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Genetic variation in egg traits and early survival in rainbow trout

Genetisk variasjon i eggegenskaper og tidlig overlevelse hos regnbueørret

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Abstract

In farmed rainbow trout a large part of fertilized and incubated eggs and alevins are lost from fertilization until first feeding. The present study was conducted to estimate the overall means of different egg traits and early survival traits and the magnitude of the genetic variation of these traits in a nucleus population of rainbow trout based on data from three year-classes (2019, 2020 and 2021) and early survival data from two year-classes (2020 and 2021). The mean egg diameter was 5.38, 5.10 and 5.01 mm for each of the three year-classes. The percentage of good eyed eggs (two clearly visible eyes at the eyed-egg stage) 45.8, 45.7 and 61.5 indicating that about half of the fertilized eggs were lost in the hatchery. The correlation between mean egg diameter and female body weight was significant and positive in year-class 2021 (0.47) but not significantly different from zero in year class 2020 (0.07). The correlation between numbers of eggs per female and female body weight was significant and positive in year 2021 (0.75) but not significantly different from zero in year class 2020 (0.14). For the trait good eggs, the estimates heritability on the liability scale based on the sire component of variance for each of the three year-classes was 0.57 ± 0.21, 0.64 ± 0.17 and 0.42 ± 0.25. The corresponding heritability estimates based on the dam component of variance were very high which may indicate that not all the females have been stripped at the appropriate time.

For year-class 2020 the average survival rate from the eyed-egg stage to hatching was 81.6% and from the eyed-egg stage to first feeding 94.7%. For year-class 2021 the corresponding averages were 90.3 and 92.1%. For each of these two traits the sire and dam components of variance were very similar. On the liability scale the estimated heritability for the two survival traits for year-class 2020 was 0.59 ± 0.12 (eyed-egg stage to hatching) and 0.32 ± 0.07 (hatching to first feeding), and for year-class 2021 0.29 ± 0.05 (eyed-egg stage to hatching) and 0.31 ± 0.04 (hatching to first feeding).

Estimates of the genetic correlations between survival of eyed-eggs and alevins in the two succeeding periods could not be obtained due to lack of convergence of the parameters. The correlation between the family mean survival in the two periods was -0.04 for year-class 2020 and 0.35 for year-class 2021.

Overall, our findings indicates that there was significant genetic variation in egg quality traits and survival of eyed eggs and fry in rainbow trout and that these traits therefore can be improved

through selective breeding. The reason for the high losses of eggs until the eyed-egg stage need further investigation.

Sammendrag

Hos oppdrettet regnbueørret går en stor del av de befruktede eggene og yngelen tapt fra befruktning fram til startfôring. Denne studien ble utført for å estimere gjennomsnitt for ulike egenskaper hos egg og yngel og størrelsen på den genetiske variasjonen for disse egenskapene i en avlskjernepopulasjon av regnbueørret basert på egg data fra tre årsklasser (2019, 2020 og 2021) og tidlig overlevelse data fra to årsklasser (2020 og 2021). Gjennomsnittlig eggdiameter for de tre årsklassene var 5,38, 5,10 og 5,01 mm. Andelen gode øyerogn (to godt synlige øyne på øyerogn stadiet) var 45,8, 45,7 og 61,5, noe som indikerer at omtrent halvparten av de befruktede eggene gikk tapt i klekkeriet. Korrelasjonen mellom gjennomsnittlig eggdiameter og vekt av hunnfiken var signifikant og positiv i årsklasse 2021 (0,47), men ikke signifikant forskjellig fra null i årsklasse 2020 (0,07). Korrelasjonen mellom antall egg per hunnfisk og vekta av hunnfisken var signifikant og positiv for årsklasse 2021 (0,75), men ikke signifikant forskjellig fra null for årsklasse 2020 (0,14). For egenskapen gode øyerogn egg var arvegradsestimatet på den underliggende skalaen basert på varianskomponenten for far0,57 ± 0,21, 0,64 ± 0,17 og 0,42 ± 0,25 for hver av de tre årsklassene. De tilsvarende estimatene basert på morkomponenten var veldig høye, noe som kan indikere at alle hunnene ikke har blitt strøket til rett tidspunkt.

For årsklasse 2020 var den gjennomsnittlige overlevelsen fra øyerogn stadiet til klekking 81,6% og fra øyrogn stadiet til startfôring 94,7%. For årsklasse 2021 var tilsvarende gjennomsnitt 90,3 og 92,1%. For hver av disse to egenskapene var varianskomponentene for far og mor veldig like. På den underliggende skalaen var den estimerte arvegraden for de to overlevelsesegenskapene for årsklasse 2020 0,59 ± 0,12 (øyerognstadiet til klekking) og 0,32 ± 0,07 (klekking til første fôring), og for årsklasse 2021 0,29 ± 0,05 (øyerognstadiet til klekking) og 0,31 ± 0,04 (klekking til første fôring).

Et estimat av den genetiske korrelasjoner mellom overlevelse av øyerogn og yngel i de to påfølgende periodene kunne ikke oppnås på grunn av manglende konvergens av parametrene. Korrelasjonen mellom familie gjennomsnittene for overlevelse i de to periodene var -0,04 for årsklasse 2020 og 0,35 for årsklasse 2021.

Totalt sett viser funnene at det var signifikant genetisk variasjon i eggkvalitetsegenskaper og overlevelse av øyerogn og yngel hos regnbueørret og at egenskapene derfor kan forbedres gjennom

selektiv avl. Årsakene til de høye tapene av egg fram til øyerogn stadiet trenger nærmere undersøkelse.

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List of abbreviation

kg	:	Kilogram
mm	:	Millimeter
d^o	:	Day-degree
^oC	:	degree Celsius
NOK	:	Norwegian Kroner
r_G	:	Genetic correlation
HUFA	:	highly unsaturated fatty acids
PUFA	:	Polyunsaturated fatty acids
mg	:	Milligram
g	:	Gram
m	:	Meter
O_2	:	Oxygen
ml	:	Milliliter
dl	:	Deciliter
No.	:	Numbers
yc	:	Year-class

1. INTRODUCTION

1.1.Background

The development of commercial aquaculture in Norway began around 1970 and has since that time developed into a major industry in coastal areas (Venvik, 2005), with Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) as the two main species with a production of 1.4 million tonnes and 83,000 tonnes, respectively in 2019 (StatisticsNorway, 2020). Rainbow trout is the only fish species in Norwegian aquaculture which is not native. It was imported from Denmark around 1900 and was cultured in freshwater until the early 1960s (Jonsson et al., 1993; Venvik, 2005). Breakthrough in intensive rainbow trout production started after they were successfully transferred to seawater following smoltification. Rainbow trout is an anadromous species, which spend their life cycle in both freshwater and saltwater phase. Hatching of egg and smolt production takes place in freshwater tanks, while in Norway, on-growing to market size are cultured in the offshore net- cages in the sea (Venvik, 2005).

In their native areas, the wild rainbow trout have flexible life history (Figure 1). After the fish reach the age of 1-3 years, they migrate to seawater and spend 1-6 years at sea before returning to freshwater to spawn (Metcalfe & Thorpe, 1990; Metcalfe & Thorpe, 1992). In nature, these fish breed in cold and larger river streams after the sexual maturing male and female reach the spawning ground. The females prepare their reds (nests) (Quinn, 2018), and they mate in the early hour of the day (Hoitsy et al., 2012).

In a farmed environment, the eggs of rainbow trout are ovulated but not oviposited; they remain in the body cavity until they are artificially stripped from the fish (Bromage & Cumaranatunga, 1988). Offspring are produced by stripping eggs and semen from the sexually mature fish during spawning season to conduct artificial insemination and incubation until hatching (Ginatullina et al., 2018). Female trout are highly fecund, producing up to 2,000-3,000 eggs per kg body weight (Hoitsy et al., 2012; Jalabert, 2005), and eggs are relatively large in diameter, i.e. 5.1 mm on average (Gjedrem, 1993; Towers, 2010; Tyler et al., 1996). After the eggs are fertilized, they are incubated. They change into an eyed egg after about 175-day degree (d^o) and hatch after about

370-day degree (d^{o}) from fertilization at a freshwater temperature of 8 ^{o}C (Gjedrem, 1993). The fish spawn once a year, in spring (January-May), however implementation of selective breeding and photoperiod adjustment has created strain that can become sexual mature and spawn all year round (Towers, 2010).

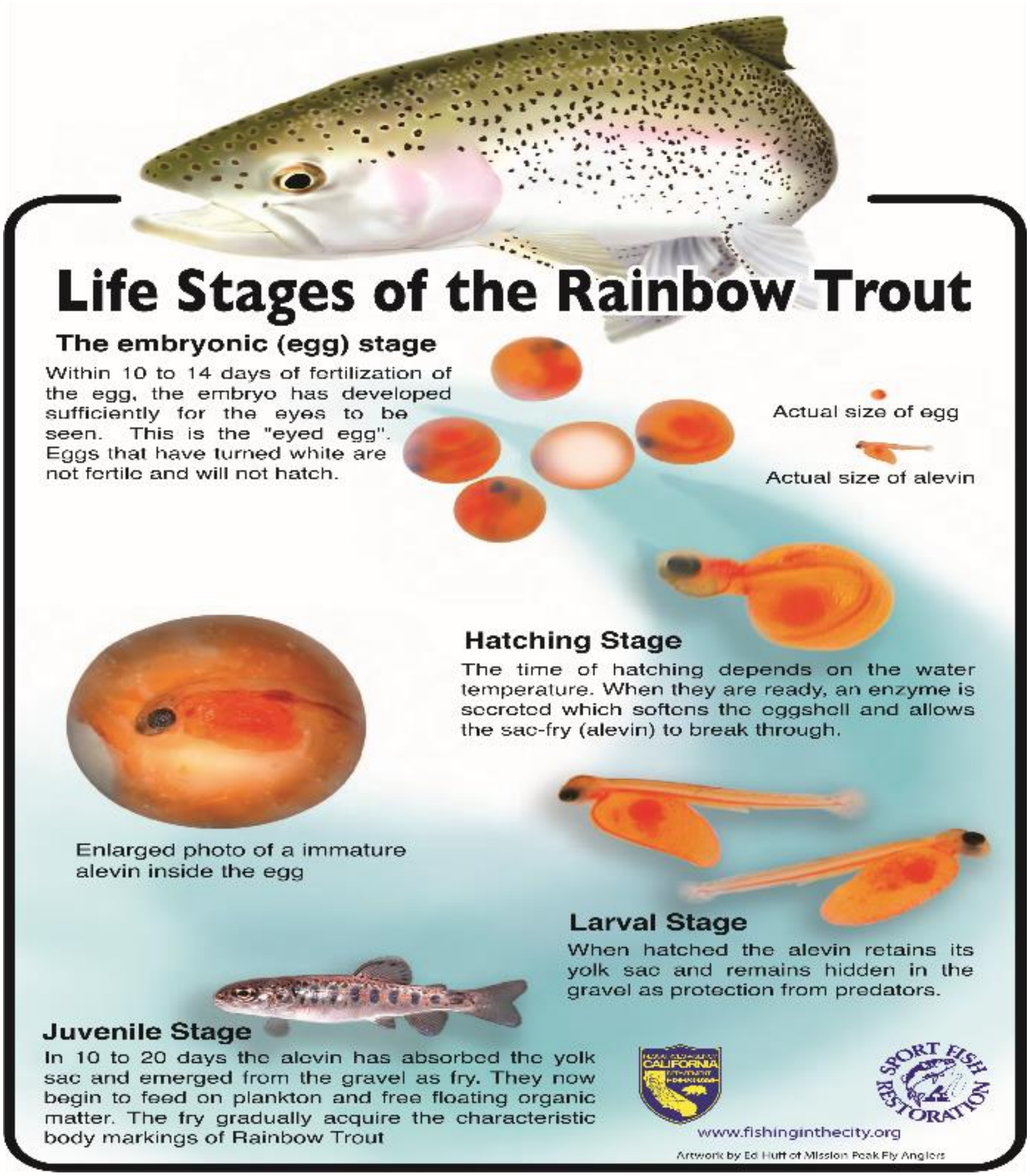

Figure 1. The early life stage of rainbow trout (Huff, 2009).

The early life stages are essential for the future performance of an individual, and effects in early events can affect the later stage of life (Pakkasmaa & Jones, 2002). In salmonid farming, a significant proportion of the incubated eggs are lost in the early freshwater periods. Few studies have been concerned with this problem, but the reasons for the high losses before the eyed egg stage and first feeding are still not well documented (Rye et al., 1990). It is therefore of interest to know whether genetic effects influence early survival traits or not. In the present study, genetic variation of different egg quality and early survival traits and their relationship with female body weight were investigated for rainbow trout (*Oncorhynchus mykiss*).

1.2. Statement of problem

More than 300 different species of finfish are cultured all over the world. Most of the fish seed comes from natural sources, which is not enough to supply the needs of farmers. Therefore, the important approach to satisfy the farmer's needs is to ensure a year-round supply of high-quality fertile eggs with high survival and individuals with high growth rates. Cultivation of broodstock that produces high-quality eggs become essential for this purpose (Watanabe et al., 1984) and one of the challenges in trout culture. The expansion of trout farming is constrained by many factors, including high mortality in early life stages as high as 50 % loss up to hatching (Brooks et al., 1997). The control of egg and sperm quality is a possible way of improving the sustainability of the system. During incubation and the early freshwater phase, survival rates ranged from 48 % to 98 %, with the lowest survival rate before the eyed-egg stage and during the first feeding (Rye et al., 1990). Briggs (1953) reviewed the work in rainbow trout hatcheries in the US and found that egg losses to the eyed egg stage were 19 % on average. Similar losses were also reported by Bromage and Cumaranatunga (1988) in their paper from the survey done on rainbow trout hatcheries in UK.

Salmonids such as rainbow trout have to be raised for several months or years before becoming sexually mature. If the final produced eggs are of poor quality, this leads to major economic losses (Bobe, 2015). The cost of one eyed-egg in Norway is 1-1.30 NOK. If the eggs are of bad quality or high mortality in the early stage, then there will be a significant economic loss to the individual farmers and fish industry. If we find out the measures to reduce mortality, this will be very beneficial to the industry.

Culture conditions like water quality, diet and feeding regimes, sex ratio, stocking density, photoperiod and water temperature are husbandry factors that may influence reproduction traits (Gebretsadik, 2007), and some genetic factors like sire and dam effects may influence reproductive traits. Several techniques have been reported to improve seed production in rainbow trout, including manipulating environmental conditions, such as temperature and photoperiod, and broodstock management strategies, such as conditioning brood fish before spawning and timely removing eggs for artificial incubation.

The shortage of egg, fry and fingerling production due to high mortality in early life stages is still one of the factors limiting the expansion of commercial trout culture. Therefore, increased knowledge of the factors with effects on broodstock reproduction is of significant importance to alleviate the problem of low egg quality and high mortality in the early stages.

The magnitude of genetic variation of different egg quality traits in farmed fish species is poorly investigated. The main objective of this study was to estimate the magnitude of the genetic variations in some egg quality and survival traits in a breeding nucleus population of rainbow trout.

1.3. Objectives of study

1.3.1. Main objective

- To estimate the genetic variation of different egg quality and survival traits in rainbow trout.

1.3.2. Specific objectives

- Estimate the magnitude of mortality in early life stages.
- Estimate the relationship of female body weight with different egg quality and egg survival traits.
- Estimate variations in different egg quality traits, and egg and fry survival rates between families.
- Discuss how to improve egg and fry survival through selective breeding.

2. LITERATURE REVIEW

2.1. Egg Quality

Good quality eggs are usually defined as the egg's ability to be fertilized, producing a normal embryo with high hatchability, and ultimately fry with increased survival and high growth rate (Bobe & Labbé, 2010; Bromage et al., 1992).

Egg quality is deeply influenced by environmental factors, husbandry practices, and the domestication level of the species. Under aquaculture conditions, poor egg quality can lead to a lack of fertilization, problems of egg activation during cell division, developmental arrests, and embryonic deformities (Bobe & Labbé, 2010; Bobe, 2015).

2.2. Reproductive traits

2.2.1. Fecundity

Fecundity is a measure of the number of offspring produced by an organism over time (Editors, 2017). It can be expressed in terms of either the number of eggs per female fish (relative fecundity) (Bromage & Cumaranatunga, 1988; Izquierdo et al., 2001) or the number of eggs contained in the ovary of a female fish (absolute fecundity) (Borthakur, 2018). For most salmonid fish like rainbow trout, the measure of relative fecundity is readily made because eggs are artificially stripped from each female at spawning (Bromage & Cumaranatunga, 1988).

Knowledge about the fecundity of a fish stock helps evaluate its commercial potential life history and cultural management of the fish (Borthakur, 2018). Fecundity and egg size increase with increasing body weight and thus the age of the fish (Sargent et al., 1987), and they are mainly used to measure egg production (Bromage & Cumaranatunga, 1988).

Egg size, usually defined in terms of the diameter of the egg or the number of eggs per unit volume or weight of eggs stripped (Bromage et al., 1992), is essential because purchasers of eggs often demand that they are of a specific size. In general, rainbow trout eggs under 4.75 mm in diameter (approx. 13,500 eggs per litre) are not acceptable for general sale (Bromage & Cumaranatunga, 1988).

It is known that egg size and larval sizes are positively correlated. Larger larvae tend to survive longer without food than those hatched from smaller eggs (Kjørsvik et al., 1990). Similarly, eggs' fecundity and size increase with the fish length (L'Abée-Lund & Hindar, 1990). Varghese (1973) found out that fecundity and ovary size are not influenced by egg size.

Bromage et al. (1990) studied 12 commercial stocks of rainbow trout and found that fecundity and egg size increase with the increasing fish size. Similarly, larger rainbow trout produce large eggs (Sargent et al., 1987), and the survival rate was higher in fry from larger than those from small eggs (Bagenal, 1969).

2.2.2. Time of stripping

Variability in egg quality of rainbow trout may be due to effects of post-ovulatory oocyte ageing. In a natural environment, mature oocytes are expelled at ovulation into the coelomic cavity, where they remain submerged in the ovarian fluid until environmental and social stimuli trigger spawning. Under farming conditions, these stimuli are absent, so oocytes remain in the body cavity until farmers manually strip them. If the fish are not stripped, the eggs degenerate and are gradually resorbed. This post-ovulatory ageing period lasts from several days to few weeks, during which changes constantly affects the egg viability and egg biochemical composition (Aegerter & Jalabert, 2004).

The time of ovulation and stripping may affect the survival rate in the early life stage of the rainbow trout offspring. The broodstock should be checked for ready to be stripped at regular intervals to achieve high fertilization rates so that eggs are stripped at optimum ripeness (Bromage & Cumaranatunga, 1988). Springate et al. (1984) demonstrated that the percentage of eggs reaching the eyed stage was 100% when stripped on the 6th-day post ovulation and that the percentage of hatchling and swim-up fry exceeded 94% when stripped at 4th day post-ovulation at 10 ^{o}C. The authors also found that larger eggs were obtained when stripped 4-6th day post ovulation. A similar result was obtained by Sakai et al. (1975) that found that the percentage of eyed eggs and hatching rate exceeded 70 % when the fish were stripped on the 10th day after ovulation. Craik and Harvey (1984) found the highest egg viability in eggs from the fish whose date of ovulation was known compared to the brood fish whose time of ovulation was unknown. Bromage et al. (1984) found that extended daylight (18L: 6D) will advance the spawning time and that the egg size was larger in short daylight (6L: 18D) treated fish as compared to controls (normal seasoned light cycle).

2.2.3. Age and body size at sexual maturity

Egg size is also affected by female parents' age and body size (Springate & Bromage, 1985). In his review paper (Briggs, 1953) mentioned that there is on average 18-19 % mortality in early life stages until fry stage of rainbow trout. He also mentioned that once the young fish is free swimming then they are safe and lively.

Female rainbow trout produced better quality eggs in the second than in the first spawning season (Brooks et al., 1997). Similar finding was reported by Bromage and Cumaranatunga (1988) who found that survival rate of eggs to the eyed egg stage from female trout from second spawning season was higher than of egg from female spawning for the first time. In contrast, brown trout eggs show similar survival rate of eggs to the eyed egg stage in both two and three-year-old fish groups (Bromage & Cumaranatunga, 1988).

In some two-year old first spawning rainbow trout groups egg size was not related to fish size (Bromage et al., 1992).Older and heavier female rainbow trout produce larger eggs and fry compared to younger broodfish (Gall, 1974; Hoitsy et al., 2012; Springate & Bromage, 1985).

Additional factors affecting egg quality are abiotic factors such as temperature, water quality and salinity and biotic factors such as availability of food during gonadal development also influence egg size (Kristjansson & Vøllestad, 1996).

2.3. Environmental effects

Environmental factors that affect the egg quality are diet of brood fish and the physiological condition of the water in which the eggs are incubated (temperature, salinity and pH). Also, the photoperiod to which the broodfish have been exposed, stress level on broodfish, fertilization process, over ripening of eggs in body cavity and bacterial colonization of fertilized eggs can affect the egg quality (Brooks et al., 1997; Davies & Bromage, 2002).

2.3.1. Temperature

Fish are poikilotherms, so, water temperature directly influences the dynamics of the reproductive cycle, age at first puberty (in relation to growth) and the quality of the eggs (Bobe, 2015; Brown et al., 2006). Water temperatures during spawning and incubation of the eggs are particularly important for the egg quality and also affect metabolism, activity and structure of the developing embryo (Kinne & Kinne, 1962).

Rainbow trout withstand vast ranges of temperature variation (0-25 ^{o}C), but the best temperature for the growth is between 16 and 18 ^{o}C and the optimum temperature required for spawning and hatching of eggs is 9-14 ^{o}C (Rai et al., 2005; Towers, 2015). The temperature during gametogenesis is important both for successful spawning and egg viability (Kjørsvik et al., 1990). In salmonid fish like rainbow trout, the temperature at which eggs are incubated has a notable impact on the survival and time of hatching. High or low temperature during incubation can results in embryo mortality and deformities during early development stages (Brooks et al., 1997). To reduce deformities in Atlantic salmon, the recommended temperature during egg rearing is less than or equal to 8 ^{o}C, and after first feeding less or equal to 12 ^{o}C (Baeverfjord et al., 1998; Ytteborg et al., 2012).

Pankhurst et al. (1996) reported that sexually matured female trout held in temperature of 9 and 15 ^{o}C for three months prior to ovulation had higher egg production, higher survival to the eyed stage as compared to female that are kept at 18 and 21 ^{o}C (almost nil). This shows that elevated holding temperature may have deleterious effect on ovulation, egg production and survival. Similar result was obtained by Güner et al. (2012).

Ahmet et al. (2010) found that brown trout incubated at 8 ^{o}C reached the eyed egg stage four days before eggs incubated at 7 ^{o}C. Similarly, at the same temperature, eggs of Black sea trout eyed 6 days ahead than eggs of brown trout while hatching of the black sea trout eggs were three days after hatching of the brown trout eggs. This indicate that incubation temperature and its variation were the main factors controlling the duration of the early development stages of fish.

Davies and Bromage (2002) studied effect of spawning time through combined photoperiod and water temperature of female rainbow trout and found that these factors have no significant effect on fecundity and egg survival until hatching but that the egg diameter was decreased as the spawning time was advanced.

Aegerter and Jalabert (2004) studied the effect of post-ovulatory oocyte ageing on the eyed egg development success in rainbow trout and found that when females were exposed to 17 ^{o}C for a few weeks before ovulation, it might lead to reduced embryo survival rate and some are no longer viable. They also found this increased the malformation rates of the fry and also in increased proportion of triploid fry (Bobe, 2015). Likewise, elevated temperature during egg rearing and start feeding stage in Atlantic salmon (*Salmo salar*) lead to increasing number of fish with skeletal abnormalities, heart failure and jaw deformities (Baeverfjord et al., 1998; Ytteborg et al., 2012). Photoperiod manipulation has proven to be a useful tool in altering the time of spawning of fish in aquaculture (Bromage et al., 1984; Pankhurst et al., 1996) and reproductive development of salmonid fish is known to be strongly influenced by photoperiod (Bromage, 2001; Dabrowski & Blom, 1994). It is recommended not to expose developing larvae to strong light, which may indirectly leads to high mortality (Hoitsy et al., 2012).

2.3.2. Nutrition

Fish nutrition can impact the egg yolk composition and also impact the egg quality (Watanabe et al., 1984). Brood fish diets should be optimized to improve egg morphology, early egg development and larval survival (Bobe, 2015; Izquierdo et al., 2001).

Salmonid broodstock should be fed a good quality diet for several months before the spawning season to enhance their reproductive performance (Izquierdo et al., 2001; Watanabe et al., 1984). Watanabe et al. (1984) demonstrated that supplying 36 % protein and 18 % lipid in broodstock diet resulted in more eyed eggs and higher hatchability as compared to a diet with 28 % protein and EFA (Essential Fatty Acid) -deficient feed. They also found that EFA-deficient diet results in low egg production and egg quality in rainbow trout.

Ascorbic acid plays an important role in salmonid reproduction (Izquierdo et al., 2001). Several studies have been conducted on ascorbic acid and its deposition in rainbow trout eggs (Blom & Dabrowski, 1995; Dabrowski & Blom, 1994). These authors have shown that vitamin C is essential for the synthesis of collagen during embryonic development of rainbow trout and they also found that deficiency of this vitamin in female broodfish diet results in higher egg mortality. The requirement of ascorbic acid was up to eight times higher in juveniles rainbow trout as compared to in adults (Izquierdo et al., 2001).

Rainbow trout broodstock that were fed a diet with high carbohydrate and low protein content resulted in high fecundity and high egg survival to the eyed egg stage and also increased hatchability compared to fish fed a high protein diet (Washburn et al., 1990).
Honeyfield et al. (2005) found that early mortality syndrome in lake trout *(Salvelinus namaycush)* was due to thiamine deficiency in the broodfish diet.

2.3.3. Salinity

In rainbow trout culture, mortality is directly proportional to the salinity. As the salinity increases, mortality increases and growth ceases. Salinities above 20 % may have detrimental effects on trout production (McKay & Gjerde, 1985). Rainbow trout grow well in both fresh and sea water. However, prior to becoming sexual mature they must be transferred to freshwater, otherwise they will suffer high mortality (Gjedrem & Gunnes, 1978). Similarly, the ovulation time of Atlantic salmon was earlier when the broodfish was transferred to fresh water 3-4 months before the expected ovulation time as compared to broodstock kept in sea (Magwood et al., 2000). In contrast, Tatum (1976) suggested that brackish water is more favorable than fresh water in rainbow trout culture as mortality was higher in fresh as compared to brackish water.

2.4. Genetic parameters

2.4.1. Genetic variation

Rye et al. (1990) found that heritability for survival in early life of Atlantic salmon based on the dam components of variance were higher than those based on the sire component which indicate dam component is biased upwards due to non-genetic effects, maternal effects and tank effect. Likewise, Kanis et al. (1976) reported that the heritability from dam components were higher than those from the sire components. Heritability estimates of mortality for eyed eggs were found to be high but rather low for alevin mortality, which indicates that magnitude of maternal effects as well as non-additive genetic effect are diminishing during development of embryo and after hatching. Moreover, Gjedrem et al. (1986) also discovered that in rainbow trout, dam components of variance were particularly high for egg diameter and body weight compared with the sire components.

It was also evident that, in rainbow trout, the fertility and lipid contents of eggs are result of variation in genetic and environmental factors (Kristjansson & Vøllestad, 1996).

2.4.2. Phenotypic correlation

Springate and Bromage (1985) reported that there was positive correlation between mean initial egg size (water-hardened egg) and survival of fry during first feeding (8 weeks after fertilization) but after 2 week of first feeding there was no correlation between egg size and fry weight. They also found that there was no significant relationship between egg size and egg survival at early development stages (eyeing, hatch, swim up and 3-month fed fry).

2.4.3. Genetic correlation

Rye et al. (1990) found positive genetic correlation between growth and survival in both rainbow trout and Atlantic salmon. Gjerde (1986) mentioned in a review paper that there is no strong unfavorable correlation between egg size, egg number, egg volume and female body weight in rainbow trout. The amount of yolk is crucial for larval growth and survival in brown trout (*Salmo trutta L),* and the egg size in brown trout (*Salmo trutta L*.) is related to the concentration of yolk present in eggs (Bagenal, 1971). It is shown by that amount of yolk is important for larval growth and survival in trout (*Salmo trutta L.)* and herring (*Clupea hurengus L.*) (Bagenal, 1969; Bagenal, 1971; Blaxter, 1963).

Gjedrem et al. (1986) mentioned that egg volume and egg number per female were both highly genetically and phenotypically correlated in both rainbow trout and Atlantic salmon. However, in Atlantic salmon, there were quite high negative genetic correlations between egg size and egg volume (r_G = 0.56) and between egg size and egg number (r_G = 0.71). Egg volume and egg number were highly correlated genetically with body weight and length in Atlantic salmon whereas, in rainbow trout, these correlations were not significant.

2.5. Salmonid species, other fish species

Some study shows that the fecundity is positively related to body weight, total length and ovary weight. For example, the number of eggs in the freshwater garfish (*Xenontedon cancila)* increased with the increase in total length, body weight and ovary weight (Borthakur, 2018). (Bromage et al., 1994) mentioned that in Atlantic halibut fertilization should be done in between 4-6 hours after ovulation because egg quality will decrease if they are fertilized after that. Similarly, McEvoy (1984) found that maximum egg viability in turbot is ensured if the fish was stripped within 10 hour after ovulation at the temperature of 12-14 ^{o}C (Jeuthe et al., 2013). In Pink salmon *(Oncorhynchus gorbuscha*) artificial light was used to delay the spawning time of pink salmon and which increased the egg mortality until the eyed egg stage from 60 to 80 % (Dabrowski & Blom, 1994; MacQuarrie et al., 1979).

Brown et al. (2006) found that rising the water temperature during the spawning season and the vitellegenous period in Atlantic halibut (*Hippoglossus hippoglossus L.*) caused delay in spawning time and reduction in quality and quantity of the eggs. Delayed spawning less than two weeks after ovulation had no effects on fertilization and survival rate in Pacific herring (*Clupea pallasii*), but progressive loss of eggs and larva viability are consequences of longer delays (Hay, 1986). Experiment on gilthead sea bream showed that fish fed a diet with squid oil (rich in n-3 HUFA) produced more viable eggs than fish fed a diet with soybean oil (rich in n-6 PUFA) and that there should be a minimum deposition of 17 mg n-3 HUFA/g dry weight in the egg to ensure good egg quality (Harel et al., 1994).

3. MATERIAL AND METHODS

3.1. Location of experiment and fish material

The experiment was conducted at Osland Havbruk AS, Bjordal, which is one of the oldest fish farming company in Norway. Bjordal is located in Høyanger Municipality in Vestland County, Norway along the Fuglsetfjorden, an arm that branches off south of the main Sognefjorden at the altitude of 3 m above sea level and latitude 61° 04′35″N 05° 49′47″E.

Figure 2. Map of location of experiment.

The Osland farmed strain of rainbow trout was established based on fish material purchased from Denmark and is one of the three farmed strains of rainbow trout in Norway. Data were obtained from nucleus families of year-class 2019 and 2020 of generation 18 and 2021 of generation 19 (Table 1). Eggs from year class 2019, 2020 and 2021 were used to study the variation among the families for number of eggs per female, egg diameter and egg diameter distribution and percentage of eyed eggs. Data from year class 2020 and 2021 was used to study survival from the eyed egg stage to hatching and from hatching to start feeding.

Table 1. Produced year classes from 1964 to 2020.

	Sub-populations		
Generation	**1**	**2**	**3**
0	1964	1965	1966
1-10	1967-1994	1968-1995	1969-1996
11	1997	1998	1999
12	2000	1999	2002
13	2003	2004	2005
14	2006	2007	2008
15	2009	2010	2011
16	2012	2013	2014
17(0)	2015	2016	2017
18 (1)	2018	2019	2020

3.2. Preparation of brood fish

About 2 ½ year old brood fish were in August/September transferred from a cage (Figure 3) in the sea (30-32 ‰ salinity) to cement tanks (Figure 4) with brackish water (8-12 ‰ salinity) 5-6 months before the breeding season in January/February. The male and female brood fish were kept in the tanks without feeding for four months prior to the stripping; consequently they use their stored nutrient in the body for gonadal development. The temperature in the tank was maintained between 10-12 ^{o}C with 80-90 % O_2 saturation and 8-12 ‰ salinity and these parameters were recorded daily. Body weight of the females were measured in October/November.

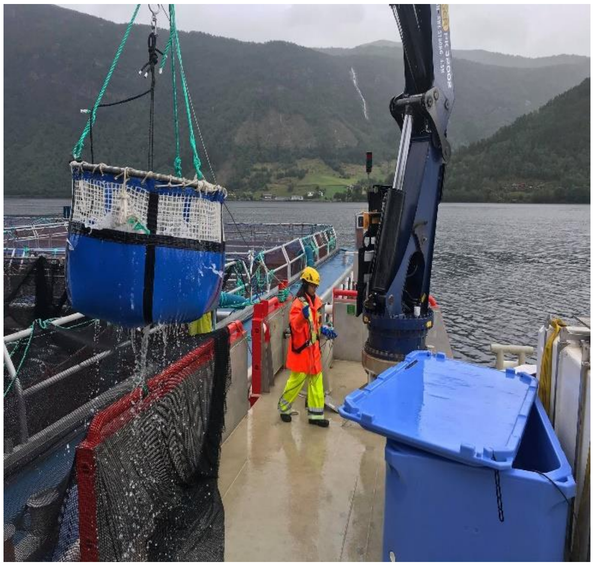

Figure 3. Transferring brood fish from sea cage to freshwater-tank

Figure 4. Cemented tank where the brood fish are kept until stripping

3.3. Stripping and fertilization

The females are checked for sexual maturity once a week, and from those found ready for stripping some eggs/ovarian fluid was collected and send it to a lab to make sure that the female is deprived of IPNV (Infectious Pancreatic Necrosis Virus).

The day after having received the results from the lab, the fish is made unconscious by putting them in water mixed with anesthesia (Tricaine) after which the fish are hung from the tail and their gills are cut so that all the blood in the body is drained out. After this, the abdomen is open and the eggs from each female are collected in a bowl (Figure 5), the ovarian fluid is rinsed off, the weight of all the eggs is recorded and then rinsed with physiological saline water (0.9 % salinity). A vet doctor check if the fish have any deformities in its body such as bent backbone, kidney stone and pale liver. If any deformity is found the egg from that female is not fertilized and discarded.

Milt from the chosen male is collected by stripping (Figure 6), or cryopreserved milt is used. The milt and activator (for frozen milt) is added to the eggs and mixed well by hand with glove so that all the egg get fertilized (Figure 7). A new glove is used for each female to prevent contamination of milt and diseases among the eggs from the different females.

Milt from one male is used to fertilize the eggs from two different females in a nested mating design. For each year classes about 120 full-sib families are produced thus being the offspring of about 60 sires and 120 dams. A procedure is developed to prevent the mating of too closely related parents (e.g. fullsibs and halfsibs) to prevent inbreeding, inbreeding depression and reduction in the genetic variance.

Two minutes after fertilization and water hardening, the fertilized eggs are again washed with physiological saline water twice and the eggs are disinfected with buffodine solution (9 ml buffodine in 10 liter physiological saline water) and left for 10 minutes. After this, 2 dl of the fertilized eggs (about 2500 eggs) from each female are kept in a separate cake (Figure 8) stacked inside a cylinder with a total of 8 such cakes (Figure 9). The conditions in all cakes within a cylinder are kept as similar as possible through a continuous flow of gently aerated freshwater in all cylinder. During the egg incubation period, water temperature is measured daily and should not exceed 8 ^{o}C and not below 4 ^{o}C. All the eggs are kept in cakes inside cylinder until they reach eyed egg stage (Figure 10).

Soon after the eggs reach the eyed egg stage (about 300 d°), they are subjected to shocking by dropping the eggs from 1.5 m height to a bucket filled with water. Shocking of eggs are done (Figure 11) so that the non-fertilized or dead eggs are turned white and can easily be separated from the fertile healthy ones. After shocking, they are run through the Rognmaskin (Egg machine) G3 (Figure 12) in which their individual diameter is measured and unfertilized and bad eggs are sorted out.

After the measurement, two samples each of 50 eggs per family was chosen randomly (Figure 13) for the survival test and kept in the hatching cabinet (Figure 14 & 15).

Figure 5. Collection of eggs by cutting the abdomen of female fish

Figure 6. Collection of milt from male by stripping.

Figure 7. Fertilizing the eggs by adding and mixing with milt.

Figure 8. 2 dl of fertilized eggs form each female kept in one cake.

Figure 9. Eight cakes stacked in each cylinder.

Figure 10. Cakes containing all type of eggs before shocking.

Figure 11. Shocking of eggs.

Figure 12. Putting the eyed egg from of each fullsib family into the Rognmaskin G3 to measure the eyed egg traits data.

Figure 13. Random sampling of 2 x 50 eyed egg from each family

Figure 14. Two samples of 50 eyed eggs from each family were kept in separate cakes in the hatching cabinets.

Figure 15. The cakes are put into hatching cabinets.

3.4.1. Egg traits

For each stripped female of each year class (2019, 2020 and 2021) the following traits were recorded: Number of eggs and egg diameter distribution._The traits were obtained from the Rognmaskin G3 (Figure 16). After the egg reach eyed egg stage, they were kept in the machine to separate good eyed egg from unfertilized and bad eggs.

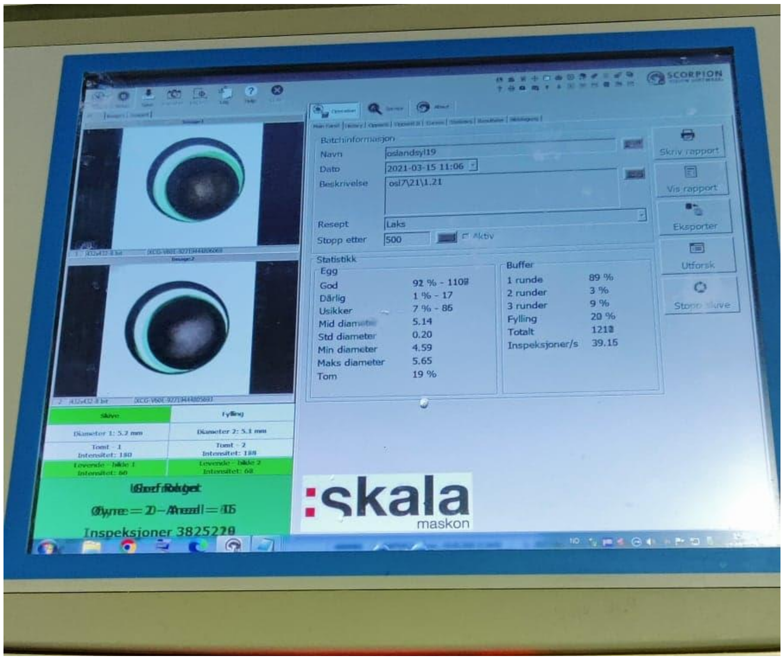

Figure 16. The egg traits were recorded by the Rognmaskin G3.

Table 2. Number of sires, dams and families for each of the three year classes.

Year class	No. of		
	Sires	Dams	Families
2019	46	59	59
2020	76	111	111
2021	57	75	75

Out of the total number of sire used in fertilization, no. of sires mated to two or more dams was for

yc2019 11 + 1 sire mated to three dams (out of in total 59 families);

yc2020 31 (out of in total 111 families);

yc2021 18 (out of in total 75 families.

Calculation of number of eggs per liter of eggs

I took 1 liter of rinsed newly stripped and unfertilized eggs from each of two females and counted the total number of eggs and found out that:

1 liter of eggs = 1.12 kg eggs = 11650 eggs

Classification of eyed eggs

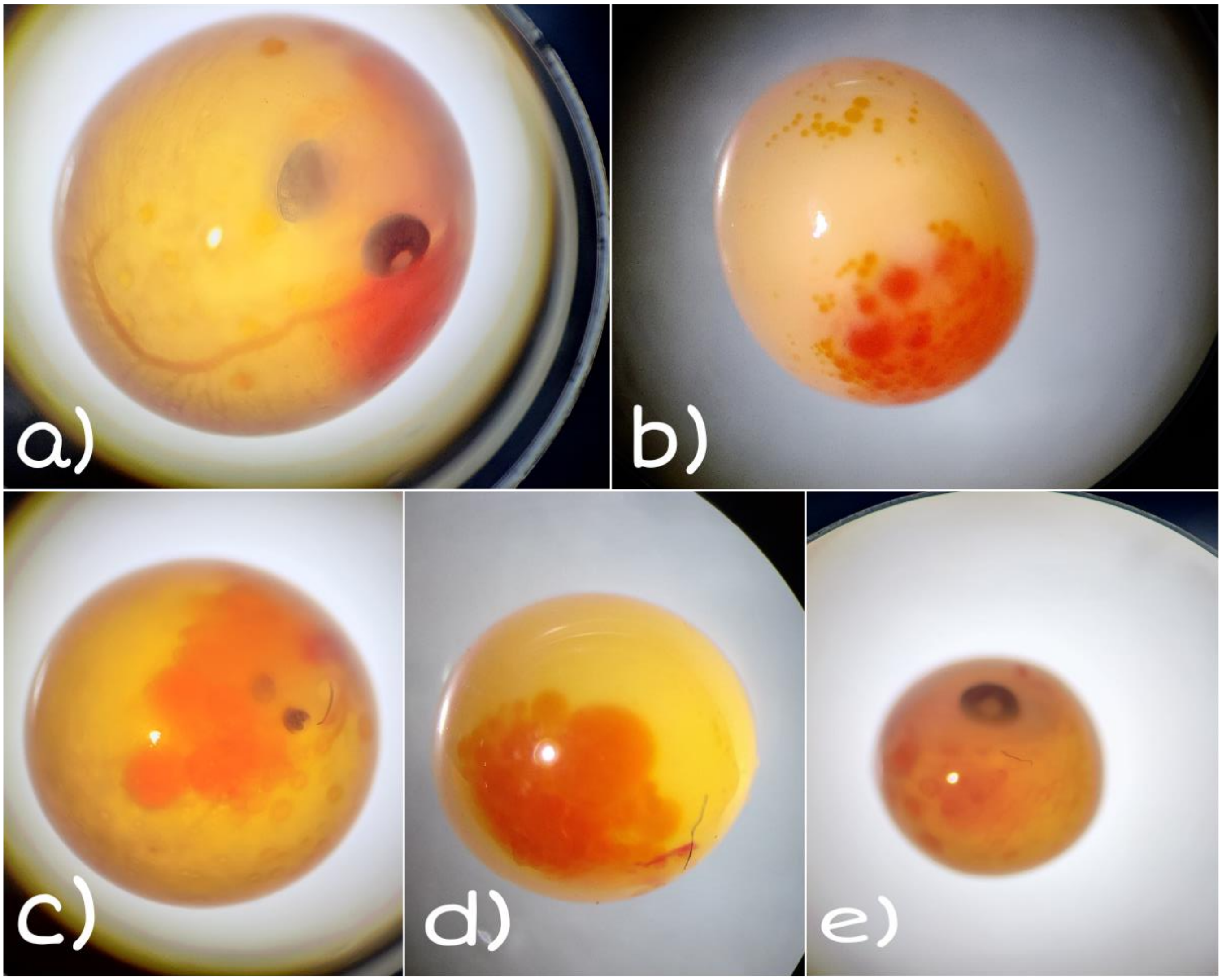

Figure 17. Classification of egg traits. **a)** Good eyed egg: Good egg is characterized by clear visible embryo with two eyes and pumping of heart under microscope. Only good eyed egg are further used for production and sale; other traits mentioned below are discarded. **b)** Bad eyed egg: Bad egg is characterized by dark white clog inside the egg and no visible embryo inside it. **c)** Too-small eyed egg: Small eyed egg is characterized by clear embryo with too small eyes but the egg

diameter is optimal (more than 4.40mm). **d)** Unfertilized egg: unfertilized egg is characterized by clogged nucleus inside the egg. **e)** Small eyed egg: small eyed egg is characterized by clear embryo with big eyes but the size of egg is small (< 4.40mm).

The following traits were calculated:

Ovasomatic Index: $\frac{\text{Total weight of eggs per female}}{\text{Body weight of female}} X\ 100\%$

Fecundity = $\frac{\text{Total no of eggs per female}}{\text{Body weight of female}}$

Eyed-eggs, % = $\frac{\text{No. of eyed eggs per female}}{\text{Total no. of eggs per female at fertilization}} \text{X}\ 100\%$

3.5. Survival of eyed-eggs and fry

For the study of survival from the eyed-egg stage to hatching and from hatching until first feeding, thirty-three full-sib families were chosen of year class 2020 and 60 full-sib families of year-class 2021. Two samples of 50 eyed egg were chosen randomly from each family (Table 2).

Table 3. Descriptive statistics of the experimental design of the survival of the eyed egg and fry study.

Year class	No. of					
	Sires	Dams	Families	Replicates	Eggs/replicate	Eggs
2020	24	33	33	2	50	3300
2021	37	49	49	2	50	4900

The following traits were calculated:

Hatched eggs, % = $\frac{\text{Total no.of alevins}}{\text{Total no. of eyed eggs}}\ X\ 100\%$

Fry-survival (%) = $\frac{\text{No. of fry}}{\text{Total no. of hatched alevins}} X\ 100\%$

3.5.1. Recordings

Water temperature in the hatchery was recorded daily. For each full-sib family (fertilization to the eyed-egg stage), and for each of the two replica within family (hatching until first feeding), the following traits were counted at the different life stages:

No. of uneyed eggs from fertilization until the eyed egg stage and the date of the recording.
No. of dead eyed eggs at hatching.
No. of alive yolk-sac fry after all eggs had hatched.
No. of dead and alive fry from hatching until start feeding.

3.6. Statistical analysis

3.6.1. The egg traits

The female traits

Variation between females for the female traits Ovasomatic Index and Fecundity are shown as bar graphs. The relationship of egg diameter and estimated no. of eggs stripped per female on body weight was studied using a liner regression of each of the two traits on the 1st and 2nd degree polynomial of body weight.

The family traits

Estimates of genetic parameters for the two either-or traits survival of eggs from the eyed-egg stage to hatching and survival of alevins from hatching to first feeding were derived from the estimated variance components of the random effects of the following single trait linear mixed sire and dam model using the ASReml software (Gilmour et al, 2009):

$$y = Xb + Z_1 u_s + Z_2 u_d + Z_3 r_t + e \qquad (1)$$

where y is the observed either-or trait dead (0) or alive (1), b is the fixed effect of year-class, u_s and u_d is the additive genetic effect of the sire and the dam, respectively, r_t is the replicated tank effect, e is the residual effect, and X, Z_1, Z_2 and Z_3 are design matrices that assign each observation to the appropriate level of the fixed effect and the three random effects sire, dam and replicated tank effect; u_s and $u_d \sim N(0, \mathrm{A}\sigma_u^2)$, where $\sigma_{u_s}^2 = \sigma_{u_d}^2 = \frac{1}{4}\sigma_a^2$; where σ_a^2 is the additive genetic variance and ***A*** is the additive genetic relationship matrix among the sires and dams and their grandsires and granddams.

For the either-or trait trait good eyed-eggs, bad eyed-eggs and good and bad eggs, for which no replicate was available, estimates of the genetic parameters was obtained from a similar model but without the replicated tank effect.

Estimates of the variance components were also obtained on the liability scale using a similar non-liner sire and dam model.

The heritability from the sire and the dam component of variances of each trait was calculated as:

$$h_s^2 = \frac{4\sigma_{us}^2}{\sigma_{us}^2+\sigma_{ud}^2+\sigma_e^2}\ , \ h_d^2 = \frac{4\sigma_{ud}^2}{\sigma_{us}^2+\sigma_{ud}^2+\sigma_e^2}$$

For the heritability estimate on the liability scale the error variance was set equal to 1.0.

4. Results

4.1.The egg quality traits

Descriptive statistics for the studied traits for each of the three year-classes are presented in Table 4. The mean egg diameter varied from 5.01 mm (2021) to 5.38 mm (2019). The mean no. of eggs per kg body weight varied considerably from 379 (2019) to 696 (2020). The percentage of good eyed egg varied from 45.7 (2020) to 61.5 (2021), while the percentage of too small eyed-eggs were low and varied from 0.2 (2019) to 2.4 (2020). From the table 4, we can say that almost half of the produced egg are discarded and not used further for production and sale.

Table 4. Descriptive statistics for the recorded egg traits of each year class.

Year-class[1] / Trait	Mean	SD	Min	Max
2019				
Female body weight, kg	7.52	1.07	4.8	9.7
No. of eggs/kg female body weight	379	114	50	804
Egg diameter (mm)	5.38	0.20	4.83	5.83
Good eyed eggs (%)	45.8	21.8	15.4	92.6
Bad eyed eggs (%)	32.1	22.0	1.5	76.7
Unfertilized eyed egg (%)	22.0	15.5	1.1	58.3
Too small eyed egg (%)	0.2	15.6	41.6	98.9
2020				
Female body weight, kg	6.06	0.77	4.5	8.2
No. of eggs/ kg female body weight	696	140	380	1202
Egg diameter (mm)	5.10	0.13	4.63	5.44
Good eyed eggs (%)	45.7	23.1	2.1	91.4
Bad eyed eggs (%)	41.3	22.7	5.3	95.1
Unfertilized eyed egg (%)	10.6	10.4	0.8	59.5
Too small eyed egg (%)	2.4	6.1	0.0	34.6
2021				
Female body weight, kg	6.61	0.98	4.4	9.5
No. of eggs/kg female body weight	560	147	274	1047
Egg diameter (mm)	5.01	0.18	4.64	5.38
Good eyed eggs (%)	61.5	25.9	2.8	92.6
Bad eyed eggs (%)	30.9	25.4	3.9	95.1
Unfertilized eyed egg (%)	6.9	6.1	0.3	38.5
Too small eyed egg (%)	0.6	1.4	0.0	7.4

[1] No. of families; 59 for yc2019, 111 for yc2020 and 75 for yc2021.

4.2. The survival traits

Table 5 shows that the mean survival of eggs from the eyed-egg stage to hatching was higher in year class 2021 (90.3 %) while the mean survival of alevin from hatching to first feeding was higher in year class 2020 (94.7 %).

The survival percentage of eyed-eggs until hatching and of alevins from hatching to first feeding for each family is presented in Figure 18 (year-class 2020) and Figure 19 (year-class 2021).

Table 5. Mean survival (%) of eggs from the eyed stage to hatching and of alevin from hatching to first feeding for the families of the two studied year classes.

Year-class	No. of fam.	Mean	SD	Min	Max
2020					
Eyed egg to hatching, %	33	81.6	15.5	42.0	100.0
Hatching to first feeding, %	33	94.7	3.6	85.8	100.0
2021					
Eyed egg to hatching, %	49	90.3	12.2	43.0	100.0
Hatching to first feeding, %	49	92.1	6.6	71.2	100.0

Genetic correlation between survivals in the two period was not possible to obtain due to lack of convergence. Correlation between family mean was -0.037 for year class 2020 and 0.35 for year class 2021.

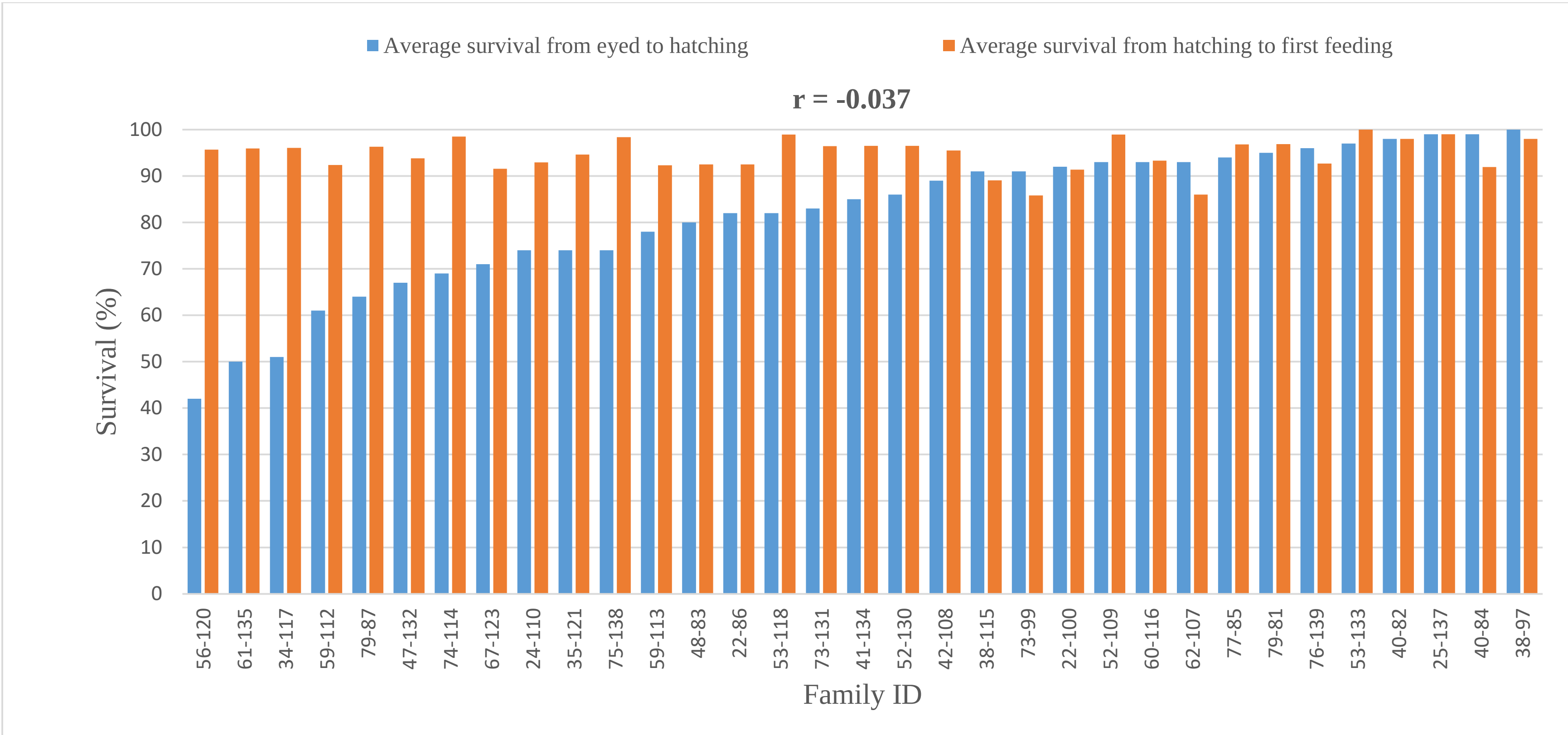

Figure 18. Survival (%) of alevin of the year-class 2020 families from the eyed egg stage to first feeding.

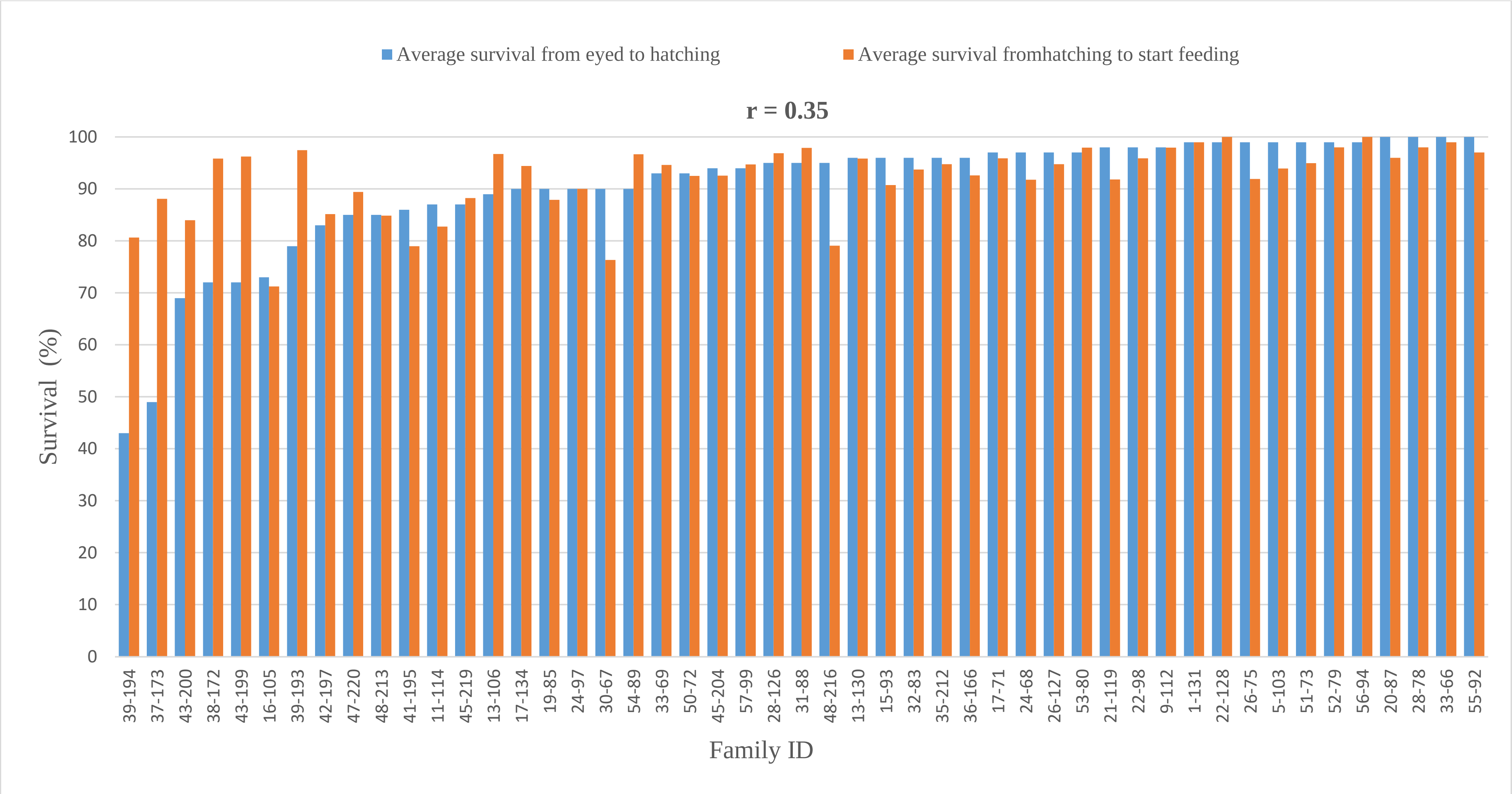

Figure 19. Survival (%) of alevin of the year-class 2021 families from the eyed egg stage to first feeding.

4.3. Regression of egg diameter on body weight of female

Figure 20 shows that the relationship between body weight of female and mean egg diameter was not significant for year-class 2020 (r = 0.068, P>0.05) but significant and positive for year class 2021 (r = 0.47, P<0.01).

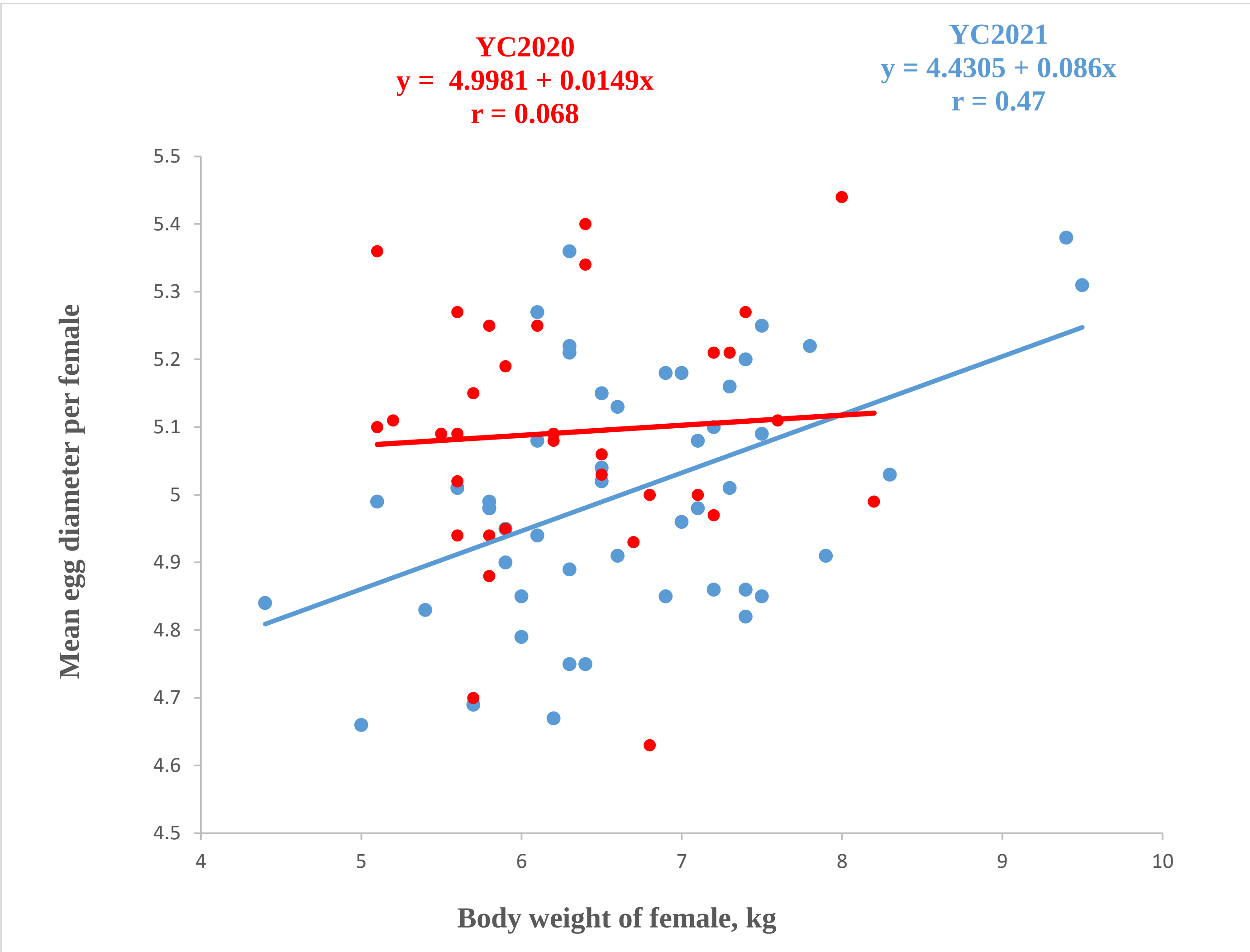

Figure 20. Linear regression of mean egg diameter on body weight of female.

4.4. Regression of total no of eggs on body weight of female

Figure 21 shows that there was no significant relationship between body weight of female and total number of eggs (r = 0.143, P>0.05) but significant and positive for year-class 2021 (r = 0.75, P<0.01).

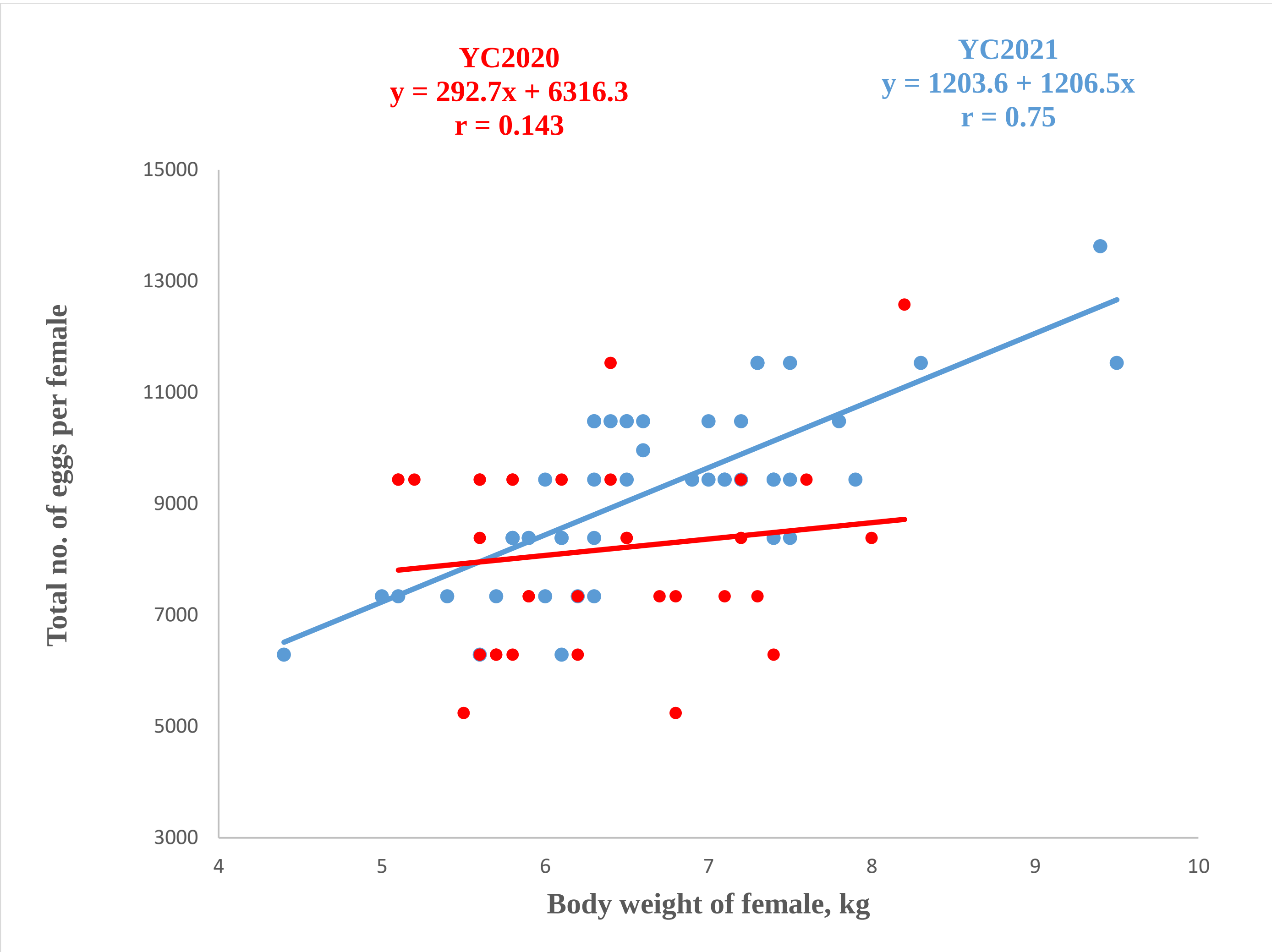

Figure 21. Linear regression of total no of eggs on body weight of female.

4.5. Regression of egg diameter on survival of eyed-eggs until hatching and of alevins from hatching to first feeding

Figure 22 shows that there was no significant relation between mean egg diameter and survival in two periods (eyed egg to hatching and hatching to first feeding; r = 0.20; r = 0.008 respectively).

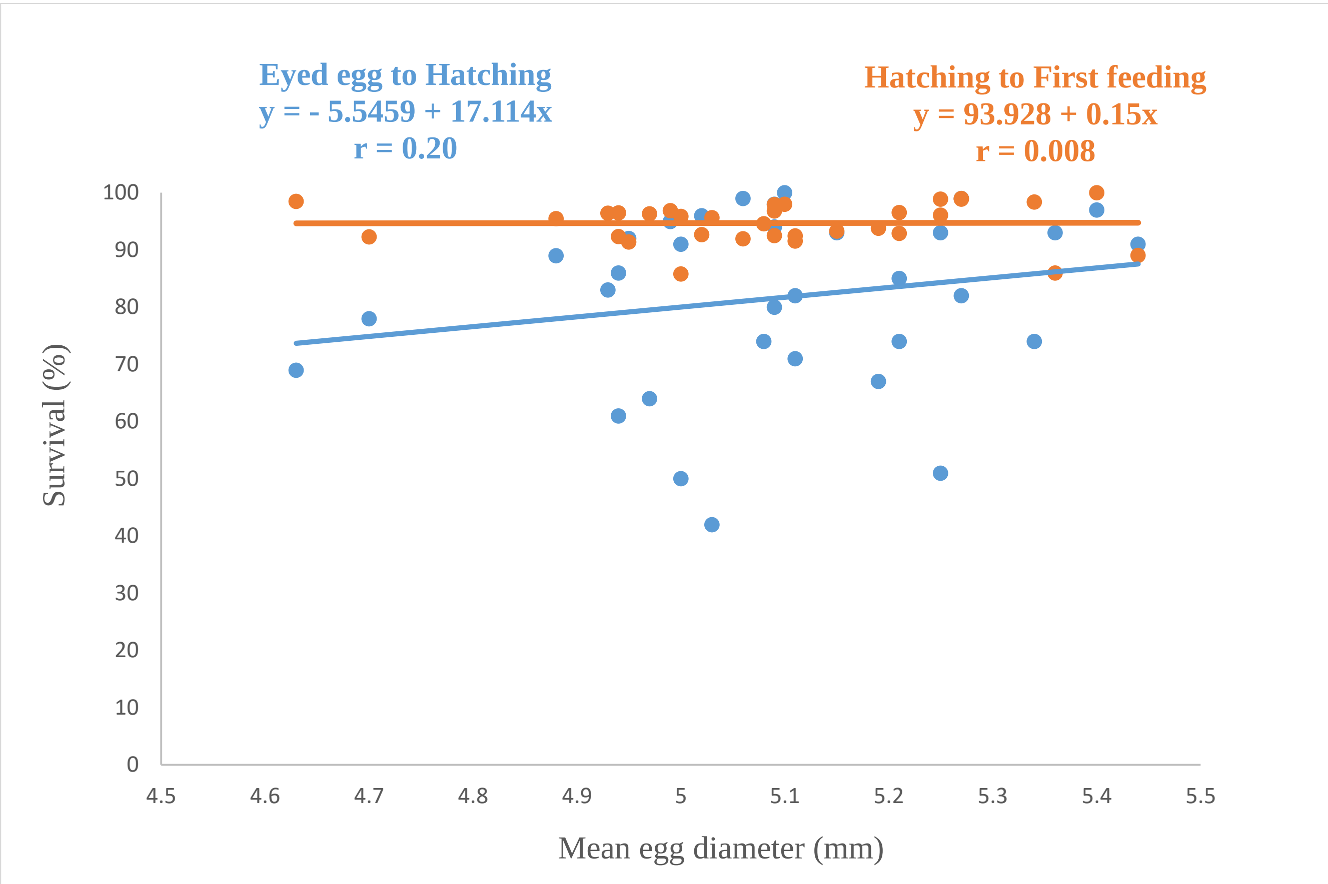

Figure 22. Regression of egg diameter (mm) on survival of eyed-eggs until hatching and of alevins from hatching to first feeding for YC2020.

Figure 23 shows that the relation between egg diameter and survival within two periods (eyed egg to hatching and hatching to first feeding) was not significant and negative (r = -0.13; r = -0.21).

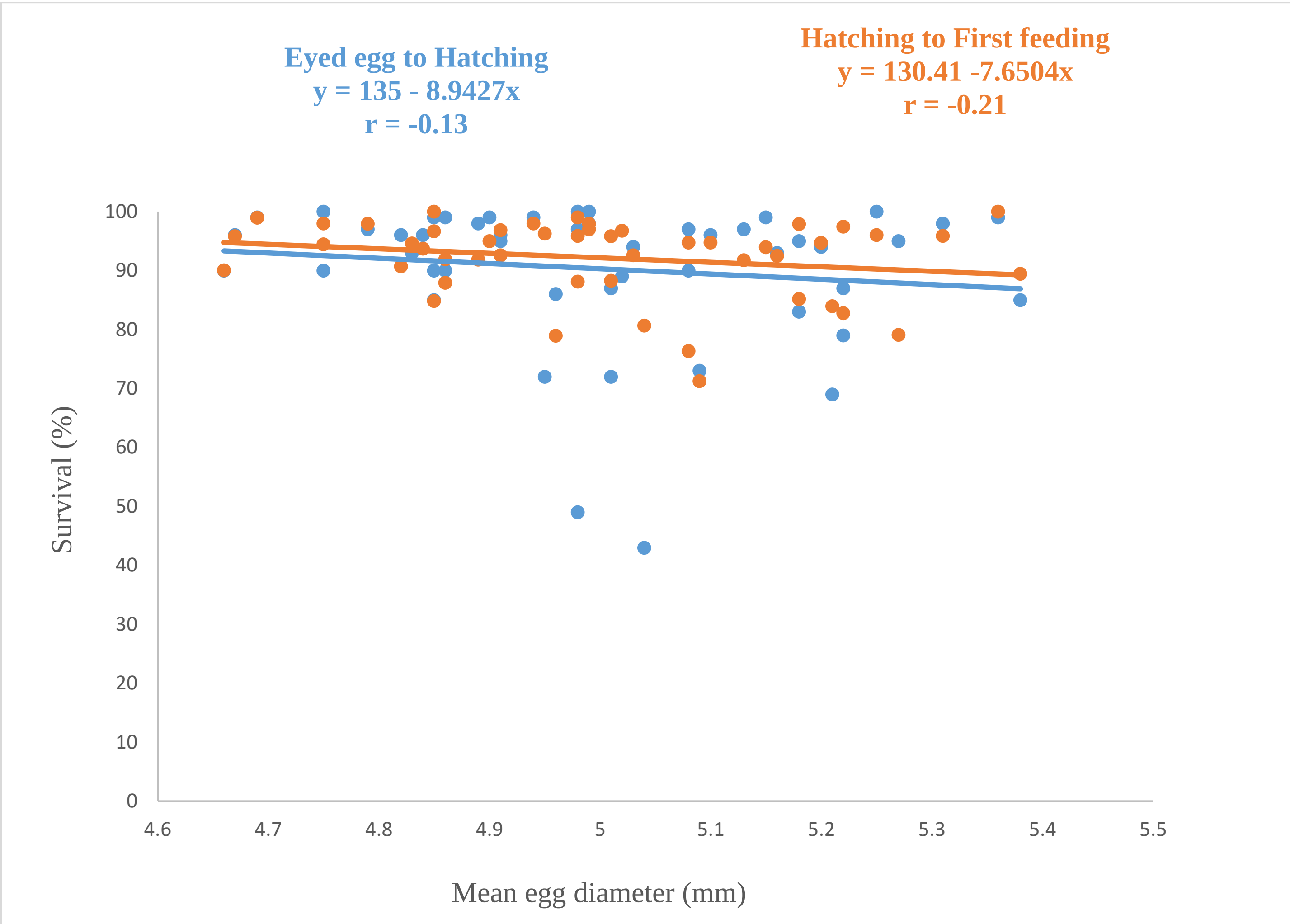

Figure 23. Regression of egg diameter (mm) on survival of eyed-eggs until hatching and of alevins from hatching to first feeding for YC2021.

4.6. Heritability for good and bad eggs

The estimated heritability for these egg quality traits for each of the three year-classes and combined are given in Table 6. In general, the estimates were high and those based on the dam component of variance higher than those based on sire component, and the liability estimates as expected higher than those on based the observed scale. The estimates for good eyed eggs were similar to those for bad eyed-eggs.

For the trait too small eyed eggs the heritability based on the sire component of variance was close to zero.

Table 6. Estimates of heritability for egg quality traits recorded using recorder machine.

Trait	Year-class	%	VarCompSire ⫲ VarCompDam			
			Observed scale		Liability scale	
			$h_s^2 \pm se$	$h_d^2 \pm se$	$h_s^2 \pm se$	$h_d^2 \pm se$
Good&Bad	2019	77.8	0.35 ± 0.11	0.23 ± 0.08	0.54 ± 0.20	0.55 ± 0.18
	2020	87.0	0.36 ± 0.09	0.33 ± 0.08	0.24 ± 0.11	0.90 ± 0.16
	2021	92.4	0.05 ± 0.05	0.25 ± 0.06	0.08 ± 0.11	0.63 ± 0.15
	All		0.31 ± 0.06	0.31 ± 0.04	0.32 ± 0.09	0.70 ± 0.09
Good	2019	45.8	0.40 ± 0.15	0.32 ± 0.12	0.57 ± 0.21	0.46 ± 0.17
	2020	45.7	0.36 ± 0.11	0.74 ± 0.13	0.64 ± 0.17	1.01 ± 0.18
	2021	61.5	0.41 ± 0.19	0.78 ± 0.19	0.42 ± 0.25	1.19 ± 0.26
	All		0.30 ± 0.08	0.78 ± 0.09	0.47 ± 0.11	1.10 ± 0.12
Bad	2019	32.1	0.45 ± 0.19	0.49 ± 0.17	0.70 ± 0.28	0.70 ± 0.24
	2020	41.3	0.31 ± 0.10	0.77 ± 0.13	0.42 ± 0.14	1.13 ± 0.18
	2021	30.9	0.49 ± 0.22	0.83 ± 0.21	0.45 ± 0.28	1.28 ± 0.29
	All		0.29 ± 0.08	0.86 ± 0.09	0.35 ± 0.10	1.26 ± 0.12
TooSmallEye	2019	0.2	0.10 ± 0.02	0.01 ± 0.01	0.67± 0.52	1.09 ± 0.76
	2020	2.4	0.00 ± 0.00	0.82 ± 0.09	0.18 ± 0.14	1.96 ± 0.23
	2021	0.6	0.10 ± 0.03	0.06 ± 0.02	0.07 ± 0.23	1.28 ± 0.29
	All		0.00 ± 0.00	0.64 ± 0.05	DNC	DNC

DNC=Did Not Converge

4.7. Heritability for survival of eggs and alevins

Estimated heritability for survival of eyed eggs until hatching and alevins from hatching to first feeding are shown in Table 7. In general, the estimates were high and those based on the dam components of variance similar to those based on the sire component, the estimates for eyed eggs to hatching similar to those from hatching to first feeding, and the liability estimates as expected higher than those based on the observed scale.

Table 7: Heritability estimates for survival of eggs from the eyed egg stage to hatching and of alevin from hatching to first feeding, and for survival of eggs and alevin over the two periods.

Traits	Year-class	% Survival	VarCompSire ≠ VarCompDam				VarCompSire = VarCompDam	
			Observed scale		Liability scale		Observed scale	Liability scale
			$h_s^2 \pm se$	$h_d^2 \pm se$	$h_s^2 \pm se$	$h_d^2 \pm se$	$h_{sd}^2 \pm se$	$h_{sd}^2 \pm se$
Eyed-eggs to hatching	**2020**	81.6	0.30±0.15	0.17±0.10	0.58±0.34	0.58±0.30	0.32±0.08	0.59±0.12
	2021	90.3	0.12±0.08	0.19±0.08	0.27±0.18	0.36±0.18	0.18±0.04	0.32±0.07
	2020+2021	86.8	0.26±0.10	0.23±0.07	0.40±0.18	0.48±0.16	0.25±0.05	0.45±0.07
Hatching to first feeding	**2020**	94.7	0.23±0.09	0.14±0.06	0.28±0.15	0.32±0.14	0.20±0.04	0.31±0.08
	2021	92.6	0.19±0.07	0.14±0.05	0.30±0.12	0.26±0.11	0.17±0.03	0.29±0.05
	2020+2021	93.4	0.22±0.06	0.14±0.04	0.31±0.10	0.30±0.08	0.19±0.02	0.31±0.04
Eyed-eggs to first feeding	**2020**	77.3	0.30±0.14	0.17±0.10	0.44±0.23	0.43±0.26	0.25±0.07	0.45±0.11
	2021	83.6	0.13±0.10	0.21±0.10	0.29±0.18	0.34±0.17	0.19±0.05	0.32±0.07
	2020+2021	81.04	0.22±0.09	0.18±0.06	0.35±0.15	0.37±0.13	0.23±0.04	0.38±0.06

The proportion of replicate tank variance was close to zero (ranging from 0.000 ± 0.006 to 0.030±0.014) which fractionally varied across models.

4.8. Other observations during experiments

During experiment, several other problem were also seen in hatchery. While collecting eggs, lots of egg were discarded and not used for fertilization due to over-ripening of eggs (Figure 24) and un-matured eggs which were not ready for fertilization. In some of the eyed egg, there were dead embryo inside the egg (Figure 25) and the reason for this is unknown. When the eggs were hatched, some of the hatching showed different types of skeletal deformities (Figure 26) which might be caused due to elevated temperature during incubation (Ytteborg et al., 2012) or vitamin deficiency (Madsen & Dalsgaard, 1999). In some of the hatchery, at yolk-sac stage there have been accumulation of fluid around the yolk sac (blue-sac disease) (Figure 27) which might be associated with the water quality (Sandoval, 2019; Wolf, 1957).

Figure 24. Over-matured eggs.

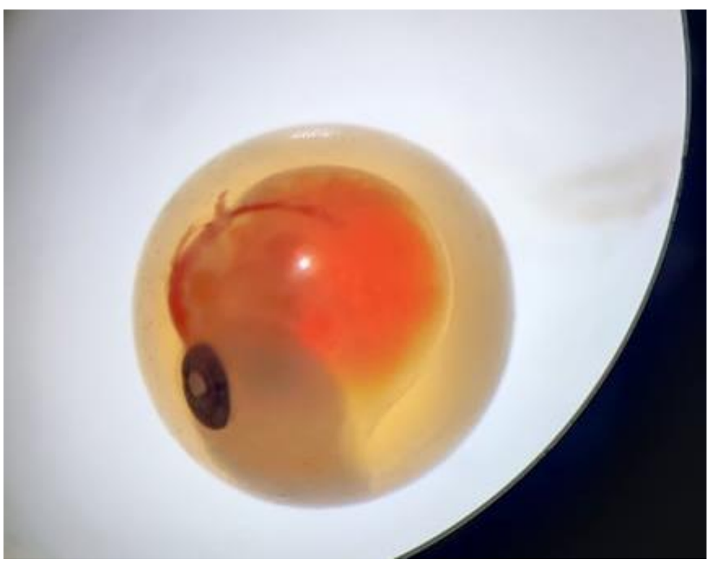

Figure 25. Eyed egg with dead embryo inside it.

Figure 26. Fry with different skeletal deformities.

Figure 27. Fry with undissolved yolk sac (blue-sac disease).

5. DISCUSSION

In Norway, rainbow trout farming has developed into a major business along the Norwegian coast but high mortality has been recorded in early life stages as compared to other species e.g. farmed Atlantic salmon (Rye et al., 1990). Specially, higher level of mortality observed from fertilization until hatching. For the experiment of year-class 2021 in this study, the plan was to produce 60 full-sib families but due to technical reasoning of high mortality, overripening, and high proportion of overripe and unmatured eggs caused the reduction in number of families to 49. The main reason for this high mortality might be the fungal attack during incubation which cause death of healthy embryo. Osland stamfisk use several layers of cakes in a cylinder to incubate the eggs and it is very difficult to monitor the eggs in such a system. If eggs from one cake are infected with fungus, this will easily spread to other cakes possibly due to limitations in monitoring of the cakes/eggs in such a system. Manipulation of incubation temperature to reach eyed egg stage of eggs fertilized at different dates might also be another reason for death of eggs during incubation. There have been cases of blue sac disease in several trout farm that is associated with water quality criteria such as temperature, pH, total nitrogen compounds and dissolved gasses which is also one of the reason of losses in trout hatchery. High mortality in early life stages implies unreliable production of eyed-eggs to meet the market demand of eggs and fry, as well as a problem in a selective breeding program to produce the planned number of full-sib families. To overcome challenges due to these losses more female breeders need to be selected and stripped to meet the required production target of eggs and fry, and thus increased work load, more expenses for the actual company and for the entire industry.

Genetic selection has significantly increased production level of livestock species but over time negative side effects have become more visible and animals in a population that have been selected for high production efficiency seems to be at risk for several reproduction, health, physiological and metabolic problems (Rauw et al., 1998; Teletchea, 2016). Selective breeding programs in fish have mainly focused on improving the growth rates, disease resistance, and flesh quality, and on an average there is about 10-14% genetic gain per generation because of higher genetic variance in fish as compared to land animals (Gjedrem et al., 2012; Olesen et al., 2003; Teletchea, 2016). However, in certain livestock breeds (Rauw et al., 1998) it was found that without proper management and selection strategies numerous breeding programs resulted in a rapid loss of genetic diversity as a consequence of inbreeding, leading to a decline of productivity, therefore, precaution should be taken to avoid inbreeding as well as the emergence undesirable side effects e.g., reduced reproductive performance. This requires that the selective breeding programs include not only market values e.g., growth rate, milk production, flesh quality, but also non-market values such as ethical, welfare, and reproductive traits for example, fecundity, egg/larvae quality traits (Teletchea, 2016). In this study, there was a huge variation among the families in egg traits, egg diameter distribution and survival traits until first feeding that might be due to environmental as well as genetic effects.

5.1. Egg traits

The number of eggs produced by single females differs and depends upon several factors such as age, body size, egg size and condition factors in rainbow trout (Springate & Bromage, 1985) and other species (Towers, 2014). As the body size of female of trout increases, the numbers of eggs and egg size both increases (Bromage et al., 1990; Bromage et al., 1992; Sargent et al., 1987) and between different year-classes as found in this study. It has been found that large egg size is associated with small number of eggs and thus a reduced number of eggs per kg body weight (Gall, 1972; Gall, 1975; Hempel & Blaxter, 1967). In the present study it was found that that number of eggs per kg female and mean egg diameter increases with increasing body size of the female rainbow trout. The present experiment also shows that only half of the produced eggs were categorized as good eggs and used further for production and sale while the rest of the eggs had to be discarded. Good eyed eggs have potential to produce more viable fry with physical characteristics including embryo inside the egg with two visible eyes and are deprived of fungi attack. Fry that are hatched from good eyed eggs are lively, healthier and deprived of any malfunctions (Woynarovich & Horváth, 1980).

In the present study, the correlation of body weight of female with mean egg diameter and total number of eggs per female was positive and significantly different from zero for year class 2021. The linear relationship of fecundity of fish and mean egg diameter increased with the weight of fish as also reported earlier (Gall, 1972; Gall, 1975; Gall & Gross, 1978; Huang & Gall, 1990; Hubbs et al., 1968; Ojanguren et al., 1996; Su et al., 1997). However, these findings were inconsistent in year class 2020, where the correlation between body weight of female with mean egg diameter and total number of eggs per female was low and not-significantly different from zero. The observed difference between the two year-classes may be due to the lower variation in female body weight in year class 2020 (CV=0.13) than for year class2021 (CV= 0.15).

5.2. Survival traits

Survival of eyed-eggs from fertilization to hatching and of alevins from hatching to first feeding varied among the different families in the two investigated year classes. Survival percentage from eyed egg to hatching was lower as compared to survival percentage from hatching to first feeding. The correlation between mean family survival up to hatching and first feeding with egg size was close to zero which indicates that egg size has no direct impact on fry survival which was in agreement with the earlier finding by Springate and Bromage (1985). The variation in survival may be due to the temperature during incubation and the optimum time of stripping. Fish used for experiment were stripped on three different dates but they reach the eyed egg stage at the same time by manipulating the incubation temperature which may affect survival as reported by Weber et al. (2016) for rainbow trout and Jeuthe et al. (2013) for Arctic charr. There was significant differences in survival from eyed-eggs to hatching between the different dates of stripping which may be caused by differences in optimum time of stripping as reported in several papers (Craik & Harvey, 1984; Sakai et al., 1975; Springate et al., 1984). Another reason for the variation in survival among families can be different types of milt (frozen and fresh) that was used for fertilization. Cryopreserved milt is normally used for production of families in the nucleus for building genetic ties across year classes. There was significant difference in mortality of eyed egg on type of milt used with higher mortality in families produced using cryopreserved milt.

Some studies showed that the survival rate to the eyed stage increases in second year of spawning (Bromage & Cumaranatunga, 1988), but in this case the female were killed while collecting the egg and are not used for second year so, in this experiment, we couldn't compare the survival rate for same female for second year. The replicate effect (two cakes per family) on survival from eyed-egg-egg stage to hatching and from hatching to first feeding was very low and can therefore be ruled out as the reason for the observed variation in survival between the families.

5.3. Heritability for egg traits

Heritability obtained from dam components for all egg traits were higher as compared to that of sire components and can be due to the fact that it includes effects common to fullsibs; i.e. , maternal effects (e.g. body size of female) and non-additive genetic effects in addition to additive genetic effects (Kanis et al., 1976). For the trait too small eyed eggs, the heritability estimates based on the sire component of variance was close to zero, which indicates no additive genetic variance, most likely due to the very low frequency of this trait. As expected the heritability estimates on the liability scale were higher than those based on the observed scale. Some of the heritability estimates from dam component of variance were outside the parameter space (Table 6). A possible reason for this is variation around the optimum time of stripping of the different females as the stripping was performed at three different dates 7-10 days apart which may not be frequent enough to obtain good egg quality from all the females.

5.4. Heritability for survival of eyed-eggs and alevins

In this experiment, I found out that there is significant additive genetic variance for the survival of eyed-eggs and alevins. The estimated heritabilities based on the dam components of variance were similar to those based on the sire components which indicates that the magnitude of maternal effects as well as non-additive genetic variance is of little importance for these two survival traits. In contrast to our results, the dam components based heritability estimates for early mortality (uneyed and eyed eggs) reported by (Kanis et al., 1976; Rye et al., 1990) were significantly higher than sire components based estimates. Moreover, they found low genetic variation for fry survival indicating that the maternal effects had considerable magnitude in early stages and possibly disappear during the development of embryo and later stages. Similar results were also found by Ayles (1974) for young splake hybrids (*Salvelinus fontinalis* × *S. namaycush*). Kristjansson and Vøllestad (1996) mentioned that early development depends on maternal effects, including the dam's genotype.

Genetic correlation between survivals in two successive periods (eyed-egg stage to hatching and from hatching to first feeding) in year class 2021 was positive and medium, which was similar to the result obtained by (Rye et al., 1990); while a close to zero negative genetic correlation was observed for year class 2020 which indicates that the survival before hatching and after hatching till start feeding are independent traits. These results need to be verified from estimates from additional year-classes.

6. Conclusion and Recommendation

6.1. Conclusion

Early life stages are of significant importance for the long-term performance of an individual. In rainbow trout farming, high mortality during an early stage of life has been a significant issue. Most of the problems that were seen during the experiment of the current study were caused due to water quality criteria such as temperature, dissolved oxygen, other dissolved gases, so they should be monitored daily. (Baeverfjord (unpublished)) suggested not to decrease temperature below 6^{o}C during incubation to prevent skeletal deformities in rainbow trout. Instead of cakes, using a vertical tray and upwelling incubator for incubation of eggs would assist in monitoring eggs and more easily remove the bad ones. To avoid stripping of overripening of eggs it is crucial to strip and fertilize the eggs at the correct time. The survival in early stages (eyed egg to hatching and hatching to first feeding) and egg quality traits showed moderate to high heritability with a strong possibility to improve these traits by selection and breeding. To better understand the implications of these results, the industry should consider the recommended solutions that might help reduce the mortality rate and thus decreased current economic loss in the trout industry.

6.2. Recommendation for future thesis

Further research is needed to estimate and investigate levels and causes of losses incurred by industry before first feeding. Well planned studies covering optimization of the time of stripping the females, effects of different temperature during incubation on the development of rainbow trout and study the causes of different skeletal deformities found in hatching should be carried out for further inspection.

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