen the hatching success at all. What a fish farmer wants to see are many and large eggs, this remains a beautiful view, but nothing more or less.

5. Bibliography

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7. Statutory Declaration

I herewith formally declare that I have written the submitted master thesis independently. I did not use any outside support expect for the quoted literature and other sources mentioned in the paper.

I clearly marked and separately listed all of the literature and all of the other sources which employed when producing this academic work, either literally or in content.

I am aware that the violation of this regulation will lead to failure of the thesis.

Julien Kocabiyik

Date, Student's signature

8. Appendix

Fig. 39. Colour scale for the JBL NH_3 test for fresh water and salt water.

Fig. 40. Colour scale for the JBL NO_2 test for fresh water and salt water.

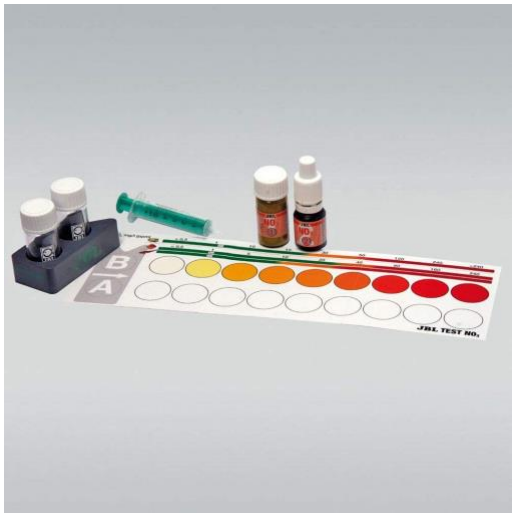

Fig. 41. Colour scale for the JBL NO_3 test for fresh water and salt water.

Table 5. Raw mortality data. *F*= female salmon, F1-3 are three-years old, F4-6 are two-years old, F7-9 are one-year old. *M*= male salmon, all males were 1-year old. The number inside the matrix shows dead/broken eggs inside the specific basket on the shown day. * *behind a number*= Overripe eggs or eggshells, which were taken out. – *in front of a number*= Number of eggs taken away on the shown day because of fungus growth and spore spread. *EP*= The first day an egg reached the eye point stage. *A*= The first day an alevin was observed. *End*= The last day of observation, where all unhatched eggs were removed.

Day		F1	F2	F3		F4	F5	F6				
1	M1	0	0	9	M4	0	0	1*-27				
	M2	0	0	4	M5	3	4	0*-71				
	M3	1	0	3	M6	0*2	0	0*-30				
3		0	0	9		0	0	1				
		0	0	5		2	4	0				
		1	0	3		0	4	0				
5		0	0	9		0	0	2				
		0	1	5		2	4	0				
		1	0	3		0	4	0				
										F7	F8	F9
7		2	1	9		3	0	2	M7	0	1	1
		1	2	5		2	4	0	M8	10	0	2
		1	1	3		0	4	0	M9	0	1	1
9		6	1	10		4	0	2		0	1	1
		1	2	5		3	4	0		10	0	2
		4	1	3		0	5	0		0	1	1
11		6	1	10		4	1	2		0	1	1
		3	2	5		3	4	2		10	0	2
		4	2	3		0	5	0		0	1	1
13		7(-4)	1	10		4	4	2		0	1	1
		6	2	5		3	6	3		10	0	2
		4	2	3		0	5	2		0	1	1
15		3	1	13-13		4	5	3		0	1	1
		15-20	3	5-2		3	8	3		10	0	2
		4	3-3	3(-1)		2	5	4-11		1	1	1
17		3(-3)	2	7		5	23	2		1	2	1
		16(-7)	5	6(-3)		1	27	4		10	0	2

		6	2	2		2	24	0		1	2	1
19		4(-5)	2	9		5(-4)	48	2*-37		1	3	1
		25(-7)	7(-17)	7(-1)		5	40	4*-87		10	0	2
		9(-14)	9(-1)	7		3(-3)	35(-7)	0*-5		1	2	1
21		6	2	14(-2)		4	58	1		1	4	1
		32(-12)	3	9		5	45(-4)	0(-17)		10	0	2
		7(-17)	9	8		0	39	0		3	2	1
23		6(-7)	2	19(-18)		4	63(-5)	5		1	5	1
		28(-11)	11(-2)	15(-1)		5	44(-3)	7		10	0	3
		7-(5)	9(-2)	14(-2)		0	45(-12)	0(-14)		3	2	2
25		5	2	17		4	62	5(-1)		2	6	1
		29(-10)	10(-3)	15(-4)		5	43	7		11	0	3
		9	8	14		2	41(-2)	0		3	2	2
27		5	2	18(-6)		4	64	7		2	6	1
		22(-23)	8(-5)	11(-3)		5	44	7		11	0	4
		9	9	16(-2)		2	42(-4)	0		3	2	2
29		5	2	17		4(-2)	64	8		2	6	1
		12(-5)	4(-3)	9		5	44	7		11(-1)	0	4
		10(-1)	9	14		5	38	0		3	2	3
31		5	2	17(-5)		2	65(-4)	8		3	6	1
		11(-8)	3(-2)	9(-5)		5	45(-6)	7		11	0	4(-3)
		10	9	14(-1)		9(-7)	38(-2)	0		3	2	3
33		5	3(-2)	13(-3)		2	61(-1)	9(-8)		3	6	1
		6(-3)	1	9		5(-1)	42	7(-2)		12	0	2
		10	9	15(-11		2	37	1		4(-1)	2	3
35		5	3	14		2	60	4		4	6	1
		6(-1)	1	12(-5)		4	42	5(-2)		12	0	2
		10	9	10(-2)		2	37	1		3(-1)	2	4
37		7	3	15(-6)		2(-1)	62(-16)	4		4	6	1
		5	1	10(-2)		4	46(-10)	3		13(-5)	0	2
		10(-2)	9	8(-3)		2	37(-5)	1		3	2	5

39		7	3	10		1	46(-9)	4		4(-2)	6(-2)	1
		5(-2)	1(-1)	11(-1)		4	38(-10)	3(-1)		9(-1)	0	2
		9(-2)	9	5(-1)		2	35(-12)	1		3	2	5
41	EP (F1,2,5)	7	3(-1)	10		1	39	5(-1)		2	4	1
		3	0	11(-1)		4	31	2		8(-2)	0	2
		7	9	4(-2)		2	24	1		4	3	5
43	EP (F3,4,6)	7	2(-1)	10(-4)		1	45(-22)	4		2	4(-2)	1
		3	1	13(-2)		4	33(-15)	2		7(-2)	0	2
		7	9(-1)	2		6	25(-11)	2		5	3	5
45		7(-2)	1	6(-3)		1	24(-11)	4		2	2	2
		3	1	12		4	21(-9)	2(-2)		5(-2)	0	2
		7(-1)	8	2		8(-6)	17(-3)	2		5	3	5
47		6	1	3		1	13(-6)	4		2	2(-1)	2(-2)
		3	1	12(-1)		5(-1)	13(-4)	0		3	0	2
		6	8	2(-1)		3	14(-7)	2		5	3	6
49		6(-1)	1	4		1	7(-3)	4		3	2	0
		3(-2)	1	11(-2)		5(-1)	9	1		5(-1)	0	2(-1)
		6(-1)	8	1(-1)		3	12(-3)	2		5(-2)	3	6(-4)
51		5	1(-1)	5		1	4	2		5(-3)	2	1
		1	1(-1)	12(-5)		4	12(-4)	1		5	0	1
		5(-2)	8	3(-2)		4(-1)	14	1		3(-1)	3	3
53		5(-1)	0	7		2	4	2		2	3	1
		1	0	7(-1)		4	12(-4)	1		6	0	1
		4	8	1		3	15	1		5	3(-1)	4(-1)
55		5(-2)	0	10(-2)		2	5	2(-1)		2	3(-1)	1
		1	0	6(-3)		4	8	1		7	0	1
		4(-1)	8(-2)	1		3	19(-2)	1		6(-2)	2	3
57	EP (F7,8,9)	3	0	8, A		2	5(-1), A	1, A		2	2	1(-1)
		1	0	3		4	9, A	1, A		9	0	1(-1)
		4	6	1		3	17	1, A		4	2	3
59		4(-1), A	0	8		2	4	1		2	2	0

		1	0	3(-1), A		4	9	1		9(-1)	0	0
		4, A	6	3, A		3(-1)	20(-2)	2		4(-1)	2(-1)	3(-2)
61		3	0	9(-2)		2	6	1(-1)		2	2(-1)	0
		2	0	3		4(-2)	9	1		8	0	0
		6	6	4		2(-1)	20	2		4	1	1
63		3(-1)	0, A	8(-1)		3(-1), A	7(-1)	0		2(-1)	1	0
		2(-1), A	0	4		2, A	13(-4)	1		9(-1)	0	0
		6	6, A	7		1(-1), A	23(-3), A	2		4	1	1
65		2	0	8(-1)		3	8	0		0	1	0
		1	0	7(-2)		2	14(-1)	1		9	0	0
		6	7(-2)	9(-1)		0	25(-3)	2(-1)		4	1	1
67		2	0	8(-4)		4	11	0		0	1	0
		1	1	5		2	17	1		9(-2)	0	0
		6(-5)	5	9		0	22(-1)	1		4	1	2(-1)
69		2	0	4(-1)		4	12	0		0	1, A	0, A
		1	1	5		2	22(-5)	2(-1)		7	0	0, A
		1	5(-5)	12		0	26(-4)	1		4	1, A	1
71		2	0	4		4	15(-2)	1		0, A	1	0
		1	1	5		2(-1)	24(-2)	1		7(-3)	0, A	0
		4	1	12(-2)		2	26	1		4	2(-1)	1, A
73		3	0	5		4	15(-2)	2		0	3	1
		1	1	6		1(-1)	31(-1)	1		4	0	0
		5	1	13		4	35(-6)	1		4(-1), A	1	1(-1)
75		3	0	6		5(-1)	19(-3)	2		1	3	1
		1	1	7(-2)		0	38(-4)	1(-1)		5	0	0
		6(-1)	1	17(-6)		6	37(-9)	1		3(-1)	1	0
77		6(-2)	0	8		5(-5)	21(-3)	3(-1)		1	3(-2)	2
		1	1, A	7		0	45	0		5(-1), A	0	0
		6	2(-1)	13(-2)		7	40(-9)	1		2	2	0
79		4	0	12(-2)		1	20(-3)	2		2	1	3
		2	1	8(-6)		1	50(-11)	0		4	0	0
		8(-5)	1	13(-4)		7	36(-4)	1		2	2	1

81		7(-2)	0	13(-4)		1	24(-9)	2		2	1	3
		2	2	4(-2)		2	48(-18)	0		4(-1)	0	0
		3	2	10(-1)		8	40(-2)	1		2	2	1
83		9	1(-1), A	16(-2)		1	18(-4)	2		2	3	3
		4	3	7		2	36(-14)	0		4	0	0
		6	2	16		10(-1)	48(-18)	1(-1)		4(-1)	2	1
85		25(-2)	0	27(-2)		1	21(-10), A	3		3	3	4
		18(-3)	4	24(-14)		2	28(-9)	1		4	0	1
		18(-2)	2	37(-6)		9(-3)	33(-13)	0		3	5(-1)	1
87		26(-6)	5	34(-6)		3	12(-3)	4		3	4	5(-4)
		20(-4)	4(-1)	19(-7)		5	28(-11)	2		4	0	1
		24(-4)	6	53		7	27(-12)	0		3	7(-3)	1
89	End (W1-6)	-79	-16	-90		-69	-24	-31		3	4	1
		-81	-59	-116		-85	-51	-17		4(-2)	0	1
		-114	-42	-151		-184	-49	-15		5	2	1(-1)
85										4(-1)	7(-3)	1
										4(-1)	0	1
										8(-2)	5(-2)	0
87										4	6	1
										8	3	1(-1)
										10(-4)	7(-2)	1
89	End (W7-9)									-17	-16	-25
										-19	-21	-18
										-26	-32	-17

Table 6. Nitrate-, nitrogen- and ammonium content (mg/l) and pH over 90 days.

Date	Time	Nitrate content (mg/L)	Nitrite content (mg/L)	Ammonium content (mg/L)	pH
01.12.2020	08:45	30	0.01	<0.05	7
02.12.2020	08:45				7
03.12.2020	08:45				7
04.12.2020	08:45				7
05.12.2020	08:45	30	0.01	<0.05	7
06.12.2020	08:45				7
07.12.2020	08:45				7
08.12.2020	08:45				7

09.12.2020	08:45	30	0.01	<0.05	7
10.12.2020	08:45				7
11.12.2020	08:45				7
12.12.2020	08:45				7
13.12.2020	08:45	30	0.01	<0.05	7
14.12.2020	08:45				7
15.12.2020	08:45				7
16.12.2020	08:45				7
17.12.2020	08:45	30	0.01	<0.05	7
18.12.2020	08:45				7
19.12.2020	08:45				7
20.12.2020	08:45				7
21.12.2020	08:45	30	0.01	<0.05	7
22.12.2020	08:45				7
23.12.2020	08:45				7
24.12.2020	08:45				7
25.12.2020	08:45	30	0.01	<0.05	7
26.12.2020	08:45				7
27.12.2020	08:45				7
28.12.2020	08:45				7
29.12.2020	08:45	30	0.01	<0.05	7
30.12.2020	08:45				7
31.12.2020	08:45				7
01.01.2021	08:45				7
02.01.2021	08:45	30	0.01	<0.05	7
03.01.2021	08:45				7
04.01.2021	08:45				7
05.01.2021	08:45				7
06.01.2021	08:45	30	0.01	<0.05	7
07.01.2021	08:45				7
08.01.2021	08:45				7
09.01.2021	08:45				7
10.01.2021	08:45	30	0.01	<0.05	7
11.01.2021	08:45				7
12.01.2021	08:45				7
13.01.2021	08:45				7
14.01.2021	08:45	30	0.01	<0.05	7
15.01.2021	08:45				7
16.01.2021	08:45				7
17.01.2021	08:45				7
18.01.2021	08:45	30	0.01	<0.05	7
19.01.2021	08:45				7
20.01.2021	08:45				7
21.01.2021	08:45				7
22.01.2021	08:45	30	0.01	<0.05	7
23.01.2021	08:45				7
24.01.2021	08:45				7
25.01.2021	08:45				7

26.01.2021	08:45	30	0.01	<0.05	7
27.01.2021	08:45				7
28.01.2021	08:45				7
29.01.2021	08:45				7
30.01.2021	08:45	30	0.01	<0.05	7
31.01.2021	08:45				7
01.02.2021	08:45				7
02.02.2021	08:45				7
03.02.2021	08:45	30	0.01	<0.05	7
04.02.2021	08:45				7
05.02.2021	08:45				7
06.02.2021	08:45				7
07.02.2021	08:45	30	0.01	<0.05	7
08.02.2021	08:45				7
09.02.2021	08:45				7
10.02.2021	08:45				7
11.02.2021	08:45	30	0.01	<0.05	7
12.02.2021	08:45				7
13.02.2021	08:45				7
14.02.2021	08:45				7
15.02.2021	08:45	30	0.01	<0.05	7
16.02.2021	08:45				7
17.02.2021	08:45				7
18.02.2021	08:45				7
19.02.2021	08:45	30	0.01	<0.05	7
20.02.2021	08:45				7
21.02.2021	08:45				7
22.02.2021	08:45				7
23.02.2021	08:45	30	0.01	<0.05	7
24.02.2021	08:45				7
25.02.2021	08:45				7
26.02.2021	08:45				7
27.02.2021	08:45	30	0.01	<0.05	7
28.02.2021	08:45				7
01.03.2021	08:45				7
02.03.2021	08:45				7
03.03.2021	08:45	30	0.01	<0.05	7
04.03.2021	08:45				7
05.03.2021	08:45				7
06.03.2021	08:45				7
07.03.2021	08:45	30	0.01	<0.05	7