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Brood-stock management and early hatchery rearing of Arctic charr (*Salvelinus alpinus* (Linnaeus))

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Abstract

Arctic charr (*Salvelinus alpinus* (Linnaeus)) is a stenothermic cold-water fish, which has been cultured in Northern Europe and North America since the 1980s. The industry has remained relatively small with an annual production between 6000 and 10 000 tonnes, and is still challenged by an unreliable offspring production. This review focuses on offspring production in Arctic charr aquaculture including holding conditions for brood-stock, fertilisation and egg rearing until hatch. Brood-stock requires low temperatures during summer (<12°C) with the optimum still unknown. The temperature maximum for egg incubation lies between 6 and 8°C. The composition of an optimal brood-stock diet is debated regarding fatty acids. A demand for a freshwater-based diet rich in omega-6 fatty acids is indicated, but results remain inconclusive. Extensive knowledge has been gained on the timing of spawning and its manipulation through photoperiod, temperature and hormone treatments; spawning can be induced by short-day photoperiod; and temperature drops to 5°C. Eggs are fertilised dry in ovarian fluid. Egg quality is highly variable and positively related to egg size and energy density. Contrary, little information is available on sperm quality and its impact on egg survival. There may also be profound differences between Arctic charr of stationary or anadromous origin regarding requirements for holding conditions of brood-stock and their diet. However, these differences have received little attention, and direct comparative studies are in demand.

Key words: brood-stock husbandry, egg rearing, fertilisation, pre-hatch survival, *Salvelinus alpinus*.

Introduction

The Arctic charr (*Salvelinus alpinus* (Linnaeus)) is an appreciated sport and household fish, especially for indigenous peoples of the north (Balikci 1980, Johnson 1984, Boivin *et al.* 1989, Power *et al.* 1989). Commercial fisheries of Arctic charr are important in Canada (Kristofferson *et al.* 1984, Dempson 1995, Dempson & Shears 1998), and it has potential to be grown in Aquaculture (e.g. Jobling *et al.* 1993). The species is suitable for aquaculture as it tolerates high stocking densities (Jorgensen *et al.* 1993), has a high fillet yield (Glandfield 1993) and is amendable to niche marketing (Sæther *et al.* 2013). It tolerates highly intensive production in recirculating systems (Summerfelt *et al.* 2004, Skybakmoen *et al.* 2009). As high growth rates have been achieved at low temperatures (Brännäs & Linnér 2000, Siikavuopio *et al.* 2009, Siikavuopio *et al.* 2010)), Arctic charr is especially productive when farmed at high latitudes or altitudes.

Commercial farming of Arctic charr started in the early 1980s (e.g. Jobling *et al.* 1993). Annual global production reached quantities between 6000 and 10 000 tonnes by 2013 with the largest producers located in Northern Europe (Sæther *et al.* 2013). During the early development of Arctic charr aquaculture, brood-stock holding conditions and egg rearing regimes analogous to those used for rainbow trout (*Oncorhynchus mykiss* (Walbaum)) or other salmonids were applied. However, experience from the

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farms revealed that species-specific conditions for brood-stock and egg rearing regimes were necessary. Some success in rearing Arctic charr under standardised conditions has been achieved, for example, in the Swedish breeding programme (Nilsson *et al.* 2010). However, the growth of the industry has not been as successful as anticipated. Fertility issues, which are manifested in highly variable gamete quality, fertilisation rates and hatching success (Jobling *et al.* 1998), are important reasons behind the limited development of the industry. Low fertilisation success and pre-hatch survival are reported in studies from around the world. In Iceland, a study showed a general egg survival of 32%, with major mortality exhibited during the first week of incubation (Leblanc *et al.* 2016). Mansour *et al.* (2011) reported on fertility of eggs ranging between 0 and 83% in a Canadian brood-stock of Arctic charr. In Sweden, a study on incubation temperature showed family variations in hatching rates between 9 and 97% (Jeuthe *et al.* 2016) while general, hatching rates between zero and 70% are the norm in Swedish hatcheries (Jeuthe *et al.* 2013).

Certainly, some of the issues connected to pre-hatch survival could be attributed to the lack of recognition of Arctic charr as a stenothermal cold-water species with a unique biology (Johnson 1980, Jobling *et al.* 1998). Hence, particular attention has been given to temperature (e.g. Krieger & Olson 1988, Jobling *et al.* 1995, Atse *et al.* 2002, Jeuthe *et al.* 2013, Jeuthe 2015, Jeuthe *et al.* 2015). Optimal holding temperatures of brood-stock during summer are estimated to be < 12°C (Jeuthe *et al.* 2013), while temperatures ~5°C are optimal during spawning in autumn (Gillet 1991). The upper temperature limit for egg incubation is 8°C (reviewed by Elliott & Elliott 2010). Several other factors influencing the reproduction of Arctic charr in aquaculture have also been investigated with the following results. The role of fatty acid composition in brood-stock nutrition is still under debate (e.g. Mansour *et al.* 2006a, Pickova & Brännäs 2006, Brännäs *et al.* 2007, Pickova *et al.* 2007, Mansour *et al.* 2011, Brännäs *et al.* 2011b). Physiological mechanisms induced by stress have been investigated (e.g. Berg 2003, Berg *et al.* 2004a, Berg *et al.* 2004b). The stress hormone cortisol (F) mainly interferes with vitellogenin (Vtg) production (Berg *et al.* 2004a) during the oocyte growth phase (Berg 2003). Extensive knowledge is available on the timing of spawning and its manipulation in Arctic charr (e.g. Gillet 1991, Gillet & Breton 1992, Jansen 1993, Gillet 1994, Gillet *et al.* 1996, Gillet & Breton 2009, Gillet *et al.* 2011). Spawning can be synchronised by shifting to short-day photoperiod (Gillet 1991, Gillet & Breton 1992, Gillet 1994, Duston *et al.* 2003, Frantzen *et al.* 2004, Gillet & Breton 2009), lowering the water temperature (Gillet 1991, Gillet & Breton 1992), or hormone treatments under suitable conditions (Gillet & Breton 1992, Jansen 1993, Gillet *et al.* 1996, Brännäs *et al.* 2007, Gillet & Breton 2009). Year-round gamete production is possible using photoperiodic manipulation (Gillet 1994). Knowledge on intrinsic egg quality traits, such as egg size, is available (e.g. Wallace & Aasjord 1984, Jónsson & Svavarsson 2000, Pakkasmaa *et al.* 2001, Valdimarsson *et al.* 2002, Mansour *et al.* 2008a, Janhunen *et al.* 2010, Leblanc *et al.* 2011, Jeuthe *et al.* 2013, Leblanc *et al.* 2016, Jeuthe *et al.* 2019). Egg size has been correlated to egg survival (Jeuthe *et al.* 2013) and a variety of traits in larvae (Wallace & Aasjord 1984, Leblanc *et al.* 2011). However, egg energy density appears to be a better predictor for egg viability than egg size (Leblanc *et al.* 2016). In addition, species-specific techniques for cryopreservation of sperm have been developed for Arctic charr (e.g. Piironen 1992, Piironen 1993, Lahnsteiner 2000b, Richardson *et al.* 2000, Mansour *et al.* 2006b, Mansour *et al.* 2008b, Richardson *et al.* 2011).

Despite the progress made, there is still major room for improvements of pre-hatch survival in Arctic charr hatcheries today. Further research is necessary to fully comprehend its limiting factors. In this review, species-specific research on fertility and pre-hatch survival of Arctic charr in hatcheries is discussed, including brood-stock husbandry, gamete quality, fertilisation process and egg incubation. The aim of this review is to summarise existing results and to outline areas of future research.

Brood-stock

Brood-stock properties

Arctic charr strains used in aquaculture are derived from various wild populations, and some are selectively bred. Strains are originally anadromous or stationary (Johnson 1980). Both types of strains are used in research, such as the anadromous Nauyuk strain (e.g. Tabachek & de March 1991), Fraser river/Labrador strain (e.g. Duston *et al.* 2003) (both Canada), or the Lake Storvatn or Hammerfest strain (Norway) (e.g. Tabachek & de March 1991, Frantzen *et al.* 2004). Stationary strains are for instance derived from Lake Geneva (France) (e.g. Gillet 1991), or constitute the basis for breeding programmes. The Arctic Superior strain, derived from Lake Hornavan (Sweden) (Nilsson *et al.* 2010), and the Hólar University College strain, partially derived from Lake Ölvesvatn (Iceland) (e.g. Leblanc *et al.* 2016), are both selectively bred.

There are many profound differences between brood-stocks of different strains of Arctic charr. They differ in age at first spawning (Nilsson 1992, Jobling *et al.* 1993, Delabbio 1995, Hatlen *et al.* 1997, Jobling *et al.* 1998), and using late maturing strains in selective breeding is advantageous to postpone first spawning (Jobling *et al.* 1998). In

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addition, resistance to fungal infections in adult fish appears to be heritable (heritability 0.34) (Brännäs *et al.* 2011b). This trait could be connected to resistance against fungal infections in eggs. It also seems to be positively correlated to the age at first spawning. However, selection for resistance to fungal infections has been evaluated as too expensive, as the resistance to fungal infections is difficult to assess (Brännäs *et al.* 2011b). Also, anadromous strains kept in sea water during summer exhibited a higher reproductive performance (e.g. Atse *et al.* 2002; further discussed under water chemistry). Such experiments have not been conducted on stationary strains. It may be reasonable to hypothesise that the beneficial effect of sea water during summer on egg and sperm quality may differ between anadromous and stationary strains.

Fecundity and egg viability also differ greatly between strains, partly caused by the origin of brood-stocks. Differing holding conditions and age of the brood-stock are highly influential to egg viability and fecundity as well (Jobling *et al.* 1998). Thus it is difficult to attribute brood-stock performance directly to its origin, as other influential parameters and requirements to the environment differ between strains. Egg survival itself does not seem to be a heritable trait according to analyses performed in the Swedish breeding programme (Brännäs *et al.* 2007). Consequently, it is unlikely that one particular strain has a higher egg viability solely based on its origin. The selectively bred Arctic Superior (Nilsson *et al.* 2010) exhibited the lowest egg survival compared with strains from Lake Hornavan, Lake Ottsjön and Lake Rensjön (Sweden) for example. This was likely caused by extrinsic factors, such as higher temperatures, and the lower age at first spawning in Arctic Superior. For brood-stocks of all strains, egg survival declined between 1986 and 2004, and the age at first spawning declined due to higher growth rates in Arctic Superior (Brännäs *et al.* 2007). Lower age at first spawning may be a cause of lower egg viability for the youngest brood-fish. In the strain based on Arctic charr from Lake Geneva (France), female age was positively linked to egg size and fecundity (Lasne *et al.* 2018). Increasing weight of the female within one age class additionally increased fecundity, but had a low impact on individual egg size. Strong correlations between female age, egg size and egg viability were documented in the Swedish Arctic charr breeding programme (Jeuthe *et al.* 2013, Jeuthe 2015). The connection between egg viability and the age of the female was a well-known phenomenon among fish farmers before (Brännäs *et al.* 2011b). Egg quality and size increased with female age up to the age of 6 years, with no subsequent age-dependent improvement (Jeuthe *et al.* 2013, Jeuthe 2015). The results relied on 9 females monitored for 4 years from age 2 + to 6 + , where the average egg survival increased from 5.7% to 65.5%. Six of the females were monitored for one additional year, but showed no change in egg survival from age 6 + to 7 + (Jeuthe *et al.* 2013). The individual variation was large at age 6 + , with survival to the eyed stage between 17.4 and 94.4% (Brännäs *et al.* 2011b).

Various properties and the quality of eggs of Arctic charr differ not only between strains, but also between individual families. An experiment using half-sib families demonstrated that parental effects are one cause of differing metabolic rates in Arctic charr embryos between families (Pakkasmaa *et al.* 2006). The differences in metabolic rate between families persisted when corrected for developmental stage and the time of the measurement taken. This indicates genetic or epigenetic effects. Metabolic rate was not correlated to hatching time in this study. Also, differences in egg viability were found to be large between families in a study on egg incubation temperature using the Arctic Superior brood-stock (Jeuthe *et al.* 2016). All treatments combined, average survival rates per family were 17%, 48%, 77%, 82%, 83% and 98% to the eyed stage and 9%, 44%, 67%, 67%, 74% and 97% until hatch, respectively. The underlying reasons for the large between-family variations in egg survival remain unknown.

Individual male fish also influence the quality of fertilised eggs through genetic paternal effects and other sperm quality traits. The influence of sperm quality and paternal genetic effects is further discussed under sperm quality. In relation to brood-stock properties, the standing of the individual male in the social hierarchy is associated with profound differences in hormone levels and consequently sperm quality (e.g. Rudolfsen *et al.* 2006, Haugland *et al.* 2009). However, this phenomenon has mainly been investigated in wild fish.

Holding temperature

Reproductive development in Arctic charr is highly dependent on ambient water temperatures. Tolerated temperature ranges for brood-stock appear to be narrow, and summer temperature requirements are low. Summer temperatures below 12°C are generally recommended for Arctic charr brood-stock (Jeuthe *et al.* 2013). Summer temperatures above 12°C are shown to delay ovulation (Jobling *et al.* 1995, Jobling *et al.* 1998). Beneficial effects of low summer rearing temperatures are further documented for brood-stock derived from northern anadromous populations, such as the Labrador strain with origins in Fraser River (Newfoundland and Labrador, Canada) (Krieger & Olson 1988, Atse *et al.* 2002). Krieger and Olson (1988) found that eggs fertilised by males which had been kept at 6.5°C survived at a higher rate than eggs fertilised by males kept at 8–17°C. Atse *et al.* (2002) compared the performance of brood-stock kept in natural freshwater, heated

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sea water (8–16°C), cooled freshwater and natural sea water (4–10°C) from May to September. Eggs of fish from colder treatments performed better. Contrary, detrimental effects of low summer temperatures were discovered in the landlocked southern population of Lake Geneva (France). Brood-stock of this population kept at 5°C during early autumn produced smaller ova than brood-stock kept at 8 or 10°C (Gillet 1991, Gillet & Breton 1992). In addition, eggs of brood-stock kept at 5°C exhibited lower survival rates compared with brood-stock kept at 8°C in early autumn (Gillet 1991, Gillet & Breton 1992, Gillet & Breton 2009, Gillet *et al.* 2011). In these studies, brood-stock kept at 5°C in early autumn was kept at the same temperature until spawning.

The effect of summer temperature on egg quality in Arctic charr has been investigated to some extent experimentally (Jobling *et al.* 1995, Atse *et al.* 2002, Jeuthe *et al.* 2015) and in observational studies (Jeuthe *et al.* 2013, Jeuthe *et al.* 2015). All studies identified elevated summer temperatures as detrimental to egg quality. Jobling *et al.* (1995) held Arctic charr brood-stock at constant temperatures of 4, 8, 12 and 16°C from mid-June to late September and found that oocytes of brood-stock held at low temperatures exhibited higher phospholipid (PL) content, lower triacylglycerol (TAG) content, higher cholesterol and docosahexaenoic acid (22:6 n-3, DHA) content, and less saturated fatty acids (SFA). Eggs of brood-stock reared at lower summer temperatures (4–10°C compared with 8–16°C) had a higher total energy content and a 5× higher survival until hatch (Atse *et al.* 2002). Mortality within the first 24 h post fertilisation was 3× higher in eggs from brood-stock reared at colder temperatures, but never exceeded 10% (Atse *et al.* 2002). By evaluating the reproductive performance of sibling brood-stock in two facilities, Jeuthe *et al.* (2015) measured a larger egg diameter and a higher survival rate when the maximum summer temperature was 15°C compared with 19.1°C. By analysing hatchery data from Kälarne (Sweden) over the course of 11 and 28 years, respectively, Jeuthe *et al.* (2013) and Jeuthe *et al.* (2015) found that lower summer temperatures increased survival until the eyed stage. Lower temperatures in July and August, and fewer warm days (>15°C) (Jeuthe *et al.* 2013), as well as lower temperatures in September (Jeuthe *et al.* 2015), resulted in higher survival to the eyed stage. Summer temperatures below 12°C seem to be optimal according to an extrapolation of the data (Jeuthe *et al.* 2013).

The effect of reduced oestradiol (E_2) plasma levels on vitellogenesis may be an important mechanism behind the detrimental effects of high summer temperatures on egg quality in Arctic charr. According to Frantzen *et al.* (1997), early vitellogenesis commences in Arctic charr from March to July, when oocytes are recruited at low E_2 plasma levels. The oocyte growth phase occurs between July and early August, and is characterised by a marked increase in plasma E_2 concentrations. From August to late September, E_2 plasma levels start to decrease slightly with an abrupt decline at ovulation. Oocytes exhibit reduced growth in this period. Similar relative patterns in hormone cycles are documented in other studies of Arctic charr (Mayer *et al.* 1992, Tveiten *et al.* 1998, Berg *et al.* 2004a). However, the accurate timing of peak E_2 plasma levels varies slightly between these studies. While Frantzen *et al.* (1997) documented an increase in E_2 plasma levels from late July towards a peak in mid-August, Berg *et al.* (2004a) found E_2 plasma levels to peak in September, and Tveiten *et al.* (1998) measured a peak from August to September. By comparing the peak E_2 plasma concentrations and maximum temperatures measured in different studies on Arctic charr, Berg *et al.* (2004a) found that maximum E_2 plasma concentrations appear to be temperature dependent. The highest maximum E_2 plasma concentrations (20 ng mL^{-1}) were recorded by Tveiten *et al.* (1998), who kept their brood-stock at 4°C during summer. Intermediate maximum E_2 plasma concentrations of 10 ng mL^{-1} and 3.5 ng mL^{-1} were measured at summer temperatures of 12°C (Frantzen *et al.* 1997) and 16°C (Mayer *et al.* 1992), respectively. At a maximum summer temperature of 17.5°C, E_2 plasma levels peaked at 1.0 ng mL^{-1} (Berg *et al.* 2004a). Accordingly, it can be hypothesised that peak E_2 plasma levels in Arctic charr are influenced by summer temperature analogous to Atlantic salmon (*Salmo salar* (Linnaeus)), as annual hormone cycles, for example E_2 plasma concentrations in Arctic charr, follow similar patterns. Mechanisms mediating the negative effect of high summer temperatures on egg viability, and the duration and timing of high temperatures required, have been more thoroughly studied in the related species Atlantic salmon in Tasmania (King *et al.* 2003, Watts *et al.* 2004, Watts *et al.* 2005, King *et al.* 2007). Vitellogenesis is negatively affected by high temperatures of 22°C, which was established by measuring decreased 17,20β-dihydroxy-4-pregnen-3-one (17,20βP), E_2 and Vtg levels. Summer holding temperatures of 22°C resulted in a smaller egg size and decreased survival (King *et al.* 2003). E_2 plasma concentrations were confirmed to be decreased in February and March, and Vtg levels were lower in February (Watts *et al.* 2004). Consequently, E_2 production was markedly reduced in February, while Vtg was found to accumulate in the blood. This indicated a decreased uptake by the oocytes, which was also reflected in the lower gonadosomatic index (GSI) of fish held at higher temperatures (22°C). Testosterone (T) was readily produced at high temperatures contrary to E_2 that led to the hypothesis that the transition from T to E_2 through cytochrome P450-aromatase ($P450_{arom}$) at the follicular level might have been impaired. Therefore, short-

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term temperature increases could be detrimental to egg development. In addition, Watts *et al.* (2005) found that E_2 was bound to its receptor to 80% at low affinity at 22°C in February. This would not occur in Atlantic salmon held at lower temperatures until approximately one month later. Subsequently, short-term exposure to elevated temperatures (22°C) has been investigated at various times by King *et al.* (2007). It was found that a 6-week exposure to 22°C in late summer and autumn was as damaging to egg quality as a 12-week exposure to the same temperature. In addition, high temperature exposure for only 4 weeks from late February to early March was found to have a pronounced negative impact on egg quality. An equally short exposure to high temperatures until mid-February or after mid-March, however, was found to be little effective.

In autumn around the time of ovulation, upper temperature limits are well documented for the Lake Geneva (France) population of Arctic charr. In general, the upper temperature limit for ovulation lies between 8 and 10°C (Gillet 1991), with temperatures of 11°C inhibiting ovulation completely (Gillet, 1991, Gillet & Breton 1992). At 8°C, ovulation is delayed (Gillet 1991), and spontaneous ovulation is inhibited at higher temperatures (Gillet & Breton 1992). The effects of temperature on ovulation are discussed under timing of spawning. Subsequent to completed ovulation, egg quality declines rapidly in eggs that are not released at temperatures > 8°C due to over-maturation (Gillet 1991), and the effect of over-maturation prevails at temperatures between 6 and 8°C (Gillet & Breton 1992, Gillet 1994). At 8°C, fertilisation success was found to decline significantly within four days after ovulation, approaching zero within seven days. Seven days after ovulation, eggs kept at 6°C did not show signs of decreased quality (Gillet 1994).

Brood-stock nutrition

Feed quantity impacts fecundity and the total investments in gametes in fish (reviewed by Izquierdo *et al.* 2001). In Atlantic salmon, even short periods of feed restriction are reported to delay or inhibit sexual maturation (Norrgård *et al.* 2014). The amount and quality of nutrients in the fertilised egg influence embryonic development in Arctic charr. Nutrients appeared to be consumed by the embryo according to their initial concentration. Especially, lipid use from early development appeared to be advantageous. The highest survival and fastest development until the eyed stage were achieved by embryos consuming lipids earlier (Atse *et al.* 2002). In this study, physiological mechanisms influenced by temperature and salinity differences caused the differences in nutritional composition of the eggs. Brood-stock from all treatments were fed the same diet. In Arctic charr, research on the nutritional content of eggs and sperm and brood-stock diet has mainly focused on fatty acid composition and antioxidants.

Fatty acids and egg quality

The role of fatty acids in brood-stock nutrition of Arctic charr is poorly understood, as there are no comprehensive results on this issue. Some studies indicate a connection between fatty acid composition and egg survival (Pickova & Brännäs 2006, Pickova *et al.* 2007), which is further elaborated in several reports (Brännäs *et al.* 2007, Brännäs *et al.* 2011b). Contrary, Mansour *et al.* (2011) claimed that there may not be a connection. The ambiguous results may be attributed to confounding factors and inadequate experimental procedures to cover the entire process from fatty acid concentrations in the feed, via fatty acid concentrations in eggs and sperm, to egg survival.

In support of the connection between fatty acid composition in Arctic charr eggs and egg survival, Pickova and Brännäs (2006) found differences in average fatty acid compositions between eggs of wild and farmed Arctic charr. Eggs of wild fish exhibited higher survival. Comparing two farmed and one wild population, Pickova *et al.* (2007) found 15× higher concentrations of arachidonic acid (20:4 n-6, ARA) and a higher ratio of n-6/n-3 fatty acids in wild eggs. Wild eggs exhibited higher survival in this study as well. However, these studies are based on comparisons between eggs of farmed and wild origin without considering confounding factors such as photoperiod, age of the brood-stock and holding temperature. Additional support for the connection between fatty acid composition of the feed and egg quality was gathered in feeding experiments in Kälarne (Sweden) and Omegalax (Sweden) (Brännäs *et al.* 2011b). Fodder enriched with 2.3% ARA was fed to 30 female and male Arctic charr, improving the number of maturing females and egg survival. However, these experiments are difficult to assess, as experimental procedures are not reported. Contrary to the hypothesis of a connection between fatty acid composition and egg quality in Arctic charr, no correlations between fatty acid content of unfertilised eggs or their chorions and egg survival until the eyed stage were found in a Canadian study (Mansour *et al.* 2011). Eggs were also grouped by fertilisation success, but low, medium and high fertilisation success groups had no significant differences in fatty acid composition. This study (Mansour *et al.* 2011) was conducted on only one domesticated brood-stock fed the same feed, and egg survival was only compared until the eyed stage. Consequently, contrasts in fatty acid composition were much lower than in comparisons between eggs of wild and farmed fish (Pickova & Brännäs 2006, Pickova *et al.* 2007, Mansour *et al.* 2011).

The hypothesis about the importance of dietary fatty acids for the reproductive success of farmed Arctic charr is theoretically based on the importance of ARA and n-3/n-6

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polyunsaturated fatty acid (PUFA) ratios in fish egg development. Both were found to differ between eggs of wild and farmed Arctic charr (Pickova & Brännäs 2006, Pickova *et al.* 2007). In fish, ARA is an important precursor for eicosanoids (Bell *et al.* 1996, Abayasekara & Wathes 1999, Farndale *et al.* 1999), which are essential to correct embryonic development (Abayasekara & Wathes 1999). Ratios between eicosapentaenoic acid (EPA, 20:5 n-3), DHA and ARA of defined magnitudes are essential to a variety of physiological functions in fish (Sargent *et al.* 1999), and the balance between these fatty acids appears more important than their concentrations per se (Bell *et al.* 1997, Tocher 2010, Holt 2011). N-3/n-6 PUFA ratios differ between the natural fresh water based diet of stationary populations of Arctic charr and the marine based diet fed in aquaculture (Pickova *et al.* 2007). Accordingly, n-3/n-6 PUFA ratios of various species are lower in freshwater than in marine habitats (Kaitaranta & Linko 1984, Henderson & Tocher 1987, Pickova *et al.* 1997, Arts *et al.* 2001, Wiegand *et al.* 2004). However, Arctic charr is a plastic species, exhibiting different adaptations in relation to diet (e. g. Skulason *et al.* 1992).

The diet of Arctic charr ranges from entirely freshwater based in stationary populations (e.g. Olk *et al.* 2016) to approximately 90% marine based, as found in an anadromous population in the Canadian Arctic (Swanson *et al.* 2011). Consequently, higher dietary n-6 PUFA and ARA contents may only be required by strains of stationary origin. All studies supporting the claim were conducted on strains of stationary origin (Pickova & Brännäs 2006, Brännäs *et al.* 2007, Pickova *et al.* 2007, Brännäs *et al.* 2011b), while investigations on anadromous Arctic charr did not support the connection between egg fatty acid composition and egg survival (Mansour *et al.* 2011).

Physiological differences in fatty acid utilisation between populations of different origin have been studied in Atlantic salmon (Rottiers 1993). By feeding an anadromous and a non-anadromous strain identical diets, resulting in higher fatty acid content in the freshwater strain, Rottiers (1993) demonstrates that physiological adaptions to freshwater habitat poorer in lipids are plausible. Further evidence was provided by Wiegand and Idler (1985), who found elevated contents of DHA and very low contents of ARA in immature gonads of non-anadromous Atlantic salmon fed a marine diet. However, these measurements were also consistent with the general lipid composition of the marine diet or a combination of both factors. Regarding ARA, strain-specific differences of its content in eggs have been proposed in lobster (*Homarus gammarus* (Linnaeus)) (Castell *et al.* 1995) and cod (*Gadus morhua* (Linnaeus)) (Pickova *et al.* 1997). Strain-dependent dietary requirements may also therefore occur in Arctic charr.

Differences in fatty acid composition by habitat have also been found in Atlantic salmon (Pickova *et al.* 1999), even though this species is not as versatile in the utilisation of different habitats and diets as Arctic charr. In this study, hatchery-reared and wild landlocked Atlantic salmon eggs were analysed for fatty acid composition and antioxidants, and compared with eggs of wild and anadromous populations previously analysed (Pickova *et al.* 1998). Higher EPA contents in the phospholipid fraction were found in eggs of cultured females, and ARA content was lower in cultured females, both in the PL fraction and the TAG fraction (Pickova *et al.* 1999). However, DHA occurred in similar concentrations in all groups. Pre-hatch survival was lower in cultured individuals (40–75%) compared with wild ones (> 95%). The eggs from cultured females resembled the fatty acid composition of the wild anadromous stock (Pickova *et al.* 1998) more closely than that of the wild non-anadromous stocks (Pickova *et al.* 1999), indicating both dietary influence, and strain-specific requirements as a plausible cause. However, not all environmental and husbandry effects are controlled for when comparing wild and cultured stocks. Differences in survival may also be caused by differing conditions for the respective brood-stock.

Besides potential strain-specific differences in dietary fatty acid requirements of Arctic charr, confounding factors, such as temperature, may have influenced physiological processes resulting in less favourable fatty acid compositions in farmed Arctic charr in the relevant studies (Pickova & Brännäs 2006, Pickova *et al.* 2007). Jobling *et al.* (1995) conducted a study on fatty acid composition of ovulated and surgically removed eggs of Arctic charr reared at different summer temperatures. Their data suggest that eggs reared at high temperatures exhibit lower n-3 PUFA contents in the phospholipid fraction than eggs reared at low temperatures. However, these interpretations were based on single average measurements of the fatty acid content. Consequently, the correlation was not entirely confirmed. However, possible temperature dependence of the incorporation of fatty acids into oocytes of Arctic charr may have been an important confounding factor. Consequently, physiological mechanisms may alter the fatty acid composition of Arctic charr eggs considerably, and they may be more important than the initial diet. This phenomenon has not been investigated in detail considering Arctic charr eggs directly to our knowledge. However, fatty acid composition in Arctic charr muscle is found to diverge from dietary fatty acid composition in several feeding experiments (e.g. Murray *et al.* 2014, Murray *et al.* 2015), and egg lipid composition is found to be less dependent on the diet than muscle fatty acid composition in other salmonids (Hardy *et al.* 1990, Rennie *et al.* 2005).

The role of lipid reserves and physiological mechanisms in determining the fatty acid composition of eggs of farmed Arctic charr requires further investigation. These mechanisms may have the potential to ensure the production of

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viable gametes based on various feeds. Lipid reserves are shown to play a major role in anadromous Arctic charr, where ca. 30–40% of the lipid storage can be used between the time of re-entry to freshwater and spawning, and ca. 25% are deposited in the gonads (Jørgensen *et al.* 1997). Lipid reserves may contain more favourable concentrations of PUFA, as, for example, EPA (Murray *et al.* 2014) and DHA (Murray *et al.* 2014, Murray *et al.* 2015) are retained at higher rates in muscle when fed a deficient diet. Important physiological mechanisms include the transportation of fatty acids into the oocytes as well as fatty acid metabolism. The availability of n-3 and n-6 PUFA is dependent on the diet, as PUFA are not synthesised *de novo* in fish (Sargent *et al.* 2002). However, PUFA requirements vary considerably by species (Tocher 2010), as some fish have the ability to elongate and desaturate C18-PUFA, such as 18:3n-3 and 18:2n-6, to long-chained PUFA (Tocher 2003). Freshwater fish can thus often meet their dietary PUFA requirements by C18-PUFA (reviewed by Glencross 2009, Tocher 2010), which may also be possible for Arctic charr. Arctic charr possesses the ability to elongate and desaturate C18 n-3 and n-6 PUFA (Olsen *et al.* 1991, Olsen & Ringø 1992, Tocher *et al.* 2001), producing EPA, DHA (e.g. Murray *et al.* 2014) and ARA (Olsen & Ringø 1992, Tocher *et al.* 2001).

The role of dietary fatty acids in egg development of farmed Arctic charr remains poorly understood. Further investigations should focus on potential strain-specific differences in fatty acid requirements and physiological processes influencing egg fatty acid composition under various conditions, for example different temperatures. Controlled feeding experiments investigating the influence of the diet directly would also be beneficial.

Fatty acids and sperm quality

Contrary to the ambiguous results on the influence of dietary fatty acids on egg quality of Arctic charr, nutritional content and diet appear to have profound effects on sperm quality. The connection between fertility and sperm fatty acid content was investigated in an anadromous, Canadian population of Arctic charr (Mansour *et al.* 2011), by grouping ejaculates based on fertilisation rate (low $\leq$ 48%, medium 49–67% and high $\geq$ 68%). Significant differences in fatty acid profile between the low and high fertility groups were detected. Highly fertile sperm exhibited lower concentrations of SFA, higher concentrations of C20:3n-6, ARA, C22:5n-3, DHA, total n-3, total n-6, and higher ratios of PUFA to SFA and of n-3 to n-6 fatty acids. Cholesterol levels did not differ between the fertility groups. C15:0, total SFA, C22:5n-3, DHA, total n-3 and the ratio between n-3 and n-6 fatty acids were significantly correlated to fertilisation success. The regression between C15:0 and fertilisation success was significant, linear and negative. All other regressions were quadratic, meaning that both extremes are associated with lower fertility. Sperm volume per kg fish, sperm density and sperm motility did not differ in correlation with gamete quality and fatty acid profiles. These results indicate that a balanced fatty acid profile results in the highest sperm quality in Arctic charr. However, all fish used in this study derived from the same stock and fed a similar diet, meaning that nutritional content likely differed based on physiological mechanisms. Further investigations on the role of different fatty acid profiles in the diet are necessary to determine, whether it is feasible to increase sperm quality in relation to fatty acid content by dietary means.

Dietary supplements

Vitamins are an important part of the brood-stock diet in farmed fish of various species. Vitamin E (α-, β-, γ- and δ-tocopherol) deficiency has detrimental effects on fertility, as vitamin E is stabilising cell membranes and an antioxidant. Vitamin E affects pre-hatch survival and survival during the early stages post hatch (Izquierdo *et al.* 2001, Rønnestad & Waagbø 2001). Vitamin E is mainly deposited in the growing oocytes during the pre-spawning starvation period (Lie *et al.* 1994, Izquierdo *et al.* 2001, Rønnestad & Waagbø 2001) and should thus be a part of brood-stock feed during early maturation. Vitamin C (ascorbic acid) is essential for steroid production in the brood-fish (Rønnestad & Waagbø 2001) and collagen production in the embryo, as well as it is an antioxidant. Hatching success and early survival in Salmonids is strongly correlated to vitamin C intake (Sandnes *et al.* 1984). Vitamin B, especially thiamine (B_1), has been linked to early mortality syndrome in lake trout (*Salvelinus namaycush* (Walbaum)), which is closely related to Arctic charr (Fitzsimons *et al.* 2009).

Some vitamins, which function as antioxidants, improve the stability of fatty acids in sperm. This is important to maintain high sperm quality, as lipid peroxidation is found to be one of the most deleterious processes during storage and cryopreservation in sperm regardless of species (Bilodeau *et al.* 2000, Cerolini *et al.* 2000, Ball *et al.* 2001, Brouwers *et al.* 2005). Lipid peroxidation can be counteracted by adding antioxidants to the diet, and α-tocopherol and astaxanthin are commonly used in Salmonid farming to improve flesh stability and marketability (Hamre *et al.* 1998, Jensen *et al.* 1998). The effects of dietary supplementation of antioxidants on Arctic charr sperm have been investigated using α-tocopherol, lowbush blueberry (*Vaccinium angustifolium* ((Aiton) Rydb.)), α-tocopherol + lowbush blueberry and α-tocopherol + astaxanthin (Mansour *et al.* 2006a). All diets containing α-tocopherol were more efficient than lowbush blueberry alone. It was found that sperm of Arctic charr fed a diet supplemented with α-tocopherol did not undergo lipid peroxidation, while lipid

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peroxidation was delayed in fish fed a diet supplemented with lowbush blueberry. Seminal plasma of Arctic charr fed supplemented diets lowered the lipid peroxidation rate measured as area under the curve (AUC) (Davis 2002) and thiobarbituric acid reactive substances (TBARS) after 120, 150 and 180 min in sperm. When large volumes of seminal plasma were added (150 μL), this effect could also be observed in chicken (*Gallus gallus domesticus* (Linnaeus)) brain. Dietary supplementation with antioxidants was also shown to increase catalase-like activity in sperm, but not in seminal plasma. Catalase-like activity in seminal plasma was low regardless of the diet. The α-tocopherol content in seminal plasma was correlated to AUC in sperm cells and chicken brain, and a slight increase of α-tocopherol in sperm as a result of the supplementation of the diet was observed. However, even slight increases in α-tocopherol levels were found to be sufficient to increase the resistance to lipid peroxidation in animal sperm (Castellini *et al.* 2003). Further improvements are likely possible. The incorporation of α-tocopherol in sperm was reported to depend on both vitamin E and C in yellow perch (*Perca flavescens* (Mitchill)) (Lee & Dabrowski 2004), but additional vitamin C was not provided in the experimental diet of Arctic charr (Mansour *et al.* 2006a). The antioxidant activity of seminal plasma in Arctic charr was found to be low, but could be increased by the addition of α-tocopherol in a dose-dependent manner (Mansour *et al.* 2006a). Catalase-like activity was low in seminal plasma of Arctic charr. It was first discovered in fish sperm in Arctic charr (Mansour *et al.* 2006a), where it is weakly, negatively correlated to lipid peroxidation rates.

Stress

Stress arises in brood-stock subjected to inadequate holding conditions or extensive handling. The term stress is used for inadequate holding conditions and physiological stress responses in the literature on the reproductive performance of Arctic charr. For instance, there are accounts of temperature stress (Jeuthe *et al.* 2013), which are further discussed in the section on direct temperature effects. In this section, we will focus on physiological stress responses and their triggers.

In fish in general, many types of stress may affect the reproductive performance by decreasing the amount of gonadotropins produced in the pituitary gland and the amount of steroids present in the plasma. Stress responses generally affect gamete quality negatively in fish (de Montalembert *et al.* 1978, Campbell *et al.* 1992). One cause of acute stress is handling, which is also known for decreasing gamete quality in farmed fish (Li & Leatherland 2012). While handling of the brood-stock is inevitable in aquaculture, one species-specific issue in Arctic charr is the prolonged spawning period, which is further discussed in the section on the timing of spawning.

These sources of stress cause physiological reactions, which affect gamete production in Arctic charr. The stress hormone F influences the production of Vtg and zona pellucida protein (ZP), despite both processes being primarily under oestrogenic control in Arctic charr (Berg *et al.* 2004a). During the natural reproductive cycle, plasma levels of F were observed to increase towards ovulation, which decreased levels of Vtg, but increased ZP production. The effect on Vtg was only observed on the protein, but not on the mRNA level, indicating that downregulation of Vtg by F occurred post transcriptionally. As levels of F increase naturally towards ovulation, oocyte development is suggested to be more sensitive to stress during the growth phase than during maturation (Berg 2003).

The egg yolk protein Vtg is hypothesised to function as a metal-ion transporter. The protein and its metabolites contain zinc, copper (Montorzi *et al.* 1994, Montorzi *et al.* 1995) and magnesium (Falchuk & Montorzi 2001). These metal ions are suggested to be transported into the oocyte by Vtg, since they are of crucial importance during oocyte and embryonic development in animals (Vallee & Falchuk 1993, Falchuk 1998). Some metal ions are necessary to ensure correct folding and stability of metalloproteins such as Vtg (Berg 2003). Consequently, the expression of metallothioneins (MT) is regulated during reproductive development, which has been investigated in rainbow trout (Olsson *et al.* 1987). MTs are a family of highly conserved heavy-metal-binding proteins (Kägi 1993), having important roles in heavy-metal detoxification and trace-metal homeostasis (Olsson *et al.* 1990). However, MT production can be induced by external stimuli, such as metal concentrations, hormones, interferons or UV light (Berg 2003). The link between Vtg and MT was established in rainbow trout, where downregulation of Vtg production coincided with upregulation of hepatic MT expression during the reproductive season. Likely, this is triggered by free zinc (Olsson *et al.* 1987). In Arctic charr, MT mRNA levels were found to be low at the beginning of reproductive maturation, and increased thereafter, peaking in November (Berg *et al.* 2004a). The natural regulation of MT is likely mediated by E_2, which indirectly inhibits MT by altering zinc utilisation in the liver during vitellogenesis in rainbow trout (Hyllner *et al.* 1989, Gerpe *et al.* 2000). Subsequent to completed Vtg synthesis, MT is induced by free zinc in the hepatocytes in rainbow trout (Olsson *et al.* 1987). The balance between E_2 and MT production can be disrupted by environmental factors. Non-essential heavy metals, such as cadmium, are documented to inhibit E_2 induced Vtg synthesis in rainbow trout (Olsson *et al.* 1995, Gerpe *et al.* 2000). Generally, cadmium induces stress responses in animals (Berg 2003), which cause an upregulation of MT

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mediated by F in rainbow trout (Hyllner *et al.* 1989). *Vice versa,* E_2 downregulates hepatic MT mRNA, even under cadmium exposure in rainbow trout (Olsson *et al.* 1987). A combination of the described physiological mechanisms occurs under stress during reproductive development, resulting in a co-exposure of E_2 and F. Both hepatic MT and Vtg synthesis are induced in this situation. In this case, newly produced MT, which is a cysteine, will bind Zn at a higher affinity than the histidine Vtg (Glover & Hogstrand 2002, Berg 2003). Consequently, the lack of zinc leads to a degeneration of Vtg, as correct folding of the protein is not possible (Berg 2003).

The effects of F and E_2 have been investigated experimentally on Arctic charr by injecting or co-injecting the respective hormones. While the injection of F alone resulted in elevated Vtg protein levels, co-injection of F and E_2 resulted in a dose-dependent decrease in Vtg plasma levels without affecting hepatic Vtg mRNA levels. Consequently, evidence for post-transcriptional regulation of Vtg following F exposure is indicated (Berg 2003, Berg *et al.* 2004a, Berg *et al.* 2004b).On the mRNA level, vitellogenesis was even induced by F, even though the induction was 70 times less effective than the induction of vitellogenesis by E_2, and it only occurred at very high doses of F (Berg *et al.* 2004a). Previously, the effects of F alone on Vtg have been investigated in other species, revealing various effects. No effects of F on Vtg synthesis were found at the protein level in catfish (*Heteropneustes fossilis* (Bloch)) (Pelissero *et al.* 1993) and rainbow trout (Sundararaj *et al.* 1982). However, F was found to diminish the binding of E_2 to the oestrogen receptor (ER) in rainbow trout (Teitsma *et al.* 1998). Contrary, F was found to upregulate Vtg expression *in vivo* in *Xenopus* (Wagler) due to crosstalk between ER and the glucocorticoid receptor (GR) (Marilley *et al.* 1998). The opposite effect is found in rainbow trout, where GR interferes with ER and antagonises Vtg induction (Lethimonier *et al.* 2000).

Under natural conditions, F increases prior to ovulation. This effect has been reported in rainbow trout (Sundararaj *et al.* 1982), Coho salmon (*Oncorhynchus kisutch* (Walbaum)) (Feist *et al.* 1990) and brown trout (*Salmo trutta* (Linnaeus)) (Pickering and Christie 1981). In Arctic charr, F is found to increase in September with a subsequent peak in October (Berg 2003, Berg *et al.* 2004a). The increase in F coincided with the most rapid growth of the ovaries. This has led to the hypothesis that F may be involved in the upregulation of some oocyte component. However, under natural conditions, no correlation between F and Vtg or ZP has been observed (Berg 2003). ZP has been observed to steadily increase between April and September with a slight decline thereafter (Berg *et al.* 2004a). Injecting F alone had no effect on ZP. However, co-injection of F and E_2 increased ZP production in a dose-dependent manner. It also induced higher levels of ZP than injection of E_2 alone, which indicates that the stress-related mechanisms regulating ZP differ from those regulating Vtg (Berg *et al.* 2004a). The exact reasons for this phenomenon remain uncertain. However, one striking difference between Vtg and ZP is that ZP do not rely on metal ions for correct folding. The sequestration of zinc by MT does not affect ZP production (Berg 2003, Berg *et al.* 2004a). In addition, effects on the mRNA level are suggested, as F and E_2 in combination were found to upregulate the ovalbumin gene in chicks (Hager *et al.* 1980). However, ZP-gene promoters in Arctic charr are not thoroughly studied, and too little information is available to confirm this hypothesis (Berg *et al.* 2004a).

In summary, the influence of acute stress during vitellogenesis likely reduces the amount of circulating Vtg, which reduces the amount of nutrients deposited in the oocytes, and may lead to lower embryonic survival caused by starvation. As ZP production increases, thicker eggshells may form, which are less permeable. Eventually, the size of the micropyle may be reduced, which would decrease the fertilisation success (Berg 2003). The physiological effects of acute stress situations have been studied by injecting F in Arctic charr (Berg *et al.* 2004a, Berg *et al.* 2004b). However, as not all physiological mechanisms are thoroughly understood, there is potential for additional research on various mechanisms. It also remains to be investigated, to what extent these mechanisms decrease egg survival when induced by actual stress, and how prolonged exposure to stress affects oocyte development.

Water chemistry

General water chemistry requirements for cultured Arctic charr are reviewed by Sæther *et al.* (2016), and they are similar to the requirements of other salmonids. Especially, dissolved oxygen concentrations, concentrations of metabolic waste products such as ammonia and CO_2, pH and the presence of toxicants, such as heavy metals and organic pollutants, are outlined as important. Mechanisms interfering with oocyte development induced by heavy metals are described in the section on stress. In addition, physiological mechanisms can be disturbed by xenobiotics that act as endocrine disrupting substances. Endocrine disrupting substances interfere with hormonal messaging connected to oocyte development and maturation. As such, o,p′-DDT and o,p′-DDD have been investigated in experiments on Arctic charr, and it has been confirmed that both substances bind to the 20,17-βP receptor in the oocytes. However, binding affinity was found to be less than 1% of the natural ligand (Berg *et al.* 2005).

Regarding gamete development in Arctic charr, research on water chemistry has mainly focused on the effects of salinity and sea water on anadromous stocks

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during summer. Holding anadromous Arctic charr brood-stock in full strength sea water (33–35 ‰) during summer is possible, and the fish exhibit good growth and quick feeding resumption (Delabbio *et al.* 1990, Arnesen *et al.* 1993b). However, there are cases where feed intake was not resumed, while the ion and water balance was retained (Arnesen *et al.* 1993a). In autumn, when the fish naturally had returned to freshwater (Jobling *et al.* 2010, Jørgensen & Johnsen 2014), growth and survival in sea water are compromised (Arnesen *et al.* 1994), as salinity tolerance is decreased (Staurnes *et al.* 1994). The loss of salinity tolerance is not thoroughly understood. However, it usually occurs when photoperiod and temperature change, and sexual maturation onsets (Delabbio *et al.* 1990, Eliassen *et al.* 1998, Duston *et al.* 2007, Jørgensen & Johnsen 2014). Sexually mature Arctic charr should thus not be held in sea water, as this can lead to osmotic imbalance, abnormal seminal plasma composition, inhibition of ovulation or milt production, and decreased fecundity and egg viability (Staurnes *et al.* 1994). Contrary, holding anadromous Arctic charr brood-stock in sea water during summer appears to be beneficial to egg quality (Atse *et al.* 2002). Salinity alone or in combination with temperature did not affect relative fecundity. However, salinity in combination with lower temperature improved egg size, survival to the eyed stage and hatch, lipid, total energy and protein content, and caused the embryos to utilise lipids and proteins before the eyed stage. Eggs from fish of the sea water group at natural temperature also hatched earlier (Atse *et al.* 2002). Salinity alone caused heavier eggs, an increased amount of spermatozoa in milt, and a higher seminal plasma osmolality (Atse *et al.* 2002).

Timing of spawning

Arctic charr usually spawn in late autumn (Brännäs *et al.* 2011a) between September in the Arctic and December towards the southern limits of the distribution. In the southernmost populations, spring spawning also occurs (Elliott & Baroudy 1995, Jeuthe 2015). A wider range of spawning periods of different populations of Arctic charr from mid-July until January is reported by Johnston (2002). Within one population, Arctic charr usually exhibit a prolonged spawning window with a duration of up to 10 weeks. However, the timing of spawning in each cohort appears to be relatively consistent from year to year, and individual females exhibit consistent relative timings of spawning. Usually, males mature prior to females during 4 to 10 weeks, while peak ripeness occurs at variable times for females. As sperm remains viable and healthy in the testes, the spawning window of females is of primary importance (Johnston 2002).

The prolonged spawning window is problematic as post-ovulatory eggs deteriorate quickly in quality (Gillet 1991, Gillet & Breton 1992, Gillet 1994) (See egg quality), and repeated handling of late spawning females results in stress (Brännäs *et al.* 2007, Jeuthe *et al.* 2013). Stress affects the reproductive ability of the brood-stock negatively in fish (Li & Leatherland 2012), and frequent determinations of ripeness are time consuming and costly (Brännäs *et al.* 2007).

The timing and synchronisation of spawning in Arctic charr are determined by environmental cues, mainly day length and temperature (Brännäs *et al.* 2011a). Spawning can be synchronised by manipulating environmental cues in aquaculture facilities. Photoperiod manipulations have been investigated to synchronise spawning and to move the spawning period in Arctic charr (Gillet 1991, Gillet & Breton 1992, Gillet 1994, Duston *et al.* 2003, Frantzen *et al.* 2004, Gillet & Breton 2009). Synchronisation of spawning has also been achieved by manipulating water temperature (Gillet 1991, Gillet & Breton 1992). Synchronous spawning can be triggered using hormone treatments under suitable conditions (Gillet & Breton 1992, Jansen 1993, Gillet *et al.* 1996, Brännäs *et al.* 2007, Gillet & Breton 2009).

Photoperiod

Day length is regarded the most important environmental cue controlling reproductive development in Salmonids (Bromage *et al.* 2001), also in Arctic charr (Jobling *et al.* 1993, Johnston 2002, Jeuthe 2015). In Arctic charr, photoperiod manipulation has been studied since the late 1980s, when abrupt changes in photoperiod and temperature from 18 h light and 12°C from March to September to 6 h light and 6°C from September to March were suggested to synchronise spawning in Norwegian (Lake Storvatn) and Canadian (Nauyuk Lake) stocks (Tabachek & de March 1991). Various photoperiod manipulations and their effects have been investigated in France (Lake Geneva) (Gillet & Breton 1992, Gillet 1994, Gillet & Breton 2009), Norway (Hammerfest strain) (Frantzen *et al.* 2004) and Canada (Fraser River, Labrador stock) (Duston *et al.* 2003) (Table 1). In general, spawning can be advanced or postponed in Arctic charr, which is particularly useful if water temperatures are more favourable for egg development outside the natural spawning season. Synchronisation of male and female fish can also be achieved by manipulating only one sex. Practically, Arctic charr reacts most on differences in light intensity, which makes it most important to keep the night as dark as possible (Brännäs *et al.* 2011a).

Long days generally provide the environmental cue to initiate gametogenesis (Gillet & Breton 1992, Gillet 1994) and have an inhibitory effect on ovulation (Gillet & Breton 1992, Gillet & Breton 2009). While Gillet and Breton (1992) did not find an effect of long-day treatment on

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Table 1 Photoperiodic regimes and their impact on the timing of spawning of Arctic charr

Photoperiod	Temperature	Hormone treatment	Timing of spawning	Duration of spawning (month)	Egg quality	Source
Natural 46°N	Ambient	None	Late November–early January	1.5	OK	Gillet and Breton (1992); Gillet (1994)
Natural 70°N	Ambient	None	Median 22.09.	2.5	60% survival	Frantzen *et al.* (2004)
17L:7D, 21.06. – 15.12.	Ambient	None	Late December–early February	1.5	OK	Gillet and Breton (1992); Gillet (1994)
17L:7D, 16.08. – spawning	Ambient	None	Mid-January–early April	2.5	OK	Gillet and Breton (1992); Gillet (1994)
17L:7D, 24.08. – spawning	Ambient	None	Early February–late April	3	NA	Gillet and Breton (2009)
17L:7D, 08.08. – 15.12.	Ambient	None	Mid-January–late February	1.5	NA	Gillet and Breton (2009)
17L:7D, 16.08. – 15.12.	Ambient	None	Early January–early February	1	OK	Gillet and Breton (1992); Gillet (1994)
17L:7D, 16.08. – 15.12.	Ambient	None	Early January–late February	1.5	OK	Gillet and Breton (1992); Gillet (1994)
14L:10D late summer, 6L:18D in December	Ambient	None	January	1	OK	Brännäs *et al.* (2011a)
17L:7D, 01.09. – 15.12.	Ambient	None	Mid-January–late February	1.5	NA	Gillet and Breton (2009)
17L:7D, 01.10. – 15.12.	Ambient	None	Mid-January–late February	1.5	NA	Gillet and Breton (2009)
17L:7D, 01.10. – 05.05., 7L:17D – spawning	5°C from 06.05.	None	Early May–October	4–6	OK	Gillet and Breton (1992); Gillet (1994)
Second spawning, natural from October	Ambient	None	February–March	2	OK	Gillet and Breton (1992); Gillet (1994)
17L:7D, 06.11. – 15.12.	Ambient	None	Mid-January–late February	1.5	NA	Gillet and Breton (2009)
17L:7D, 04.01. – June, 7L:17D, 01.07 – spawning	5°C from 01.07.	None	Early September–early November	2	OK	Gillet and Breton (1992); Gillet (1994)
17L:7D, 01.04. – 30.06., 7L:17D, 01.07. – spawning	5°C from 01.09	None	Late September–early November	1.5	OK	Gillet and Breton (1992); Gillet (1994)
24L:0D, February – May, 6L:18D May	Ambient	None	Median 16.07.	0.75	17% survival	Frantzen *et al.* (2004)
24L:0D, February – June, 6L:18D June	Ambient	None	Median 16.07.	2	NA	Frantzen *et al.* (2004)
24L:0D, late February – spawning	Ambient	None	Median 22.09.	3.75	97% survival	Frantzen *et al.* (2004)
17L:7D, September – 23.01.	5°C from 23.01.	28.02. sGnRHa + pimozide	March	1	NA	Gillet and Breton (2009)
17L:7D, September – 23.01.	10° from 23.01.	28.02. sGnRHa + pimozide	March	1	NA	Gillet and Breton (2009)
17L:7D, September – 23.01., 7L:17D, 23.01. – spawning	5°C from 23.01.	28.02. sGnRHa + pimozide	March	1	NA	Gillet and Breton (2009)
17L:7D, September – 23.01., 7L:17D, 23.01. – spawning	10° from 23.01.	28.02. sGnRHa + pimozide	March	1	NA	Gillet and Breton (2009)

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gametogenesis in early summer, Gillet (1994) found that an earlier exposure of Arctic charr to long days advanced spawning. He hypothesised that long days triggered gametogenesis. Gametogenesis can already be initiated right after spawning, as demonstrated by Gillet and Breton (1992), who manipulated a group of Arctic charr to ovulate in May by exposing them to a long-day treatment from October to May followed by a short-day treatment in May. Subsequently, these fish were exposed to ambient photoperiod starting with long days in summer and ovulated again in February. By this experiment, further evidence for the initiation of gametogenesis by long days was provided, and the length of the reproductive cycle in Arctic charr was estimated to 8 month. In late summer and autumn, prolonging the exposure of Arctic charr to long days postpones spawning (Brännäs *et al.* 2011a). Long days from October onwards for instance delay spawning by 8 month (Gillet & Breton 1992). These observations suggested that long days exhibit an inhibitory effect on ovulation (Gillet 1994). This inhibitory effect reached its maximum after a long-day treatment of more than 40 days. Arctic charr exposed to different durations of long-day treatments in autumn prior to its natural spawning period delayed spawning less when exposed to long days for 40 days, than when exposed to long days for 77, 107 or 130 days (Gillet & Breton 2009). Short-day treatments reversed the inhibitory effect of long days on ovulation, and spawning usually occurred approximately one month after the light regime had changed (Gillet & Breton 2009). However, the inhibitory effect of long days did not prevent ovulation completely. Prolonged long-day treatments during winter rather delayed ovulation and extend the spawning period (Gillet & Breton 2009). This was earlier demonstrated, as a lack of short days after a long-day treatment until October extended the spawning period over five month (Gillet & Breton 1992). Asynchrony of ovulation caused by a lack of short-day treatment was also observed by Frantzen *et al.* (2004).

Short-day treatments synchronised spawning in rainbow trout (Bromage *et al.* 1984), and a similar effect was demonstrated to act on Arctic charr when short-day treatments were applied several weeks prior to the natural time of ovulation (Gillet & Breton 1992, Gillet 1994). Physiologically, short days cause the pituitary to be highly responsive to sGnRHa regardless of water temperature (Gillet & Breton 2009). Short-day treatments were also applicable to advance spawning in Arctic charr when administered after mid-summer (Brännäs *et al.* 2011a), and ovulation could be advanced by approximately two month (Gillet & Breton 1992).

While the timing of spawning can be manipulated by photoperiod in repeat spawners, the number of maturing fish (repeat spawners, 3+) was not influenced by photoperiodic manipulations that included ambient photoperiod, continuous light from February to March or June and continuous light during the entire gamete development (Frantzen *et al.* 2004). However, the number of maturing juveniles can be affected by photoperiodic manipulation (Gillet & Breton 1992, Gillet 1994, Duston *et al.* 2003). This technique is particularly promising in southern Norway, as selective breeding for later maturation is not permitted (Brännäs *et al.* 2011a). For this purpose, long days can be applied from February to mid-March, followed by ambient photoperiod or an abrupt change to short days, the latter being more effective (Duston *et al.* 2003). Maturation can also be advanced in females by subjecting them to a constant long-day treatment (Duston *et al.* 2003). When juveniles are subjected to long-day treatments in winter, first spawning can be advanced by six month (Gillet & Breton 1992). However, in this case, the fish are required to be in good condition and receptive to the manipulation. Gillet (1994) attempted to advance ovulation by subjecting juveniles to a long-day treatment from October, which resulted in fish ovulating from May to September. He concluded that juveniles became receptive to the treatment during winter at different times, which likely caused the asynchrony in ovulation.

It is possible to produce viable gametes of Arctic charr at all times of the year (Gillet 1994). However, sufficient time for complete gametogenesis is required to ensure acceptable egg quality. Advanced females, for instance, have been observed to produce smaller eggs (Gillet 1994). Applying continuous light from February to May followed by a short-day period from May until spawning has also been demonstrated to cause gametogenesis to occur more rapidly. In this case, peaks in sex steroid levels (E_2 and 11-ketotestosterone (11-KT)) were more short-lived, and low fertilisation success caused by incomplete gametogenesis was another consequence of the treatment. It was concluded that long-day treatments of more than 10 weeks are required to ensure satisfactory sperm and egg quality (Frantzen *et al.* 2004). However, these results might also have been affected by high temperatures during spawning and egg development, as spawning occurred in summer at 8°C.

Temperature

Temperature was shown to affect the timing of spawning in fish by influencing plasma hormone levels and gonadal development (Rombough 1997, Van Der Kraak & Pankhurst 1997, Jobling *et al.* 1998). Maintaining sufficiently low temperatures constitutes a major challenge to the Arctic charr aquaculture industry, and failure to do so often results in asynchronous spawning (Jobling *et al.* 1998). Asynchronous spawning caused by elevated temperatures at ovulation can be avoided by hormone treatments with sGnRHa and a dopamine antagonist (Gillet & Breton 1992, Jansen 1993, Gillet *et al.* 1996, Jobling *et al.* 1998).

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Table 2 Temperature regimes and their effect on the timing of spawning and egg viability of Arctic charr

Temperature	Timing of spawning	Egg quality	Source
Natural fluctuation 12°C summer, 4°C winter	Mid-November–early January	Increased with decreasing temperature	Gillet (1991); Gillet and Breton (1992)
Natural fluctuation 12°C summer, 4°C winter	Late November–early January	Increased with decreasing temperature	Gillet (1991); Gillet and Breton (1992)
5°C, 15.07 – spawning	October–late January	Comparable to wild fish	Gillet (1991)
5°C, 15.07 – spawning	Late November–mid-January	Comparable to wild fish	Gillet and Breton (1992)
5°C, 01.09. – spawning	Late November–mid-January	Comparable to wild fish	Gillet and Breton (1992)
5°C, 15.09. – spawning	Early November–early January	Comparable to wild fish, lower weight	Gillet (1991)
5°C, 15.09. – spawning	Late November–mid-January	Comparable to wild fish, lower weight	Gillet and Breton (1992)
5°C, 01.10. – spawning	Mid-November–mid-January	Comparable to wild fish	Gillet and Breton (1992)
8°C, early December – spawning	Mid-Decembe –late January	37% ovulated, lower viability	Gillet (1991)
8°C, early December – 15.12., 5°C – spawning	Mid-Decembe –late January	Highest survival of comparable treatments	Gillet (1991); Gillet and Breton (1992)
8°C, early December – 05.01., 5°C – spawning	Early January–late February	Intermediate in comparable treatments	Gillet (1991); Gillet and Breton (1992)
8°C, early December – 15.01, 5°C – spawning	Mid-January–late March	80% ovulated, reduced quality	Gillet and Breton (1992)
8°C, early December – 25.01, 5°C – spawning	Late January–mid-March	80% ovulated, lowest survival in comparable groups	Gillet (1991)
10°C, 01.09. – January	Almost inhibited	Only one female ovulated	Gillet and Breton (1992)
10°C, 01.10. – 29.12., 5°C – spawning	Early January–mid-January	85% ovulated	Gillet and Breton (1992)
10°C in Autumn – December, 8°C – spawning	Mid-December–late January	37% ovulated, reduced quality	Gillet and Breton (1992)
11°C, early December – late March	Inhibited	NA	Gillet (1991)
11°C, early Autumn – March	Inhibited	NA	Gillet and Breton (1992)
Wild fish caught ovulating (ca. 5.5°C)	Late November–early January	Control group, good quality	Gillet (1991); Gillet and Breton (1992)

However, given an adequate temperature regime throughout the entire reproductive cycle, such treatments should not be necessary (Jobling *et al.* 1995, Tveiten *et al.* 1996, Jobling *et al.* 1998).

Elevated temperatures during vitellogenesis may delay ovulation. Arctic charr held at 12–16°C from mid-June to September ovulated three to four weeks later than conspecifics held at 4°C, despite low autumn temperatures of 4°C in all treatments (Jobling *et al.* 1995). Contrary, Atse *et al.* (2002) could not confirm the effect of summer temperature *per se* on the timing of spawning. Elevated summer temperatures decreased vitellin deposition and lipid metabolism in other teleosts, resulting in decreased activity of vitellogenesis (Cossins & Bowler 1987, Sargent *et al.* 1989). Lipid deposition is also shown to be influenced by summer temperatures in Arctic charr (Jobling *et al.* 1995). This was interpreted as a result of delayed oocyte development by Jobling *et al.* (1998), as lipid class composition and fatty acid profiles changed significantly during vitellogenesis. PL is primarily incorporated in the oocytes during summer, resulting in an net increase in the PL content, and a decreasing TAG:PL ratio over time in teleosts (Nassour & Léger 1989, Wiegand 1996, Jobling *et al.* 1998). TAG:PL ratios in Arctic charr held at high summer temperatures exhibited higher values (1.2) compared with fish held at 4°C (0.76), when measured in September (Jobling *et al.* 1995, Jobling *et al.* 1998).

The main effect of temperature on the timing of spawning occurs in autumn (Table 2). High temperatures ≥ 11°C completely inhibit ovulation (Gillet 1991), and 10°C results in an almost complete inhibition (Gillet 1991, Gillet *et al.* 1996, Gillet & Breton 2009). The temperature induced inhibition onsets within a few days subsequent to a transfer from 5 to 10°C (Gillet *et al.* 2011), and also, males are affected by temperatures ≥ 10°C through decreased milt quality and fewer spermatozoa mL^{-1} compared with males held at 5°C (Brännäs *et al.* 2011a). Temperatures as low as 8°C delay ovulation compared with 5°C (Gillet 1991, Gillet & Breton 1992), and maintenance of the brood-stock at 8°C in the natural spawning period is only feasible for a limited duration (Gillet 1991). The quality of well-developed ovaries decreases at 8°C, which is likely an effect of oocyte atresia (Gillet 1991). Oocyte atresia at elevated temperatures has previously been observed in the related species brook trout (Henderson 1963). Consequently, high temperatures are an inadequate mean to artificially delay

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spawning. Contrary, decreasing water temperature around the timing of spawning can be used to synchronise and stimulate ovulation, which has been demonstrated for transfers from 8 to 5°C (Gillet 1991, Gillet & Breton 1992) and from 10 to 5°C (Gillet & Breton 1992). However, advancing the spawning period by a reduction in ambient water temperatures does not seem to be possible (Gillet & Breton 1992), as fish transferred to 5°C in July did not ovulate prior to fish transferred in September (Gillet 1991). Temperatures around 5°C only seem to be required during the last weeks prior to ovulation (Gillet 1991).

Experiments, comparing physiological functions at 10 and at 5°C, have revealed several physiological mechanisms inhibiting spontaneous ovulation in Arctic charr at high temperatures (Gillet & Breton 1992, Gillet *et al.* 1996, Gillet & Breton 2009, Gillet *et al.* 2011). Biochemical differences in Arctic charr oocytes related to temperature were reported by Gillet and Breton (1992), who discovered that oocytes exhibited higher levels of cyclic adenosine monophosphate (cAMP) in autumn when fish were held at higher temperatures. A prolonged decline in cAMP was identified as a potential precondition for ovulation (Gillet & Breton 1992). Subsequently, a dopamine-induced inhibition of ovulation at high temperatures was discovered, as diphenylbutylpiperidines increased the effectiveness of sGnRHa stimulation on LH secretion at the end of vitellogenesis at 10°C, but not at 5°C (Gillet *et al.* 1996, Gillet & Breton 2009). However, it was also hypothesised that final maturation may be inhibited at 10°C due to a lack of a spawning trigger, as eggs ovulated by hormonal treatments often exhibited low quality at that temperature (Gillet *et al.* 1996). By comparing Arctic charr held under long- and short-day photoperiods at 5 and 10°C in all possible combinations, Gillet and Breton (2009) documented the effects of photoperiod and temperature on LH levels in Arctic charr. LH secretion seemed to be reduced by high temperature and long-day photoperiod. Stimulation of LH did not seem to be persistent at 10°C either (Gillet & Breton 2009).

When ovulation commences, 17,20βP levels increase naturally in Arctic charr (Gillet *et al.* 2011), and already ovulating females exhibit similar levels when transferred to either 5 or 10°C. The surge in 17,20βP is caused by the stimulation of several steroidogenic enzymes, ultimately initiated by LH in vertebrates (Jalabert *et al.* 1991, Bobe *et al.* 2008, Nagahama & Yamashita 2008). However, one or several steps in the synthesis of 17,20βP are hypothesised to be impaired by high temperatures in Arctic charr (Gillet *et al.* 2011), analogous to a similar suggestion for rainbow trout (Pankhurst & Thomas 1998). In addition, there is a direct influence of reduced LH levels at higher temperatures in Arctic charr (Gillet *et al.* 2011). As 17,20βP secretion is stimulated immediately after a transfer from 10 to 5°C, a limiting effect of high temperatures on 20β-hydroxysteroid-dehydrogenase activity is likely (Gillet & Breton 2009). A decline in the responsiveness of the ovary to LH stimulation caused by elevated temperatures has also previously been observed in grass carp (*Ctenopharyngodon idella* (Valeuciennes)) (Glasser *et al.* 2004). To investigate the responsiveness of the ovary to LH and 17,20βP, Gillet *et al.* (2011) stimulated four groups of ovaries from fish reared at 5 or 10°C, which were either kept at 5 or 10°C *in vitro*, with either LH or 17,20βP. The stimulation of LH was both dependent on rearing temperature of the fish and holding temperature of the ovary, while the effect of 17,20βP was only dependent on the holding temperature of the ovary. Consequently, the responsiveness of the ovary to LH stimulation is dependent on the previous temperature regime, but a direct effect of temperature on ovulation cannot be excluded (Gillet *et al.* 2011). The holding temperature of the ovary affected the effectiveness of 17,20βP, which proves a direct, immediate effect of high temperature on ovulation. However, as rearing temperature of the female did not affect stimulation by 17,20βP, long-term effects of high temperature occurred upstream, that is before the production of 17,20βP, and might be linked to failed LH stimulation at 10°C (Gillet *et al.* 2011). Regardless of the physiological mechanism, the competence to resume meiosis and ovulate is lost at high temperatures. However, it can also be regained gradually in response to repeated LH stimulation, which enables follicular somatic cells to produce 17,20βP and oocytes to respond to it (Gillet *et al.* 2011).

Hormone treatments

Ovulation in salmonids can be induced by pituitary preparations and injections of GnRH (Jalabert *et al.* 1978, Crim *et al.* 1983, Sower *et al.* 1984, Breton *et al.* 1990). This is also possible in Arctic charr using GnRHa, eventually combined with a dopamine inhibitor (Table 3). However, as only the gamete release is stimulated, egg quality may be low under unfavourable conditions. Consequently, successful hormone treatments are carried out on sexually mature fish under favourable spawning conditions. Hormone treatments can nevertheless be advantageous to reduce stress and costs associated with a prolonged spawning season (Brännäs *et al.* 2011a).

Under favourable conditions, hormone treatments were successful in Arctic charr and resulted in good egg quality at 5°C (Gillet & Breton 1992) and at 7°C when no photoperiodic spawning trigger was provided (Jansen 1993). At elevated temperatures (10°C), egg quality of hormone treated fish exhibited lower values and generally more variation, and stimulation did not seem to be as persistent (Gillet & Breton 1992). In addition, the type of treatment becomes crucial at elevated temperatures, as, for example, D-Arg^6-sGnRHa induced significantly lower plasma gonadotropin

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Table 3 Hormone treatments tested to induce spawning in Arctic charr

Hormone treatment	Temperature	Photoperiod	Timing of spawning	Egg quality (% fertilisation)	Source
Saline control	5°C	Ambient	75% within 38 days	ca. 70%	Gillet and Breton (1992)
Saline control	5°C	Ambient	100% within 35 days	ca. 70%	Gillet and Breton (1992)
Saline control	10°C	Ambient	No ovulation	No ovulation	Gillet and Breton (1992)
Saline control	7°C	Constant	24.5% within 13 days	> 90% fertility	Jansen (1993)
Saline control	5°C	Ambient	90% within 40 days, 36% in 14 d	73.6 ± 2.7%	Gillet *et al*. (1996)
Saline control	10°C	Ambient	1/49 females ovulated	5.0%	Gillet *et al*. (1996)
D-Trp^6, Des-Gly^{10}LH-Rha (30 μg kg^{-1})	5°C	Ambient	60% in 14 days, 100% in 21 days	72.6 ± 7.2%	Gillet and Breton (1992)
D-Trp^6, Des-Gly^{10}LH-Rha (30 μg kg^{-1})	10°C	Ambient	No ovulation	No ovulation	Gillet and Breton (1992)
D-Trp^6, Des-Gly^{10}LH-Rha (30 μg kg^{-1})	5°C	Ambient	75% in 14 days	72.6 ± 7.2%	Gillet *et al*. (1996)
D-Trp^6, Des-Gly^{10}LH-Rha (30 μg kg^{-1})	10°C	Ambient	1/49 females ovulated	25.8%	Gillet *et al*. (1996)
D-Trp^6, Des-Gly^{10}LH-Rha (30 μg kg^{-1}) slow release	10°C	Ambient	100% in 12 days	50.3 ± 13.3%	Gillet and Breton (1992)
D-Trp^6, Des-Gly^{10}LH-Rha (30 μg kg^{-1}) slow release	5°C	Ambient	100% within 26 days	76.7 ± 7.6%	Gillet and Breton (1992)
D-Trp^6, Des-Gly^{10}LH-Rha (20 μg kg^{-1}) slow release	5°C	Ambient	80% within 14 days	71.0 ± 6.2%	Gillet *et al*. (1996)
D-Trp^6, Des-Gly^{10}LH-Rha (20 μg kg^{-1}) slow release	10°C	Ambient	80% within 15 days	44.4 ± 5.6%	Gillet *et al*. (1996)
D-Trp^6, Des-Gly^{10}LH-Rha (20 μg kg^{-1}) slow release	5°C	Ambient	60% in 14 days, 100% in 21 days	51.68 ± 13.8%	Gillet and Breton (1992)
D-Trp^6, Des-Gly^{10}LH-Rha (20 μg kg^{-1})	10°C	Ambient	No ovulation	No ovulation	Gillet and Breton (1992)
D-Trp^6, Des-Gly^{10}LH-Rha (20 μg kg^{-1}) slow release	10°C	Ambient	67% in 21 days	34.6 ± 8.2%	Gillet and Breton (1992)
D-Trp^6, Des-Gly^{10}LH-Rha (60 μg kg^{-1})	5°C	Ambient	50% within 15 days	NA	Gillet and Breton (1992)
D-Trp^6, Des-Gly^{10}LH-Rha (60 μg kg^{-1}) slow release	10°C	Ambient	100% within 12 days	36.2 ± 12.4%	Gillet and Breton (1992)
D-Ala^6, Des-Gly^{10}LH-RHa (20 μg kg^{-1})	5°C	Ambient	90% within 17 days	76.5 ± 5.6%	Gillet and Breton (1992)
D-Arg^{6}sGnRHa (20 μg kg^{-1})	5°C	Ambient	50% in 10 days, 80% in 14 days	69.4 ± 4.5%	Gillet *et al*. (1996)
D-Arg^{6}sGnRHa (20 μg kg^{-1})	10°C	Ambient	37% in 50 days	43.4 ± 14.0%	Gillet *et al*. (1996)
D-Arg^{6}sGnRHa (20 μg kg^{-1})	5°C	Ambient	90% within 9 days	73.0 ± 3.6%	Gillet and Breton (1992)
D-Arg^{6}sGnRHa (20 μg kg^{-1})	10°C	Ambient	50% within 7 days	39.8 ± 20.2%	Gillet and Breton (1992)
D-Arg^{6}sGnRHa (20 μg kg^{-1}) + pimozide 5 mg kg^{-1}	5°C	Ambient	50%, 10 days, 80%, 14 days, 100%, 50 days	45.0 ± 6.4%	Gillet *et al*. (1996)
D-Arg^{6}sGnRHa (20 μg kg^{-1}) + pimozide 5 mg kg-1	10°C	Ambient	51% within 50 days	34.8 ± 5.7%	Gillet *et al*. (1996)
D-Arg^{6}sGnRHa (60 μg kg^{-1})	10°C	Ambient	100% within 9 days, 80 within 4 days	40.6 ± 12.6%	Gillet and Breton (1992)
D-Arg^{6}sGnRHa (100 μg kg^{-1})	10°C	Ambient	80% within 15 days	39.1 ± 10.0%	Gillet *et al*. (1996)
Pimozide	5°C	Ambient	50% within 12 days	62.5 ± 5.8%	Gillet *et al*. (1996)
Pimozide	10°C	Ambient	3/13 ovulated	72.9 ± 6.4%	Gillet *et al*. (1996)
Ovaprim 0.1 mL kg^{-1} day 1, 0.4 mL kg^{-1} day 3	7°C	Constant	87.8% within 7–11 days	> 90%	Jansen (1993)
FSsH (preliminary results)	NA	NA	Synchronized within 7 days	No improvement	Brännäs *et al*. (2007)

levels at 10°C than at 5°C (Gillet & Breton 1992). At 10°C, only sustained release forms or high dosages of the same treatment induced comparable ovulation rates as at 5°C, and diphenylbutylpiperidine alone or in combination with other treatments induced ovulations at this temperature (Gillet *et al*. 1996). At 5°C, there may be a tendency of reduced egg quality caused by diphenylbutylpiperidine (Gillet *et al*. 1996). At 10°C, the combination of sGnRHa and diphenylbutylpiperidine was most effective at stimulating ovulation, while sGnRHa resulted in a higher percentage of ovulation than diphenylbutylpiperidine alone. The effectiveness of combining diphenylbutylpiperidine with sGnRHa at 10°C was also reflected in plasma LH levels. However, long-day photoperiod rendered this treatment

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ineffective (Gillet & Breton 2009). LH stimulation was also found to be less effective at 10°C than at 5°C *in vitro*, and elevated temperatures several weeks prior to ovulation interfered with the effectiveness of the treatment (Gillet *et al.* 2011). Regarding hormone treatments at suitable temperatures (≈5°C), Gillet *et al.* (1996) found no correlation between the time of first ovulation and maximum ovulation, and concluded that different treatments also exhibited differences in effectiveness at 5°C. While all treatments resulted in a high percentage of ovulation and good egg quality, there may be treatments that are superior at synchronising a relatively large proportion of the spawners within few days and others that result in a higher cumulative percentage of ovulation over time.

Other factors

Few additional factors have been investigated regarding their effect on the timing of spawning in Arctic charr. The condition of the fish has been reported to influence timing of spawning (Johnston 2002), and Tabachek and de March (1991) claimed that '[i]t is well known that diet can have an impact on egg survival, fecundity and the timing of spawning'. However, profound examples, quantifications or physiological explanations have not been provided regarding impacts of the diet on timing of spawning. Hydrostatic pressure and conditions of captivity did not seem to affect the timing of ovulation (Gillet 1991, Gillet & Breton 1992).

Identification of ripeness

Captive Arctic charr are stripped, as they usually retain their eggs. Stripping has to occur within 4 days post ovulation, as stripping overripe eggs results in reduced egg quality (Brännäs *et al.* 2011a). Consequently, ripe females need to be identified at the appropriate time, which is most effectively achieved by sorting the brood-stock during the spawning season (Brännäs *et al.* 2011a).

Determining the sex of the fish is challenging (Johnston 2002). Morphology only differs during the spawning season (Brännäs *et al.* 2007). The use of genetic markers has resulted in a correct sex determination in 88% of the investigated cases (Brännäs *et al.* 2007). Ultrasonic scanning was impractical (Brännäs *et al.* 2011a). Morphological characteristics during the spawning season appear to be the most appropriate trait to determine sex in Arctic charr. Males have bright red or orange spawning colouration, especially the bright colouration on their bellies and flanks, and their snouts become more pointed, turn light brown and develop a hooked kype. However, also females develop spawning colouration and may develop a small kype (Johnston 2002).

As soon as the spawning period commences, ripe males and females should be kept in separate tanks and stripped as soon as possible (Johnston 2002). In general, males are more manageable, as they are often more synchronised (Johnston 2002), and milt can be cryopreserved. As the spawning window spans over several weeks for most brood-stocks, females should be sorted by ripeness to avoid unnecessary stress. Unripe females can be identified by their white, hard and un-swollen bellies, and nearly ripe females exhibit soft, slightly protruding bellies with mottled flanks and slightly swollen genital papillae. Ripe females can be distinguished by their distended, soft and dark bellies, and the release of eggs which can be triggered by light pressure (Johnston 2002). Once identified, unripe females can be kept in a separate tank and be checked for ovulation every other week. Nearly ripe females should be checked at least once every week at temperatures below 6°C (Johnston 2002). At temperatures above, they should be checked twice every week (Brännäs *et al.* 2011a).

Effect on egg quality

Poor management during the spawning period may greatly affect egg quality. In general, farmed Arctic charr brood-stock are subjected to a variety of unnatural conditions during this period, such as unnatural photoperiod and light intensity, unnatural temperature and the separation of males and females (Jeuthe 2015). However, the relationship between timing of spawning and egg quality remains to be quantified. In an exploratory factor analysis (EFA) on hatchery data, which were collected between 2000 and 2011 in Sweden, the date of fertilisation was included, but did not exhibit a statistically significant correlation to egg quality (Jeuthe *et al.* 2013). The lack of significant correlation might be due to several confounding factors, such as the repeated handling stress for late ovulating females, which were checked for ovulation twice every week over a prolonged period (Jeuthe *et al.* 2013). Contrary, late ovulating females might have benefitted from lower water temperature towards the end of the season (Jeuthe *et al.* 2013). Evidence for increased egg quality in females that spawn at intermediate dates is also provided by Srivastava *et al.* (1991), who investigated a variety of egg properties in eggs sampled early in the season, mid-season and late in the season. Eggs sampled mid-season exhibited highest nutrient content and better survival and growth during embryonic development, measured as highest wet weight from stripping to one month after first feeding, greatest mean body length after hatch until one month after first feeding, largest mean yolk sac volume, highest survival from fertilisation until one month after hatch, highest protein and lipid content until one month post hatch, highest carbohydrate content until the eyed stage and highest total energy content from fertilisation until first feeding. However, the experimental design of this study was not thoroughly described, and differences did not exceed 10% and might thus be caused by confounding factors, despite the statistical

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significance of the differences (Johnston 2002). In addition, Johnston (2002) criticised that also egg size differed between the groups and that egg size may be linked to the measured qualities. However, as egg size also is a property of the egg and not a primary cause of egg quality, it might also be influenced by the timing of spawning.

Fertilisation process

Hygiene

Eggs of Arctic charr may be contaminated with blood, mucus, broken eggs or faeces, and have to be checked while stripping. While badly contaminated batches need to be discarded, some contamination can be removed with the tip of a paper towel (Johnston 2002). Also, albumen from broken eggs needs to be removed, as it can clog the micropyle of viable eggs and inhibit sperm motility in fish (Piper *et al.* 1982). Contamination with urine may be problematic when stripping milt, and its effects on milt quality in fish in general are reviewed by Rurangwa *et al.* (2004). There are no species-specific hygienic concerns for Arctic charr; thus, the general literature on hygiene during fertilisation in aquaculture can be consulted (e.g. Piper *et al.* 1982, Rurangwa *et al.* 2004).

Sperm quality

Sperm quality parameters in Arctic charr can be assessed by a variety of techniques that are not unique to this species (Rurangwa *et al.* 2004, Fauvel *et al.* 2010, Migaud *et al.* 2013). Sperm quality is foremost associated with fertilisation success in fish, but can also influence embryonic survival after fertilisation by epigenetic and transcriptional mechanisms (Cabrita *et al.* 2014). When eggs of Arctic charr are fertilised in aquaculture, excessive amounts of milt and minimal amounts of water are usually used to ensure fertilisation, and milt of several males is often used to dilute the effects of low quality milt (Jeuthe 2015). Fertilisation of pooled egg and milt batches is also performed for logistical reasons. However, mixed-milt fertilisation may be disadvantageous, as sperm velocity and fertilisation success depend on social status of the male and may favour subordinate individuals (Haugland *et al.* 2009). Sperm quality related to social status may also quickly change depending on other fish held in the same tank (Rudolfsen *et al.* 2006). Milt quality, and consequent fertilisation success, may also be influenced by artificial spawning and holding conditions. Spawning conditions and the treatment of the milt may also induce haploid selection, thus changing the genetic makeup of the offspring generation in fish (Gavery & Roberts 2017, Gavery *et al.* 2018). However, this effect has not been studied directly in Arctic charr to our knowledge. A decrease in the number of motile sperm cells and increase in swimming velocity were observed within four generations of hatchery-reared Arctic charr (Kekäläinen *et al.* 2013). These results suggest that advantageous traits related to sperm quality can be rapidly lost under artificial selection, while counteracting selection on related traits may delay the overall decline in fertility in Arctic charr males (Kekäläinen *et al.* 2013). However, the decreased number of motile sperm in Arctic charr milt is of concern and should be further investigated. It also remains unknown, whether this effect generally occurs in Arctic charr. Changes in sperm velocity and longevity may also be induced by unintended haploid selection due to the choice of fertilisation method. Haploid selection could also be used for breeding purposes (Alavioon *et al.* 2017). However, this has not been attempted in Arctic charr to our knowledge.

Sperm quality has been shown to be a limiting factor for reproductive success in farmed Arctic charr. Sperm swimming velocity was positively correlated to fertilisation rate when an excessive amount of milt was used, indicating that this likely reflects maximum fertilisation potential (Jeuthe *et al.* 2019). The motility parameter beat cross frequency (BCF) and milt density were both correlated with egg mortality, but not with fertilisation rate (Jeuthe *et al.* 2019). It is unclear how these parameters could be connected to offspring viability. BCF is generally not included or recommended as a quality indicator in sperm motility studies. One could speculate that these two sperm quality parameters may be linked to another, possibly genetic or epigenetic, quality parameter. Linear regression using the highest fertilisation rate achieved by each individual male revealed that BCF and 17, 20β-P levels in males were sufficient to explain 65% of the variation in egg survival up to the eyed stage in this study (Jeuthe *et al.* 2019).

Damage to paternal DNA can negatively affect fertilisation and hatching rates, while damage up to 25% was shown to not necessarily do so (Devaux *et al.* 2011). However, malformations occurred up to twice as often during the eyed stage compared to controls, and they have a negative effect on post-hatch survival. In this study, paternal DNA damage was induced by the model genotoxic substance methyl methanesulfonate (MMS). Consequently, sperm quality in Arctic charr both directly affects fertilisation success, and it affects embryonic survival through epigenetic and genetic effects. However, the mechanisms and correlations connecting different traits remain poorly elucidated, and more research is necessary on this topic.

Sperm cryopreservation

Cryopreservation of sperm as a technique to store gametes and to allow for a more flexible timing of fertilisation has been suggested for Arctic charr (Brännäs *et al.* 2011b). Relatively high fertilisation rates have been achieved (Table 4). However, the state of the art protocols still has potential for improvement. In addition, suboptimal conditions under

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Table 4 Most successful protocols for cryopreservation of sperm of Arctic charr

	Cryoprotectant	Diluent	Container	Freezing	Thawing	Fertilisation success	Survival to the eyed stage	Reference
Absolute success reported	15% Methanol	0.3 M glucose	0.5 mL straw	5 cm above liquid nitrogen	Combined results; 3.3°C s^{-1} and 11.6°C s^{-1}	70.7 ± 3.3%	65.7 ± 2.9%	Richardson *et al*. (2011)
	10% Methanol + 7% egg yolk	0.3 M glucose	0.5 mL straw	4 and 5 cm above liquid nitrogen	25°C for 30 s	69.5 ± 6.4%	70.4 ± 6.5%	Mansour *et al*. (2006b)
	10, 12.5 and 15% Methanol	0.3 M glucose	0.5 mL straw	5 cm above liquid nitrogen	25°C for 17 s (11.6°C s^{-1})	62.1 ± 2.7%	57.0 ± 2.4%	Richardson *et al*. (2011)
	10% Methanol	0.3 M glucose	0.5 mL straw	5 and 6 cm above liquid nitrogen	25°C for 30 s	61.2 ± 7.4%	62.2 ± 6.0%	Mansour *et al*. (2006b)
	12.5% Methanol	0.3 M glucose	0.5 mL straw	5 cm above liquid nitrogen	Combined results; 3.3°C s^{-1} and 11.6°C s^{-1}	57.4 ± 3.3%	54.1 ± 2.9%	Richardson *et al*. (2011)
	10, 12.5 and 15% Methanol	0.3 M glucose	0.5 mL straw	5 cm above liquid nitrogen	5°C for 60 s (3.3°C s^{-1})	56.4 ± 2.8%	55.3 ± 2.4%	Richardson *et al*. (2011)
Relative success reported	10% Methanol	Lahnsteiner's diluent	0.5 mL straw	2.5 cm above liquid nitrogen	25°C for 30 s	76.2% of control		Lahnsteiner *et al*. (1997)
	20% glycerol	0.3 M glucose	Pellets				75% of control	Piironen (1993)
	20% glycerol	Described by Mounib 1978	Pellets				75% of control	Piironen (1993)
	10% Methanol	Lahnsteiner's diluent	1.2 mL straw	1.5 cm above liquid nitrogen	30°C for 30 s	73.5% of control		Lahnsteiner *et al*. (1997)

cryopreservation are not the cause for poor reproductive success of Arctic charr in general, as survival rates are also observed to be low when fresh milt is used for fertilisation (e.g. Jeuthe *et al*. 2013, Jeuthe *et al*. 2015). However, well-designed protocols for cryopreservation are useful to produce high-quality spawns of Arctic charr, and optimal diluents for cryopreservation of sperm are species-specific (Stein & Bayrle 1978, Piironen 1993).

Milt that is cryopreserved is mixed with an extender at 4°C at a ratio of 1:3 or 1:2 resulting in a concentration of 8.0×10^8–2.5×10^9 cells mL^{-1} (Lahnsteiner 2000b). The milt can be frozen in straws holding 0.5 or 1.2 mL, while larger straws of 5 mL resulted in inconsistent freezing (Richardson *et al*. 2000). The milt is frozen over liquid nitrogen, either on a fixed rack (Lahnsteiner *et al*. 1997) or in a Styrofoam box floated in liquid nitrogen (Richardson *et al*. 2000). When frozen, straws are placed in liquid nitrogen for storage (Lahnsteiner *et al*. 1997). Temperature endpoints during thawing have a greater impact on fertilisation success than thaw rates (between 3.3°C s^{-1} and 11.6°C s^{-1}) and should be placed just above 0°C (Richardson *et al*. 2011). Eggs are fertilised by dispersing sperm over the eggs with a minimal amount of ovarian fluid and gently mixing the gametes. After one minute, 15 mL of water is added, and the eggs are left for another minute. To activate the sperm, 0.12 M NaCl is added (Richardson *et al*. 2000).

The extender for cryopreservation generally consists of a diluent and a cryoprotectant. The diluent may be glucose (Piironen 1993, Richardson *et al*. 2000, Mansour *et al*. 2006b, Richardson *et al*. 2011), a combination of NaCl, KCl, $Na_2HPO_4{\cdot}7H_2O$ and L-α-lecithin at pH 7.5 (Richardson *et al*. 2000), or Lahnsteiner's diluent (Lahnsteiner *et al*. 1997, Lahnsteiner 2000b, Mansour *et al*. 2006b). Lahnsteiner's diluent consists of NaCl, KCl, $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$ and HEPES sodium salt, adjusted to PH 7.8 using NaOH and HCl (Lahnsteiner 2000b). The most common cryoprotectants used are as follows: dimethyl sulfoxide (DMSO) (Richardson *et al*. 2000, Mansour *et al*. 2006b), glycerol (Piironen 1993, Richardson *et al*. 2000), dimethylacetamide (DMA) (Richardson *et al*. 2000, Mansour *et al*. 2006b) and methanol (Lahnsteiner *et al*. 1997, Mansour *et al*. 2006b, Richardson *et al*. 2011). High fertilisation success of cryopreserved sperm was achieved using 0.3 M glucose as diluent and 10% methanol as cryoprotectant (56.2 ± 5.2% fertilised; 55.5 ± 5.0% survival to the eyed stage) (Mansour *et al*. 2006b). Ca. 60% fertilisation success could also be achieved using DMSO and 0.3 M glucose in 0.5 mL straws, and DMA in 0.3 M glucose or 0.3 M glucose and 0.011 M KCl (Richardson *et al*. 2011). The best results of 70.7 ± 3.3% fertilised eggs and 65.7 ± 2.9% eyed eggs were achieved using 15% methanol and 0.3 M glucose in a 0.5 mL straw, frozen 5 cm above

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liquid nitrogen (Richardson *et al.* 2011). Using 15% methanol as cryoprotectant resulted in higher fertilisation success than using 12.5% and 10% methanol, respectively (Richardson *et al.* 2011). Consequently, even higher concentrations of methanol as cryoprotectant should be investigated, as they may result in even higher fertilisation success of cryopreserved milt.

The addition of egg yolk (7%) (Mansour *et al.* 2006b) and different fractions of isolated Arctic charr seminal plasma proteins (Mansour *et al.* 2008b) to the extender was also investigated, but did generally not improve post-thaw fertilisation success.

Egg quality and fecundity

Egg quality in cultured Arctic charr is often highly variable, and low, and it is reflected in egg survival, which is additionally influenced by the rearing environment. Comprehensive data on egg survival are available from the Swedish breeding programme at Aquaculture Centre North in Kälarne, where egg survival was on average 42 ± 25% for single sire/dam fertilisations between 2000 and 2011, excluding zero success batches. Of the 540 analysed batches, 106 had no success at reaching the eyed stage, and average survival including these batches was 33 ± 28% (Jeuthe *et al.* 2013). These figures demonstrate low and highly variable survival in eggs of Arctic charr. However, the effect of egg quality *per se* is more distinguishable when comparing eggs of different families, which are reared under identical conditions. Varying fertilisation rates between 59 and 100% and survival rates between 9 and 98% have been detected under these circumstances (Jeuthe 2015). Differences in egg fertility between females reared under similar conditions varied between 0 and 83% in another study (Mansour *et al.* 2011), where it was not correlated to the number of ovulated eggs or egg size. In both cases, variation between families existed despite similar holding conditions for female brood-stock (Mansour *et al.* 2011, Jeuthe 2015, Jeuthe *et al.* 2016). Egg quality may be even more variable under varying conditions.

Eggs of Arctic charr exhibit differences in regularity in size and shape and the distribution of lipid droplets depending on their quality. Egg size in Arctic charr usually varies between 4 and 5 mm in diameter (Delabbio 1995, Jobling *et al.* 1995, Frantzen *et al.* 1997, Jobling *et al.* 1998), and eggs usually weigh between 50 and 60 mg (Gillet 1994). Good quality eggs of Arctic charr can be identified by their solid pale yellow or golden colour, a size between 4 and 5 mm in diameter and the lack of concave or dimpled surfaces. When stripped, they are in a mass of touching eggs with moderate amounts of ovarian fluid (Johnston 2002). Mansour *et al.* (2008a) classified eggs that are uniform in size and shape with evenly distributed lipid droplets as good. Eggs with some coalesced lipid droplets towards one pole, which are otherwise uniform in shape and size, are classified as fair. In eggs of poor quality, all lipid droplets are coalesced towards one or both poles, and the eggs are of irregular size and shape. Also heterogeneous eggs with varying characteristics occur in Arctic charr. Heterogeneous eggs and eggs of poor quality could not be successfully fertilised in this study.

Egg retention in the abdominal cavity (over-maturation) is identified as one of the main factors determining ultimate egg quality in salmonids (Papst & Hopky 1984, Springate *et al.* 1984, Bromage *et al.* 1992) including Arctic charr (Gillet 1991, Gillet & Breton 1992, Gillet 1994, Atse *et al.* 2002). During over-maturation, various chemical and physical properties of the eggs deteriorate, and eggs of heterogeneous quality are likely a result of over-maturation. They were found frequently in Arctic charr held at 7°C (Mansour *et al.* 2008a). Contrary, eggs of heterogeneous quality were not observed in large quantities in brown trout (Mansour *et al.* 2007). Eggs of heterogeneous quality exhibited an ovarian fluid pH below 8, which could be a sign of increased ovarian fluid secretion (Mansour *et al.* 2008a), or leakage from damaged eggs, as shown for rainbow trout (Lahnsteiner 2000a, Dietrich *et al.* 2007). Lower adenosine triphosphate (ATP) content and an increased concentration of catabolic enzymes also indicated post-ovulatory ageing in this study (Mansour *et al.* 2008a).

There is a positive connection between female age, egg size and egg viability (Jeuthe *et al.* 2013, Lasne *et al.* 2018). In some instances, egg survival has shown a stronger correlation with egg size than with female age (Jeuthe *et al.* 2013, Jeuthe 2015). Improvement of egg survival by size was seen in eggs between 4 and 5 mm, but not below, even within female age groups (Jeuthe *et al.* 2013). These results point to an additional individual factor affecting size-dependent egg viability, independent of female age. However, egg size does not always correlate to egg viability and pre-hatch survival, and many effects are first revealed post hatch. A comparison of average egg weight, ranging from 59.0 to 113.0 mg, between Arctic charr families in Iceland, revealed no correlation between egg weight and survival until the eyed stage or first feeding, or the frequency of abnormally shaped alevins (Jónsson & Svavarsson 2000). However, egg size has been positively connected to survival of alevins from emergence to first feeding (Wallace & Aasjord 1984). Mortality associated with low egg size usually manifested as pinhead mortality, which is caused by starvation, and alevins from smaller eggs were generally smaller and had smaller yolk sacs. Larvae from larger eggs were also found to be larger in a different study. They exhibited a more active foraging behaviour, in addition to be generally more active (Leblanc *et al.* 2011). However, larvae from the largest eggs were also found to be deformed at a higher

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frequency (Wallace & Aasjord 1984). Egg size also influences embryonic development, and embryos in small eggs are found to develop more quickly than embryos in large eggs. For instance, a significant negative correlation between egg weight and the developmental index of melanin formation in the eyes has been found, also when eggs of similar size classes were pooled. However, in this study, no correlation between egg size and the time of hatching was found (Valdimarsson *et al.* 2002).

In individual eggs, ranging from 4.0 mm and 28.9 mg to 4.8 mm and 44.7 mg in size and weight, a lower energy content was detected in smaller eggs. However, it was not correlated to pre-hatch survival (Leblanc *et al.* 2016). Energy density measured in dry weight varied from 18647 to 28873 J g^{-1}, but was not correlated to egg size or female size. It did not differ by female. However, energy density was more variable in smaller than in larger eggs (Leblanc *et al.* 2016). Contrary, egg energy density was evaluated as a better predictor of egg quality than weight or size. Egg size and weight are variable, and change depending on environmental factors. Egg size was found to decrease when brood-stock was held at high summer temperatures. Egg weight was found to increase when brood-stock was held in sea water during summer (Atse *et al.* 2002).

Even though final egg size is largely determined by initial egg size in Arctic charr, additional variation arises through swelling. Parental effects depending on males and females, and interactive effects between males and females are found to determine final egg size after swelling. All of these effects were demonstrated by comparing several matrices of egg size depending on males and females in a cross-fertilisation experiment based on 7 stocks of hatchery-reared Arctic charr (Pakkasmaa *et al.* 2001). However, the underlying mechanisms remain unknown in Arctic charr, and both genetic and environmental effects are considered. Either way, different egg swelling is supposedly connected to differences in egg quality and viability (Pakkasmaa *et al.* 2001). This correlation has been demonstrated in lake trout (*Salmo trutta lacustris* (Linnaeus)) (Lahnsteiner *et al.* 1999). In that regard, it also remains uncertain whether little egg swelling causes lower viability or *vice versa* (Pakkasmaa *et al.* 2001).

In Arctic charr, considerable variations in fecundity between different brood-stocks were documented. Fecundity also varies by the age of the fish. Generally, a fecundity of 3–4000 eggs per kg fish may be expected (Jobling *et al.* 1998). However, fecundity can also be altered by environmental conditions, such as photoperiod (Gillet 1994). Compared to the natural photoperiod at ca. 46 °N, keeping fish at long-day photoperiod (17L:7D) from October to August, followed by an abrupt change to short-day (7L:17D), resulted in relatively higher fecundity and a lower average egg mass.

Ovarian fluid

Fertilisation in Arctic charr can be conducted dry, with no water added, and spermatozoa moving in ovarian fluid. In this setting, sperm can be motile for approximately two minutes (Johnston 2002). Ovarian fluid aids the fertilisation process, as it increases sperm longevity, which has been investigated on a gradient between 0% and 50% ovarian fluid. Sperm motility lasted for 43 and 128 s, respectively (Turner & Montgomerie 2002). Also, swimming speed and linearity index of the sperm movement increased with an increasing amount of ovarian fluid. However, at a low concentration of ovarian fluid (5%), the swimming speed of spermatozoa was lower than in freshwater. This was likely because of the higher viscosity of ovarian fluid, which may have a larger effect at lower concentrations. The enhancing effect on swimming speed also increased with time, as there was relatively little enhancement of swimming speed at 10s post activation (6% difference between freshwater and 50% ovarian fluid). At 20s post activation, this effect increased to a 48% difference between freshwater and 50% ovarian fluid. Consequently, ovarian fluid is an advantageous medium for fertilisation in general, at least up to a concentration of 50% (Turner & Montgomerie 2002). However, ovarian fluid of Arctic charr also seems to influence the outcome of the fertilisation process. Differences have been found in the enhancement of sperm velocity between females and between combinations of females and males (Urbach *et al.* 2005). It has been concluded that different combinations of males and females may be more compatible and achieve higher fertilisation rates.

Egg incubation

Incubation temperature

The upper temperature limit for survival of eggs of Arctic charr is commonly referred to as 8°C (Elliott & Elliott 2010). Drastically elevated mortality has been reported at incubation temperatures > 10°C (Jungwirth & Winkler 1984, Steiner 1984, Gillet 1991). However, reduced hatching success is also reported for temperatures above 6°C (de March 1995, Janhunen *et al.* 2010). Hatching success is generally highest at incubation temperatures between 1 and 5°C (Humpesch 1985, Elliott & Elliott 2010). However, different temperature optima are reported for different populations of Arctic charr. de March (1995) reported a higher hatching success at an incubation temperature of 3°C than at 6°C for northern anadromous strains with origins in Fraser River (Newfoundland and Labrador, Canada) and Lake Storvatn (Nordland, Norway). In a landlocked Austrian strain, Steiner (1984) reported the highest egg survival rates at incubation temperatures of 5–7°C and adequate hatching success between 3 and 8°C. Below 2°C, hatching success

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was considerably lower in this population (Steiner 1984). Temperature fluctuations between 3.5 and 6°C during the eyed stage did not seem to affect embryonic development or survival (Jeuthe *et al.* 2016). The lower temperature limit for eggs is also found to vary by developmental stage. Jeuthe *et al.* (2016) found that Arctic charr eggs of the Arctic Superior strain (origins in Lake Hornavan, Sweden; (Nilsson *et al.* 2010), stationary, but closely related to northern Norwegian anadromous strains (Mayer *et al.* 1992, Schmitz 1992)) exhibited higher survival rates when incubated at temperatures around 6°C compared with 2.8°C during early incubation. The lower temperature limit of eggs of Arctic charr during the first few weeks of incubation could thus be placed between 2.8 and 4°C, and temperatures between 4 and 7°C are recommended at that stage (Jeuthe *et al.* 2016).

Thermal stress during egg incubation was previously indicated to cause some of the poor egg quality in farmed Arctic charr in Sweden (Jeuthe *et al.* 2013). Incubation of eggs at low temperatures (2.7°C) during early incubation led to considerably higher mortality rates before and during the eyed stage, as well as an increased frequency of spinal malformations (Jeuthe *et al.* 2016). Consequently, sensitivity to cold incubation temperatures may be one cause of high pre-hatch mortality in cultured Arctic charr, and the temperature limit is relatively high (≥2.8°C). However, the initial higher sensitivity to low temperatures is restricted to the first week of incubation or less, which coincides with the developmental stages up to gastrulation or the start of epiboly (Jeuthe *et al.* 2016).

Temperature effects during incubation were first suspected to be independent of intrinsic properties of the egg (de March 1995). Incubation temperatures of 3 and 6°C were investigated in 18 families and 3 mixed groups of Arctic charr eggs in this study, and eggs survived at a higher rate at low incubation temperatures (65 ± 30% compared with 47 ± 30%, respectively). Eggs incubated at lower temperatures were also better developed in terms of size and pigmentation. Larvae from eggs incubated at lower temperatures exhibited curved spines that straightened out after a few days post hatch and smaller yolk sacs, meaning that they were larger and in a later developmental stage. However, as the variation around average egg survival was similar at both temperatures, no evidence for intrinsic properties influencing temperature tolerance was detected (de March 1995). When comparing survival and development between eggs reared at 2 and 7°C, similar effects on survival and growth were detected, such as higher survival, and larger larvae, which were less variable in size and had smaller yolk sacs at lower temperatures (Janhunen *et al.* 2010). However, in this study, relative variation around family-specific means in survival increased at higher incubation temperatures and was 30.6% and 66.1% at 2 and 7°C, respectively. Parental effects had an influence on egg survival, yolk sac volume and larval size, dependent on temperature. Embryonic survival was mainly determined by the dam effect, while sire–dam interactions were significant at an egg rearing temperature of 2°C, but not at 7°C. At 7°C, the total variance in egg survival was higher, and especially, the error variance increased in proportion. The proportions of the variance of parental effects on egg survival were estimated to 82.7 and 72.4% for the dam effect at 2 and 7°C, respectively. Sire–dam interactions explained 16% and 3.5% of the variance at 2 and 7°C, respectively, and sire effects were not detectable on egg survival. Dam, sire and sire–dam effects were found to influence yolk sac volume and larval size. As the same parents were used at both temperatures, evidence for temperature influencing the expression of genetic effects was found in this study (Janhunen *et al.* 2010). Consequently, intrinsic properties of the egg likely interact with rearing temperature, influencing pre-hatch survival and other properties.

Water chemistry

Water chemistry requirements for egg incubation have not been evaluated in detail for Arctic charr in particular. However, general requirements for salmonids can be used. Especially, exposure of eggs of salmonids to nitrate is found to be detrimental, and concentrations should not exceed 1 mg L^{-1} during egg incubation (MacIntyre *et al.* 2008). Eggs of Arctic charr are also found to tolerate a wide range of oxygen concentrations at different temperatures. Low mortality was recorded at P_{O2} of 20% and 30% at 4 and 8°C, respectively, and at P_{O2} of 50 and 100% at both temperatures (Gruber & Wieser 1983). However, oxygen consumption, growth rates and food conversion increased with increasing oxygen concentrations, and post-hatch mortality was highest at P_{O2} of 30% and 8°C (Gruber & Wieser 1983), indicating detrimental effects of low oxygen concentrations in combination with high temperature.

Hygiene

Eggs of Arctic charr are routinely surface disinfected in many hatcheries, using a buffered iodine solution when they are moved to an incubator. Concentrations of 100 ppm are used, and eggs are submerged in a bath of buffered iodine solution for 10 min. This procedure is especially important when water with low pH is used for incubation (Johnston 2002). In the incubator, *Oomycetes* such as *Saprolegnia* (Nees) may settle on dead organic matter, such as eggs, eggshells or blood, and may spread to adjacent eggs. This can be avoided by removing all dead material and dead eggs, filtering incoming water, chemical treatments and regular hand-picking of dead eggs. Usually,

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chemical treatments are conducted before the eyed stage, and hand-picking is used during the eyed stage (Johnston 2002, Olk et al. 2019).

Conclusion

The most severe shortcomings in relation to reproduction of Arctic charr in hatcheries seem to be related to the holding conditions of the brood-stock. The holding conditions of the brood-stock have also been shown to be influential in relation to the timing of spawning, which has been thoroughly investigated, and adequate knowledge to produce high-quality gametes in relation to the timing of spawning is available. Temperature is identified as one of the most important issues in Arctic charr aquaculture, and the detrimental effects of elevated summer temperatures remain to be investigated, also regarding the identification of an optimal summer temperature. In addition, results regarding the effect of the fatty acid composition of the brood-stock diet remain equivocal. It also remains unclear, whether the fatty acid composition of the diet is problematic in Arctic charr aquaculture in general. This is likely only problematic in freshwater stocks, which are fed a marine-based diet.

The effect of handling and confinement stress remains to be investigated *in situ* in Arctic charr brood-stock. Stress interferes with oocyte development, especially vitellogenesis. However, these results were obtained injecting cortisol (F). Another brood-stock-related factor that may negatively influence egg quality is the age of the brood-stock. Age group differences in reproductive performance are a result of age-dependent (positive) stress tolerance. This problem could be resolved by improving the holding conditions. Regarding the fertilisation process, adequate knowledge is available. 'Dry' fertilisation in ovarian fluid appears to be the most suitable option for Arctic charr. While egg quality of Arctic charr has been studied to a large extent, there are few accounts on sperm quality parameters and their relation to fertility or holding conditions of the brood-stock. Sperm quality in Arctic charr should thus be investigated further, especially in light of the decreased number of motile sperm cells discovered in one hatchery. Fertilisation protocols may have to be revised to account for haploid selection. Genetic and epigenetic effects of sperm remain to be investigated in Arctic charr. Regarding egg incubation, temperature has been most thoroughly studied. Large differences in hatching success between families, and differences between strains are concerning and demand further investigation. It appears advantageous to treat anadromous and stationary strains differently regarding salinity during summer, fatty acid composition of the diet and potentially temperature. Direct comparative studies between stationary and anadromous stocks are lacking and in demand.

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