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Arctic charr (*Salvelinus alpinus*) farming in southern Norway

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Abstract

Arctic charr (*Salvelinus alpinus*) is a stenothermic cold-water fish with a circumpolar distribution. The species features large variations in life history, as populations are anadromous, riverine or lake dwelling. It occurs in various different morphs, which differ in physical traits, such as body size, head shape, and mouth position. The ecological niches of different morphs exhibit great variation as well, e. g. in terms of feeding ecology. Arctic charr has potential as a cultured species, and interest in Arctic charr farming existed since the 1970s. The global production of Arctic charr in aquaculture was between 6000 and 10 000 tonnes in 2013, with all the largest producers located in northern Europe. Arctic charr is suitable for aquaculture, as it grows rapidly at low temperatures, and tolerates high stocking density. Its high fillet yield and amenability to niche markets are also advantageous. However, Arctic charr aquaculture is a relatively small industry, which has made slow progress during its development. Some of the main problems are connected to obtaining viable eggs and juveniles in terms of egg quality, fertilisation rates, and survival through first feeding. There have also been challenges connected to variable growth and flesh pigmentation, early maturation, and marketing. Despite the large volumes of freshwater available in Norway, freshwater fish farming in general, and Arctic charr farming in particular have remained small industries here. One underlying reason are strict environmental regulations connected to freshwater aquaculture in Norway. Parasites may cause harm to Arctic charr aquaculture by reducing growth rates and marketability, as well as causing mortalities in fish held in cages in freshwater. There is little information available on freshwater parasites in southern Norway, as well as parasites threatening Arctic charr aquaculture.

The aim of this thesis is to identify challenges, and propose solutions to problems connected to freshwater aquaculture of Arctic charr in southern Norway. As a general problem in Arctic charr aquaculture, pre-hatch survival has been investigated by reviewing relevant literature about brood-stock management, fertilisation, and egg rearing in aquaculture. A common disinfection protocol for eggs featuring chemical disinfection with formalin before the eyed stage, and hand-picking during the eyed

stage, was re-examined. The protocol was tested on fish from a local population in Vestfold and Telemark County. Four different treatments were tested, formalin treatment before the eyed stage only, hand-picking during the eyed stage only, a combination of formalin treatments and hand-picking, and one untreated control group. The hatching percent of these four treatments was compared using a two-way ANOVA. Both formalin treatments and hand-picking increased the proportion of hatched larvae. Hand-picking was more effective, likely because of its timing during the eyed stage. The use of formalin during the entire incubation period is advised until more information on the dynamics of water mould infections is available. Such information allows timing the application of chemical treatments to times of higher infection risk.

To successfully produce offspring of Arctic charr in aquaculture, summer holding temperatures of the brood-stock should be kept low (< 12 °C). Results on the dietary fatty acid composition and its influence on egg survival remain inconclusive. Extensive knowledge has been gained on the timing of spawning, and its manipulation using photoperiod, temperature and hormone treatments. Year round spawning is possible in cultured Arctic charr, and the synchronisation of spawning is important for good egg quality. Suitable routines for fertilisation and egg incubation are being developed, and important factors are the identification of ripe females before spawning, “dry” fertilisation in ovarian fluid, and low incubation temperatures (< 8 °C). There are numerous studies on egg quality parameters, that identify e.g. egg size as important. Egg size is also correlated to female age up to six years. However, not all studies confirm egg size as a suitable quality parameter. Energy density is shown to be more variable in smaller eggs, and may be a better egg quality parameter. Not much information is available on sperm quality parameters, but sperm quality has been identified as limiting factor for reproductive success.

To test conditions for Arctic charr aquaculture in the region, parasites were identified in a southern Norwegian lake, and factors determining their abundance were identified. The lake is located in Telemark and Vestfold County, in a different watercourse than the population of Arctic charr for the formalin experiment. Macro-parasites were identified,

and their abundance was modelled in relation to age, length, weight, $\delta^{13}C$, $\delta^{15}N$, C/N-ratio, location, season, and sex in negative binomial generalised linear models. The most prevalent parasites in the investigated host species were determined by the habitat of the host. Arctic charr was the most pelagic species investigated, followed by European whitefish (*Coregonus lavaretus*) and European perch (*Preca fluviatilis*), according to stable isotope signatures. The abundance of parasites were most often increasing with increasing age or length of the host, and short-lived parasites exhibited seasonal variations.

Keywords: brood-stock husbandry, egg rearing, formalin, freshwater parasites, pre-hatch survival, *Salvelinus alpinus*

List of papers

Article 1

Olk, T. R., J. Wollebæk, and E. Lydersen. 2019. Formalin treatments before eyeing and hand-picking of Arctic charr (*Salvelinus alpinus*) eggs; re-evaluating the timing of antifungal treatments. Vann **54**(1)**:** 21 – 32. URL: https://vannforeningen.no/wp-content/uploads/2019/06/Olk-Wollebæk-Lydersen.pdf

Article 2

Olk, T. R., H. Jeuthe, H. Thorarensen, J. Wollebæk, and E. Lydersen. 2020. Brood-stock management and early hatchery rearing of Arctic charr (*Salvelinus alpinus* (Linnaeus)). Reviews in Aquaculture **12**(3): 1595 - 1623. doi: 10.1111/raq.12400

Article 3

Olk, T. R., A.-C. Henriksen, S.I. Dolven, M.L. Haukø, E. Lydersen, and T.A. Mo. 2020. Factors determining parasite abundance in three freshwater fish, European perch (*Perca fluviatilis*), European whitefish (*Coregonus lavaretus*), and Arctic charr (*Salvelinus alpinus*), in an oligotrophic lake, southern Norway. Fauna Norvegica **40**: 109 – 129. doi: 105324/fn.v40i0.3444

Papers summary

Paper I: Formalin treatments before eyeing and hand-picking of Arctic charr (*Salvelinus alpinus*) eggs; re-evaluating the timing of antifungal treatments

A common protocol for the disinfection of Arctic charr eggs in hatcheries is applying chemical treatments with formalin before the eyed stage, and hand-picking of eggs during the eyed stage. In this study, we re-evaluated this practice by comparing the hatching proportion of eggs in four groups, hand-picked and formalin treated eggs, hand-picked eggs, formalin treated eggs, and an untreated control group. The hatching proportions were compared using a two-way ANOVA with hand-picking and the use of formalin as factors. Hand-picking and formalin treatments both increased the proportion of hatched eggs, with the former being more effective. This was likely caused by the timing of hand-picking during the eyed stage, when there is a higher risk for water mould infections due to accumulation of water moulds in the incubator.

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

Arctic charr is a stenothermic cold-water fish, which has been cultured since the 1980s. The industry has remained relatively small, with an annual production between 6000 and 10 000 tonnes. One of the major challenges for the industry is unreliable offspring production. In this paper, factors affecting pre-hatch survival from brood-stock holding via fertilisation and egg quality, to egg rearing were reviewed. Brood-stock requires relatively low temperatures during summer (< 12 °C), with the optimum still unknown. The temperature maximum for egg incubation lies between 6 and 8 °C. Results concerning an optimal brood-stock diet in relation to fatty acid composition remain inconclusive. Extensive knowledge has been gained on the timing and synchronisation of spawning, and its manipulation by photoperiod, temperature, and hormone treatments. Eggs of Arctic charr are usually fertilised “dry” in ovarian fluid. Egg quality is highly variable, and positively correlated to egg size and energy density. Little information is available on sperm quality, even though it has been identified as a limiting

factor for fertilisation success. There may be profound differences between populations of Arctic charr, especially differences between stationary and anadromous Arctic charr, regarding optimal holding conditions and diet. These differences have received little attention, as there are no direct comparative studies.

Factors determining parasite abundance in three freshwater fish, European perch (*Perca fluviatilis*), European whitefish (*Coregonus lavaretus*), and Arctic charr (*Salvelinus alpinus*), in an oligotrophic lake, southern Norway.

This study identifies macroparasites found in a southern Norwegian lake, and factors that determine their abundance are investigated. Arctic charr (*Salvelinus alpinus*), European whitefish (*Coregonus lavaretus*), and European perch (*Perca fluviatilis*) were fished in spring, summer and autumn at three different locations in 2018 using gill nets. The age, length, weight, sex, and stable isotope ratios $\delta^{13}C$ and $\delta^{15}N$ in white muscle, along with the C/N-ratio were determined in each fish. Macro-parasites, both ecto- and endoparasites, were identified and counted. Prevalence and mean abundance of the parasites were calculated. The abundance of individual parasites was modelled for each host species using negative binomial generalised linear models. The most prevalent parasites in each species were determined by the habitat and diet of the host. European perch seemed to have the most littoral niche, and were predominantly infected by acanthocephalans. European whitefish fed on both littoral and pelagic resources, and were mainly infected by acanthocephalans and *Proteocephalus* sp. (Cestodes). Arctic charr were the most pelagic species in this study and mainly infected by cestodes. European perch were infected by parasites transmitted by benthic animals. European whitefish were infected by parasites transmitted by benthic animals and pelagic copepods. Arctic charr were mainly infected by pelagic copepod transmitted parasites. The origin of the diet was determined according to stable isotope signatures. Individual parasite abundances often increased with increasing age or length of the host, due to accumulation of parasites. Seasonal variations were predominantly found in the abundance of short-lived parasites.

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Abbreviations

17,20 β-P – 17,20 β-dihydroxy-4-pregnen-3-one

AIC – Akaikes Information Criterion

ARA – Arachidonic Acid

E_2 - Oestradiol

GSI – Gonadosomatic Index

PCR – Polymerase Chain Reaction

PKD – Proliferative kidney disease

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1 Introduction

1.1 Arctic charr

Arctic charr (*Salvelinus alpinus*) is distributed throughout the arctic, subarctic, boreal and temperate regions of the Holarctic (Klemetsen et al. 2003). There are estimated to be approximately 50 000 populations world-wide, most of which are located in Scandinavia. There are 30 000 populations in Norway, 13 000 in Sweden, 3 500 in Canada, 1 000 in Russia, 1 000 in Iceland and Greenland, 500 in the USA, 200 in the UK, and < 100 populations elsewhere (Maitland 1995). However, estimates for Canada and Russia are evaluated as too low (Klemetsen et al. 2003). Due to its occurrence at high latitudes, Arctic charr experiences large seasonal changes in environmental conditions, and the species is well adapted to exploit resources in this changing environment (Johnson 1980; Jørgensen & Johnsen 2014; Sæther et al. 2016). Arctic charr is a stenothermic cold-water fish (Johnson 1980). It has the lowest area of the temperature tolerance polygon (410 – 460 °C^2) recorded in Salmonids, and the upper incipient lethal temperature is about 21 – 22 °C (Baroudy & Elliott 1994). The temperature limits appear consistent for the species as a whole, as there are no indications for higher temperature limits of southern populations (Klemetsen et al. 2003), and there are negligible differences in temperature tolerance among larvae (alevins) and 0+-parr (Baroudy & Elliott 1994; Lyytikäinen et al. 1997; Thyrel et al. 1999; Elliott & Klemetsen 2002).

Arctic charr utilises a variety of habitats. It occurs in anadromous populations, migrating to full strength sea water (e.g. Maitland 1995), riverine populations towards the northern edge of its distribution (Curry-Lindahl 1957; Power 1973), but most populations are found in oligotrophic and ultra-oligotrophic lakes (Klemetsen et al. 2003). In many of those lakes, Arctic charr occurs at depths of over 200 m, e.g. in Attersee (Brenner 1980), and Lake Constance (Hartmann 1984) in the Alps, in Loch Ness in Scotland (Shine et al. 1993; Klemetsen et al. 2003), in Gander Lake in Newfoundland, Canada (O'Connell & Dempson 2002; Klemetsen et al. 2003), and in Lake Tinnsjøen (Norway) (Østbye et al. 2020). Arctic charr is well adapted to the profundal habitat of

very deep lakes due to its ability to push ecological barriers (Klemetsen et al. 2003). During summer, epilimnetic waters may also become too warm, causing Arctic charr to move to profundal waters (Klemetsen et al. 2003). Anadromy is complex in Arctic charr, and both sexually mature and immature individuals perform seasonal migrations between river systems and the sea (Nordeng 1983; Johnson 1989). All migrants, including juveniles, return to freshwater every year, as opposed to Atlantic salmon (*Salmo salar*) (Klemetsen et al. 2003). Migratory life history traits occur in the northern parts of the distribution of Arctic charr, while southern populations are strictly freshwater resident (Gross et al. 1988). Extremely northern populations could be characterised as facultatively anadromous according to Radtke et al. (1996). Most river systems with anadromous Arctic charr also have resident Arctic charr, which belongs to the same population, as demonstrated for the Salangen river system in Northern Norway (Nordeng 1961; 1983). Anadromous and stationary Arctic charr have been found in the same watercourse on many accounts (Johnson 1980; Jonsson & Jonsson 2001; Klemetsen et al. 2003; Rikardsen et al. 2004; Klemetsen 2013; Hammar 2014; Sæther et al. 2016). Breeding experiments resulted in a small fraction of resident Arctic charr smoltifying, and transforming into anadromous Arctic charr. However, more offspring of anadromous parents smoltified, indicating some genetic influence on life history in Arctic charr (Nordeng et al. 1989). In relation to anadromy, Arctic charr seem to display a life-history dichotomy, with migratory forms maturing at older ages, resulting in higher fecundity and longevity (Tallman et al. 1996).

In many watercourses of the Holarctic, Arctic charr coexists in different morphs and variants, and commonly there are two to four sympatric morphs. Morphs differ in body size, spawning colouration, external and internal morphological structures, parasite fauna, growth rate, feeding habitat and diet, life history traits, and behaviour (Jonsson & Jonsson 2001; Østbye et al. 2020). Arctic charr display extreme variations in body size for vertebrate species, as the weight of sexually mature individuals varies between 3 and 12 000 g. In addition, Arctic charr is found in various habitats, and many populations undergo ontogenetic niche shifts or migrate. Consequently, Arctic charr seems very flexible in its genetic constitution, which may be an adaption to the unpredictable

environment of the far north (Klemetsen et al. 2003). As a consequence, Arctic charr often occupies vacant niches, especially in glacial lakes with few fish species (Klemetsen et al. 2003). There is great variation in feeding ecology in Arctic charr (Snorrason et al. 1994), especially in lake dwelling forms (Jonsson & Jonsson 2001; Klemetsen et al. 2003; Klemetsen 2013; Hammar 2014; Knudsen et al. 2014; Sæther et al. 2016). Adapting to this variation, morphs differ in mouth position, fin size, head shape, gill raker number, and length (Jonsson & Jonsson 2001). Also, cannibalism is common in Arctic charr (Klemetsen et al. 2003), and has been observed in Arctic Canada (Johnson 1980; Hobson & Welch 1995), Greenland (Sparholt 1985; Riget et al. 1986), Jan Mayen (Skreslet 1973), Bear Island (Klemetsen et al. 1985), Svalbard (Gullestad 1975; Svenning & Borgstrøm 1995), and mainland Norway (Amundsen 1989; 1994). Cannibalism is not commonly observed in temperate lakes (Amundsen et al. 1999), and it appears to increase with latitude (Griffiths 1994). Sympatric morphs may thus differ in most traits, and they are in many cases reproductively isolated, spawning at different times of the year in different locations (Frost 1965; Skúlason et al. 1989; Elliott & Baroudy 1995; Hesthagen et al. 1995; Klemetsen et al. 1997; Præbel et al. 2016).

In addition to feeding ecology, morphs differ in growth rate, age and size at maturation, and reproductive traits, such as fecundity (Jonsson & Jonsson 2001). Within morphs, fast growing individuals mature at a younger age and are smaller than slow growing conspecifics, which is the norm in salmonid fishes (Jonsson et al. 1984; Forseth et al. 1995; Jonsson & Jonsson 1993; 2001), but age and size at maturation also differs between morphs. For example in Thingvallavatn, Iceland, small benthivorous and planktivorous Arctic charr mature at a younger age compared to piscivorous and large benthivorous Arctic charr (Jonsson et al. 1988). Fecundity and egg size vary between morphs, and are influenced by body size, as typical for salmonids (Jonsson et al. 1996; Jonsson & Jonsson 1999). In Lake Vangsvatnet, Norway, individual fecundity and egg size in Arctic charr increased with body length and age for two morphs, while dwarf charr had fewer and smaller eggs than normal Arctic charr (Jonsson & Hindar 1982). In addition, large sized anadromous Arctic charr exhibit higher age-specific fecundity than corresponding freshwater residents (Tallman et al. 1996). Relative gonadal investment

is also found to differ between morphs in Lake Thingvallavatn, Iceland, with planktivorous and piscivorous morphs investing more into gametes than benthivorous morphs. As a result, gonadosomatic indices (GSI) differ between morphs in Lake Thingvallavatn (Jonsson et al. 1988). Egg size may also differ between morphs of the same lake. In Lake Windermere (UK), spring spawning Arctic charr lay smaller eggs than autumn spawning Arctic charr (Elliott & Baroudy 1995), which is likely linked to the different environmental conditions related to incubation period, that the eggs encounter (Jonsson & Jonsson 2001).

In conclusion, Arctic charr is a stenothermic cold-water species, which exhibits large variation in terms of morphology, habitat, feeding ecology, life-history, and reproductive traits.

1.2 Artctic charr as a food source

Arctic charr is a valuable sport and household fish, especially for indigenous peoples of the north (Balikci 1980; Johnson 1984; Boivin et al. 1989; Power et al. 1989). Commercial Arctic charr fisheries are important in Canada (Kristofferson et al. 1984; Dempson 1995; Dempson & Shears 1998), and Arctic charr has potential in aquaculture (e.g. Jobling et al. 1993).

1.3 Arctic charr in Aquaculture

There has been interest in aquaculture of Arctic charr since the 1970s (Jobling et al. 1998). The yearly global production of Arctic charr reached 6 000 – 10 000 tonnes in 2013, with the largest producers located in northern Europe (Sæther et al. 2013). In 2011, 3 200 tonnes were produced in Iceland, 2 300 tonnes were produced in Sweden, and 700 tonnes were produced in Norway (Brännäs et al. 2011b). In addition, 800 – 900 tonnes of unspecified finfish were produced in Canada each year, which are likely Arctic charr (Rogers & Davidson 2001). In Iceland, the country producing most Arctic charr in Aquaculture, there are 22 farms. Flow through systems are used in 21 of these farms, while one farm uses cages in a brackish lagoon. Over 70 % of the Icelandic production of Arctic charr comes from two farms, producing over 1 000 tonnes annually. In addition,

there are six Arctic charr farms of intermediate size, producing 100 – 700 tonnes per year each (Sæther et al. 2013). The production of Arctic charr constitutes a major fraction of the annual production of farmed fish in Iceland. Until 2006, Atlantic salmon was the main cultured species in Iceland. However, in 2010, the production of Arctic charr and Atlantic cod (*Gadus morhua*) has surpassed that of Atlantic salmon. Arctic charr aquaculture contributed with 47.2 % to the total aquaculture production in 2010 in Iceland (Paisley et al. 2010). This fraction has increased since, and a fraction of over 50 % of the Icelandic aquaculture production being Arctic charr has been reported (Troell et al. 2017).

Arctic charr aquaculture has potential, which has been promoted during the 1980s and 1990s. Small scale culture has been carried out at the time in the northern temperate zone. Until the late 1990s however, the same techniques used for rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon were in use, rendering the culture of Arctic charr less successful (Jobling et al. 1998). However, despite the fallacies of early Arctic charr aquaculture, it is still evaluated to have good potential in the Nordic countries (Paisley et al. 2010). This is due to many advantageous traits of Arctic charr for culture, such as the ability to grow rapidly at low temperatures (Brännäs & Linner 2000; Siikavuopio et al. 2009; Siikavuopio et al. 2010; Sæther et al. 2013), which makes the species suitable for fish farming at high latitudes and altitudes. In addition, Arctic charr has a high tolerance for high density culture conditions, so intense systems can be used (Summerfelt et al. 2004; Sæther et al. 2013). The fillet yield of Arctic charr is high, and it is amendable for niche marketing (Summerfelt et al. 2004; Skybakmoen et al. 2009; Jobling et al. 2010). Also, the inherent plasticity of the species can be advantageous in aquaculture, as Arctic charr has the ability to adapt to various culture regimes (Sæther et al. 2016).

The development of Arctic charr aquaculture has progressed slowly, due to problems obtaining viable eggs and juveniles. Particularly, egg quality, fertilisation rates, hatching rates, and survival through first feeding are problematic (Jobling et al. 1998). In addition, there have been challenges with variable growth and flesh pigmentation during grow-

out, year-round culture in sea water, early maturation, and challenges connected to the marketing of portion sized fish (Jobling et al. 1998). Some of these problems were caused by the lack of recognition of Arctic charr as a stenothermic cold-water species (Johnson 1980). However, also the plasticity of the species has its disadvantages, as growth rates, and size and age at maturation differ between individuals (Sæther et al. 2016). A good rearing environment is additionally hard to define, as farmed fish face temporarily variable combinations of hazards. Important water quality parameters include dissolved oxygen, metabolic waste products, such as ammonia and carbon dioxide, pH, and toxicants, such as heavy metals and organic pollutants. The requirements of Arctic charr in relation to temperature, dissolved gas concentrations, and ionic concentrations varies additionally by life-stage. In addition, interactions between environmental parameters, that cause physiological or behavioural responses, have to be taken into account (Jobling 1994; Colt 2006; Lekang 2007; Branson 2008; Harmon 2009; Jobling et al. 2010; Sæther et al. 2016). As a result, the environment provided to the farmed fish will always be a compromise between what is desirable for the fish, and what is feasible at the location, and for the producer (Sæther et al. 2016).

1.4 Freshwater Aquaculture in Norway and Sweden

In Norway, there is a large potential for freshwater aquaculture, due to large volumes of water. Norwegian watercourses cover over 16 000 km^2 or ca. 5 % of the area, which is more than the area covered by fertile soil. There are 440 000 lakes over 60 m^2, and 250 000 km of river length transferring volumes > 1 $m^3 s^{-1}$ (Haug et al. 2006). Freshwater aquaculture is a small industry in Norway, producing mainly Arctic charr and brown trout (*Salmo trutta*) in cages in lakes or in land-based facilities (Haug et al. 2006). There are only 38 officially registered licence holders allowed to produce Arctic charr (Norwegian Directorate of Fisheries, 52 single licences), of which 17 are active, 13 commercial licences and 4 research facilities (Sæther et al. 2013). In 2006, there were only few smaller facilities farming freshwater species in Norway, with Arctic charr farming increasing in volume the years before. The industry is characterised by small actors with limited access to capital resources, and commercial networks are absent (Haug et al.

2006). There was a political goal of establishing more profitable aquaculture facilities in freshwater, which could supply the market with resources of stable quality (Haug et al. 2006); However, regulations were also a challenge to new establishments. The legal framework of 2006 required large investments to establish a fish farm, as facilities needed to be secured against contamination, and wastewater treatment was mandatory. Fish were also required to be of local origin to prevent spreading of diseases, parasites, and foreign species (Haug et al. 2006). This legislation hampers the development of freshwater aquaculture in Norway, as the establishment of a national breeding program is not possible (Haug et al. 2006). Many lakes may additionally be inhabited by fish that are not suitable for production in aquaculture (Haug et al. 2006). To limit contamination, it was decided to limit the production in cages in freshwater. However, a licence to produce 330 tonnes in cages in Lake Fyresvatn (Telemark) was granted in 2011, following the standards of organic production (Sæther et al. 2013). Legislation is known to hamper the development of Arctic charr aquaculture in Norway, which is mainly of concern for new establishments. Existing farms, however, do not seem to be influenced, and farmers have few objections to the strict environmental protection, as it takes off pressure from NGOs and aids marketing (Sæther et al. 2013).

In Sweden, there were 14 – 15 Arctic charr farms, of which seven use cages in freshwater rivers or lakes. The remaining farms use flow through systems (Sæther et al. 2013). Between 2003 and 2010, Arctic charr production has increased in Sweden, and there are 31 farms producing smolts all over the country, most of which cover the whole production chain until fattened fish (Paisley et al. 2010). Eggs are produced in a national breeding program by the Swedish University of Agricultural Sciences (Paisley et al. 2010). Ca. 70 % of the Swedish fish farms are located in the continental zone, and the main species produced are rainbow trout (70 %), brown trout, and Arctic charr (4.6 %) (Paisley et al. 2010). Most of the Swedish Arctic charr, approximately 1000 tonnes per year or 91 %, is produced in cages in freshwater. As cage technology does not allow for active waste management, these farms are located in oligotrophic reservoirs of hydroelectric power production. Discharges thus provide a positive input of nutrients, which support biological productivity (Eriksson et al. 2010). This approach is practical in Sweden, as the

majority of the largest Swedish rivers has been developed for hydroelectric power production. In each watercourse, there is a series of dams, and the upper regions have thus considerable seasonal variations in water levels. As a result, movements of water and ice along the shoreline move nutrients and carbon, and deplete the nutrients of lakes within 15 – 30 years (Stockner et al. 2000). In addition, spawning grounds of Arctic charr and other fish species are being destroyed, rendering the already unproductive waters extremely unproductive (Eriksson et al. 2010). Re-eutrophication has been attempted using fossil phosphorus (Stockner et al. 2000), and organic waste products from fish farms contribute to this endeavour (Eriksson et al. 2010). Arctic charr is particularly interesting for this purpose, as it thrives in the good water quality of oligotrophic lakes (Paisley et al. 2010). Fish farms producing 50 000 to 70 000 tonnes annually are necessary to restore pre-regulation nutrient levels in each of the five biggest rivers in northern Sweden (Larsson et al. 2009). Consequently, aquaculture in cages is allowed and encouraged in Sweden (Haug et al. 2006). However, some limitations exist regarding the spread of diseases. Movement of fish from the coastal to the inland zone is forbidden, for instance (Paisley et al. 2010). The main production strategy in Sweden is keeping eggs, fry, and pre-growing stages in tanks on land, and moving fish to cages for on-growing (Sæther et al. 2013). Swedish Arctic charr farmers are mainly concerned about eggs, early life stages and the pre-growing phase, as egg survival has been low in Sweden. This concern has also been voiced in Iceland, although at a later occasion (Sæther et al. 2013).

1.5 Reproductive success of Arctic charr in Aquaculture

One of the largest issues regarding Arctic charr aquaculture is the production of viable offspring, which is expressed in highly variable gamete quality, variable and low fertilisation rates, and variable and low hatching success (Jobling et al. 1998). Low and variable reproductive success of Arctic charr in aquaculture is reported from most countries involved in the industry. An Icelandic study reports a general egg survival of only 32 %, with the highest mortality during the first week of incubation (Leblanc et al. 2016). In a Canadian study, fertility of eggs between 0 and 83 % was reported (Mansour

et al. 2011). Large family-wise differences in hatching rates were discovered in a Swedish study, ranging between 9 and 97 % (Jeuthe et al. 2016). Usual hatching rates in Sweden have previously been measured between 0 and 70 % (Jeuthe et al. 2013).

1.6 Approaches to improve reproductive success

One main focus of research, conducted on pre-hatch survival of Arctic charr in aquaculture, is temperature from brood-stock holding until hatch (Krieger & Olson 1988; Jobling et al. 1995; Atse et al. 2002; Jeuthe 2015; Jeuthe et al. 2013; 2015, Olk et al. 2019). Especially during the final stages of the reproductive cycle, during egg development, and during the development of hatchlings, low temperature is required. To improve egg survival, water temperatures, e. g. in lakes, need to be low at appropriate times of the year (< 12°C in summer (Jeuthe et al. 2013)), which means that aquaculture facilities would need to be placed at suitable locations fulfilling these conditions. Alternatively, well bore water at a suitable temperature can be used (Jobling et al. 2010; Gillet et al. 2011; Jeuthe et al. 2013; 2015). In addition, improvements were attempted regarding the fatty-acid composition of brood-stock feed. However, the results remain controversial (Pickova & Brännäs 2006; Pickova et al. 2007; Brännäs et al. 2007; 2011a; Mansour et al. 2011). Intrinsic egg and sperm quality traits have been investigated (Wallace & Aasjord 1984; Jónsson & Svavarsson 2000; Pakkasmaa et al. 2001; Valdimarsson et al. 2002; Mansour et al. 2008; Janhunen et al. 2010; Leblanc et al. 2011; Jeuthe et al. 2013; Leblanc et al. 2016; Jeuthe et al. 2019), e. g. egg size. The timing of spawning can be manipulated using photoperiod, temperature, and hormone treatments (Gillet 1991; 1994; Gillet & Breton 1992; 2009; Jansen 1993; Gillet et al. 1996; Gillet et al. 2011).

1.7 Some parasites in freshwater aquaculture

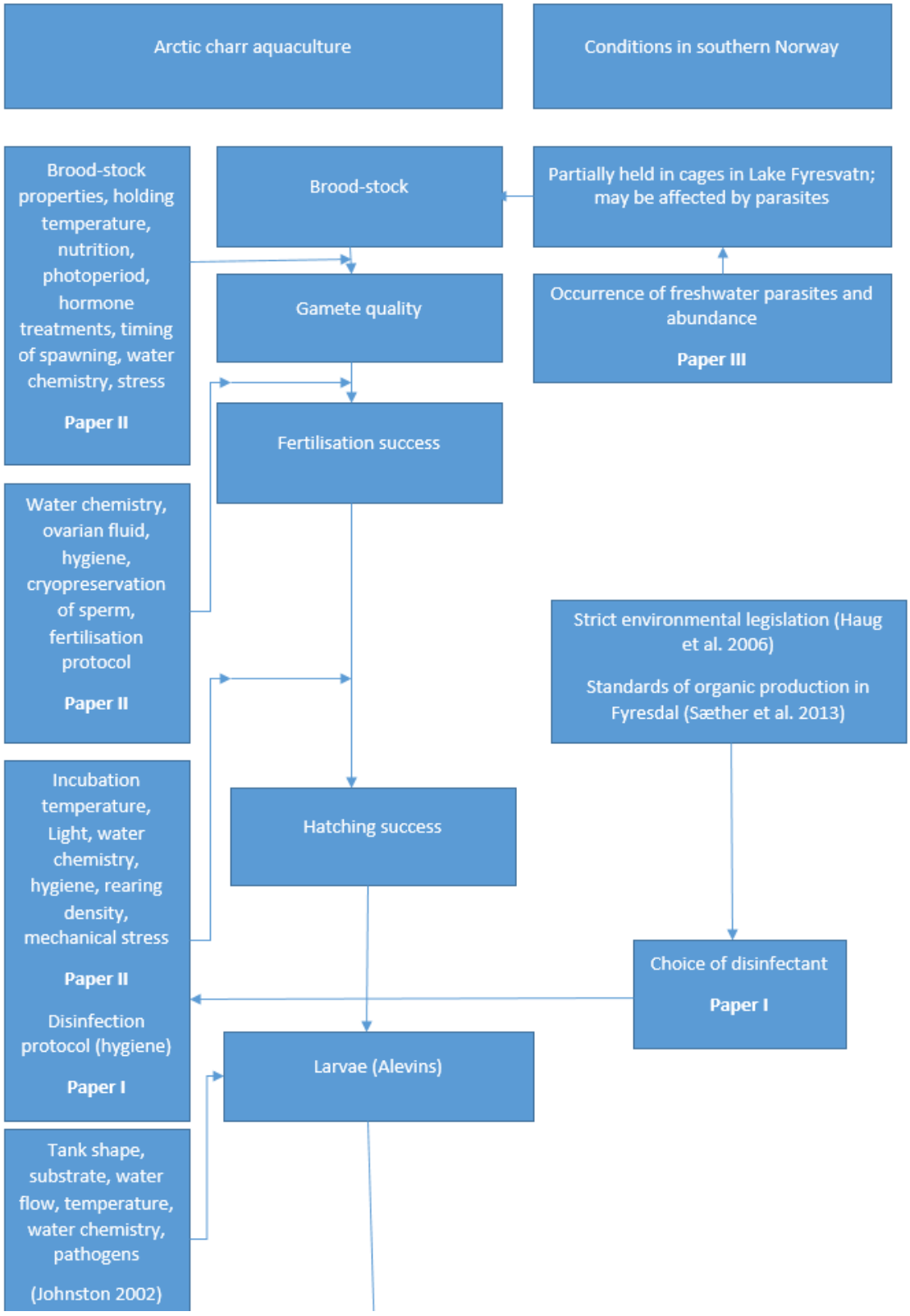
Several species of macroparasites can cause problems in freshwater aquaculture. Outside of Norway, problems with farmed or wild fish have been reported regarding the parasites *Eubothrium salvelini,* and *Salmincola edwardsii* (Bykhovskaya-Pavlovskaya et al. 1964; Post 1987). Wild salmonids have been demonstrated to struggle with the ecto-

parasite *Argulus coregoni* (Huitfeldt-Kaas 1912). Parasites that cause mortalities or pathogenic effects on farmed Atlantic salmon in Norway are e.g. *Triaenophorus nodulosus* (Bristow 1993), *Dibothriocephalus dendriticus* and *Dibothriocephalus ditremus* (Bristow 1993), and *Argulus coregoni* (Johannessen 1990; Bristow 1993). Pathogenic effects caused by parasites may also act in combination with stress, and cause mortalities. In farmed Atlantic salmon, infections with *Dibothriocephalus dendriticus* may become lethal in combination with temperature stress, or stress caused by transportation of the fish. In this case, *Dibothriocephalus dendriticus* penetrates various tissues or organs, and mortalities may rise to 10 % day^{-1}, or 100 % at intensities of only four parasites per fish (Bristow 1993).

Freshwater parasites may also pose a threat to Arctic charr aquaculture in southern Norway. The largest freshwater farm of Arctic charr in southern Norway, Telemarkrøye in Fyresdal (Sæther et al. 2013), operates using cages in Lake Fyresvatn for on-growing and holding of some brood-stock. These fish may be exposed to freshwater parasites through feeding on zooplankton (Johnston 2002), or to ecto-parasites (Piasecki et al. 2004). Parasites that are found in wild Arctic charr may be of concern for Arctic charr aquaculture, when Arctic charr is farmed in cages in lakes. However, most parasites so far identified in farmed Arctic charr, Proliferative kidney disease (PKD), *Gyrodactylus salaris*, and *Caligus elongatus* (Dick 1984; Galbreath et al. 1994; Due & Curtis 1995; Kolasa & Curtis 1995; Johnston 2002), were rather linked to other cultured species (Johnston 2002). PKD, caused by the myxozoan *Tetracapsuloides bryosalmonae* was responsible for a die-off amongst cultured Arctic charr in Newfoundland in 1996 (Khan 2009). PKD occurs seasonally, and is temperature dependent, occurring at high temperatures (Sterud et al. 2007; Khan 2009), and it also occurs in Skienselva (Mo & Jørgensen 2017), the watercourse where my parasitological study (Paper III) is situated. Among parasites found in wild Arctic charr, the ecto-parasite *Salmincola edwardsii* has caused fish health problems in cultured Arctic charr in Quebec and New Brunswick (Piasecki et al. 2004). In addition, the tapeworm *Triaenophorus* spp. has been found in European aquaculture facilities and wild fish, and caused high mortality in the Arctic charr population of Lake Königssee (Germany) (Schäperclaus 1992; Piasecki et al. 2004).

2 Objectives

The aim of this thesis was to identify challenges, and propose solutions to problems connected to Arctic charr farming in southern Norway. This includes general challenges linked to Arctic charr aquaculture, particularly pre-hatch survival, and environmental conditions in southern Norway, namely the abundance of freshwater parasites. In addition paper I is an attempt to reduce the use of formalin in egg disinfection to adapt Arctic charr aquaculture to the strict environmental legislation in Norway. The process of farming Arctic charr from brood-stock management, egg incubation, hatching, start feeding, to grow-out and slaughter, and factors influencing each step are discussed in Johnston (2002). Figure 1 places my studies within this framework, and highlights their relevance to Arctic charr aquaculture in southern Norway in particular.


Arctic charr aquaculture
Conditions in southern Norway
Brood-stock properties, holding temperature, nutrition, photoperiod, hormone treatments, timing of spawning, water chemistry, stress
Paper II
Brood-stock
Partially held in cages in Lake Fyresvatn; may be affected by parasites
Gamete quality
Occurrence of freshwater parasites and abundance
Paper III
Fertilisation success
Water chemistry, ovarian fluid, hygiene, cryopreservation of sperm, fertilisation protocol
Paper II
Strict environmental legislation (Haug et al. 2006)
Standards of organic production in Fyresdal (Sæther et al. 2013)
Incubation temperature, Light, water chemistry, hygiene, rearing density, mechanical stress
Paper II
Hatching success
Choice of disinfectant
Paper I
Disinfection protocol (hygiene)
Paper I
Larvae (Alevins)
Tank shape, substrate, water flow, temperature, water chemistry, pathogens
(Johnston 2002)

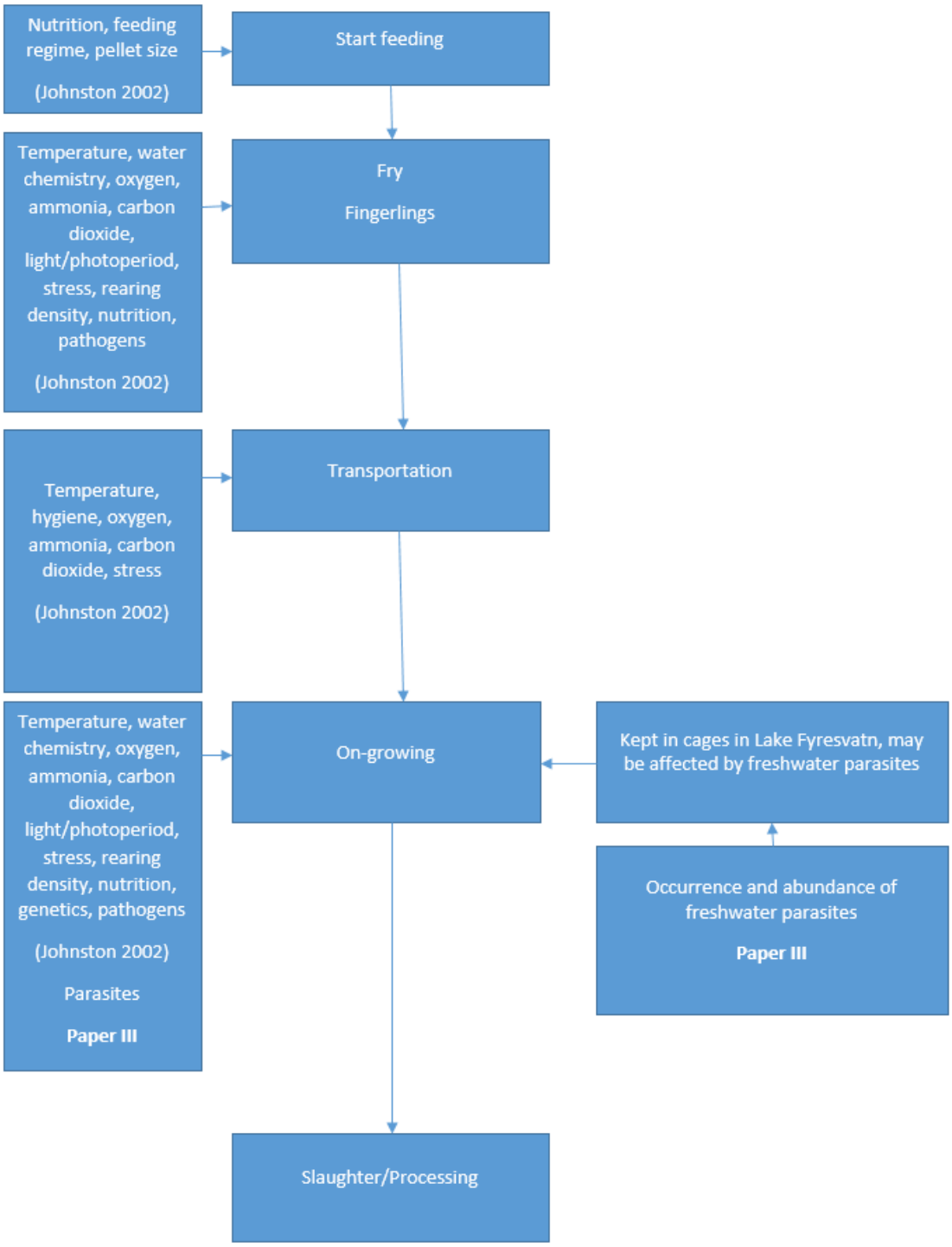


Figure 1. The process of farming Arctic charr, and factors influencing its success with references to southern Norway.

Paper I. Formalin treatments before eyeing and hand-picking of Arctic charr (*Salvelinus alpinus*) eggs – re-evaluating the timing of antifungal treatments

Eggs of Arctic charr in aquaculture are disinfected by formalin treatments before the eyed stage, and hand-picking of dead eggs during the eyed stage. Disinfection is necessary to avoid the spread of infections by water moulds, and to reduce mortalities. However, formalin is toxic to aquatic organisms (Kitchens et al. 1976) and carciogenous to hatchery workers (reviewed by Swenberg et al. 2013). As there are strict environmental legislations on freshwater aquaculture in southern Norway (Haug et al. 2006), and Telemarkrøye AS operates by the principles of organic production (Sæther et al. 2013), this study is designed to test whether the use of formalin can be omitted, maintaining sufficient survival to hatch. To achieve this, the protocol was divided into its components of formalin treatment and hand-picking, and their relative efficiency was estimated using a factorial design. Given the efficiency of formalin treatments previously shown for the disinfection of eggs of rainbow trout (Marking et al. 1994; Schreier et al. 1996; Barnes et al. 2000; Arndt et al. 2001) and fall Chinook salmon (*Oncorhynchus tshawytscha*) (Waterstrat & Marking 1995; Barnes et al. 1997; Barnes et al. 2003), and the increased effectiveness of formalin treatments compared to hand-picking (Barnes et al. 1997; Barnes et al. 2000; Barnes et al. 2003), I hypothesise, that formalin treatments connot be omitted without a significant reduction in survival until hatch. This means, that the formalin treated only group should exhibit a higher survival until hatch than the untreated control group, and that the group that is both treated with formalin and hand-picked should exhibit a higher survival until hatch than the hand-picked only group. Furthermore, the increased efficiency of formalin compared to hand-picking results in an expected increase in survival to hatch in the formalin treated only group compared to the hand-picked only group.

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

This paper is based on a literature review on Arctic charr in aquaculture from brood-stock management, through fertilisation, and egg incubation, until hatching. The aim of

the study is to gather and compile available information regarding factors influencing pre-hatch survival.

Paper III. Factors determining parasite abundance in three freshwater fish, European perch (*Perca fluviatilis*), European whitefish (*Coregonus lavaretus*), and Arctic charr (*Salvelinus alpinus*), in an oligotrophic lake, southern Norway

The aim of this study was to identify freshwater macroparasites, which are found in southern Norway, and can pose a threat to freshwater aquaculture of Arctic charr. In addition, their abundance was related to various properties of the infected fish, and differences in abundance by season and location were considered. To achieve a compelling overview over parasites present in a lake that resembles lakes that are suitable for Arctic charr aquaculture, parasites were identified in three fish species, Arctic charr, European perch (*Perca fluviatilis*), and European whitefish (*Coregonus lavaretus*). These species were chosen as they inhabit a broad range of habitats from littoral to pelagic/profundal in Lake Norsjø. The host properties age, length, weight, stable isotope signatures $\delta^{13}C$ and $\delta^{15}N$ and sex, as well as location and season were recorded and related to parasite abundance, as parasite abundance is previously demonstrated to vary with diet and habitat (Knudsen et al. 2008) (measured by stable isotope signatures in this study), age (Khan 2012), body size (e. g. Poulin 2000), and sex (Balling & Pfeiffer 1997). In addition, seasonal variations in parasite-host systems occur (Andersen 1978; Scott & Smith 1994). Based on this knowledge on freshwater parasite abundance, I hypothesise, that a variety of freshwater parasites previously found in Norway, as catalogued by Sterud (1999), can be found in Lake Norsjø. In addition, I expect to find increases in individual parasite abundance by age, length and weight, and potentially a higher abundance of parasites in female fish, as found for *Proteocephalus* sp. by Balling & Pfeiffer (1997). In addition, especially short lived parasites, such as *Argulus coregoni*, are expected to vary in abundance by season. I also hypothesise, that the species composition of the parasite fauna varies by habitat, thus by fish species in this study.

3 Material and Methods

3.1 Ethical statement (Paper I and paper III)

No ethical consent was required for these studies. In the experiment of paper I, adult fish and hatched larvae were handled by trained hatchery personnel at Telemarkrøye AS in Fyresdal, following their standard protocols for production. Eggs of Arctic charr were handled by the researchers after fertilisation until hatch. This life-stage in fish is not covered by the Norwegian legislation on the use of animals in research (Lovdata 2018). According to §2, larvae of vertebrates are first included in the regulation when they feed independently. Persistent harm or pain in later life stages was not expected as a result of this study, as all experimental procedures were usual husbandry practice. The study in paper III was conducted on wild-caught fish, which were immediately killed upon collection.

3.2 Study area (Paper I and paper III)

The experiment on disinfection protocols described in paper I was conducted in the facilities of the commercial Arctic charr farm Telemarkrøye AS in Fyresdal (South-eastern Norway), only using indigenous Arctic charr from Lake Fyresvatn in their production (Figure 1). The study of parasite abundance in three fish species, described in paper III, was implemented in Lake Norsjø about 70 km east of Lake Fyresvatn (Figure 1). Lake Norsjø is a large (55.48 km^2), oligotrophic lake (Holtan 1968), with a mean depth of 87 m, and a maximum depth of 171 m (Vann-Nett 2019). It is located at 15 m. a. s. l., and is part of the Telemark watercourse, draining mountain areas in the north west of Vestfold and Telemark County (Vann-Nett 2019). Three main rivers enter Lake Norsjø, and it is drained by the river Skienselva in the south, with a minor regulation height of < 1 m (Vann-Nett 2019). The lake is moderately impacted by human activities, such as agriculture, industry, and recreational activities (Vann-Nett 2019). Lake Norsjø is home to a rich fish-fauna, consisting of 14 different species, including Arctic charr (Jensen 1954).

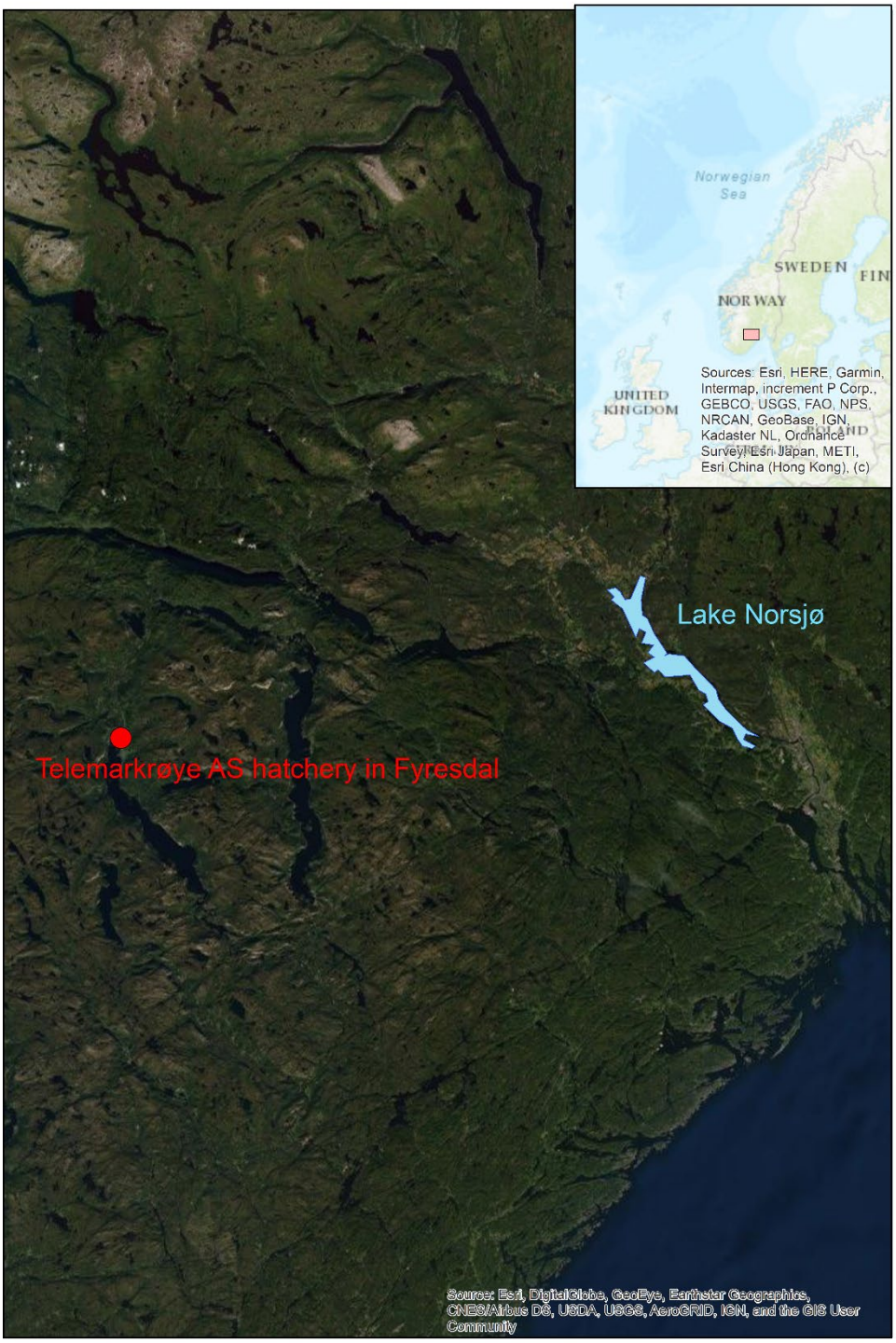


Figure 2. Map of the study area. The study of disinfection protocols for eggs of Arctic charr (Paper I) was conducted in the hatchery of Telemarkrøye AS in Fyresdal. The study on the abundance of freshwater macro-parasites was conducted in Lake Norsjø. Source: ArcGIS (ESRI, 2012).

3.3 Fish stock (Paper I)

All fish used in the experiment on disinfection protocols (Paper I) are derived from the hatchery brood-stock at Telemarkrøye AS. This brood-stock originates from the nearby Lake Fyresvatn. The brood-stock includes fish that were wild-caught in 2011, and offspring of these fish. The offspring were hatched in captivity, and bred without artificial selection. Two half-sib and one full-sib family were used in the experiment. All females were five years old at the time and hatched in captivity. Eggs of half-sib families were fertilised by two wild-caught males. The male of the full-sib family was hatched in captivity, and five years old at the time.

3.4 Fertilisation routine (Paper I)

All fish of the brood-stock were checked weekly for ripeness during the spawning period. Ripe fish were sedated using clove oil (Scan Aqua AS, Årnes, NO) in a bathing treatment at concentrations of 0.32 g L^{-1}. The genital papillae were wiped dry, and gametes were extracted by gentile abdominal massage (stripping). Care was taken to avoid contamination by mucus, faeces, and urine. Subsequently, unfertilised eggs were rinsed in physiological saline solution. Milt was stripped into a beaker and transferred to rinsed eggs using a syringe. The eggs were fertilised in darkness for two minutes. Fertilised eggs were again rinsed in physiological saline solution and disinfected using a buffered iodophore solution (PHARMAQ Ltd, Fordingbridge, Hampshire, UK) following the manufacturers guidelines. Subsequently, eggs were moved to the incubator.

3.5 Egg incubation and disinfection (Paper I)

The eggs were incubated in darkness in a vertical flow incubator (Alvestad Marin, Oslo, NO). The incubator compartments were disinfected using a buffered iodophore solution (PHARMAQ Ltd, Fordingbridge, Hampshire, UK) prior to use, and they were rinsed by hatchery water. Tap water from Fyresdals municipal water plant was used for incubation at a flow rate of 1.5 L min^{-1}. Water temperatures during incubation ranged from 6.2 to 7.7 °C. All work during the incubation period was carried out using a head lamp with red light.

3.6 Experimental setup and treatment (Paper I)

Eggs were incubated in two compartments (drawers), which were divided into 30 rectangular plots of ca. 100 cm^2 each (Figure 2). The plots were separated by 6 cm high PVC-plates (Fishtech, Vestby, NO) with circular perforations with a diameter of 2 mm. The plates were attached using a toxicant free silyl-modified polyether (Relekta, Oslo, NO). The eggs were divided into four groups filling 15 plots each. In each group, the same amount of plots were filled by eggs of all families, to reduce family-wise differences between the treatments. Each group was assigned one of the following treatments: untreated control, formalin treatment only, hand-picking only, and formalin treatment combined with hand-picking. Both formalin treated and un-treated groups were kept in their respective compartments. One compartment was treated with 20 mL formalin solution (formaldehyde: 380 mg mL^{-1} with 10.8 – 13.2 Vol-% methanol; Cenavisa S. L., Reus, ESP) added to the upstream end of the compartment. This treatment was applied three times weekly before the onset of the eyed stage, equivalent to a formalin treatment of 380 ppm (mg L^{-1}) for 13 minutes. Hand-picking was conducted weekly during the eyed stage using forceps. The number of dead eggs, empty eggshells, and hatched larvae were counted upon removal during hand-picking, or at the end of the experiment for unpicked groups. The larger number of either hatched larvae or empty eggshells was used as the number of hatched larvae, as hatched larvae were escaping into the hatching substrate beneath the plots (Figure 3). At the end of the experiment, infected eggs were collected, and stored in either hatchery water or 70 Vol-% ethanol. These eggs were sent to the Norwegian Veterinary institute (Veterinærinstituttet) for taxonomical identification of water moulds by morphology and genetic markers. Eight eggs, which were infected by hyphae visible to the unaided eye, were used for cultivation of water moulds using glucose yeast extract agar. Four eggs were analysed for genetic markers using direct polymerase chain reaction (PCR) and gene sequencing with specific ITS-primers for oomycetes.

Figure 3. Setup of the experimental plots in one compartment for the experiment on disinfection protocols (paper I)

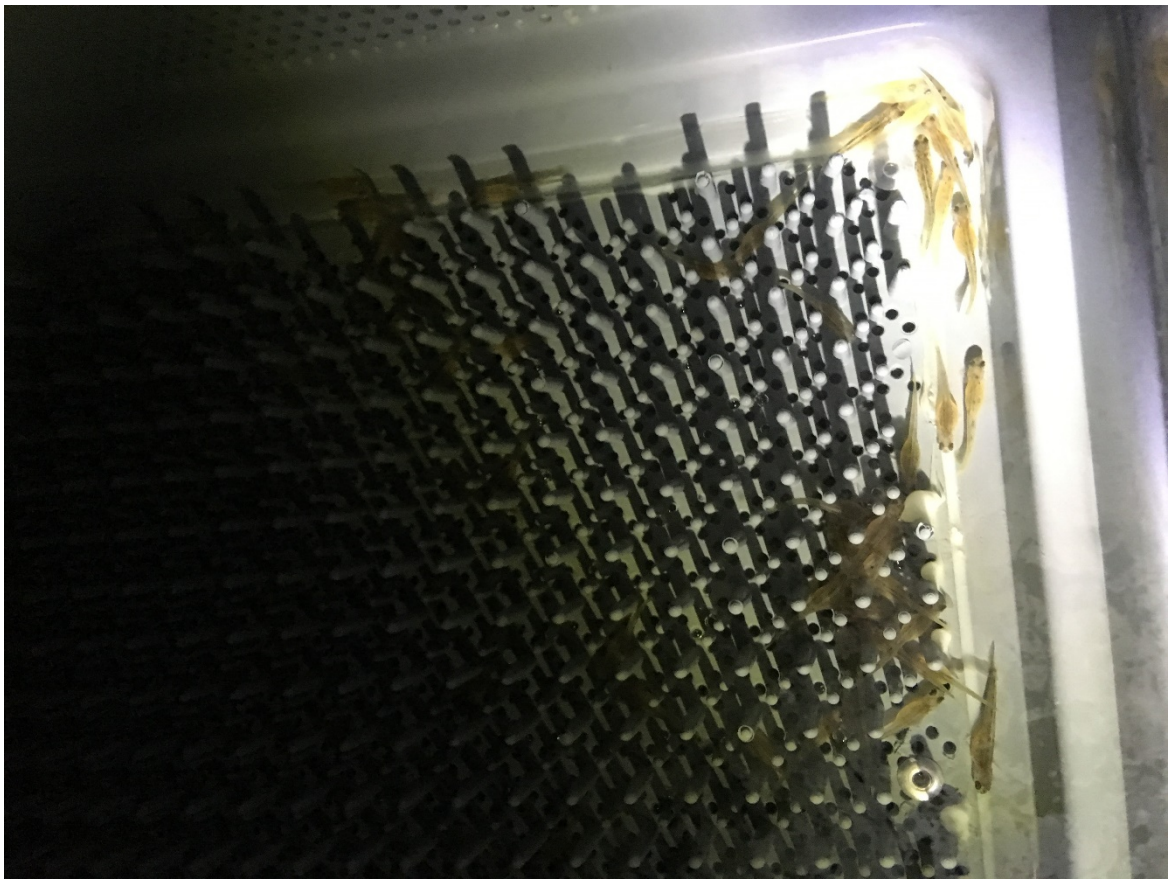


Figure 4. Hatched larvae that have escaped into the hatching substrate beneath the experimental plots (paper I)

3.7 Data analysis (Paper I)

For each hand-picked plot, the total number of eggs, dead eggs, and hatched larvae were calculated according to the following formulae:

$$N_{TotalDead} = \sum_{i=1}^{8} N_{Deadi}$$

$$N_{TotalHatched} = \sum_{i=1}^{8} N_{Hatchedi}$$

$$N_{total} = N_{TotalDead} + N_{TotalHatched}$$

for dead eggs, hatched larvae, and the total number of eggs, respectively. Observation (i) refers to the individual observations on each of the eight sampling dates. Accumulated hatch at the end of the experiment was calculated as proportion of the

total amount of eggs, and proportions were arcsine-transformed to stabilise their variances (Ott & Longnecker 1984). The proportion of hatched eggs were compared between the four treatment groups using a two-way ANOVA in R (R Core Team 2019). The explanatory factors used were the use of formalin and hand-picking, and interactions between these factors were included in the model. Individual between-group differences were assessed by Tukey's multiple comparison post-hoc test in R (R Core Team 2019). A significance level of $\alpha = 0.05$ was chosen for this analysis.

3.8 Fish sampling (Paper III)

The fish for the study of parasite abundance (paper III) were sampled at three locations, north (59.371806 °N, 9.191388 °E), in the middle (59.288811 °N, 9.280637 °E), and south (59.214154 °N, 9.472426 °E) in Lake Norsjø (Figure 4). The sampling site in the north was located in Årnes Bay, a shallow area surrounded by wetland vegetation. The sampling site in the middle of Lake Norsjø was located near the town of Ulefoss at the inlet of River Eidselva, while the sampling site in the south was located where the lake divides into two areas, the deeper Fjærekilen Bay, and the discharge bay at Skotfoss. All fish were sampled in 2018 at three occasions: late May (spring), late July (summer), and September (autumn). The fish were caught in standard bottom-set gill nets (1.5 m * 25 m) with mesh sizes between 13.5 and 45 mm. Six series of eight gill nets were created, and two series were deployed at each sampling location for each sampling date. The nets were set in the morning and collected the following day, sampling for approximately 24 hours. All nets were set from the shore in a 90° angle out in the lake. For this study, a randomly selected sub-sample of the collected fish was used. The sub-sample consisted of 75 European perch, 75 Arctic charr, and 50 European whitefish.

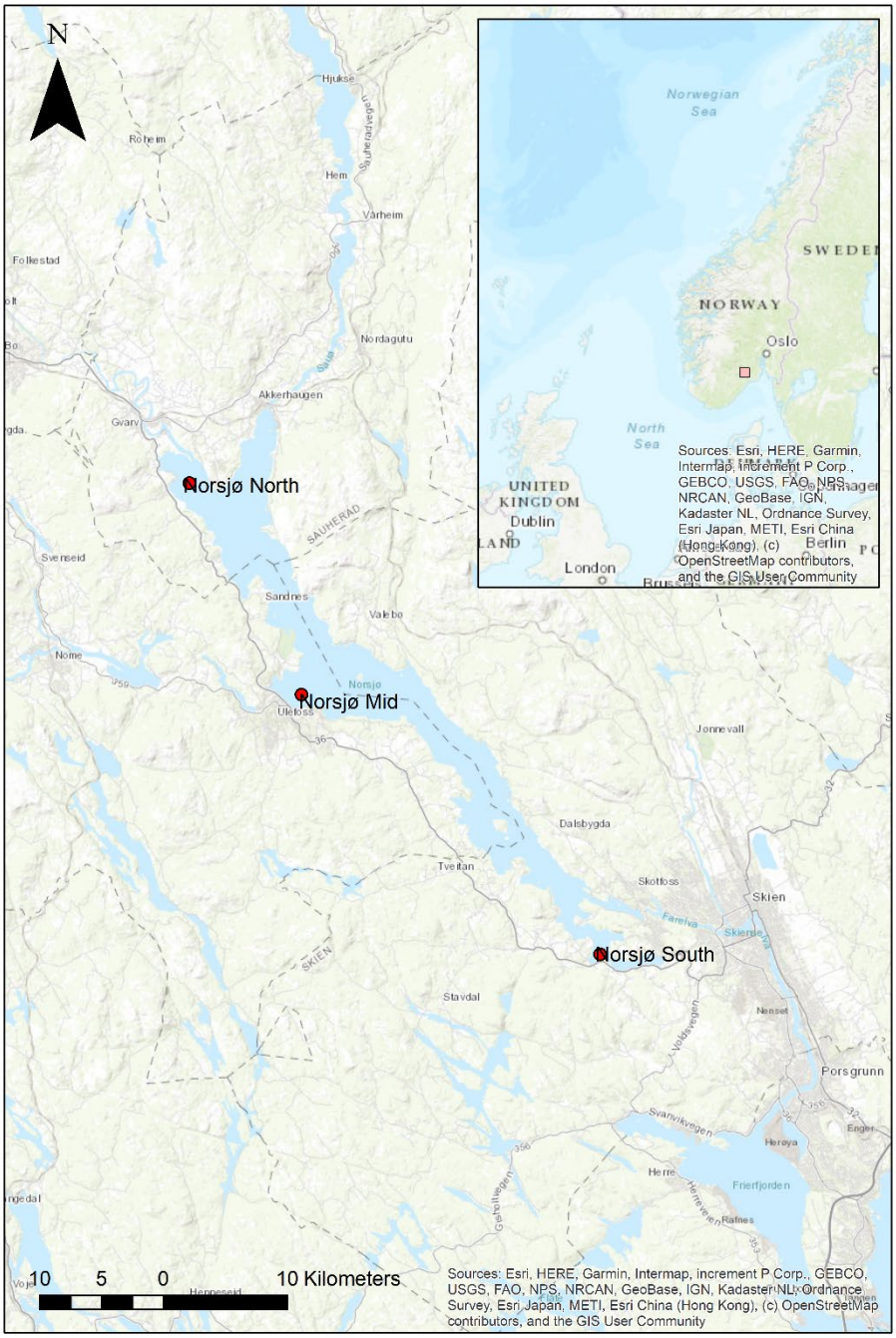


Figure 5. Map of Lake Norsjø with the sampling sites for the parasite abundance study (paper III). Source: ArcGis (ESRI, 2012).

3.9 Fish properties (Paper III)

Fish, sampled in the study on parasite abundance in Lake Norsjø (paper III), were measured to the nearest mm in a measuring cone, and weighted to the nearest g on a

scale. Age was determined using the otoliths. The otoliths were removed, and burned over a propane stove, until they had a light brown colour. Burned otoliths were divided transversally using a scalpel, and age readings were conducted on the cut surface under a stereomicroscope at a magnification of 40x. For stable isotope analysis, ca. 2 g of muscle was removed below the dorsal fin. Muscle samples were freeze-dried using a Heto LyoLab 3000 (Heto-Holten A/S, Allerod, DK) freeze drier. Subsequently, muscle samples were ground to a fine powder using mortar and pestle, and ca. 2 mg were weighted in and placed in tin capsules. The tin capsules were sent to the Norwegian Institute for Energy Technology (IFE) for analyses of stable isotope ratios ($\delta^{13}C = {}^{13}C/{}^{12}C$ and $\delta^{15}N = {}^{15}N/{}^{14}N$) and C/N ratio. To determine the stable isotope ratios the tin capsules were combusted in the presence of O_2 and Cr_2O_3 at 1 700 °C in an NCS 2500 elemental analyser (Thermo Fisher Scientific, Waltham, MA, USA). NO_x was reduced to N_2 in a Cu oven at 650 °C, and H_2O was removed in a chemical trap of $Mg(ClO_4)_2$. Subsequently, N_2 and CO_2 were separated on a 2 m Poraplot Q GC column (Agilent J&W, Santa Clara, CA, USA). N_2 and CO_2 were directly injected on-line to a DeltaXP plus isotope mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) to determine $\delta^{13}C$ and $\delta^{15}N$ along with the C/N-ratio.

3.10 Parasite sampling and identification (Paper III)

Ecto-parasites were sampled directly in field and stored in glass vials containing 96 % ethanol. Endoparasites were sampled in the laboratory by opening the fish from gills to gut. First, all cysts containing plerocercoid larvae of cestodes were registered on the intestinal tract and other tissue. A sample of encysted parasites was identified prior to the removal of organs. The intestinal tract was removed and placed in a petri dish in 9 % saline solution. The intestinal tract was opened from oesophagus to anus. All macroscopic parasites found in the intestinal tract were collected. Parasites were identified according to morphological features under a stereomicroscope at magnifications between 20 and 240 x. All parasites were counted after identification, and the number of cestodes was based on the number of heads (scolexes). Identification was conducted according to the following taxonomical keys: Bykhovskaya-Pavlovskaya

et al. (1964) was used for the genera *Argulus*, *Triaenophorus*, *Eubothrium*, and *Proteocephalus*. Kabata (1969) was used to identify species of the genus *Salmincola*. Plerocercoid larvae of *Dibothriocephalus* (formerly *Diphyllobothrium*, revised genus name according to Waeschenbach et al. (2017)) were identified according to Bykhovskaya-Pavlovskaya et al. (1964) and Andersen & Gibson (1989). Andersen & Gibson (1989) has some uncertainties related to frozen samples, as morphological features become less clear.

3.11 Data Analysis (Paper III)

Descriptive statistics, such as the mean, median, and standard deviation were calculated for all numerical variables describing fish properties. In addition to analyses on individual species of parasites, parasites with similar transmission routes and ecology were pooled in groups. *Eubothrium salvelini*, *Proteocephalus* sp.*, Dibothriocephalus dendriticus, Dibothriocephalus ditremus,* and *Triaenophorus nodulosus* were pooled as copepod transmitted parasites. *Dibothriocephalus dendriticus, Dibothriocephalus ditremus,* and *Triaenophorus nodulosus* were grouped as plerocercoid larvae of cestodes. For each parasite species or group, prevalence and mean abundance was calculated according to Bush et al. (1997). Prevalence is defined as the percentage of fish of one host species infected with at least one parasite, and calculated according to the following formula:

$\frac{N_{Infected\ Fish}}{N_{Total\ Fish}} * 100\ \%$, where $N_{Infected\ Fish}$ is the number of fish infected with at least one parasite of the corresponding species, and $N_{Total\ Fish}$ is the total number of fish of the host-species caught. Mean abundance is calculated according to the following equation:

$\frac{N_{Parasite}}{N_{host}}$, where $N_{Parasite}$ is the total number of a species or group of parasites found in the host species, and N_{host} is the total number of fish caught of the host species. The abundance of individual parasites and groups of parasites were modelled, if the prevalence of the parasite exceeded 10 %. As parasites occur in highly aggregated distributions, negative binomial generalised linear models were used (Wilson & Grenfell 1997). These models were created using the glmmTMB-package (Brooks et al. 2017) in

R (R Core Team 2019). Due to a relatively small sample of host-fish, models with one explanatory variable were created first. The candidate models featured the variables length, weight, age, $\delta^{13}C$, $\delta^{15}N$, the C/N-ratio, sex, location, and season. The candidate models were compared using Akaikes Information Criterion (AIC). The model with the lowest AIC was selected, and it was attempted to add one additional explanatory variable of the variables named above to the model. The more advanced models were compared to the base model by maximum likelihood using the anova-command in R (R Core Team 2019). If several more advanced models were significantly better than the base model, the model with the lowest AIC was selected. Residuals of the final model were checked using simulated residuals in the DHARMa-package (Hartig 2019) in R (R Core Team 2019). Graphs of the models were created using the ggplot2-package (Wickham 2016), and the ggeffects-package (Lüdecke 2018). In some cases, residuals exhibited curved patterns. Then, the inclusion of quadratic terms was attempted, using the numerical variables length, weight, age, $\delta^{13}C$, $\delta^{15}N$, and the C/N-ratio. Quadratic terms were included in the models using the poly-function in R (R Core Team 2019). Models with quadratic terms were handled as individual base models and compared and advanced in the same manner as linear models. Residuals of quadratic models were also checked using the DHARMa-package (Hartig 2019). For all tests, a significance level of $\alpha = 0.05$ was defined.

4 Results

4.1 Formalin treatments before eyeing and hand-picking of Arctic charr (*Salvelinus alpinus*) eggs – re-evaluating the timing of antifungal treatments (Paper I)

The average proportion of hatched larvae in all four groups was extremely low. Average proportion of hatched larvae were 5 ± 4, 3 ± 3, 1 ± 1, and 0 ± 0 % for formalin treated and hand-picked, hand-picked, formalin treated, and control groups, respectively (Figure 5). There was a significant difference in the proportion of hatched larvae between hand-picked and unpicked groups (ANOVA, df = 1, F = 27.19, $p < 0.001$). Formalin treated and untreated groups also differed significantly in the proportion of hatched larvae (ANOVA, df = 1, F = 4.43, $p = 0.040$). No significant interaction between hand-picking and formalin treatment was detected (ANOVA, df = 1, F = 0.083, $p > 0.100$). Both formalin treatments and hand-picking increased the proportion of hatched larvae. The difference in proportion of hatched larvae between formalin treated and untreated groups was estimated to 0.2 % (Tukey's multiple comparison test: 95 % confidence interval: 0.0004 – 0.8 %), and the difference between hand-picked and unpicked groups was estimated to 1.3 % (Tukey's multiple comparison test: 95 % confidence interval: 0.5 – 2.5 %). One genetic sample of water moulds exhibited 99 % correspondence to *Pythium monospermum*, and one genetic sample of water moulds showed 96 % correspondence to *Aphanomyces stellatus*.

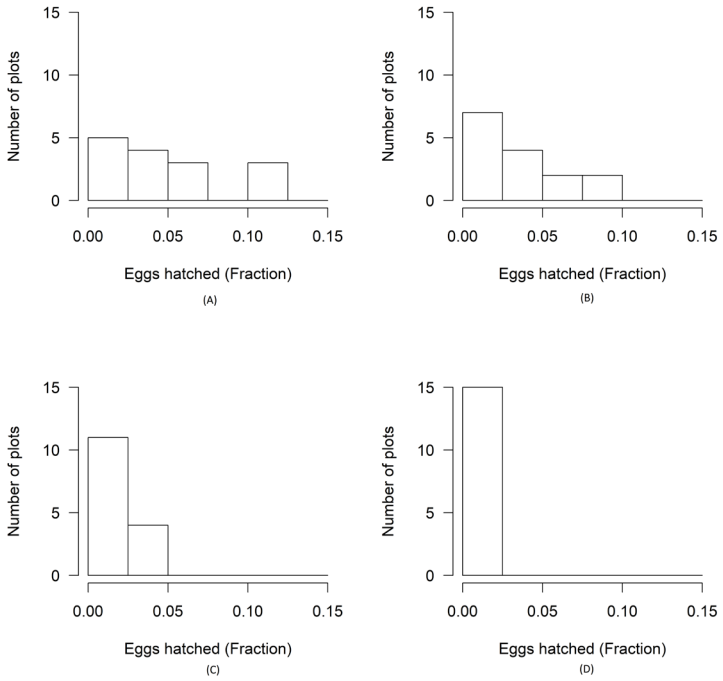


Figure 6. Proportions of hatched larvae for every experimental plot. (A) Hand-picked and formalin treated plots; (B) Hand-picked untreated plots; (C) unpicked, formalin treated plots; (D) untreated control plots.

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

Pre-hatch survival of eggs of Arctic charr has been determined by various factors, acting from the period of maturation of gametes in the brood-stock until hatch. These factors are both extrinsic, such as environmental conditions, and intrinsic, such as egg quality traits.

Temperature was identified as one of the most important variables. Arctic charr brood-stock has a narrow temperature tolerance during summer, and requires low temperatures. Temperatures below 12 °C are generally recommended during this period (Jeuthe et al. 2013). High summer temperatures have been identified as detrimental to egg quality in several experimental (Jobling et al. 1995; Atse et al. 2002; Jeuthe et al. 2015) and observational studies (Jeuthe et al. 2013; 2015). High summer temperatures seem to have an impact on hormone levels in Arctic charr, and reduce oestradiol (E_2), which is important for egg production. E_2 levels were lower in Arctic charr held at higher

temperatures during summer (Mayer et al. 1992; Frantzen et al. 1997; Tveiten et al. 1998; Berg et al. 2004a). Similar effects have been documented for Tasmanian Atlantic salmon (King et al. 2003; Watts et al. 2004; Watts et al. 2005; King et al. 2007).

The effects of brood-stock diet on egg and sperm quality have been investigated, especially regarding fatty-acid composition, but results for egg quality remain inconclusive. Different fatty-acid compositions for eggs were correlated to differences in egg survival when comparing eggs of wild and farmed Arctic charr (Pickova & Brännäs 2006; Pickova et al. 2007). In these studies, eggs of hatchery origin had lower concentrations of long-chained fatty acids, Arachidonic acid (ARA), and the ratio between n-6 and n-3 fatty acids differed between eggs of wild and farmed fish (Pickova & Brännäs 2006; Pickova et al. 2007). However, many confounding factors that occur when comparing eggs of wild and farmed fish, such as different rearing temperatures, render the results inconclusive. Within one hatchery-reared population of anadromous Arctic charr, no correlations between individual fatty-acid concentrations and egg survival were found (Mansour et al. 2011). All fish in this study were fed the same feed, and differences in fatty-acid composition of the eggs were lower. Contrary, differences in fatty-acid composition of sperm was found to influence fertilisation success in the same study (Mansour et al. 2011). In theory, differences in n-3/n-6 fatty-acid ratios between wild and farmed eggs show differences between the natural freshwater feed of non-anadromous populations of Arctic charr and the marine based feed in aquaculture (Pickova & Brännäs 2006; Pickova et al. 2007). N-3/n-6 ratios of various species were previously found to be lower in freshwater than in marine environments (Kaitaranta & Linko 1984; Henderson & Tocher 1987; Pickova et al. 1997; Arts et al. 2001; Wiegand et al. 2004). However, this may not influence egg quality negatively, as Arctic charr is a versatile species in relation to diet (Skulason et al. 1992), and anadromous populations are found to consume up to 90 % marine feed (Swanson et al. 2011). Consequently, the differences in the results of the studies by Pickova & Brännäs (2006) and Pickova et al. (2007) on one hand, and Mansour et al. (2011) on the other hand, may be linked to population differences. Mansour et al. (2011) used an anadromous population in their experiment, while Pickova & Brännäs (2006) and Pickova et al. (2007)

used a stationary population (Nilsson et al. 2010). Population specific differences in ARA in eggs have for instance been proposed for lobster (*Homarus gammarus*) (Castell et al. 1995) and Cod (*Gadus morhua*) (Pickova et al. 1997). Contrary to equivocal results for the influence of fatty-acid composition on egg quality of Arctic charr, it has profound effects on sperm quality. Good quality sperm of Arctic charr has lower concentrations of saturated fatty acids, higher concentrations of C20:3n-6, ARA, C22:5n-3, C22:6n-3, total n-3, total n-6, higher ratios of polyunsaturated fatty acids to saturated fatty acids, and higher ratios of n-3 to n-6 (Mansour et al. 2011).

Extensive knowledge has been gained on the timing of spawning and its manipulation using photoperiod, temperature, and hormone treatments. Spawning occurs between mid-July and January under natural conditions (Johnston 2002). Within single populations, spawning occurs during an extended period of ten weeks. This is governed by the periodical ripening of females, as males are ripe throughout the season (Johnston 2002). Females have a short window of ripeness, as post-ovulatory eggs deteriorate quickly in quality (Gillet 1991; 1994; Gillet & Breton 1992). This proves to be a major challenge in Arctic charr aquaculture, and one goal of manipulating the timing of spawning is the synchronisation of females. Photoperiodic manipulations can be used to synchronise spawning or to move the spawning period to different times of the year (Gillet 1991; 1994; Gillet & Breton 1992; 2009; Duston et al. 2003; Frantzen et al. 2004). Day length proves to be the most important cue to control reproductive development in Salmonids (Bromage et al. 2001), and Arctic charr in particular (Jobling et al. 1993; Johnston 2002; Jeuthe 2015). Long days stimulate the initiation of gametogenesis (Gillet & Breton 1992; Gillet 1994), and have an inhibitory effect on ovulation (Gillet & Breton 1992; 2009). Early exposure to long days can thus be used to advance the spawning period (Gillet 1994), and a new reproductive cycle can be triggered by exposure to long days right after ovulation (Gillet & Breton 1992). The length of the reproductive cycle in Arctic charr is estimated to eight months (Gillet & Breton 1992). Long day treatment can be continued in late summer and autumn, which leads to postponed spawning, and a prolonged spawning period, showing the inhibitory effect of long days to ovulation (Gillet & Breton 1992; Gillet 1994). The inhibitory effect of long day treatment on

ovulation can be reversed by short day treatment. Spawning occurs approximately one month after the onset of short day treatment (Gillet & Breton 2009). The short day treatment is important to synchronise spawning, as the lack of it can lead to extended spawning periods over several months (Gillet & Breton 1992). Frantzen et al. (2004) also observed asynchrony of ovulation when short day treatments were not applied. When applying short day treatments several weeks before natural time of ovulation, a synchronisation of spawning has been observed (Gillet & Breton 1992; Gillet 1994), and short day treatments could also be used to advance spawning by approximately two months (Gillet & Breton 1992). By combining long day and short day treatments, photoperiod manipulations allow the production of viable gametes of Arctic charr year round. However, sufficient time for oocyte development needs to be provided (Gillet 1994). Water temperature has also been used to manipulate the timing of spawning in Arctic charr, especially in order to synchronise spawning (Gillet 1991; Gillet & Breton 1992). The main effect of water temperature on the timing of spawning occurs in autumn, when temperatures ≥ 11 °C inhibit ovulation completely (Gillet 1991), and temperatures of 10 °C result in an almost complete inhibition of ovulation (Gillet 1991; Gillet et al. 1996; Gillet & Breton 2009). Temperatures of 8 °C still delay ovulation compared to 5 °C (Gillet 1991; Gillet & Breton 1992), and maintaining a brood-stock at 8 °C during the spawning period is only feasible for a short duration (Gillet 1991). A decrease in water temperatures can be used as a trigger to synchronise spawning in Arctic charr, which was demonstrated for transfers from 8 °C to 5 °C (Gillet 1991; Gillet & Breton 1992), and for transfers from 10 °C to 5 °C (Gillet & Breton 1992). While a temperature decrease can be used to synchronise spawning, it is not possible to advance the spawning period by reducing water temperature. A transfer to 5 °C in July did not result in earlier spawning than a transfer in September (Gillet 1991). Consequently, temperatures around 5 °C only seem to be required during the last weeks prior to ovulation (Gillet 1991). High temperatures during the spawning period cause dopamine induced inhibition of ovulation, as hormone treatments at high temperatures can stimulate ovulation under the addition of a dopamine inhibitor (Gillet et al. 1996; Gillet & Breton 2009). Hormone treatments can be used in Arctic charr to synchronise

spawning (Gillet & Breton 1992; 2009; Jansen 1993; Gillet et al. 1996; Brännäs et al. 2007). Ovulation in salmonids can be induced by pituitary preparations and injection of GnRH (Jalabert et al. 1978; Crim et al. 1983; Sower et al. 1984; Breton et al. 1990). In Arctic charr, hormone treatments are most successful under favourable conditions on sexually mature fish, when only gamete release is stimulated. The advantage of hormone treatments is more synchronised spawning, and a less stressful spawning period (Brännäs et al. 2011b). Consequently, hormone treatments have been successful under favourable conditions at 5 °C (Gillet & Breton 1992), and at 7 °C (Jansen 1993), even without additional spawning triggers. Under unfavourable conditions (10 °C), hormone treatments trigger spawning, but egg quality is lower and more variable. The stimulation of spawning is not as persistent under those conditions (Gillet & Breton 1992). In addition, high doses of hormones or sustained release forms, and a dopamine inhibitor are necessary at high temperatures to achieve high ovulation rates (Gillet et al. 1996). Hormone treatments also differ in effectiveness, and some treatments are better suited to synchronise a large proportion of spawners, while others lead to a higher cumulative ovulation rate (Gillet et al. 1996).

Suitable routines for the fertilisation process and egg incubation are being developed in Arctic charr aquaculture. Common routines are summarised in Johnston (2002). Captive Arctic charr have to be stripped, as they otherwise retain their eggs, which has to be done within four days of ovulation. Ripe eggs deteriorate quickly. Consequently, ripe females need to be identified before spawning (Brännäs et al. 2011b). Sex determination is challenging, and mostly conducted using morphological characteristics (Johnston 2002). Males and females are kept in separate tanks during the spawning period, and females are sorted by ripeness. Nearly ripe females are checked for ovulation at least once a week at 5 °C, while unripe females are checked bi-weekly (Johnston 2002). During the stripping process, care has to be taken to avoid contamination of eggs and sperm. However, no species specific concerns exist for Arctic charr, and the general literature on hygiene in hatcheries can be consulted (Piper et al. 1982; Rurangwa et al. 2004). Fertilisation is ensured by using excessive amounts of milt, and minimal amounts of water. Usually, the milt of several males is used to fertilise one batch of eggs, and also

pooled egg batches may be used for logistical reasons (Jeuthe 2015). Fertilisation can also be conducted without the addition of water in ovarian fluid (Johnston 2002), which aids the fertilisation process (Turner & Montgomerie 2002). During incubation, eggs should be kept at temperatures below 8 °C (Elliott & Elliott 2010), and the highest hatching success has been achieved between 1 °C and 5 °C (Humpesch 1985; Elliott & Elliott 2010). However, during the first week of incubation, a warmer temperature of 6 °C compared to 2.8 °C lead to higher survival (Jeuthe et al. 2016). To avoid infections during egg incubation, surface disinfection with buffered iodophore solutions, chemical antifungal treatments, and hand-picking of dead eggs are used (Johnston 2002).

Egg quality parameters, such as egg size, have been investigated in several studies. Egg size has been linked to viability of eggs, and increases with female age up to six years (Jeuthe et al. 2013; Jeuthe 2015; Lasne et al. 2018). However, not all studies confirm an association between egg size and survival. Jónsson & Svavarsson (2000) found no correlation between egg weight, survival to the eyed stage or first feeding, or the number of abnormally shaped larvae. Contrary, egg size was positively correlated to the survival of larvae from emergence to first feeding, and small eggs resulted in starvation more often in another study (Wallace & Aasjord 1984). Larger larvae were found to come from larger eggs. Larger larvae are more active and show more active foraging behaviour (Leblanc et al. 2011). However, larvae from the largest eggs also showed a higher frequency of malformations (Wallace & Aasjord 1984). Smaller eggs on the other hand were shown to have a lower energy density, which was not correlated to pre-hatch survival (Leblanc et al. 2016). Energy density was also shown to be more variable in smaller eggs, and it seemed to be a better predictor of egg quality than size or weight (Leblanc et al. 2016). In addition to their initial size, eggs exhibited differences in egg swelling, or the size increase due to the intake of water. This difference is supposedly connected to egg quality and ultimately viability (Pakkasmaa et al. 2001).

In general, egg survival differs greatly between different populations and families, while the differences between families remain unexplained. From Iceland, general egg survival of 32 %, with the largest mortalities during the first week of incubation, is reported

(Leblanc et al. 2016). In a Canadian study, fertility ranged between 0 and 83 % (Mansour et al. 2011). Large family-wise differences in egg survival are reported from Sweden, where hatching rates were found to range between 9 and 97 % (Jeuthe et al. 2016). Usual hatching rates in Sweden lie between 0 and 70 % (Jeuthe et al. 2013). Some of the differences in egg survival may be attributed to differences in the brood-stocks used and the respective culture conditions they are subjected to (Jobling et al. 1998). Egg survival in itself does not appear to be heritable or population-specific (Brännäs et al. 2007). Arctic charr populations are either anadromous or stationary (Johnson 1980), and both types of populations are used in aquaculture and research (Gillet 1991; Tabachek & de March 1991; Duston et al. 2003; Frantzen et al. 2004; Nilsson et al. 2010; Leblanc et al. 2016). The adaption of culture conditions to the type of population used is crucial in determining egg survival. However individual differences between families remain unexplained (Jeuthe et al. 2016). However, some of these differences may be linked to parental effects, that have been shown to influence reproductive performance (Pakkasmaa et al. 2006).

Less information is available on sperm quality parameters and their impact on egg survival. Sperm quality parameters are assessed by standard techniques in Arctic charr that are used for fish in general (Rurangwa et al. 2004; Fauvel et al. 2010; Migaud et al. 2013). Sperm quality is mainly of concern when assessing fertilisation success, but it can also be influential by epigenetic or transcriptional mechanisms (Cabrita et al. 2014). In Arctic charr, sperm quality has been shown to be a limiting factor for reproductive success. Of the investigated sperm quality parameters, beat cross frequency and 17, 20β-dihydroxy-4-pregnen-3-one (17,20β-P) levels in males were sufficient to explain 65 % of the variation in egg survival to the eyed stage in one study. However, underlying mechanisms remain to be elucidated (Jeuthe et al. 2019).

4.3 Factors determining parasite abundance in three freshwater fish, European perch (*Perca fluviatilis*), European whitefish (*Coregonus lavaretus*), and Arctic charr (*Salvelinus alpinus*), in an oligotrophic lake, southern Norway (Paper III)

In European whitefish, the parasites Trematoda, *Dibothriocephalus* spp., *Proteocephalus* sp., acanthocephalans, *Argulus coregoni*, *Salmincola* sp., and Nematoda were found. Arctic charr were infected with *Argulus coregoni*, *Salmincola edwardsii*, *Eubothrium salvelini*, *Triaenophorus nodulosus*, *Dibothriocephalus ditremus*, *Dibothriocephalus dendriticus*, *Proteocephalus* sp., and acanthocephalans. In European perch, the parasites *Triaenophorus nodulosus*, *Eubothrium* sp., *Dibothriocephalus* spp., *Proteocephalus* sp., and acanthocephalans were identified. In European whitefish, the most prevalent parasites were acanthocephalans (Prevalence: 36 %, mean abundance 4.4 (Table 1) and *Proteocephalus* sp. (Prevalence: 24 %, mean abundance; 0.68). Trematoda and Nematoda were only found in European whitefish, and *Salmincola edwardsii* and *Eubothrium salvelini* were exclusively found in Arctic charr. The most prevalent parasites in Arctic charr were encysted parasites (plerocercoids) (Prevalence: 93 %, mean abundance: 20.15), while the most prevalent single parasite species in Arctic charr were *Eubothrium salvelini* (Prevalence: 68 %, mean abundance: 2.57) and *Dibothriocephalus ditremus* (Prevalence: 43 %, mean abundance: 0.96). European perch was mainly infected with acanthocephalans (Prevalence: 79 %, mean abundance: 4.69).

Table 1. Prevalence and mean abundance of all parasite species and groups found in European whitefish, Arctic charr, and European perch in Lake Norsjø.

Host species	Parasite species/group	Prevalence (%)	Mean abundance
European whitefish (*Coregonus lavaretus*)	Trematoda	2	0.06
	Argulus coregoni	20	0.38
	Salmincola extensus	12	0.14
	Dibothriocephalus spp.	4	0.04
	Proteocephalus sp.	24	0.68
	Acanthocephala	36	4.4
	Nematoda	2	0.02
	Copepod transmitted	28	0.72
	Plerocercoids	4	0.04
Arctic charr (*Salvelinus alpinus*)	*Argulus coregoni*	1.33	0.01
	Salmincola edwardsii	16	0.17
	Cysts (plerocercoids)	93	20.15
	Eubothrium salvelini	68	2.57
	Triaenophorus nodulosus	36	0.64
	Dibothriocephalus ditremus	43	0.96
	Dibothriocephalus dendriticus	25	0.29
	Proteocephalus sp.	7	0.29
	Acanthocephala	3	0.04
	Copepod transmitted	99	24.91
	Plerocercoids	99	22.04
European perch (*Perca fluviatilis*)	*Triaenophorus nodulosus*	4	0.04
	Eubothrium sp.	3	0.04
	Dibothriocephalus spp.	4	0.05
	Proteocephalus sp.	5	0.08
	Acanthocephala	79	4.69
	Copepod transmitted	12	0.17
	Plerocercoids	7	0.09

Seasonal variations determined the abundance on *Argulus coregoni* in European whitefish (Δspring = -20.234, SE = 17083.729, z = - 0.001, $p > 0.1$; Δsummer = 2.249, SE = 1.111, z = 2.024, $p < 0.05$), which occurred at higher abundances in summer. Season was also included in the model for *Salmincola* sp. in European whitefish, but there were

no significant differences in abundance by season (Δspring = 21.07, SE = 27213.70, z = 0.001, p > 0.1; Δsummer = 20.88, SE = 27213.70, z = 0.001, p > 0.1). The abundance of *Proteocephalus* sp. in European whitefish differed by sex (Δmale = -1.6702, SE = 0.8506, z = -1.964, p < 0.05), and females exhibited higher abundances of the parasite. In European whitefish, the relationships between age and the abundance of *Proteocephalus* sp. (β poly age 2 = -8.1682, SE = 2.9387, z = -2.780, p < 0.01; β poly age 1 = 8.8081, SE = 3.5408, z = 2.488, p < 0.05), acanthocephalans (β poly age 2 = -6.3996, SE = 2.7692, z = -2.311, p < 0.05; β poly age 1 = 10.2080, SE = 3.0930, z = 3.300, p < 0.001), and copepod transmitted parasites (β poly age 2 = -7.2901, SE = 2.6623, z = -2.738, p < 0.01; β poly age 1 = 9.9698, SE = 3.4119, z = 2.922, p < 0.01) were quadratic and significant. The highest abundances of *Proteocephalus* sp., acanthocephalans, and copepod transmitted parasites in European whitefish occurred between the age of 5 and 8 years. Age was also included in the model for the abundance of *Salmincola edwardsii* in Arctic charr with no significant effect of age detected (β = 0.02457, SE = 0.04639, z = -0.530, p > 0.1). The abundance of encysted parasites (plerocercoids) was positively correlated to age in Arctic charr (β = 0.04927, SE = 0.01784, z = 2.762, p < 0.01). Age was also positively correlated to the abundance of *Eubothrium salvelini* in Arctic charr (β = 0.07495, SE = 0.02265, z = 3.309, p < 0.001), and there was a non-significant tendency towards higher abundance in spring (Δspring = 0.67294, SE = 0.35945, z = 1.872, p > 0.05; Δsummer = -0.49272, SE = 0.36182, z = -1.362, p > 0.1). The abundance of *Triaenophorus nodulosus* had a non-significant tendency to increase by age (β = 0.03862, SE = 0.02962, z = 1.304, p > 0.1). The abundance of *Dibothriocephalus ditremus* in Arctic charr differed by sex (Δmale = 0.7419, SE = 0.3685, z = 2.014, p < 0.05), with higher abundances in male fish. The abundance of *Dibothriocephalus dendriticus* varied by season (Δspring = 1.5041, SE = 0.6667, z = 2.256, p < 0.05; Δsummer = 1.3863, SE = 0.6583, z = 2.106, p < 0.05), with lower abundance in autumn. Length determined the abundance of plerocercoid larvae of cestodes in Arctic charr (β = 0.007730, SE = 0.002632, z = 2.937, p < 0.01), and age was included in the model, although the relationship was insignificant (β = - 0.009221, SE = 0.024309, z = -0.379, p > 0.1). Length also determined the abundance of copepod transmitted parasites in Arctic charr (β = 7.088 * 10^{-3}, SE = 2.57

* 10^{-3}, z = 2.758, p < 0.01), and age was included in the model without being significant (β = -5.728 * 10^{-5}, SE = 2.367 * 10^{-2}, z = -0.002, p > 0.1). The abundance of acanthocephalans in European perch varied by season (Δspring = 1.00851, SE = 0.37483, z = 2.691, p < 0.01; Δsummer = 1.41060, SE = 0.38679, z = 3.647, p < 0.001) with fewer individuals found in autumn. There was also a not significant, positive trend of the abundance of acanthocephalans in European perch by $\delta^{15}N$ (β = 0.13224, SE = 0.09457, z = 1.398, p > 0.1). The abundance of copepod transmitted parasites in European perch was linked to age by a quadratic equation (β poly age 2 = -1.8552, SE = 2.7481, z = 0.675, p > 0.1; β poly age 1 = 10.1687, SE = 4.0685, z = 2.409, p < 0.05) and the highest abundances occurred at age > 6.

5 Discussion

5.1 Formalin treatments before eyeing and hand-picking of Arctic charr (*Salvelinus alpinus*) eggs – re-evaluating the timing of antifungal treatments (Paper I)

Eggs in this study exhibited extremely low survival, which is likely linked to egg quality, the fertilisation process, but also extensive water mould infections, as the frequency and intensity of anti water mould treatments was low compared to other studies (Barnes et al. 1997; Barnes et al. 2000; Barnes et al. 2003). Both forms of anti water mould treatments, namely formalin treatments and hand-picking, increased the percentage of hatched eggs in this study. Formalin has previously been shown to increase survival of eggs in rainbow trout (Marking et al. 1994; Schreier et al. 1996; Barnes et al. 2000; Arndt et al. 2001) and fall Chinook salmon (Waterstrat & Marking 1995; Barnes et al. 1997; Barnes et al. 2003). Contrary to the hypothesised larger effect of formalin treatments, the benefits of hand-picking exceeded the benefits of formalin treatment in our study, even though the effect of hand-picking may be underestimated due to the possibility of cross-contamination from unpicked plots. This is inconsistent with previous results, where daily formalin treatments increased survival by 3 - 5 % compared to daily hand-picking (Barnes et al. 1997; Barnes et al. 2000; Barnes et al. 2003). This may be caused by the differences between the disinfection protocols used, as formalin was only applied before the eyed stage in our study. The extent of water mould infections likely varied at different developmental stages, with higher pressure by water mould infections during the eyed stage. Higher mortalities in hand-picked only fall Chinook salmon during the eyed stage have previously been observed (Barnes et al. 1997). Higher occurrences of water mould infections during the eyed stage may be caused by the accumulation of water mould spores and hyphae during incubation, which is demonstrated for *Saprolegnia ferax* in field experiments on amphibian egg masses (Kiesecker & Blaustein 1997). Consequently, hand-picking appears more effective than formalin treatments in our study, as it is applied when infections are more intense. Contrary, it has been

claimed empirically, that the majority of water mould infections occur before the eyed stage (Johnston 2002). However, no details about experiments or disinfection protocols used are provided. In addition to the effect of timing of disinfection, our experiment is characterised by a lower total effort of disinfection compared to previous studies (Barnes et al. 1997; Barnes et al. 2000; Barnes et al. 2003). This may have had a larger effect on the effectiveness of formalin treatments compared to hand-picking. More extensive water mould infections in our study may also have influenced the results. The applied dose of formalin (380 ppm for 13 min) is likely only inhibiting water mould growth, but does not interfere with the active presence of water moulds (Marking et al. 1994). This treatment is merely prophylactic. However, it was also applied before the onset of extensive water mould infections, and was likely sufficient to prevent the growth of water moulds before the eyed stage.

Evidence of the presence of multiple species of water moulds was provided by our experiment. *Pythium monospermum* and *Aphanomyces stellatus* or a closely related species were identified. *Pythium monospermum* is primarily known as a plant pathogen, which is also found in river water (Matsiakh et al. 2016), while *Aphanomyces* sp. are found in soil and water as saprotrophs, or parasites of root, algae, aquatic fungi, insects, crayfish or fish (Markovskaja 2007). Species of both genera have previously been found in Polish fish farms (Czeczuga & Woronowicz 1993), and the pH (7.53 – 7.76) was within a similar range as in our study (7.5 – 8.35).

Our results indicate that water mould infections cause higher mortalities in eggs during the eyed stage. To maximise the survival of eggs, it can thus be advised to use formalin treatments during the entire incubation period until hatch, when large amounts of dead organic matter are present. The replacement of hand-picking by formalin treatments would also be economically beneficial, as hand-picking is a laborious procedure (Leitritz & Lewis 1976). On the other hand, the use of formalin is connected to risks, concerning the environment, as formalin is toxic to aquatic organisms (Katz 1989; Marking et al. 1994; GESAMP 1997; Leal et al. 2016). Formalin is also carcinogenic, and may pose risks to hatchery workers (reviewed by Swenberg et al. 2013). Consequently, it is beneficial

to limit the use of formalin where that is possible. The demand for further research into the dynamics of water mould infections in hatcheries is highlighted. Better understanding of the dynamics of water mould infections would allow to time formalin treatments to the periods of the highest risk of infection.

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

There is extensive literature describing brood-stock management, fertilisation, and egg rearing conditions of Arctic charr in aquaculture, but also a demand for further research. The most severe problems causing low and variable hatching success in Arctic charr aquaculture seem to be related to the holding conditions of the brood-stock. The holding conditions of the brood-stock also influence the timing of spawning, which has been thoroughly investigated (e. g. Gillet 1991; 1994; Gillet & Breton 1992; 2009; Gillet, et al. 1996; Gillet et al. 2011). Temperature seems to be important (e. g. Jeuthe et al. 2013; 2015), and effects of elevated summer temperature require further investigation. In addition, optimal summer temperatures for brood-stock remain unknown. The effects of dietary fatty acid composition on egg quality remain to be investigated, as results on this topic remain equivocal (Pickova & Brännäs 2006; Pickova et al. 2007; Mansour et al. 2011). Effects of handling stress remain to be investigated *in situ*, as all studies on this topic are based on injections of the stress hormone cortisol (Berg 2003; Berg et al. 2004a; Berg et al. 2004b). Adequate knowledge is available on the fertilisation process, and "dry" fertilisation using ovarian fluid may be most advantageous (Turner & Montgomerie 2002). Egg quality has been studied in some detail (e. g. Jónsson & Svavarsson 2000; Leblanc et al. 2016). However, there are few studies investigating the impact of sperm quality on the reproductive success of Arctic charr (Mansour et al. 2011; Jeuthe et al. 2019), and further investigations are in demand. The temperature requirements during egg incubation are known (Elliott & Elliott 2010; Jeuthe et al. 2016). Large differences in hatching success and fertilisation rates between families (Jeuthe et al. 2016) require further investigation. Differences between populations also require further investigation. It appears advantageous to treat anadromous and stationary

populations differently regarding temperature, water chemistry, and diet. However, direct comparative studies are lacking.

5.3 Factors determining parasite abundance in three freshwater fish, European perch (*Perca fluviatilis*), European whitefish (*Coregonus lavaretus*), and Arctic charr (*Salvelinus alpinus*), in an oligotrophic lake, southern Norway (Paper III)

The results of this study are consistent with the hypothesis of finding parasites previously identified in Norway, and parasite abundance was influenced by various properties of the fish. All parasites that were identified during our study have previously been described in Norway (Sterud 1999). The most common parasite species found in each host species were likely determined by the habitat and diet of the host. European perch was mainly infected by acanthocephalans, and acanthocephalans are transmitted via ostracods, isopods or amphipods (Woo 2006). In Lake Norsjø, ostracods and the isopod *Asellus aquaticus* have previously been found in stomach samples of fish (Olk et al. 2016). All of these prey items are benthic animals, that indicate a littoral diet for European perch. European whitefish had acanthocephalans and *Proteocephalus* sp. as the most prevalent parasites. *Proteocephalus* sp. is transmitted by copepods (Scholz 1999). Consequently, European whitefish appears to feed on a combination of littoral and pelagic resources. Arctic charr was mainly infected by various copepod transmitted parasites, indicating that this species primarily feeds on a pelagic diet. This is supported by the average δ^{13}C-signatures, which are most negative for Arctic charr (-29.4 ± 1.1 ‰), followed by European whitefish (-28.3 ± 2.3 ‰) and European perch (-24.9 ± 2.5 ‰) with the least negative δ^{13}C-signatures. Littoral δ^{13}C-signatures are less negative than pelagic or profundal signatures (Vander Zanden & Rasmussen 1999).

The abundances of individual parasites or groups of parasites in their respective hosts depended on sex, age, length, and season in our study. Seasonal variations were observed in *Argulus coregoni* infecting European whitefish, which was more abundant during summer. This matches its distribution found in studies on the parasite in

aquaculture (Shimura 1983; Hakalahti & Valtonen 2003; Hakalahti et al. 2004b). The ecto-parasite *Argulus coregoni* overwinters as eggs (Shimura 1983), which are unable to develop or hatch until temperatures rise to 8 – 10 °C (Mikheev et al. 2001; Hakalahti & Valtonen 2003). Eggs hatch over an extended period (Hakalahti et al. 2004a), e. g. from May to July (Shimura 1983), or during early summer (Hakalahti & Valtonen 2003). From July onwards, the number of *Argulus coregoni* infecting fish decreases again, as females detach from their hosts to lay eggs (Hakalahti & Valtonen 2003; Hakalahti et al. 2004b). Consequently, the number of *Argulus coregoni* found on European whitefish is highest in summer. The cestode *Dibothriocephalus dendriticus* exhibited seasonal variations in its abundance in Arctic charr. Fewer individuals were found in autumn. The reasons behind this seasonal variation remain unclear, as the parasite survives for several years in fish (Halvorsen & Andersen 1984). In another study, no seasonal variation in the abundance of *Dibothriocephalus dendriticus* was found (Henriksen et al. 2019). Seasonal variations were also found in the abundance of acanthocephalans in European perch. Fewer individuals were found in autumn. Acanthocephalans are recruited in spring and live for approximately one season (Woo 2006). Fewer individuals may remain in autumn.

The abundance of *Proteocephalus* sp. in European whitefish varied by sex. Higher abundances of the parasite were found in female hosts. Higher abundances of *Proteocephalus percae* in females have previously been found during the spawning season in European perch (Balling & Pfeiffer 1997). However, in our study, variations by sex did not depend on season, and may have been caused by different mechanisms. Variations by sex were also found in *Dibothriocephalus ditremus* in Arctic charr, which occurred in greater numbers in males. The underlying reasons for this remain unclear.

The abundance of several parasite groups and species in our study increased with age, or showed quadratic relationships to age, with maxima at relatively high ages. Some of these species or groups are *Proteocephalus* sp. in European whitefish, copepod transmitted parasites in European whitefish and European perch, and encysted parasites (plerocercoid larvae of cestodes) in Arctic charr. Parasite abundance generally increases with age (Khan 2012), as long-lived hosts provide a more stable habitat for

parasites, and parasites may accumulate over time (Bell & Burt 1991). Acanthocephalans in European whitefish also increased in abundance by age. Acanthocephalans can be transmitted by predation on infected fish (Woo 2006), which may support the finding of higher abundances of the parasite in older fish. Older European whitefish may to a larger extent be predatory. In Arctic charr, *Eubothrium salvelini* increased in abundance with age. This parasite is specifically shown to accumulate with age (Smith 1973; Hanzelová et al. 2002). *Triaenophorus nodulosus* also increased in abundance with age in Arctic charr. This parasite can survive in its intermediate host Arctic charr for several years, and it thus accumulates over time (Dick & Rosen 1982; Rosen & Dick 1984; Hoffmann et al. 1986).

Copepod transmitted parasites and plerocercoid larvae of cestodes in Arctic charr increased in abundance with increasing length of the host. Increases in parasite abundance by body size are commonly found (Bell & Burt 1991; Poulin 1995; 2004; Poulin & Morand 2000; Valtonen et al. 2010; Poulin & Leung 2011; Timi et al. 2011; Timi & Lanfranchi 2013; Anegg et al. 2014). Larger hosts provide a greater diversity of niches to parasites, and there is generally more space for parasites as well (Poulin 1995; Poulin & Leung 2011). Larger hosts also consume more potentially infected prey (Poulin 1997). In case of plerocercoid larvae of cestodes in Arctic charr, the increase in abundance with increasing length of the host is likely a result of accumulation of larvae over time. *Triaenophorus* sp. is able to survive in its intermediate host Arctic charr for several years, and it is able to accumulate with age or size (Dick & Rosen 1982; Rosen & Dick 1984; Hoffmann et al. 1986). The same applies to plerocercoid larvae of *Dibothriocephalus* spp. (Halvorsen & Andersen 1984; Henriksen et al. 2016). In addition, *Dibothriocephalus* spp. has the ability to re-establish in predatory fish (Hammar 2000; Henriksen et al. 2016), and larger Arctic charr may be piscivorous to some extent.

6 Conclusions and future perspectives

The aim of this thesis was to analyse a variety of challenges associated with the establishment of Arctic charr aquaculture in southern Norway. As reproduction of Arctic charr in aquaculture results in low and variable hatching success, underlying factors from brood-stock holding until hatch were reviewed (Paper II), and a common disinfection protocol was investigated (Paper I). In addition, the presence of potentially problematic parasites in the region was mapped (Paper III). The research allows to draw the following conclusions:

- The low reproductive success of Arctic charr in aquaculture is likely a result of inadequate holding conditions for brood-stock. Summer temperature, stress, diet, and poor synchronisation of spawning may have detrimental effects on egg quality in Arctic charr. Routines for fertilisation and egg incubation appear better developed, and are not of primary concern. However, these routines may also be improved in the future.

- Even though Arctic charr has its species specific requirements for culture conditions, for instance by being a stenothermic cold-water species or by tolerating high density culture, many biological processes in Arctic charr are analogous to other farmed Salmonids, and knowledge from other species is transferrable. The reproductive cycle in Arctic charr is controlled by photoperiod and temperature in the same manner as for other salmonids (reviewed by Wang et al. 2010), and high temperatures negatively influence vitellogenesis according to mechanisms found in Tasmanian Atlantic salmon at higher temperatures (King et al. 2003; Watts et al. 2004; Watts et al. 2005; King et al. 2007, Jeuthe et al. 2013).

- When disinfecting eggs of farmed Arctic charr, hand-picking during the eyed stage increased the hatching percent more than formalin treatments before the eyed stage. This was likely due to the timing of the treatments, as more water mould infections occur during the eyed stage.

- The most abundant parasite species and groups in each host species are determined by the habitat and diet of the host.

- Increases in parasite abundance were often related to increasing length or age of the host, due to the accumulation of parasites over time.

Further research on pre-hatch survival of farmed Arctic charr is encouraged, as various knowledge-gaps have been identified. The effects of summer temperature during oocyte development remain to be investigated. Temperature optima for this period remain unknown. The results of studies on the dietary fatty-acid composition and its influence on egg quality in Arctic charr remain equivocal, and there may be profound differences between anadromous and stationary populations. In addition, the effects of confinement and handling-stress on oocyte development remain to be investigated *in situ*. There are few studies concerning sperm quality parameters in Arctic charr, and suitable sperm quality parameters remain to be identified. Their effect on fertilisation success and egg survival remains to be investigated. There are also large unexplained differences in egg survival between families and populations, which require further investigation. Direct comparative studies between anadromous and stationary populations of Arctic charr could shed light on differences in temperature requirements, water chemistry requirements, and dietary requirements. Regarding water mould infections, further investigations on their dynamics in hatcheries are required to better time chemical anti water mould treatment to the time when infections are most intense. Prior to the acquisition of such knowledge, eggs can be treated with formalin during the entire incubation period to maximise hatching success. Relatively little knowledge is available on the occurrence and abundance of parasites in the region, and further investigations would be useful. There is also little information available on parasites affecting Arctic charr aquaculture in general, and parasitological studies on cultured Arctic charr would be particularly useful. For Arctic charr aquaculture, most parasites are likely of minor concern, as only ecto-parasites and copepod transmitted parasites are able to infect farmed fish. Farmed fish are kept in cages for few years, meaning that parasite accumulation over time is limited, but few parasites can reduce the market value of the meat significantly. Ecto-parasites may also become problematic, as they can infect farmed fish in large numbers. Regarding ecto-parasites, *Salmincola* edwardsii,

which has caused problems in Arctic charr aquaculture before (Piasecki et al. 2004), was indentified in Lake Norsjø infecting wild Arctic charr (Paper III).

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Article 1

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Formalin treatments before eyeing and hand-picking of Arctic charr (*Salvelinus alpinus*) eggs; re-evaluating the timing of antifungal treatments

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Summary

Arctic charr (*Salvelinus alpinus*) eggs in hatcheries are treated for fungal infections using formalin before, and hand-picking during the eyed stage. The relative effectiveness of these two treatments was evaluated using a factorial design with the application of weekly hand-picking, and formalin treatments at 380 ppm for 13 minutes three times weekly as factors. The effects of the treatments on total mortality and hatching success were compared using a two-way ANOVA. Both treatments exhibited significant, positive effects on egg survival. Hand-picking during the eyed stage (+ 0.5 to 2.5 % survival to hatch) was found to be more effective than formalin treatments before (+ 0.0004 to 0.8 % survival to hatch). This is likely due to differences in the timing of administration of the treatments. The probability of fungal infections varied with time, and a peak seemed to occur during hatching.

Sammendrag

Formalinbehandling i forkant av øyerognsstadiet og plukking av røyeegg (Salvelinus alpinus); re-evaluering av tidsrammen for soppbehandling. Egg av røye (*Salvelinus alpinus*) blir behandlet for soppinfeksjoner ved å bruke formalin før øyerognsstadiet, og ved å plukke død rogn i øyerognsstadiet. Den relative effektiviteten for disse to behandlingsformene ble bedømt i et faktorialt eksperiment, med ukentlig plukking av egg og formalin behandlinger med 380 ppm formalin i 13 minutter og tre ganger i uken som faktorer. Effekten på dødeligheten av egg ble sammenlignet i en to-veis ANOVA. Begge behandlingene hadde en signifikant, positiv effekt på eggoverlevelse. Plukking i øyerognsstadiet (+ 0.5 til 2.5 % overlevelse til klekking) var mer effektivt enn formalinbehandlingen i forkant (+ 0.0004 til 0.8 % overlevelse til klekking). Dette skyldes sannsynligvis tidsmessige forskjeller i eggutviklingen når behandlingene ble utført. Sannsynligheten for utbrudd av soppinfeksjoner er indikert til å variere over tid, og den synes å være høyest under klekking.

Introduction

Commercial freshwater fish farming in Norway is a small industry despite abundant freshwater resources and its potential to generate wealth in sparsely populated areas. The slow growth of Norwegian freshwater fish farming is largely a result of restrictive policies, which are anchored in environmental concerns connected to eutrophication, escaped fish and pathogens (Haug et

al. 2006). One of the most commonly farmed species in Norwegian freshwater is Arctic charr (*Salvelinus alpinus*), due to its low temperature requirements (Brännäs & Linnér 2000; Siikavuopio et al. 2009; Siikavuopio et al. 2010), its tolerance to high density conditions, and its amenability to niche markets (Johnston 2002; Summerfelt et al. 2004; Skybakmoen et al. 2009; Jobling et al. 2010). Arctic charr farmers have few objections to the strict regulations, as an environmentally friendly image suits the market (Sæther et al. 2013). However, some environmental impact is unavoidable to maintain the entire production cycle of Arctic charr. Disinfection and disease control for instance require some use of hazardous chemicals.

Fungal infections of eggs are a common problem in salmonid hatcheries (e.g. Barnes et al. 1997). They are also abundant at our study site, Telemarkrøye AS (an Arctic charr hatchery in Fyresdal, southern Norway). Formalin is still widely used in aquaculture (Boyd & McNevin 2015; Leal et al. 2016). Formalin is effective at treating fungal infection of salmonid eggs (Burrows 1949; Marking et al. 1994; Waterstrat & Marking 1995; Schreier et al. 1996; Barnes et al. 1997; Barnes et al. 2000; Arndt et al. 2001; Barnes et al. 2001). However, formalin is toxic to aquatic organisms, such as fish, amphibians, invertebrates and microorganisms (Kitchens et al. 1976), if the effluent is insufficiently treated (Katz 1989; Marking et al. 1994; GESAMP 1997; Leal et al. 2016). Formalin is also carcinogenic in humans (reviewed by Swenberg et al. 2013), thus poses a risk to hatchery workers if not properly handled. As an alternative to formalin treatments, dead eggs can be removed by hand-picking. It can be performed safely during the eyed stage (Barnes et al. 1997). Chemical fungal infection treatments are necessary before the eyed stage (Piper et al. 1982; Post 1987).

To balance the negative environmental and health effects from formalin treatment with its benefits of reducing the amount of eggs lost to fungal infections, the current disinfection protocol at our study site combines formalin treatment before the eyed stage with hand-picking during the eyed stage. This protocol is generally recommended for Arctic charr (Johnston 2002). The combination of the different approaches, however, obscures their relative effectiveness. In this study, a factorial design under high exposure to fungal infections is used to determine the relative effectiveness of the factors formalin treatment and hand-picking in this particular protocol. In addition, mortality and the presence of fungal infections over time are monitored to generate further insight to the dynamics of fungal infections and egg mortality. In light of the findings, possible adaptions to the protocol are proposed.

Material and Methods

Ethical Statement

No ethical consent was required for this study. Adult fish and hatched larvae were handled by trained personnel of Telemarkrøye AS, following their standard procedures for production. Eggs of Arctic charr were used in the experiment, and handled by the researchers after fertilization until hatch. This life stage is not covered by the Norwegian legislation on the use of animals in research (Lovdata 2018). According to §2, larvae of vertebrates are first included in the regulation when they feed independently. Persistent harm or pain in later life stages was not expected as a result of this study. All experimental procedures were usual husbandry practice.

Fish

All gametes used in this study derive from the hatchery brood-stock at Telemarkrøye AS. The brood-stock fish primarily originate from the nearby Lake Fyresvatn, and were caught in 2011. Their offspring hatched in captivity are included in the brood-stock without any direct artificial selection. Two half-sibling (half-sib) families and one full-sib family were used in our study. The first two families derived from one five-year-old female each, and were fertilized by two males. The females were hatched in captivity. The two males used in these two families were wild fish. In the third family, only one female

and one male were used, both hatchery reared five-year-old individuals.

Fertilization protocol

All brood-stock individuals used in the experiment were checked weekly for ripeness. Ripe fish were sedated by clove oil (Scan Aqua AS, Årnes, NO), using a bathing treatment at 0.32 g L^{-1}. The genital papilla was wiped dry, and gametes were removed by gentile abdominal massage (stripping), carefully avoiding contamination with urine, faeces or mucus. Unfertilized eggs were rinsed using physiological saline solution. Milt was stripped into a beaker and transferred to the eggs by a syringe. Fertilization occurred in plastic buckets containing physiological saline solution for 2 minutes in darkness. The fertilized eggs were rinsed in physiological saline solution, and disinfected in a buffered iodophore solution (PHARMAQ Ltd, Fordingbridge, Hampshire, UK) following the manufacturers protocol.

Incubation environment

All eggs were incubated in a vertical flow incubator (Alvestad Marin, Oslo, NO) in two compartments (drawers), which were divided into 30 rectangular plots of ≈ 100 cm^2 each using 6 cm high PVC-plates (Fishtech, Vestby, NO) with circular perforations with a diameter of 2 mm (Figur 1). The plates were attached on the 20.10.2017 using a silyl-modified polyether glue (Relekta, Oslo, NO), certified as toxicant free. The compartments were disinfected using buffered iodophores (PHARMAQ Ltd, Fordingbridge, Hampshire, UK) and rinsed with hatchery water before transfer to the incubator on the 26.10.2017. The water flow rate in the incubator was 1.5 L min^{-1}. Incubation occurred at temperatures between 6.2 and 7.7 °C, recorded once daily (Supplement 3). The accumulation of degree days (dd) were calculated as the sum of all daily temperature measurements up to, and including, the corresponding day. Major water chemistry was analyzed weekly during this study to indicate growth conditions of the water moulds identified (Table 1).

Figur 1 Experimental plot for one chemical treatment group. Photographer: Tom Robin Olk.

Experimental setup

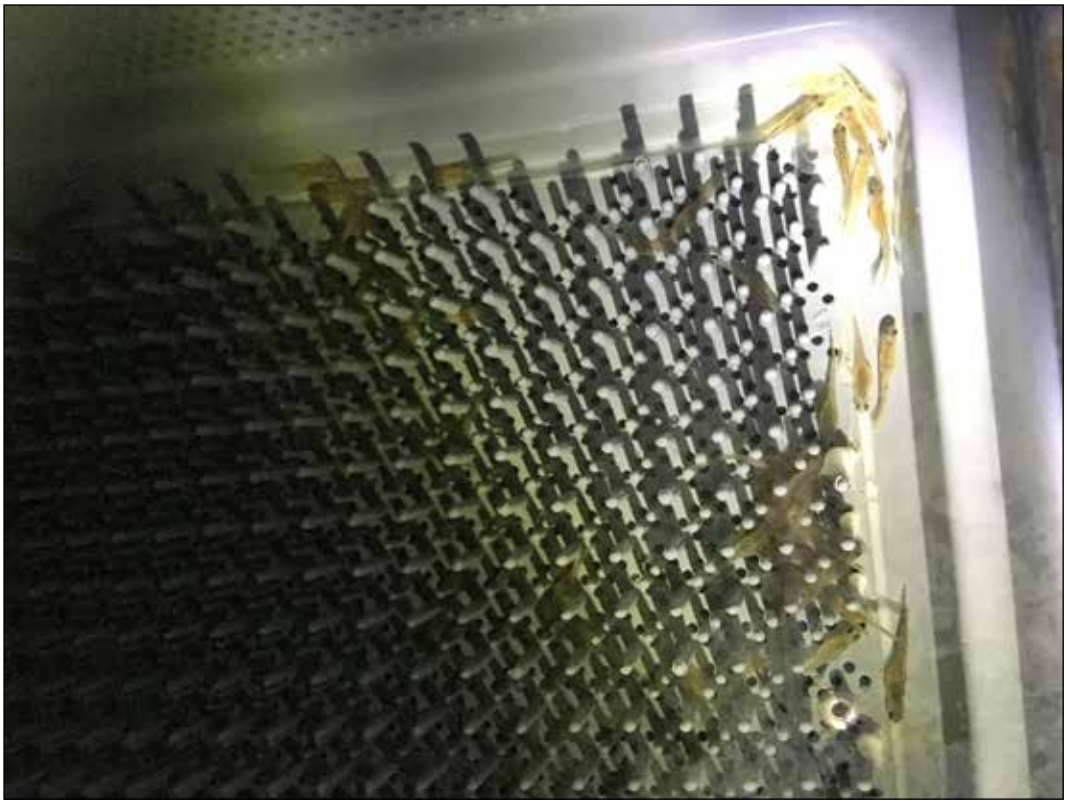
The disinfected eggs were submerged in physiological saline solution and transferred to counting plates for 100 eggs (originally designed for rainbow trout (*Oncorhynchus mykiss*)) (Fiap Gmbh, Ursensollen, GER). The plates were rinsed in physiological saline solution until each depression contained eggs with no additional eggs on the plate. As eggs of Arctic charr are relatively small compared to eggs of rainbow trout, there were either one or two eggs in each depression. Two counting plates, i.e. between 200 and 400 eggs, were transferred to each plot. The eggs were allowed to swell, using the municipal freshwater source in the hatchery (Table 1). Both fertilization and stocking of the incubator were conducted on the 26.10.2017. Three treatments were assigned to 15 plots each, formalin treatment only, formalin treatment and hand-picking, and hand-picking only. One untreated control group was assigned to 15 plots. One of the incubator compartments was treated with 20 mL formalin solution (formaldehyde: 380 mg mL^{-1} with 10.8 – 13.2 Vol-% methanol; Cenavisa S. L., Reus, ESP) added at the upstream end of the compartment. The formalin concentration was 380 ppm (mg L^{-1}). The formalin added was

Table 1: Water chemistry data for hatchery water during autumn.

Date	pH	H^+ (µeq L^{-1})	Alkalinity (µeq L^{-1})	Conductivity (µS cm^{-1})	Turbidity NTU	Ca^{2+} (mg L^{-1})	Mg^{2+} (mg L^{-1})	Na^+ (mg L^{-1})
26.10.2017	7.53	0.03	999	NA	NA	NA	NA	NA
01.11.2017	7.72	0.02	1016	95.9	0.14	17.84	0.45	1.56
09.11.2017	7.57	0.03	979	94.0	0.32	17.82	0.44	1.49
16.11.2017	7.62	0.02	1077	110.7	0.33	20.70	0.51	1.88
23.11.2017	7.73	0.02	1036	100.9	0.24	18.94	0.49	1.87
30.11.2017	7.72	0.02	1017	97.6	0.37	18.38	0.45	1.77
07.12.2017	7.60	0.03	1002	103.5	0.25	18.94	0.47	1.69
14.12.2017	7.76	0.02	967	98.5	0.26	18.26	0.46	1.65
21.12.2017	7.71	0.02	1035	100.0	0.23	18.80	0.45	1.53

K^+ (mg L^{-1})	NH_4^+ (µg L^{-1})	SO_4^{2-} (mg L^{-1})	NH_3 (mg L^{-1})	TOC (mg L^{-1})	TotP (µg L^{-1})	TotN (µg L^{-1})
NA	< 50	NA	NA	NA	NA	NA
0.47	< 50	2.38	1.56	0.8	3.05	167.5
0.43	< 50	2.34	1.49	0.7	0.99	160.5
0.49	< 50	3.23	1.88	0.9	4.21	178.5
0.49	< 50	3.33	1.87	0.9	0.19	109.5
0.39	< 50	2.84	1.77	1.1	0.59	123.0
0.44	< 50	2.84	1.69	0.9	3.33	150.0
0.44	< 50	2.66	1.65	1.1	2.64	142.0
0.44	< 50	2.40	1.53	1.3	0.39	108.5

transported out of the compartment by the ordinary effluent with a water residence time of about 13 minutes. The formalin treatment was conducted three times every week before the eyed stage. The treatments were implemented at 13, 15, 18, 20, 22, 25, 27, 29, 32 and 34 days post fertilization (dpf). Both formalin treated groups were kept in one compartment. The other compartment was not treated chemically. Hand-picking, by using forceps, was conducted weekly, beginning at the onset of the eyed stage at 35, 42, 49, 56, 63, 70, 77 and 84 dpf. During hand-picking, dead eggs, empty egg shells, and larvae were counted. The larger number of either hatched larvae or empty egg shells present was registered as a measurement for the number of hatched larvae. Subsequently, empty eggshells and dead larvae were removed. Living larvae escaped the plots through slits in the bottom of the tray into the hatching substrate (Figur 2), and were only counted once. In the plots, which were not hand-picked, dead eggs, larvae and empty egg shells were counted at 84 dpf. The presence of fungal infections was recorded for each plot, defined as visible hyphal infection on at least one egg, larvae or empty eggshell (Figur 3). All eggs were incubated in darkness, and hand-picking was conducted with a headlamp (Black Diamond, Salt Lake City, UT, USA). At the end of the experiment, infected eggs were collected, stored in either hatchery water or 70 Vol-% ethanol, and sent to the Norwegian Veterinary Institute (Veterinærinstituttet) for taxonomical identification of the water mould by morphological traits and genetic markers, respectively. Water moulds from eight eggs, that were infected by hyphae visible to the unaided eye, were cultivated using glucose yeast extract



Figur 2. Hatched larvae that have escaped into the hatching substrate beneath the experimental plots were collected at the end of the experiment (18.01.2018). Photographer: Tom Robin Olk.

(GY) agar, which is especially suitable for cultivation of Saprolegnia. The medium also contained antibiotics. Four eggs were analyzed by direct polymerase chain reaction (PCR) and gene sequencing, using specific ITS-primers for oomycetes.

Data analysis

The total number of eggs, dead eggs, and hatched larvae were calculated for each hand-picked plot by the following equations:

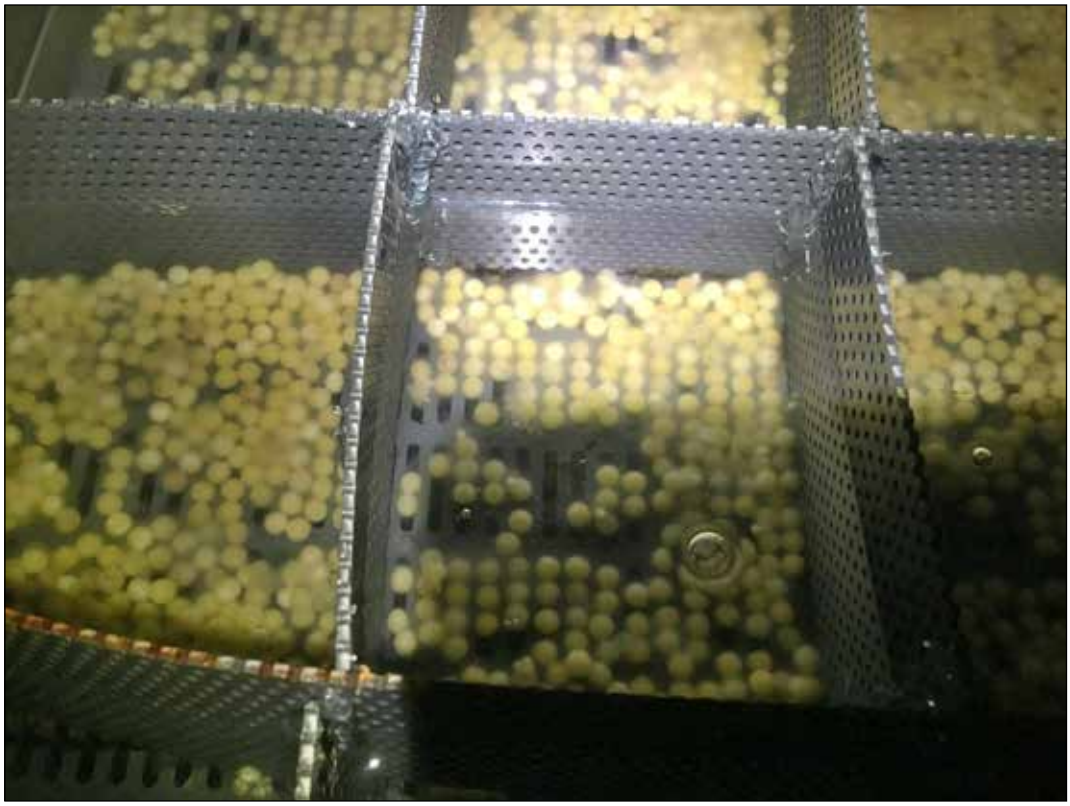
$$N_{TotalDead} = \sum_{i=1}^{8} N_{Deadi} \quad \text{Equation 1}$$

$$N_{TotalHatched} = \sum_{i=1}^{8} N_{Hatchedi} \quad \text{Equation 2}$$

and,

$$N_{Total} = N_{TotalDead} + N_{TotalHatched} \quad \text{Equation 3}$$

The calculations were performed for dead, hatched and total number, respectively. Observation *i* refers to the individual observation on the corresponding sampling date. There were eight observations per plot. The proportions of accumulated hatch at the end of the experiment (84 dpf) were arcsine-transformed to stabilise their variances (Ott & Longnecker 1984), and compared using a two-way ANOVA in R (R Core Team 2017). As explanatory factors, the use of formalin, hand-picking and an interaction between these two factors were included. Individual between-group differences were evaluated with Tukey's multiple comparison test in R. Results at a significance level of $\alpha = 0.05$ were accepted as significant. Results are reported as percentages, where the total number of eggs incubated in the respective plot(s) represents 100 %.



Figur 3. Arctic charr eggs in the unpicked experimental plots towards the end of the experiment (04.01.2018). There are some eyed eggs in the lower middle plot. Large accumulations of water mould hyphae make egg masses appear blurry, especially in the upper middle plot. Photographer: Tom Robin Olk.

Results

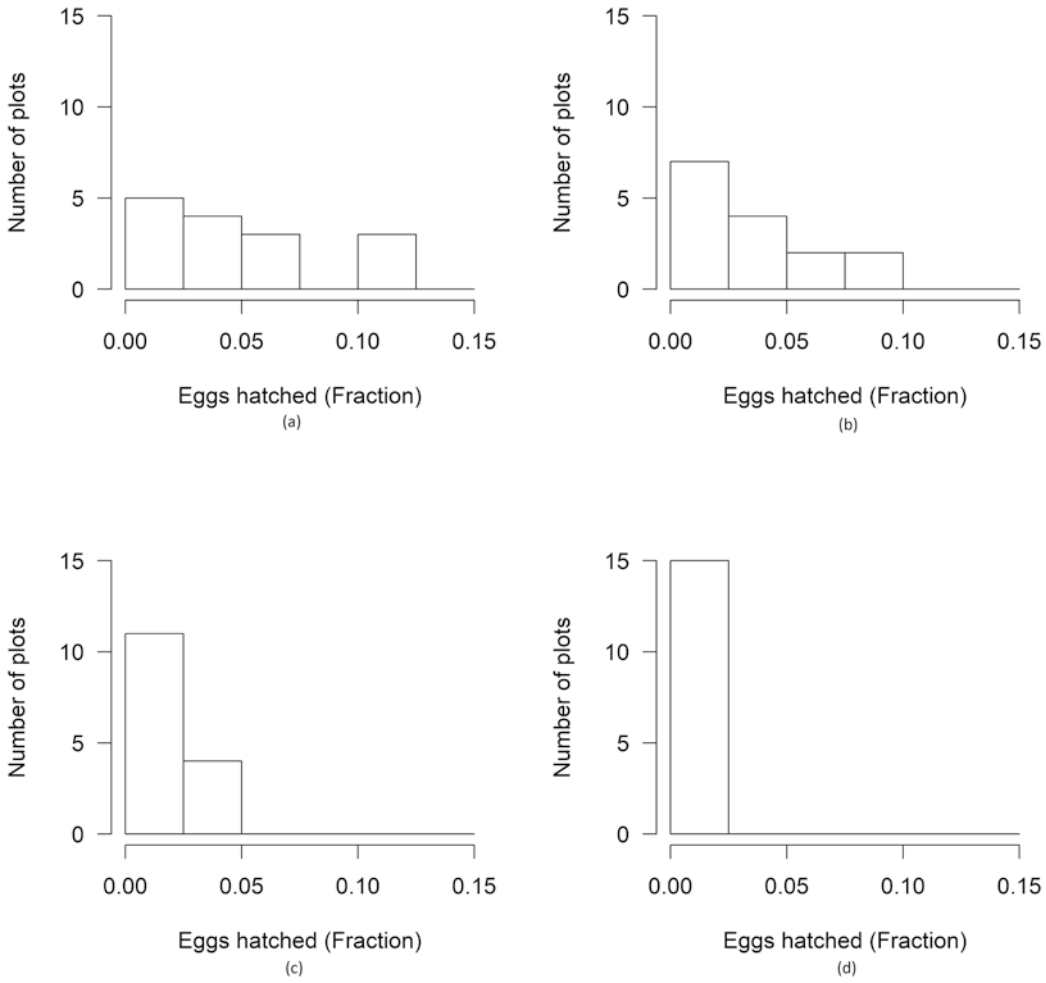
Total percentage hatched

The average proportion of hatched larvae for the entire incubation was low, and exhibited values of 5 ± 4, 3 ± 3, 1 ± 1, and 0 ± 0 % for formalin treated and picked, picked untreated, formalin treated not picked, and control groups, respectively (Figur 4).

The proportion of hatched larvae for the entire incubation differed significantly between the hand-picked and non-hand-picked groups (ANOVA, df = 1, F = 27.19, $p < 0.001$), and between groups treated with formalin and non-treated groups (ANOVA, df = 1, F = 4.43, p = 0.040). Both treated groups and hand-picked groups exhibited higher hatching rates than groups that were not treated with formalin or hand-picked. No significant interaction between hand-picking and formalin treatment was found (ANOVA, df = 1, F = 0.983, $p > 0.100$). Group differences in hatching proportion between formalin treated and non-treated groups were estimated to 0.2 % (Tukey's multiple comparison test: 95 % Confidence interval: 0.0004 – 0.8 %). Group differences in hatching proportion between picked and non-picked groups were estimated to 1.3 % (Tukey's multiple comparison test: 95 % Confidence interval: 0.5 – 2.5 %) (Tab. 2).

Identification of water moulds

No axenic culture of a single water mould species could be separated, and identified by morphological features. None of the water moulds that grew in culture resembled any species of the genus *Saprolegnia*. Instead, unidentified mould and bacteria grew. The analysis using PCR and gene sequencing revealed several different PCR



Figur 4. Number of plots of the different treatments exhibiting various hatching success measured as fraction of the total number of eggs. (a) Hand-picked and formalin treated plots; (b) Hand-picked not formalin treated plots; (c) Not hand-picked formalin treated plots; (d) Not hand-picked and not formalin treated plots.

Table 2: Differences in the proportions of hatched eggs between plots that were either not treated with formalin and not hand-picked, not treated with formalin and hand-picked, treated with formalin and not hand-picked, or treated with formalin and hand-picked.

Formalin Group 1	Picking Group 1	Formalin Group 2	Picking Group 2	Difference Hatch Proportion (%)	Lower Limit (%)	Upper Limit (%)	P
Yes	No	No	No	0.5	0.0	2.2	> 0.100
No	Yes	No	No	1.8	0.3	4.7	< 0.001
Yes	Yes	No	No	2.6	0.6	5.8	< 0.001
No	Yes	Yes	No	0.5	0.0	2.3	> 0.100
Yes	Yes	Yes	No	0.9	0.0	3.0	0.021
Yes	Yes	No	Yes	0.1	-0.3	1.1	> 0.100

bands for two of the samples, meaning that several unidentified species of water moulds were present. The two remaining samples could be identified, and exhibited 99 % correspondence to *Pythium monospermum* and 96 % correspondence to *Aphanomyces stellatus* as the closest matches in the gene bank, respectively.

Discussion

Total percentage hatched

Hatching success of all egg batches in this study is extremely low, with an average egg survival of 5 ± 4 % in the treatment group, and 12.2 % in the plot with the highest performance, respectively. The underlying reasons for the low survival are likely linked to egg quality and the fertilization process. In addition, some additional mortality is caused by fungal infections, as the effort invested in antifungal treatments was low compared to other studies (Barnes et al. 1997; Barnes et al. 2000; Barnes et al. 2003). Plots of all treatments were stocked with eggs of similar origin, meaning that egg quality and the fertilization process influenced total egg survival, but not the relative differences between treatments. The incubation environment of the eggs was also consistent between treatments regarding the type of incubator, stocking density, temperature, and water chemistry.

According to the comparison between all four treatments, both the application of formalin treatment three times weekly before the onset of the eyed stage, and hand-picking of dead eggs during the eyed stage, increased the number of hatched eggs. Formalin treatments at various stages, times of exposure and concentrations, have previously been shown to enhance survival of eggs of rainbow trout (Marking et al. 1994; Schreier et al. 1996; Barnes et al. 2000; Arndt et al. 2001), and fall Chinook salmon (*Oncorhynchus tshawytscha*) (Waterstrat & Marking 1995; Barnes et al. 1997; Barnes et al. 2003). Contrary, studies on rainbow trout and blueback salmon (*Oncorhynchus nerka*) (Burrows 1949), and brown trout (*Salmo trutta*) (Barnes et al. 2001) did not exhibit any clear improvement in survival caused by the use of formalin. However, the authors concluded in both cases, that this was due to absence of extensive fungal infections. Despite the agreement between the results of our study with previous studies on the effectiveness of formalin treatments *per se*, the benefit of hand-picking appeared to be much greater than the effect of formalin treatment in our study. In addition, the effect of hand-picking on egg survival in Arctic charr is likely underestimated in our study, as hand-picked and unpicked plots were kept in the same compartments, and spores may have spread from unpicked to picked plots. Contrary, formalin treated and untreated plots were kept in different compartments with separate in- and outflows, rendering cross-contamination unlikely. This is inconsistent with the results of previous studies comparing the effectiveness of the daily administration of formalin to hand-picking, which resulted in an increase in hatched eggs by 3 – 5 % in the formalin treated groups (Barnes et al. 1997; Barnes et al. 2000; Barnes et al. 2003).

Theoretically, formalin treatments should be more effective, as microscopic zoospores and hyphae, that would not be removed by hand-picking (Smith et al. 1985; Rand & Munden 1993), could weaken additional egg membranes, and ultimately result in an increased number of failed hatches in the picked groups (Burrows 1949). Fungicides, such as formalin, would likely remove large proportions of these microorganisms (Willoughby & Roberts 1992). However, this theory could not be confirmed in later investigations on fungal infections in hatcheries on eggs of rainbow trout (Kitancharoen & Hatai 1996) and Atlantic salmon (*Salmo salar*) (Thoen et al. 2011). Effects of egg mortality caused directly by the treatment are expected to be larger for hand-picking than for formalin treatments, as hand-picking may cause physical harm to living fish eggs as hypothesized for brown trout and Atlantic salmon (Sutela et al. 2007). Formalin did not produce toxic effects to rainbow trout eggs at an exposure of 5000 ppm for 15 to 30 minutes (Marking et al. 1994). Consequently, such effects are expected to bias the results in favour of more effective formalin treat-

ments, thus they are not the underlying reason for more effective hand-picking found in our study.

However, several underlying reasons for the discrepancy between the results of our study and previous studies can be outlined. In our study, formalin treatments were administered before the onset of the eyed stage, while hand-picking was conducted at weekly intervals throughout the eyed stage until hatch. This may strongly influence the results, as the extent of fungal infections likely varied throughout development. Barnes et al. (1997) observed higher mortalities during the eyed stage in groups of fall Chinook salmon eggs that were only hand-picked, indicating that fungal infections may cause lower mortality rates before. One underlying reason might be the accumulation of fungal spores and hyphae throughout the incubation period, also observed in all not hand-picked plots in this study. The accumulation of spores of *Saprolegnia ferax* throughout the breeding season has previously been demonstrated in field experiments on egg masses of amphibians (Kiesecker & Blaustein 1997). In our experiment, the plots were only separated by perforated PVC-walls. Spores and freely floating hyphae may have contaminated other plots, while infected eggs were too large to cross the barriers. Contamination between plots by vegetative growth of hyphae was not observed. Consequently, hand-picking was likely more effective in this particular protocol, as it was administered when infections were most intense.

Contrary, Johnston (2002) empirically claimed, that problematic fungi mainly occur during the pre-eyed stage. As no experimental evidence was provided, this may also be the result of different protocols or other confounding factors. Differences in the susceptibility to physical damage to eggs during hand-picking or the resistance to formalin between species may also be a cause for the conflicting results. We are not aware of previous studies investigating the use of formalin to disinfect eggs of Arctic charr. In addition, the previous studies documenting increased survival in formalin treated groups compared to hand-picked groups (Barnes et al. 1997; Barnes et al. 2000; Barnes et al. 2003), were based on daily formalin treatments and hand-picking. In our study, treatments were conducted less frequently. The lower total effort invested in our treatments may have had a larger impact on the effectiveness of formalin than on the effectiveness of hand-picking.

Fungal infections in our study were more intensive than in other studies, which may also have influenced the relative effectiveness of hand-picking and formalin treatments. Hand-picking effectively removes infected eggs as soon as the infection becomes visible by the unaided eye. Contrary, a formalin treatment at a concentration of 380 ppm formalin for ca. 13 min was likely only inhibiting fungal growth without eliminating their active presence in water (Marking et al. 1994). This means that it was merely effective as a prophylactic measure prior to the establishment of visible hyphae. However, as the formalin treatment in our study was applied prior to the onset of excessive fungal growth in the treated compartment, higher concentrations of formalin would not have been necessary to prevent fungal growth.

Identification of water moulds

Evidence for the presence of several different species of water moulds in eggs of Arctic charr was found. Two samples exhibited multiple PCR bands, which matched different oomycetes. In addition, unidentified mould could be grown in culture, and hyphae were frequently observed in the trays during incubation of the eggs. In one sample, *Pythium monospermum* was identified using PCR and gene sequencing. The sequenced DNA exhibited a 99 % match to this species. A 96 % match to *Aphanomyces stellatus* was detected in another sample, meaning that either *Aphanomyces stellatus*, or a closely related species, was identified. *P. monospermum* is primarily known as a plant pathogen, also found in river water (Matsiakh et al. 2016), while *Aphanomyces* sp. are found in soil or water as saprotrophs or parasites of roots, algae, aquatic fungi, insects, crayfish or fish (Markovskaja 2007). Species of both genera were previously found on

fish eggs in Polish hatcheries (Czeczuga & Woronowicz 1993). Data on water chemistry in hatcheries with fish eggs infected by the identified species of oomycetes remains insufficient to draw general and detailed conclusions on the conditions promoting fungal growth. However, the pH was similar in our study (7.53 – 7.76) and the hatcheries investigated by Czeczuga & Woronowicz, (1993), where either *Pythium* sp. or *Aphanomyces* sp. were found (7.5 – 8.35).

Possible adaptions to the protocol

This study demonstrates, that both formalin treatment before the eyed stage and hand-picking during the eyed stage increased survival until hatch of hatchery reared eggs of Arctic charr, even under severe pressure of fungal infections. However, the discrepancy between the relative effectiveness of formalin and hand-picking between our study and previous studies (Barnes et al. 1997; Barnes et al. 2000; Barnes et al. 2003) indicates, that the limitation of the use of formalin to the period before the eyed stage allows for additional mortality during the eyed stage. This is especially problematic, as higher mortalities caused by fungal infections were reported during the eyed stage in fall Chinook salmon (Barnes et al. 1997). The results of our study indicated the possibility of a higher risk of fungal infections during hatch. Consequently, the use of formalin during the entire incubation period, eventually combined with hand-picking is one alternative, maximising survival until hatch. It would also be economically beneficial to combine prolonged formalin treatment with reduced hand-picking effort, as hand-picking is a laborious procedure (Leitritz & Lewis 1976) generating high operating expenses at the hatchery. This adaption to the protocol is designed to maximise egg survival without considering the environmental and health concerns regarding formalin. Thus it should only be applied until sufficient information on the dynamics of fungal infections in Arctic charr eggs is available to develop appropriate protocols, which synchronise the application of formalin to stages of higher infection risk.

Conclusions

Combining formalin treatment before the onset of the eyed stage with hand-picking during the eyed stage to treat fungal infections in hatchery reared eggs of Arctic charr does not balance the environmental and health concerns connected to formalin and its efficiency as a fungicide in an adequate way. Antifungal treatment during the eyed stage appears to have the largest total impact on egg survival through hatch, meaning that formalin is inefficiently used in this protocol. Based on our current knowledge on the dynamics of fungal infections, formalin should be administrated during the entire incubation period to maximise hatching success and reduce labour expenses. Further research on the dynamics of fungal infections in hatcheries is encouraged to render chemical treatments more effective by synchronising their administration to peaks in infection risk. Our study indicates that one major infection peak may occur during hatch.

Supplementary material

Supplements, data, software code, and model figures are available at dx.doi.org/10.23642/usn.7334573.

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Article 2

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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

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

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

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 land-locked 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-

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

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

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

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

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

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

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)

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).

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

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

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

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

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

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

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

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

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,

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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Article 3

Olk, T. R., A.-C. Henriksen, S.I. Dolven, M.L. Haukø, E. Lydersen, and T.A. Mo. 2020. Factors determining parasite abundance in three freshwater fish, European perch (*Perca fluviatilis*), European whitefish (*Coregonus lavaretus*), and Arctic charr (*Salvelinus alpinus*), in an oligotrophic lake, southern Norway. Fauna Norvegica **40**: 109 – 129. doi: 105324/fn.v40i0.3444

Factors determining parasite abundance in European perch, *Perca fluviatilis*, European whitefish, *Coregonus lavaretus*, and Arctic charr, *Salvelinus alpinus*, in an oligotrophic lake, southern Norway.

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Olk TR, Henriksen A-C, Dolven SI, Leithe Haukø M, Lydersen E and Mo TA. 2020. Factors determining parasite abundance in European perch, *Perca fluviatilis*, European whitefish, *Coregonus lavaretus*, and Arctic charr, *Salvelinus alpinus*, in an oligotrophic lake, southern Norway. Fauna norvegica 40: 109–129.

Temporal and spatial variations in macroparasite status were investigated in European perch, *Perca fluviatilis*, European whitefish, *Coregonus lavaretus*, and Arctic charr, *Salvelinus alpinus* in Lake Norsjø (Southern Norway), based on gillnet fishing in three locations in the spring, summer, and fall 2018. In addition, length, weight, age, sex, $\delta^{13}C$, and $\delta^{15}N$ were determined. Parasite abundance was modelled using negative binomial generalized linear models in relation to fish metrics, season, and sampling location. The most prevalent parasite species were determined by the diet and habitat of the host. European perch was mainly infected by acanthocephalans, European whitefish mainly infected by acanthocephalans and cestodes, and Arctic charr mainly infected by cestodes. The most prevalent parasites in European perch are transmitted by benthic animals. Parasites in European whitefish are transmitted by both benthic animals and copepods, while the most prevalent parasites in Arctic charr are copepod transmitted. This corresponds well with the $\delta^{13}C$ signatures in the three species, indicating that European perch primarily fed in the littoral zone ($\delta^{13}C$: -24.9 ± 2.5 ‰), Arctic charr in the pelagic and profundal zone ($\delta^{13}C$: -29.4 ± 1.1 ‰), while European whitefish both fed in the littoral and pelagic zone ($\delta^{13}C$: -28.3 ± 2.3 ‰) of Lake Norsjø. Individual abundances of parasites depended on host age, length, sex, $\delta^{15}N$, and season. Positive correlations between parasite abundance and host age, length, or trophic level measured as $\delta^{15}N$ were most common, and occurred in all three host species. Many parasites accumulate with age, and larger hosts provide more diverse habitats for parasites.

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INTRODUCTION

Species composition of parasite communities and parasite abundance in freshwater fish are determined by various biotic and abiotic factors. Abundance, which is the number of parasites in an individual host, is subject to higher fluctuations due to stochastic factors than species composition (Timi *et al*. 2010; Timi & Lanfranchi 2013; Locke *et al*. 2013). One of the most important determinants of parasite community structure and abundance is host ecology (Kennedy 1978; Bush *et al*. 1990; Poulin 1995; Locke *et al*. 2014), and parasite communities are highly dependent on habitat and diet of the host (Knudsen *et al*. 2008). Parasite abundance can be linked to information on niche use, for instance by analysis of stable carbon isotope signatures ($\delta^{13}C$), stomach samples or head shape (Knudsen *et al*. 2014). Parasite abundance is also influenced by the age of the host (Khan 2012), as longevity provides a more stable habitat for parasites and allows for accumulation of parasites over time (Bell & Burt 1991). Increasing host body size also increases parasite abundance (e. g. Poulin 2000, 2004; Valtonen *et al*. 2010; Timi & Lanfranchi 2013; Anegg *et al*. 2014). Larger hosts provide a greater diversity of niches and more space for parasites (Poulin 1995; Poulin & Leung 2011), as well as they consume more food, increasing the chance of contracting a parasite infection (Poulin 1997). Larger fish also feed on larger prey, with higher diversity of potential parasites (Timi *et al*. 2011). However, not all studies confirm higher parasite abundance for larger hosts (Balling & Pfeiffer 1997; Poulin 2007; Luque & Poulin 2008). Another factor influencing parasite community structure and abundance is the trophic position of the host (Luque & Poulin 2008; Timi *et al*. 2011; Alarcos & Timi 2012), with higher diversities of parasites observed at higher trophic levels (Chen *et al*. 2008; Knudsen *et al*. 2008).

Especially piscivorous fish have higher abundances of parasites (Valtonen *et al*. 2010), as parasites accumulate in predators through the food chain (Valtonen & Julkunen 1995), and some parasites are able to reestablish in predatory fish (Bérubé & Curtis 1984; Sandlund *et al*. 1992). However, not all studies reveal a correlation between parasite abundances and trophic position (Knudsen *et al*. 2014). In some studies, parasite abundance is also shown to vary by sex (Balling & Pfeiffer 1997). In addition, some parasite-host systems exhibit seasonal fluctuations in parasite abundance (Andersen 1978; Scott & Smith 1994).

Studies on parasites of freshwater fish in southern Norway have mainly been conducted during the 1950s to 1970s, and focused on the parasites development and lifespan (Lien 1970), single parasite-host interactions (Halvorsen 1970), the occurrence of parasites (Vik 1959, 1963; Borgström & Lien 1973), and the occurrence of parasites combined with seasonal variations in abundance (Halvorsen 1968, 1972; Skorping 1977, 1981; Andersen 1978). However, there is one recent publication investigating parasite community structure and abundance related to trophic niche and habitat in southern Norway (Paterson *et al*. 2019). In this study, we have explored factors determining the abundance of numerous macroparasites in three species of freshwater fish, Arctic charr, *Salvelinus alpinus* (Linnaeus, 1758), hereafter: charr, European perch, *Perca fluviatilis* Linnaeus, 1758, hereafter: perch and European whitefish, *Coregonus lavaretus* (Linnaeus, 1758), hereafter: whitefish, in a large, oligotrophic lake in southern Norway.

MATERIAL AND METHODS

Study Site

Lake Norsjø is a large (55.48 km^2) lake in Vestfold and Telemark county, South-Eastern Norway. The lake is 30 km long and has an average width of 3 km and a lake volume of 5.1 km^3. Its maximum depth is 171 m, with a mean depth of 87 m. Lake Norsjø is located at 15 m. a. s. l. as part of the Telemark watercourse (Vann-Nett 2019). Three main rivers enter Lake Norsjø, which are all draining mountain areas north west in the county. In south, Lake Norsjø is dammed at Skotfoss, the outlet of the lake, where the River Skienselva starts. The lake regulation is minor, only < 1 m (Vann-Nett 2019). Lake Norsjø is classified as an oligotrophic Norwegian lake (Lyche Solheim & Skotte 2016). Human activities with moderate impacts on the lake are agriculture, industry, and recreational activities (Vann-Nett 2019). According to Jensen (1954), Lake Norsjø houses charr, brown trout, *Salmo trutta* Linnaeus, 1758, Northern pike, *Esox lucius* Linnaeus, 1758, perch, whitefish, Atlantic salmon, *Salmo salar* Linnaeus, 1758, European smelt, *Osmerus eperlanus* (Linnaeus, 1758), European eel, *Anguilla Anguilla* (Linnaeus, 1758), Crucian carp, *Carassius carassius* (Linnaeus, 1758), river lamprey, *Lampetra fluviatilis* (Linnaeus, 1758), Eurasian minnow, *Phoxinus phoxinus* (Linnaeus, 1758), tench, *Tinca tinca* (Linnaeus, 1758), and three-spined sticklebacks, *Gasterosteus aculeatus* (Linnaeus, 1758).

Fish were sampled in three locations north, in the middle, and south of Lake Norsjø (Figure 1). The northern sampling site is located in Årnes Bay close to the inlets of River Bøelva and River Sauarelva. Årnes Bay is mostly shallow (< 10 m depth) with a maximum depth about 30 m. The area is surrounded by wetland vegetation. The sampling site in the middle of the lake is located by the inlet of River Eidselva close to the town Ulefoss. This area is deeper with a maximum depth up to 60 m. The southern sampling site is located

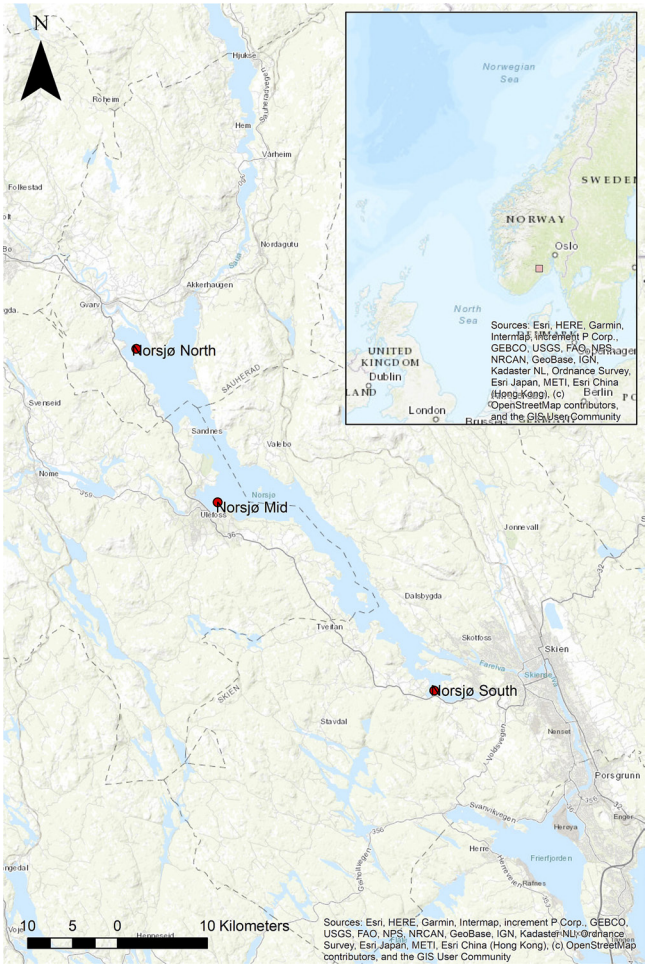

Figure 1. The study area Lake Norsjø with three study locations, Norsjø North (59.371806°N 9.191388°E), Norsjø Mid (59.288811°N 9.280637°E), and Norsjø South (59.214154°N 9.472426°E). Source: ArcGIS (ESRI 2012).

where the lake divides into two areas. One of the areas is the relatively shallow (< 30 m depth) outlet area at Skotfoss. The other area is Fjærekilen Bay, which is deeper (> 60 m depth). The sampling site is located in Fjærekilen Bay.

Sampling

Fish were sampled three times in 2018, late May (spring), late July (summer), and mid-September (fall). Standard bottom-set gill nets (1.5 m ∗ 25 m) with mesh sizes between 13.5 and 45 mm were used. Six series of eight gill nets were created. Two series were deployed at each location for each sampling season (Table 1). The nets were set in the morning, and collected 24 h later. The nets were set from the shore in a 90° angle. Linked nets were 200 m in length, and sampling occurred between depths of 2 m and 40–60 m, covering both the epilimneon and hypolimneon. In the south of Lake Norsjø, maximum sampling depth was approximately 20 m. Totally, 258 perch, 101 whitefish, and 173 charr were caught. The catches per season and location are compiled in Table 2. A sub-sample of 75 perch, 75 charr, and 50 whitefish was randomly selected for further analysis.

Fish

The fish were frozen individually in labelled plastic bags until analyzed. After thawing, the fish were measured to the nearest mm, and weighted to the closest gram. Otoliths were removed for age determination. The otoliths were burned over a propane stove, and divided using a scalpel. Age was determined under a stereo

Table 1. Mesh sizes of gill nets used at the study locations.

Location	Mesh size (mm)							
North	21	21	26	29	29	30	35	39
North	21	21	26	29	29	35	39	45
Mid	21	21	26	26	29	29	35	45
Mid	21	21	26	29	29	32	35	45
South	16.5	21	21	29	29	39	39	45
South	13.5	21	21	21	29	32	36	39

Table 2. Fish catches per season and location.

Species	Location	Season	Catch
Arctic charr	North	Spring	30
(*Salvelinus alpinus*)	North	Summer	30
	North	Fall	30
	Midt	Spring	23
	Midt	Summer	30
	Midt	Fall	30
European whitefish	North	Spring	30
(*Coregonus lavaretus*)	North	Summer	23
	North	Fall	14
	Midt	Spring	7
	Midt	Summer	11
	Midt	Fall	6
	South	Spring	2
	South	Summer	5
	South	Fall	3
European perch	North	Spring	21
(*Perca fluviatilis*)	North	Summer	30
	North	Fall	30
	Midt	Spring	27
	Midt	Summer	30
	Midt	Fall	30
	South	Spring	30
	South	Summer	30
	South	Fall	30

microscope at 40x magnification by counting opaque winter zones. Approximately 2 g of skeletal muscle was sampled below the dorsal fin of the fish. Muscle samples were freeze-dried for approximately 24 h using a Heto LyoLab 3000 (Heto-Holten A/S, Allerod, DK) freeze drier, and subsequently ground to fine powder using mortar and pestle. About 2 mg of the freeze-dried powder of each fish was weighted, and placed in a tin capsule before sent to the Norwegian Institute for Energy Technology (IFE) for determination of stable isotope ratios of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$). At IFE, the samples were combusted in the presence of O_2 and Cr_2O_3 at 1700 °C in a NCS 2500 elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA). NO_x was reduced to N_2 in a Cu oven at 650 °C. H_2O was removed in a chemical trap of $Mg(ClO_4)_2$ before separation of N_2 and CO_2 on a 2 m Poraplot Q GC column (Agilent J&W, Santa Clara, CA, USA). N_2 and CO_2 were directly injected on-line to a DeltaXP plus isotope ratio mass spectrometer (IRMS) (Thermo Fisher Scientific, Waltham, MA, USA) to determine $\delta^{13}C$ and $\delta^{15}N$ along with the C/N ratio.

Parasites

The fish were visually examined for the presence of metazoan ectoparasites, especially on the gills and fins, in the field just after the fish had been taken out of the gillnets. Sampled ectoparasites were stored in labelled glass vials containing 96% ethanol. Endoparasites were sampled in thawed fish in the laboratory. For endoparasite sampling, fish were opened from gills to anus. All cysts containing a cestode plerocercoid on the intestinal tract and other tissue were registered. A sample of encysted parasites was identified prior to the removal of organs. The intestinal tract was subsequently removed and placed in a petri dish in 9% saline solution. Saline solution was used to prevent parasites from disintegrating, which is common for cestodes in freshwater. The intestinal tract was cut open from oesophagus to anus, and all macroscopic parasites were collected. All parasites were identified according to morphological features under a stereomicroscope at 20–240x magnification. The number of cestodes was based on the number of heads (scolexes). The genera *Argulus* Müller, 1785, *Triaenophorus* Rudolphi, 1793, *Eubothrium* Nybelin, 1922, and *Proteocephalus* Weinland, 1858 were identified according to Bykhovskaya-Pavlovskaya *et al*. (1964). *Salmincola* Wilson, 1915 was identified according to Kabata (1969). Plerocercoid specimen of *Dibothriocephalus* Lühe, 1899 (formerly *Diphyllobothrium* Cobbold, 1858, revised genus name according to Waeschenbach *et al*. (2017)) were identified using Bykhovskaya-Pavlovskaya *et al*. (1964) and Andersen and Gibson (1989). It has to be noted, that Andersen and Gibson (1989) has some uncertainties related to frozen samples, as morphological features become less clear.

Data analysis

For all numerical variables describing fish metrics, mean, median, standard deviation and range were calculated. Prevalence and mean abundance of each parasite species were calculated according to Bush *et al*. (1997). *Eubothrium salvelini* (Schrank, 1790), *Proteocephalus* sp., *Dibothriocephalus dendriticus* (Nitzsch, 1824) Lühe, 1899, *Dibothriocephalus ditremus* (Creplin, 1825) Lühe, 1899, and *Triaenophorus nodulosus* (Pallas, 1781) were additionally pooled as copepod transmitted parasites, and prevalence and mean abundance was calculated for this group. *D. dendriticus*, *D. ditremus*, and *T. nodulosus* were pooled as plerocercoid larvae of cestodes, and prevalence and mean abundance calculated.

For each parasite group or species with a prevalence above 10%, the abundance was modelled. Due to highly aggregated distributions of parasites, negative binomial generalized linear models were used (Wilson & Grenfell 1997). The models were created using the glmmTMB-package (Brooks *et al*. 2017) in R (R Developer Core Team 2019). First, separate negative binomial generalized linear models using each of the following variables as explanatory variables were created. The candidate variables were length, weight, age, $\delta^{13}C$, $\delta^{15}N$, C/N-ratio, sex, location, and season. The AIC for all of these models was calculated using the AIC-function in R (R Developer Core Team 2019). The model with the lowest AIC was selected for further analysis. Subsequently, it was attempted to add one additional explanatory variable to the selected model. Extended models were created using the selected model, adding each of the remaining explanatory variables to separate models. The extended models were compared to the original model one by one, using maximum likelihood. This step was included to avoid selecting more complicated models with marginally lower AIC over the most

parsimonious model. The AIC was calculated for the original model, and for each extended model that was significantly better than the original model according to maximum likelihood. The model with the lowest AIC was selected. The model selection process for each individual model is elaborated in Appendix 1. The residuals of the selected model were checked using simulated residuals in the DHARMa-package in R (R Developer Core Team 2019). Graphs of the model results were created using the ggplot2-package (Wickham 2016), and the ggeffects-package (Lüdecke 2018). Some of the selected models exhibited curved patterns in the residuals, and were thus not viable. In these cases, quadratic terms for numerical variables were used. The numerical variables length, weight, age, $\delta^{13}C$, $\delta^{15}N$, and the C/N-ratio were included in new models as quadratic terms in the form $aX^2 + bX$, where X is the respective numerical variable, and a and b are coefficients, using the poly-function in R (R Developer Core Team 2019). These models were selected, advanced, and checked in the same manner as the models using linear terms. For all tests, a significance level of $\alpha = 0.05$ was used.

RESULTS

Descriptive statistics

Whitefish exhibited an average length of 279.2 mm, and average weight of 198.7 g, and a median weight of 177.0 g (Table 3). The average age of whitefish was 4.9 yrs. Whitefish had intermediate $\delta^{13}C$-signatures (average -28.3 ‰), and the lowest $\delta^{15}N$-signatures (average 8.0 ‰). The average length of charr was 272.5 mm, at an average weight of 231.2 g, and a median weight of 170.0 g. Charr had an average age of 12.0 yrs. Charr exhibited the most negative $\delta^{13}C$-signatures (average -29.4 ‰), and the highest $\delta^{15}N$-signatures (average 11.5 ‰). Perch had an average length of 212.2 mm, and an average weight of 141.3 g, with a median weight of 83 g. On average, perch was 4.2 yrs of age. Perch exhibited the least negative $\delta^{13}C$-signatures (average -24.9 ‰), and intermediate $\delta^{15}N$-signatures (average 9.0 ‰). Age and size distributions and growth curves are presented in Appendix 2.

Parasite species occurrence, prevalence and mean abundance

In Whitefish in Lake Norsjø, Trematoda Rudolphi, 1808, *Dibothriocephalus* spp., *Proteocephalus* sp., Acanthocephala Koelreuter, 1771, *Argulus coregoni* Thorell, 1865, *Salmincola* sp., and Nematoda (Diesing, 1861) were found. Trematodes, and nematodes were only found in whitefish. The most prevalent parasites in whitefish were acanthocephalans (Prevalence: 36%, mean abundance: 4.4) (Table 4) and *Proteocephalus* sp. (Prevalence: 24%, mean abundance: 0.7).

In charr, *A. coregoni*, *Salmincola edwardsii* (Olsson, 1869), *E. salvelini*, *T. nodulosus*, *D. ditremus*, *D. dendriticus*, *Proteocephalus* sp., and acanthocephalans were identified. *S. edwardsii*, and *E. salvelini* were only found in charr. In charr, encysted parasites (plerocercoids) had a prevalence of 93%, and a mean abundance of 20.2. *E. salvelini* was the most prevalent species (Prevalence: 68%, mean abundance: 2.6), followed by *D. ditremus* with a prevalence of 43%, and a mean abundance of 1.0.

Perch was infected with *T. nodulosus*, *Eubothrium* sp., *Dibothriocephalus* spp., *Proteocephalus* sp., and acanthocephalans. No parasite species was exclusively found in perch. Perch was mainly infected by acanthocephalans with a prevalence of 79%, and a mean abundance of 4.7.

Abundance

In whitefish, the abundance of *Proteocephalus* sp., Acanthocephalans, *A. coregoni*, *Salmincola* sp., and copepod transmitted parasites were modelled. The abundance of *A. coregoni* in whitefish varied by season with significantly higher abundance in the summer (Table 5). Season was also included in the model for *Salmincola* sp. in whitefish, with no apparent seasonal patterns. The abundance of *Proteocephalus* sp. in

Table 3. Descriptive statistics of numerical fish properties of fish selected for parasite sampling.

Species	Variable	Mean	Median	SD	Range
European whitefish (*Coregonus lavaretus*)	Length (mm)	279.2	282.5	47.4	186–440
	Weight (g)	198.7	177.0	120.0	47–757
	Age (yrs)	4.9	4.5	2.1	2–9
	$d^{13}C$ (‰)	-28.3	-28.6	2.3	-31.3– -21.8
	$d^{15}N$ (‰)	8.0	8.3	2.3	3.6–12.3
	C/N ratio	3.2	3.2	0.1	3.1–3.4
Arctic charr (*Salvelinus alpinus*)	Length (mm)	272.5	259.0	62.9	153–438
	Weight (g)	231.2	170.0	188.3	28–981
	Age (yrs)	12.0	10.0	6.6	4–31
	$d^{13}C$ (‰)	-29.4	-29.5	1.1	-32.1– -27.1
	$d^{15}N$ (‰)	11.5	11.6	1.8	6.61–14.8
	C/N ratio	3.4	3.4	0.2	3.1–4.4
European perch (*Perca fluviatilis*)	Length (mm)	212.2	197.0	55.1	112–356
	Weight (g)	141.3	83.0	141.3	13–633
	Age (yrs)	4.2	4	1.7	1–10
	$d^{13}C$ (‰)	-24.9	-25.1	2.5	-29.6– -19.3
	$d^{15}N$ (‰)	9.0	9.2	1.5	5.6–12.2
	C/N ratio	3.2	3.2	0.05	3.1–3.4

Table 4. The parasites prevalence and mean abundance in their hosts.

Host species	Parasite species/group	Prevalence (%)	Mean abundance
European whitefish (*Coregonus lavaretus*)	Trematoda	2	0.1
	Argulus coregoni	20	0.4
	Salmincola sp.	12	0.1
	Dibothriocephalus spp.	4	0.0
	Proteocephalus sp.	24	0.7
	Acanthocephala	36	4.4
	Nematoda	2	0.0
	Copepod transmitted	28	0.7
	Plerocercoids	4	0.0
Arctic charr (*Salvelinus alpinus*)	*Argulus coregoni*	1.33	0.0
	Salmincola edwardsii	16	0.2
	Cysts (Plerocercoids)	93	20.2
	Eubothrium salvelini	68	2.6
	Triaenophorus nodulosus	36	0.6
	Dibothriocephalus ditremus	43	1.0
	Dibothriocephalus dendriticus	25	0.3
	Proteocephalus sp.	7	0.3
	Acanthocephala	3	0.0
	Copepod transmitted	99	24.9
	Plerocercoids	99	22.0
European perch (*Perca fluviatilis*)	*Triaenophorus nodulosus*	4	0.0
	Eubothrium sp.	3	0.0
	Dibothriocephalus spp.	4	0.1
	Proteocephalus sp.	5	0.1
	Acanthocephala	79	4.7
	Copepod transmitted	12	0.2
	Plerocercoids	7	0.1

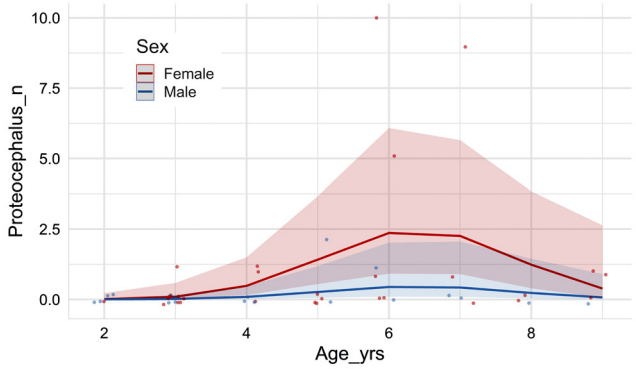

Figure 2. The abundance of *Proteocephalus* sp. in whitefish depending on the age of the host.

whitefish was affected by sex, with significantly higher abundances in females (Figure 2). The relationships between age and the abundance of *Proteocephalus* sp., Acanthocephalans (Figure 3), and copepod-transmitted parasites (Figure 4) in whitefish were all significant and quadratic, with the highest abundances found between the age of 5 and 8 years.

For charr, models were created for the parasites and groups *S. edwardsii*, encysted parasites (plerocercoids), *E. salvelini*, *T. nodulosus*, *D. ditremus*, *D. dendriticus*, plerocercoids, and copepod transmitted parasites. Age was included in the model of the abundance of *S. edwardsii* in charr, but no significant correlation or trend was found. The abundance of encysted parasites (plerocercoids) was significantly positively correlated to age in charr (Figure 5). This was also the case for the abundance of *E. salvelini* in charr. It was also a tendency towards higher abundances in the spring, although not significant (Figure 6). The abundance of *T. nodulosus* in charr tends to increase by age, but not significantly. *D. ditremus* in charr depended on sex, with significantly higher abundances in male fish. The abundance of *D. dendriticus* in charr varied by season, with significantly fewer individuals found in the fall. The abundance of plerocercoids in charr increased significantly by length, and had a non-significant tendency to decrease by age (Figure 7). In charr, the abundance of copepod transmitted parasites increased significantly by length, with a non-significant tendency to decrease by age (Figure 8).

Acanthocephalans, and copepod transmitted parasites were modelled in perch. In perch, the abundance of acanthocephalans varied by season, with significantly fewer individuals found in the fall. In addition, there was a positive trend of acanthocephalans by $\delta^{15}N$, which was not significant. The abundance of copepod transmitted parasites in perch depended on age following a quadratic equation, with the highest abundances at age > 6 (Figure 9).

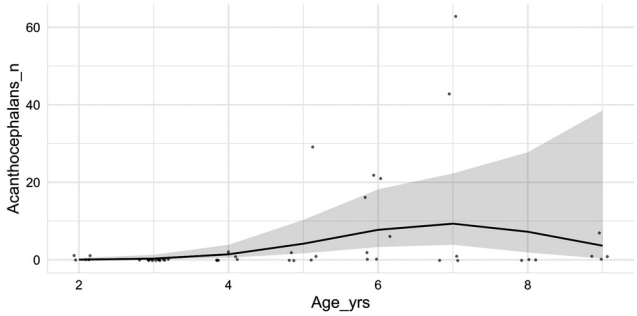

Figure 3. The abundance of acanthocephalans in whitefish depending on the age of the host.

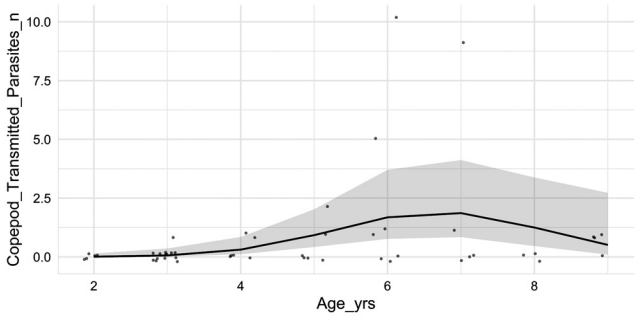

Figure 4. The abundance of copepod transmitted parasites in whitefish depending on the age of the host.

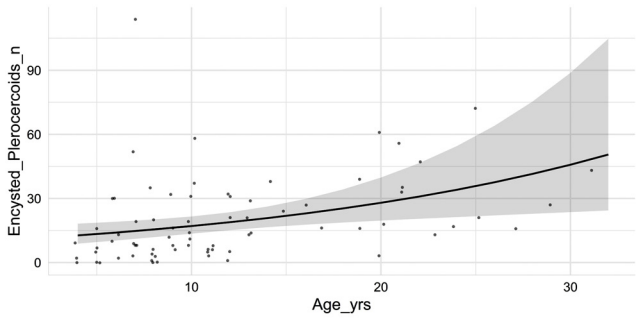

Figure 5. The number of encysted plerocercoid larvae of cestodes found in charr in relation to the age of the host.

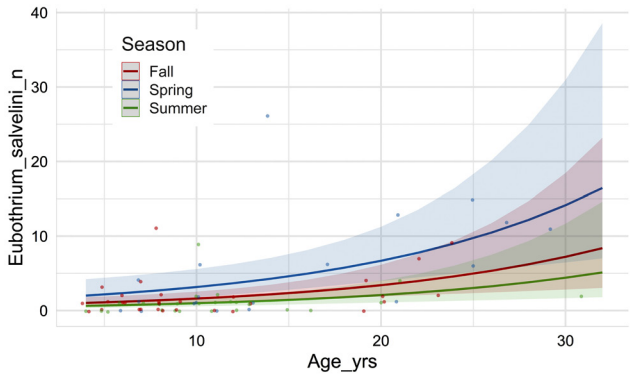

Figure 6. The number of *Eubothrium salvelini* found in charr depended on the age of the host and season.

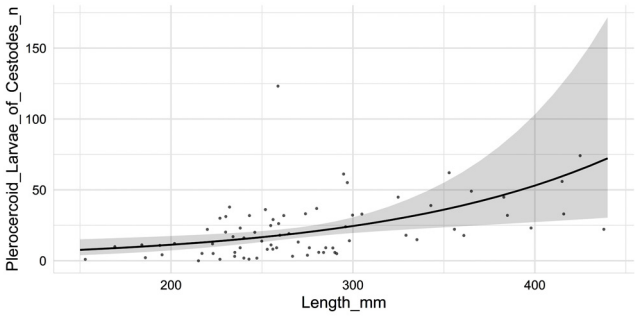

Figure 7. The number of plerocercoid larvae of cestodes in charr depending on the length of the host.

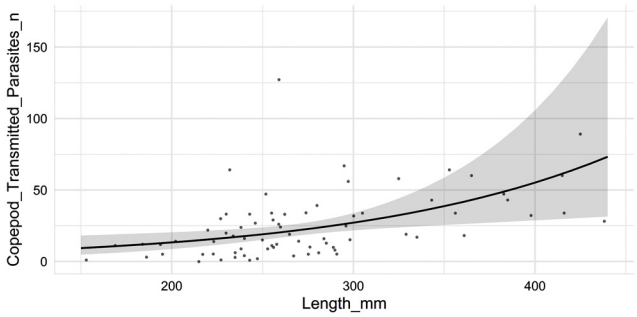

Figure 8. The length of charr was the best predictor for the abundance of copepod transmitted parasites in this species.

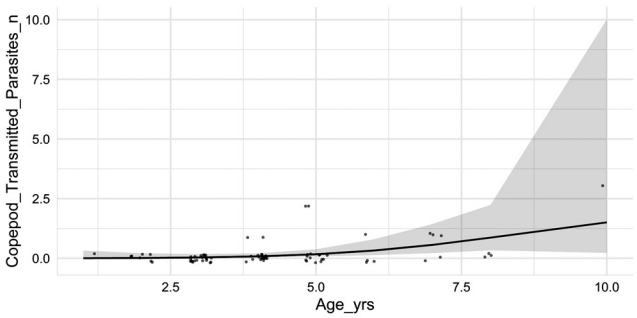

Figure 9. The number of copepod transmitted parasites found in perch depended on the age of the host.

DISCUSSION

Excepting the acanthocephalans and nematodes in whitefish, all the parasites found in our study have previously been described in Norway, and to parasitize the respective host species (Sterud 1999 and references therein). Acanthocephalans have been shown to parasitize whitefish in Italy (Dezfuli *et al.* 2009), the Bothnian Bay (Baltic Sea) (Wayland *et al.* 2004), and Finland (Karvonen & Valtonen 2004), and nematodes are found in whitefish, for instance in Finland (Pulkkinen *et al.* 1999; Karvonen & Valtonen 2004). These parasite groups may not have been found in whitefish in Norway before, as there are limited studies on parasites in southern Norway. The most common parasites found in our study depend on the general diet and habitat of the host species. Perch was mainly infected with Acanthocephalans, which are transmitted via ostracods, isopods or amphipods (Woo 2006). In Lake Norsjø, ostracods and the isopod *Asellus aquaticus* Linnaeus, 1758 have previously been found in stomach samples of fish (Olk *et al.* 2016). As these are benthic animals, the high occurrence of acanthocephalans in perch indicates a littoral diet. Perch has a high dependence on the littoral zone (Zamora & Moreno-Amich 2002; Jacobsen *et al.* 2015), and it prefers the littoral zone during spring and summer according to a telemetric study (Westrelin *et al.* 2018). In whitefish, both Acanthocephalans and *Proteocephalus* sp. were the most common parasites. *Proteocephalus* sp. are transmitted by

Table 5. Model results for macroparasite abundance in the host species Arctic charr (*Salvelinus alpinus*), European whitefish (*Coregonus lavaretus*), and European perch (*Perca fluviatilis*).

Host	Parasite	Metric	Term	Estimate	SE	z	p
European whitefish	*Argulus coregoni*	Season	Δspring	-20.234	17083.729	-0.001	> 0.1
(*Coregonus lavaretus*)			Δsummer	2.249	1.111	2.024	< 0.05
	Salmincola sp.	Season	Δspring	21.07	27213.70	0.001	> 0.1
			Δsummer	20.88	27213.70	0.001	> 0.1
	Proteocephalus sp.	Sex	Δmale	-1.6702	0.8506	-1.964	< 0.05
		Age	βAge^2	-8.1682	2.9387	-2.780	< 0.01
			βAge	8.8081	3.5408	2.488	< 0.05
	acanthocephala	Age	βAge^2	-6.3996	2.7692	-2.311	< 0.05
			βAge	10.2080	3.0930	3.300	< 0.001
	copepod transmitted	Age	βAge^2	-7.2901	2.6623	-2.738	< 0.01
			βAge	9.9698	3.4119	2.922	< 0.01
Arctic charr	*Salmincola edwardsii*	Age	βAge	0.02457	0.04639	-0.530	> 0.1
(*Salvelinus alpinus*)	encysted plerocercoids	Age	βAge	0.04927	0.01784	2.762	< 0.01
	Eubothrium salvelini	Age	βAge	0.07495	0.02265	3.309	< 0.001
		Season	Δspring	0.67294	0.35945	1.872	> 0.05
			Δsummer	-0.49272	0.36182	-1.362	> 0.1
	Triaenophorus nodulosus	Age	βAge	0.03862	0.02962	1.304	> 0.1
	Dibothriocephalus ditremus	Sex	Δmale	0.7419	0.3685	2.014	< 0.05
	Dibothriocephalus dendriticus	Season	Δspring	1.5041	0.6667	2.256	< 0.05
			Δsummer	1.3863	0.6583	2.106	< 0.05
	plerocercoids	Length	βLength	0.007730	0.002632	2.937	< 0.01
		Age	βAge	0.009221	0.024309	-0.379	> 0.1
	copepod transmitted	Length	βLength	$7.088 * 10^{-3}$	$2.57 * 10^{-3}$	2.758	< 0.01
		Age	βAge	$-5.728 * 10^{-5}$	$2.367 * 10^{-2}$	-0.002	> 0.1
European perch	acanthocephala	Season	Δspring	1.00851	0.37483	2.691	< 0.01
(*Perca fluviatilis*)			Δsummer	1.41060	0.38679	3.647	< 0.001
		$\delta^{15}N$	$\beta\delta^{15}N$	0.13224	0.09457	1.398	> 0.1
	copepod transmitted	Age	βAge^2	-1.8552	2.7481	0.675	> 0.1
			βAge	10.1687	4.0685	2.409	< 0.05

copepods (Scholz 1999). This indicates, that whitefish in Lake Norsjø feed on both pelagic and littoral resources. Charr was mainly infected by copepod transmitted parasites, indicating a pelagic diet. However, sampling was conducted relatively close to the shore, meaning that charr was found below the littoral zone, but not necessarily in the open waters of the pelagic zone. Charr was not found in the pelagic zone of Lake Norsjø during a previous survey (Sandlund *et al.* 2016). This is also supported by the $\delta^{13}C$-signatures, which were most negative for charr, followed by whitefish. The least negative $\delta^{13}C$-signatures were found in perch. Stable isotope signatures are used to trace energy flow ($\delta^{13}C$) and trophic position ($\delta^{15}N$) in food webs (Peterson & Fry 1987; Cabana & Rasmussen 1996; Post 2002). Heavier isotopes form more stable bonds, and compounds including lighter isotopes are more readily metabolized (Hoefs 2013). As a consequence, $\delta^{15}N$ increases on average with 3.4 ‰ per trophic level (Minagawa & Wada 1984; Post 2002), and $\delta^{13}C$ can be used to trace the dietary carbon source (Post 2002). The stable isotope signature of carbon ($\delta^{13}C$) averagely varies with habitat. Littoral signatures are generally less negative than pelagic or profundal signatures (Vander Zanden & Rasmussen 1999).

While the occurrence of the most common parasites depends on the dietary niche of the respective species, abundance of the individual parasite species in individual host species was found to depend on sex, age, length, season and trophic levels measured as $\delta^{15}N$ in our study. *A. coregoni* had a higher abundance in the summer in whitefish than in the other seasons. This matches the seasonal occurrence of *A. coregoni*, as found in several studies on the parasite in aquaculture (Shimura 1983; Hakalahti & Valtonen 2003; Hakalahti *et al.* 2004b). *A. coregoni* overwinters as eggs (Shimura 1983), and eggs are not able to develop and hatch at temperatures below 8–10 °C (Mikheev *et al.* 2001; Hakalahti & Valtonen 2003). When temperatures exceed this critical value, eggs hatch over an extended period (Hakalahti *et al.* 2004a), from May to July (Shimura 1983), or during early summer (Hakalahti & Valtonen 2003). From July onwards, female *A. coregoni* detach from their fish hosts to lay eggs (Hakalahti & Valtonen 2003), and egg laying proceeds from mid-July to mid-October (Hakalahti *et al.* 2004b). Consequently, the highest abundance of *A. coregoni* on its host whitefish was found in the summer.

The abundance of *Proteocephalus* sp. in whitefish was highest

in fish aged 5–8 yrs, and in females. Whitefish between the age of 5 and 8 yrs may have consumed higher numbers of copepods. This is also supported by the higher abundance of copepod transmitted parasites in whitefish aged 5–8 yrs in Lake Norsjø. However, stomach contents have not been analyzed in our study. Higher abundance of *Proteocephalus percae* (Müller, 1780) in females has previously been demonstrated in perch during the spawning season (Balling & Pfeiffer 1997). However, the higher abundance of *Proteocephalus* sp. in female whitefish in Lake Norsjø did not differ by season, and may have been caused by a different mechanism. Acanthocephalans were also most abundant in whitefish between the age of 5 and 8 yrs, meaning that whitefish between the age of 5 and 8 yrs may also consume more amphipods, isopods and ostracods (Woo 2006). In addition, acanthocephalans can be transmitted by predation on fish (Woo 2006), and since whitefish aged 5–8 yrs are towards the older end of the ages recorded, these whitefish may consume some fish.

Charr was infected by a variety of parasites in our study, most of which copepod transmitted. The abundance of the ectoparasite *S. edwardsii* showed no apparent relationship to the age of the fish, even though age was included in the model. *S. edwardsii* directly infects its host, and its abundance is not affected by the diet (Kabata 1969). Infective copepodids of *S. edwardsii* are usually found towards the bottom of the lake (Poulin *et al.* 1990), indicating that charr utilizes benthic habitat. Our results are different from previous studies, which indicate a greater risk of infection with *S. edwardsii* with increasing size and age of the host (Black *et al.* 1983; Amundsen *et al.* 1997). In addition, host size is suggested to be the most important predictor of ectoparasite loads, as larger fish represent larger targets for the parasite, and more water is circulated over the gills of large fish (Poulin *et al.* 1991). However, usually *S. edwardsii* is found to infect the gill region (Conley & Curtis 1993; Amundsen *et al.* 1997), while it was found in other microhabitats in our study. The parasite was found attached to the skin and fins in our study, which has previously been observed in small fish (Black *et al.* 1983; Conley & Curtis 1993). This may result in the more uniform distribution of the parasite related to age in charr. However, attachment to the fins may also be a local adaption of the parasite (Amundsen *et al.* 1997), as the fins were the exclusive attachment site in a study on *S. edwardsii* in Ennerdale Water in Britain (Fryer 1981). The abundance of encysted parasites (plerocercoids) in charr was positively correlated to the age of the host. Encysted parasites belong to a variety of species, and in general, parasite abundance is shown to increase by age (Khan 2012), as long lived hosts provide a more stable habitat for parasites, and may accumulate parasites over time (Bell & Burt 1991). The abundance of *E. salvelini* was also positively correlated with the age of charr, and it is specifically shown to accumulate with age (Smith 1973; Hanzelová *et al.* 2002). This parasite is host-specific to charr in Europe, and has a life-cycle using copepods as intermediate host, and charr as definite host (Andersen & Kennedy 1983). Low infection rates in copepods (0.001–0.002%) are common for *E. salvelini* (Boyce 1974; Hanzelová *et al.* 2002). Thus, the high prevalence of this parasite in charr in Lake Norsjø could indicate heavy feeding on copepods. However, as this parasite may alter the behavior of copepods, charr may selectively feed on infected copepods, and this may also explain high abundances in fish (Poulin *et al.* 1992). *E. salvelini* was more abundant in the spring in our study, although not significantly so. Previously, no seasonal variation in abundance are reported for this parasite (Hoffmann *et al.* 1986a; Hernandez & Muzzall 1998; Hanzelová *et al.* 2002), as they continuously emit eggs (Hanzelová *et al.* 2002). However, peaks in egg shedding are reported in the spring (Boyce 1974; Kennedy 1978; Hernandez & Muzzall 1998), which may cause seasonal variations in *E. salvelini* in Lake Norsjø. However, peaks in egg shedding in the spring do not cause higher abundances of the parasite observed in fish the same spring, as more time is needed for *E. salvelini* to infect copepods, to be consumed by fish, and to establish in fish to the point that the parasite is visible to the unaided eye.

Plerocercoid larvae of cestodes were found to increase in abundance by length in charr in our study. Specifically, plerocercoid larvae of *T. nodulosus*, *D. ditremus*, and *D. dendriticus* were identified. The increase in abundance by size is likely caused by accumulation of plerocercoids over time. *Triaenophorus* sp. is able to survive in its intermediate fish host for several years, and thus able to accumulate with increasing size or age (Dick & Rosen 1982; Rosen & Dick 1984; Hoffmann *et al.* 1986b). This is also reflected in the individual trend of increasing abundance of *T. nodulosus* by age in our study. *Dibothriocephalus* spp. also survives in their intermediate fish hosts for several years, and may accumulate (Halvorsen & Andersen 1984; Henriksen *et al.* 2016). In addition, *Dibothriocephalus* spp. may re-establish in predatory fish (Hammar 2000; Henriksen *et al.* 2016), and feeding on smaller infected fish may result in higher abundances of *Dibothriocephalus* spp. in larger charr. In Lake Norsjø, three-spined sticklebacks and European smelt are present (Jensen 1954), which are both intermediate hosts for *Dibothriocephalus* spp. (Andersen *et al.* 1986; Andersen & Valtonen 1992; Anikieva & Ieshko 2017). *D. ditremus* also exhibited a higher abundance in male charr, with no apparent relationship between sex and any other variable that could explain the higher occurrence of *D. ditremus* in males. As the reasons behind this remain obscure, this may be caused by feeding specialization of male charr on the infected copepod species. Charr exhibit individual feeding-specialization, and are persistent in their diet over long periods (Knudsen *et al.* 2004, 2009). The abundance of *D. dendriticus* exhibited seasonal variations, with fewer specimen found in the fall. This is also unusual, as the parasite lives for several years in fish (Halvorsen & Andersen 1984), and other studies show no significant seasonal variations in its abundance (Henriksen *et al.* 2019). As the first intermediate host of *D. dendriticus* are copepods (Halvorsen 1966; Marcogliese 1995; Scholz *et al.* 2009), seasonal trends may be linked to new infections and fluctuations in copepod abundance.

The abundance of copepod transmitted parasites in charr in general was positively correlated to fish length. Increases in parasite abundance with body size are common (Bell & Burt 1991; Poulin 1995, 2000, 2004; Valtonen *et al.* 2010; Poulin & Leung 2011; Timi *et al.* 2011; Timi & Lanfranchi 2013; Anegg *et al.* 2014). Larger hosts provide a greater diversity of niches, more space for parasites (Poulin 1995; Poulin & Leung 2011), and consume more potentially infected food (Poulin 1997).

In perch, acanthocephalans had a non-significant tendency to increase with trophic position measured as $\delta^{15}N$, and exhibited seasonal variations with lower abundance in the fall. Acanthocephalans are transmitted by isopods, amphipods and ostracods (Woo 2006), which is not necessarily consistent with higher trophic levels. However, generally more parasites are found at higher trophic levels (Luque & Poulin 2008; Timi *et al.* 2011; Alarcos & Timi 2012), and perch in Lake Norsjø may mainly feed on littoral benthic animals. Acanthocephalans are recruited in the spring, and live approximately one season (Woo 2006), which may result in fewer specimen remaining in the fall. The abundance of copepod transmitted parasites in perch was highest in fish older than 6 yrs. This may be a general positive relationship between parasite abundance and age of the host (Khan 2012), due to accumulation and a more stable habitat in long-living hosts (Bell & Burt 1991). In addition, it may be caused by older perch consuming more pelagic prey, such as copepods in Lake Norsjø. However, this

would need to be confirmed using stomach content analysis.

In Lake Norsjø, the host species, perch, whitefish and charr, exhibit different parasite communities, which could be related to their respective dietary niches. Habitat appeared to be important in defining which parasites were most prevalent in their respective hosts. Differences in parasite community by habitat have previously been found in charr (Henricson & Nyman 1976; Frandsen *et al*. 1989; Dorucu *et al*. 1995; Knudsen *et al*. 1997; Siwertsson *et al*. 2016; Paterson *et al*. 2019) and whitefish (Knudsen *et al*. 2003; Karvonen *et al*. 2013). When modelling individual parasite abundances, increasing abundances by age and size of the fish host were most commonly found in our study.

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Appendix 1. Model selection.

The base model *Argulus coregoni* in whitefish was selected according to the lowest AIC. Candidate models are presented in Table A1-1. The model including season as explanatory variable was considered the model with the best fit.

Table A1-1. Candidate models for the base model for *Argulus coregoni* in Whitefish.

Explanatory variable	AIC
Length	75.05866
Weight	75.81601
Age	80.14130
$\delta^{13}C$	72.40155
$\delta^{15}N$	79.48613
Sex	80.79960
Location	79.59549
Season	65.92807
C/N-ratio	79.15692

Subsequently, length, weight, $\delta^{13}C$, $\delta^{15}N$, sex, age, location, and the C/N-ratio were added in separate models to the base model containing season. These models, which are nested in the base model were compared to the base mode using maximum likelihood. Comparisons to the base model are presented in Table A1-2.

Table A1-2. Advanced models and the results of the comparison to the base model by maximum likelihood.

Base Model	Explanatory variable	p
Season	Length	> 0.10
Season	Weight	> 0.10
Season	$\delta^{13}C$	> 0.05
Season	$\delta^{15}N$	> 0.10
Season	Sex	> 0.10
Season	Age	> 0.10
Season	Location	> 0.10
Season	C/N-ratio	> 0.05

As no more advanced model was significantly better than the base model, the base model only containing season as explanatory variable was chosen as the final model.
The AICs for different base models for the number of *Salmincola* sp. in whitefish is shown in Table A1-3.

Table A1-3. AIC table for the model selection of the base model for *Salmincola* sp. in whitefish.

Explanatory variable	AIC
Length	48.44164
Weight	48.43219
Age	48.44905
$\delta^{13}C$	48.44557
$\delta^{15}N$	48.30059
Sex	48.10730
Location	48.40397
Season	47.49582
C/N-ratio	48.00397

The model including season as explanatory variable performed best. However, its performance was similar to all the other models. The model including season was advanced, and results of maximum likelihood tests of the more advanced models are presented in Table A1-4. None of the more advanced models was significantly better than the base model, and the base model was thus selected.

Table A1-4. Comparison of more advanced models to the base model of *Salmincola* sp. in whitefish by maximum likelihood.

Base Model	Explanatory variable	p
Season	Length	> 0.10
Season	Weight	> 0.10
Season	$\delta^{13}C$	> 0.10
Season	$\delta^{15}N$	> 0.10
Season	Sex	> 0.10
Season	Age	> 0.10
Season	Location	> 0.10
Season	C/N-ratio	> 0.10

The base model selection for the number of *Proteocephalus* sp. in whitefish is presented in Table A1-5. Four of the candidate models could not be calculated. Length was included in the base model.

Table A1-5. AIC table for base model selection for *Proteocephalus* sp. in whitefish.

Explanatory variable	AIC
Length	94.37454
Weight	97.29397
Age	96.21320
$\delta^{13}C$	NA
$\delta^{15}N$	NA
Sex	97.30856
Location	NA
Season	NA
C/N-ratio	101.38057

The comparison to more advanced models is shown in Table A1-6. The model including length and season was the only model significantly better than the base model. This model was initially selected. However, the model was not viable according to curved patterns in the residuals.

Table A1-6. Model comparison of more advanced models for *Proteocephalus* sp. in whitefish to the base model by maximum likelihood.

Base Model	Explanatory variable	p
Length	Sex	> 0.10
Length	Location	> 0.10
Length	Season	< 0.05
Length	Weight	> 0.05
Length	Age	> 0.10
Length	$\delta^{13}C$	> 0.10
Length	$\delta^{15}N$	> 0.10

Appendix I continued.

Adding quadratic terms to the model for the number of *Proteocephalus* sp. in whitefish was attempted. New base models were created containing quadratic terms of the numerical variables. An AIC table for their comparison is presented in Table A1-7.

Table A1-7. AIC table for the selection of the quadratic base model for *Proteocephalus* sp. in whitefish.

Explanatory variable	AIC
Length (quadratic)	93.09508
Weight (quadratic)	96.15357
Age (quadratic)	91.78830
$\delta^{13}C$ (quadratic)	102.95684
$\delta^{15}N$ (quadratic)	103.79494
C/N-ratio (quadratic)	97.30856
Sex	102.44419
Location	100.89044
Season	103.33786

The base model containing the quadratic term of age was selected, and more advanced models adding the other variables one by one were created. The comparison of the more advanced models to the base model by maximum likelihood is shown in Table A1-8. None of the more advanced models were significantly better than the base model, and the base model containing the quadratic term for age was selected. The residuals for this models did not exhibit curved patterns.

Table A1-8. Results of the comparison of more advanced models to the quadratic base model for *Proteocephalus* sp. in whitefish by maximum likelihood.

Base Model	Explanatory variable	p
Age (quadratic)	Length	> 0.10
Age (quadratic)	Weight	> 0.10
Age (quadratic)	$\delta^{13}C$	> 0.10
Age (quadratic)	$\delta^{15}N$	> 0.10
Age (quadratic)	C/N-ratio	> 0.10
Age (quadratic)	Sex	> 0.05
Age (quadratic)	Location	> 0.10
Age (quadratic)	Season	> 0.10

Different base models for acanthocephalans in whitefish are compared according to the AIC in Table A1-9. The model including age as explanatory variable was selected. It performed similarly to the model containing length.

Table A1-9. AIC table for base models of the number of acanthocephalans in whitefish.

Explanatory variable	AIC
Length	172.6141
Weight	176.6409
Age	172.4202
$\delta^{13}C$	179.1248
$\delta^{15}N$	182.3797
C/N-ratio	176.6081
Sex	179.3307
Location	183.4828
Season	177.8922

The base model was compared to more advanced models using maximum likelihood (Table A1-10). Both advanced models containing season and the C/N-ratio were significantly better than the base model. The advanced models performing better than the base model were compared by AIC (Table A1-11), and the model containing age and season as explanatory variables was selected. However, as the residuals exhibited curved patterns, the inclusion of quadratic terms in a new base model was attempted.

Table A1-10. Model comparisons for acanthocephalans in whitefish to the base model only containing age as explanatory variable by maximum likelihood.

Base Model	Explanatory variable	p
Age	Length	> 0.05
Age	Weight	> 0.10
Age	$\delta^{13}C$	> 0.05
Age	$\delta^{15}N$	> 0.10
Age	Sex	> 0.10
Age	Season	< 0.01
Age	Location	> 0.10
Age	C/N-ratio	< 0.05

Table A1-11. AIC table for model comparison of more advanced models for acanthocephalans in whitefish and the base mode using AIC.

Explanatory variable	AIC
Age	172.4202
Age + Season	166.4647
Age + C/N-ratio	168.3035

The model containing a quadratic term of age as explanatory variable performed best according to the AIC (Table A1-12). According to maximum likelihood tests, more advanced models containing Season and the C/N-ratio performed better than the base model (Table A1-13). These models were compared using the AIC (Table A1-14), and the model containing a quadratic term for age and season was performing best. However, the residuals of both more advanced models exhibited curved patterns, and the base model was selected based on the best residual fit.

Appendix 1 continued.

Table A1-12. AIC table for the comparison of quadratic models for the number of acanthocephalans in whitefish.

Explanatory variable	AIC
Length (quadratic)	171.0887
Weight (quadratic)	172.1426
Age (quadratic)	170.6091
$\delta^{13}C$ (quadratic)	177.0976
$\delta^{15}N$ (quadratic)	184.2953
C/N-ratio (quadratic)	178.6064
Sex	179.3307
Location	183.4828
Season	177.8922

Table A1-13. Tests between the quadratic base model for acanthocephalans in whitefish to more advanced models using maximum likelihood.

Base Model	Explanatory variable	p
Age (quadratic)	Length	> 0.10
Age (quadratic)	Weight	> 0.10
Age (quadratic)	$\delta^{13}C$	> 0.10
Age (quadratic)	$\delta^{15}N$	> 0.10
Age (quadratic)	C/N-ratio	< 0.05
Age (quadratic)	Sex	> 0.10
Age (quadratic)	Location	< 0.05
Age (quadratic)	Season	> 0.10

Table A1-14. Comparison of more advanced quadratic models for the abundance of acanthocephalans in whitefish using AIC.

Explanatory variable	AIC
Age (quadratic)	170.6091
Age (quadratic) + Season	166.2518
Age (quadratic) + C/N-ratio	167.6942

For the number of copepod transmitted parasites in whitefish, the base model containing length as explanatory variable had the lowest AIC (Table A1-15). The model containing season as aditional explanatory variable was the only higher performing more advanced model according to maximum likelihood (Table A1-16). However, as this model showed curved patterns in its residual distribution, new base models were created using quadratic terms.

Table A1-15. AIC table for base models of copepod transmitted parasites in whitefish.

Explanatory variable	AIC
Length	101.5716
Weight	104.8061
Age	102.7190
$\delta^{13}C$	109.1295
$\delta^{15}N$	109.5603
C/N-ratio	108.9059
Sex	105.1300
Location	110.5478
Season	108.5875

Table A1-16. Comparison of more advanced models to the base model of copepod transmitted parasites in whitefish by maximum likelihood.

Base Model	Explanatory variable	p
Length	Weight	> 0.05
Length	Age	> 0.10
Length	$\delta^{13}C$	> 0.10
Length	$\delta^{15}N$	> 0.10
Length	Sex	> 0.10
Length	Season	< 0.05
Length	Location	> 0.10
Length	C/N-ratio	> 0.10

The base model for copepod transmitted parasites containing the quadratic term for age was the best model according to the AIC (Table A1-17). None of the more advanced models were significantly better than the base model according to maximum likelihood (Table A1-18). Consequently, the base model was selected.

Table A1-17. AIC table for quadratic base models for copepod transmitted parasites in whitefish.

Explanatory variable	AIC
Length (quadratic)	99.61478
Weight (quadratic)	103.14450
Age (quadratic)	97.50996
$\delta^{13}C$ (quadratic)	110.39990
$\delta^{15}N$ (quadratic)	111.55580
C/N-ratio (quadratic)	110.86819
Sex	105.12996
Location	110.54780
Season	108.58752

Table A1-18. Comparison of more advanced models to the quadratic base model for copepod transmitted parasites in whitefish using maximum likelihood.

Base Model	Explanatory variable	p
Age (quadratic)	Length	> 0.10
Age (quadratic)	Weight	> 0.10
Age (quadratic)	$\delta^{13}C$	> 0.10
Age (quadratic)	$\delta^{15}N$	> 0.10
Age (quadratic)	C/N-ratio	> 0.10
Age (quadratic)	Sex	> 0.10
Age (quadratic)	Location	> 0.05
Age (quadratic)	Season	> 0.10

The base model for *Salmincola edwadsii* in charr using age as explanatory variable had the lowest AIC (Table A1-19). However, it performed similarly to models containing length and $\delta^{13}C$. None of the more advanced models was significantly better than the base model according to maximum likelihood (Table A1-20). Consequently, the base model including age was selected.

Appendix 1 continued.

Table A1-19. AIC table for base models of the number of *Salmincola edwardsii* in charr.

Explanatory variable	AIC
Length	78.68604
Weight	NA
Age	78.30467
$\delta^{13}C$	78.87065
$\delta^{15}N$	NA
C/N-ratio	NA
Sex	NA
Season	80.84004
Location	NA

Table A1-20. Model comparison of more advanced models for *Salmincola edwardsii* in charr to the base model containing age by maximum likelihood.

Base Model	Explanatory variable	p
Age	Length	> 0.10
Age	Weight	> 0.10
Age	$\delta^{13}C$	NA
Age	$\delta^{15}N$	> 0.10
Age	Sex	NA
Age	Location	> 0.10
Age	Season	NA
Age	C/N-ratio	NA

The base model for cysts in charr with the lowest AIC used age as explanatory variable (Table A1-21). More advanced models containing length and weight were both significantly better than the base model according to maximum likelihood (Table A1-22). Comparing the more advanced models to the base model by AIC revealed, that the model containing age and length was the best model (Table A1-23). However, as age and length exhibited colinearity, and age was not significant in this model, the base model was selected.

Table A1-21 base model comparison for the number of cysts in charr using AIC.

Explanatory variable	AIC
Length	598.4953
Weight	598.6042
Age	592.5246
$\delta^{13}C$	609.3290
$\delta^{15}N$	608.9781
C/N-ratio	609.4156
Sex	609.7253
Season	610.8502
Location	609.5858

Table A1-22. Comparing more advanced models to the base model for cysts in charr using maximum likelihood.

Base Model	Explanatory variable	p
Age	Length	< 0.01
Age	Weight	< 0.05
Age	$\delta^{13}C$	> 0.10
Age	$\delta^{15}N$	> 0.05
Age	C/N-ratio	> 0.10
Age	Sex	> 0.10
Age	Season	> 0.10
Age	Location	> 0.10

Table A1-23. AIC table for more advanced models and base model for cysts in charr.

Explanatory variable	AIC
Age	592.5246
Age + Length	587.1827
Age + Weight	590.5388

The selected base model for the number of *Eubothrium salvelini* in charr with age as explanatory variable had the lowest AIC (Table A1-24). Only the more advanced model containing age and season was significantly better according to maximum likelyhood (Table A1-25). Consequently, the model containing age and season was selected.

Table A1-24. AIC table comparing base models for *Eubothrium salvelini* in charr.

Explanatory variable	AIC
Length	306.2600
Weight	300.6607
Age	295.8292
$\delta^{13}C$	313.4788
$\delta^{15}N$	311.8414
C/N-ratio	316.9909
Sex	314.4400
Season	304.0997
Location	315.0706

Table A1-25. Comparison of more advanced models for *Eubothrium salvelini* in charr to the base model containing age by maximum likelyhood.

Base Model	Explanatory variable	p
Age	Length	> 0.10
Age	Weight	> 0.10
Age	$\delta^{13}C$	> 0.10
Age	$\delta^{15}N$	> 0.10
Age	C/N-ratio	> 0.10
Age	Sex	> 0.10
Age	Season	< 0.05
Age	Location	> 0.05

Appendix 1 continued.

The base model containing age was the best model for the abundance of *Triaenophorus nodulosus* in charr according to the AIC (Table A1-26). No additional variable seemed to improve the model according to comparisons to the base model by maximum likelihood (Table A1-27). The base model was selected.

Table A1-26. AIC table for base models of *Triaenophorus nodulosus* in charr.

Explanatory variable	AIC
Length	168.8717
Weight	169.0970
Age	164.1332
$\delta^{13}C$	166.9320
$\delta^{15}N$	168.1660
C/N-ratio	168.4137
Sex	167.7276
Season	167.4710
Location	165.5015

Table A1-27. Comparison of more advanced models to the base model of *Triaenophorus nodulosus* in charr using maximum likelihood.

Base Model	Explanatory variable	p
Age	Length	> 0.10
Age	Weight	> 0.10
Age	$\delta^{13}C$	> 0.10
Age	$\delta^{15}N$	> 0.10
Age	C/N-ratio	> 0.10
Age	Sex	> 0.10
Age	Season	> 0.10
Age	Location	> 0.10

The best base model for *Dibothriocephalus ditremus* in charr contained sex as explanatory variable according to the AIC (Table A1-28). The addition of one extra explanatory variable did not improve this model according to maximum likelihood (Table A1-29). Consequently, the model only containing sex as explanatory variable was selected.

Table A1-28. AIC table for base models for *Dibothriocephalus ditremus* in charr.

Explanatory variable	AIC
Length	206.6559
Weight	207.7512
Age	206.4372
$\delta^{13}C$	207.9316
$\delta^{15}N$	207.8016
C/N-ratio	207.2861
Sex	204.0079
Season	208.4528
Location	207.0390

Table A1-29. Comparison of more advanced models to base model for *Dibothriocephalus ditremus* in charr according to maximum likelihood.

Base Model	Explanatory variable	p
Sex	Length	> 0.10
Sex	Weight	> 0.10
Sex	$\delta^{13}C$	> 0.10
Sex	$\delta^{15}N$	> 0.10
Sex	C/N-ratio	> 0.10
Sex	Age	> 0.10
Sex	Season	> 0.10
Sex	Location	> 0.10

The abundance of *Dibothriocephalus dendriticus* in charr was best explained by season according to the AIC (Table A1-30). Extending this model with either season or sex did not improve the model according to maximum likelihood (Table A1-31). None of the other variables produced viable models. The base mode using season as explanatory variable was selected.

Table A1-30. Base model comparison for *Dibothriocephalus dendriticus* in charr using AIC.

Explanatory variable	AIC
Length	107.9485
Weight	108.1215
Age	NA
$\delta^{13}C$	106.5658
$\delta^{15}N$	NA
C/N-ratio	108.0964
Sex	NA
Season	102.6733
Location	108.0871

Table A1-31. Model extension for *Dibothriocephalus dendriticus* in charr by maximum likelihood.

Base Model	Explanatory variable	p
Season	Length	NA
Season	Weight	NA
Season	$\delta^{13}C$	NA
Season	$\delta^{15}N$	NA
Season	C/N-ratio	NA
Season	Age	> 0.10
Season	Sex	> 0.10
Season	Location	NA

The abundance of plerocercoid larvae of cestodes in charr was best predicted by age in the base model (Table A1-32). More advanced models containing length and weight as additional explanatory variables were significantly better than the base model according to maximum likelihood (Table A1-33). The model containing age and length was the best of these models according to the AIC (Table A1-34), and was consequently selected.

Appendix 1 continued.

Table A1-32. AIC table for base models for plerocercoid larvae of cestodes in charr.

Explanatory variable	AIC
Length	607.4484
Weight	608.2406
Age	602.2455
$\delta^{13}C$	619.7730
$\delta^{15}N$	619.8596
C/N-ratio	620.1091
Sex	620.0495
Season	621.2955
Location	620.2163

Table A1-33. Model comparisom using maximum likelihood between the base model for plerocercoid larvae of cestodes in charr and more advanced models.

Base Model	Explanatory variable	p
Age	Length	< 0.01
Age	Weight	< 0.05
Age	$\delta^{13}C$	> 0.10
Age	$\delta^{15}N$	> 0.05
Age	C/N-ratio	> 0.10
Age	Season	> 0.10
Age	Sex	> 0.10
Age	Location	> 0.10

Table A1-34. AIC table of advanced models for plerocercoid larvae of cestodes in charr and the base model containing age.

Explanatory variable	AIC
Age	602.2455
Age + Length	596.2323
Age + Weight	600.3266

Age was the best single predictor of the abundance of copepod transmitted parasites in charr according to the AIC (Table A1-35). More advanced models additionally including length and weight were significantly better according to maximum likelihood (Table A1-36). Of these three models, the model containing age and length had the lowest AIC (Table A1-37), and was selected.

Table A1-35. AIC table for base models for the abundance of copepod transmitted parasites in charr.

Explanatory variable	AIC
Length	623.5514
Weight	623.4335
Age	617.9401
$\delta^{13}C$	636.9653
$\delta^{15}N$	638.1782
C/N-ratio	638.0415
Sex	637.2904
Season	637.6381
Location	638.3867

Table A1-36. Model comparison for advancing the model for copepod transmitted parasites in charr according to maximum likelihood.

Base Model	Explanatory variable	p
Age	Length	< 0.01
Age	Weight	< 0.05
Age	$\delta^{13}C$	> 0.10
Age	$\delta^{15}N$	> 0.10
Age	C/N-ratio	> 0.10
Age	Season	> 0.10
Age	Sex	> 0.10
Age	Location	> 0.10

Table A1-37. AIC table for base model and advanced models that were significantly better, modelling the abundance of copepod transmitted parasites in charr.

Explanatory variable	AIC
Age	617.9401
Age + Length	612.8513
Age + Weight	615.5954

The base model containing season had the lowest AIC for models of the abundance of acanthocephalans in perch (Table A1-38). The model containing both season and $\delta^{15}N$ was the only model significantly better than the base model (Table A1-39). The more advanced model was selected.

Table A1-38. AIC table for base models for the abundance of acanthocephalans in perch.

Explanatory variable	AIC
Length	393.9637
Weight	397.0874
Age	397.0297
$\delta^{13}C$	397.3429
$\delta^{15}N$	392.4103
C/N-ratio	397.1748
Sex	393.8278
Season	384.4378
Location	399.0303

Table A1-39. Comparison of base model for acanthocephalans in perch to more advanced models using maximum likelihood.

Base Model	Explanatory variable	p
Season	Length	> 0.05
Season	Weight	> 0.10
Season	$\delta^{13}C$	> 0.10
Season	$\delta^{15}N$	< 0.05
Season	Sex	> 0.10
Season	Location	> 0.10
Season	Season	> 0.10
Season	C/N-ratio	> 0.10

Appendix 1 continued.

Age was the best predictor for copepod transmitted parasites in perch according to AIC (Table A1-40). According to maximum likelihood, the addition of season improved this model (Table A1-41). However, the model containing age and season as explanatory variables exhibited curved patterns in the residuals, and did not fit trends in the data well. The creation of quadratic models was attempted.

Table A1-40. AIC table for base models of copepod transmitted parasites in perch.

Explanatory variable	AIC
Length	74.14675
Weight	71.90901
Age	65.70433
$\delta^{13}C$	77.29767
$\delta^{15}N$	75.53130
C/N-ratio	77.26851
Sex	74.03819
Season	70.86179
Location	70.55145

Table A1-41. Model extension for copepod transmitted parasites in perch using maximum likelihood.

Base Model	Explanatory variable	p
Age	Length	> 0.10
Age	Weight	> 0.10
Age	$\delta^{13}C$	> 0.10
Age	$\delta^{15}N$	> 0.10
Age	Sex	> 0.10
Age	Location	NA
Age	Season	< 0.05
Age	C/N-ratio	NA

The model including age as quadratic term for the abundance of copepod transmitted parasites in perch was the best model according to AIC (Table A1-42). No additional term improved this model according to maximum likelihood (Table A1-43), and the quadratic base model was selected.

Table A1-42. AIC table for quadratic base models for the abundance of copepod transmitted parasites in perch.

Explanatory variable	AIC
Length (quadratic)	76.03101
Weight (quadratic)	72.77307
Age (quadratic)	67.18371
$\delta^{13}C$ (quadratic)	75.17302
$\delta^{15}N$ (quadratic)	77.24605
C/N-ratio (quadratic)	79.02974
Sex	74.03819
Season	70.86179
Location	70.55145

Table A1-43. Model comparisons of more advanced models to the quadratic base model for the abundance of copepod transmitted parasites in perch.

Base Model	Explanatory variable	p
Age (quadratic)	Length	> 0.10
Age (quadratic)	Weight	> 0.10
Age (quadratic)	$\delta^{13}C$	> 0.10
Age (quadratic)	$\delta^{15}N$	> 0.10
Age (quadratic)	Sex	> 0.10
Age (quadratic)	Location	> 0.10
Age (quadratic)	Season	NA
Age (quadratic)	C/N-ratio	> 0.10

Appendix 2. Age and length distribution.

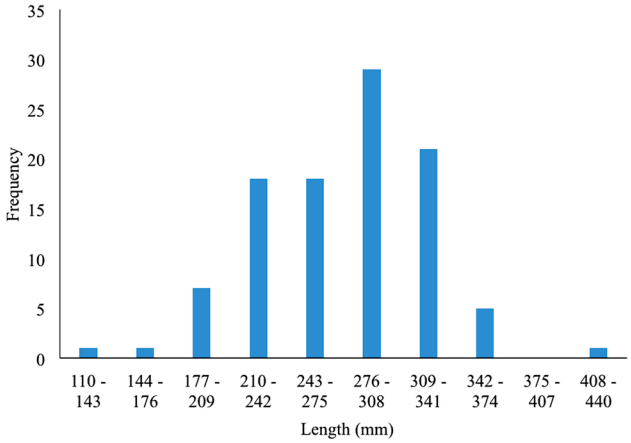

Figure A2-1. Length distribution of whitefish caught.

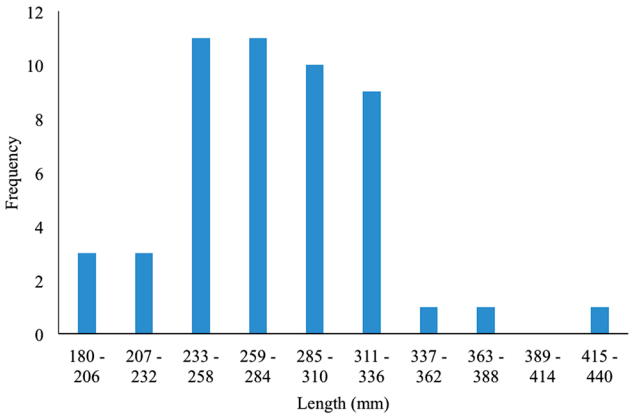

Figure A2-2. Length distribution of whitefish selected for parasite sampling.

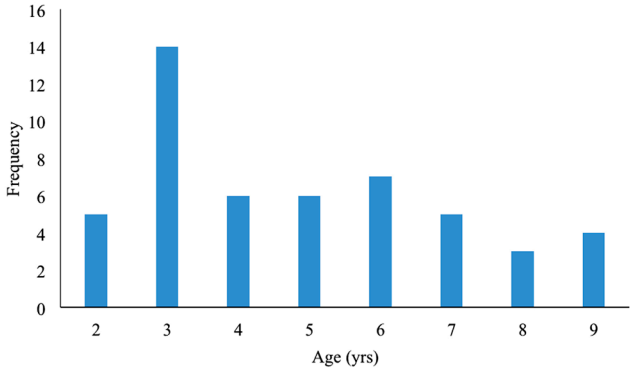

Figure A2-3. Age distribution of whitefish selected for parasite sampling.

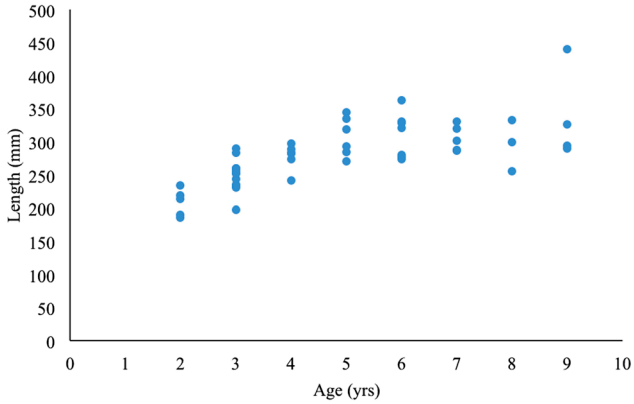

Figure A2-4. Length of whitefish selected for parasite sampling related to age.

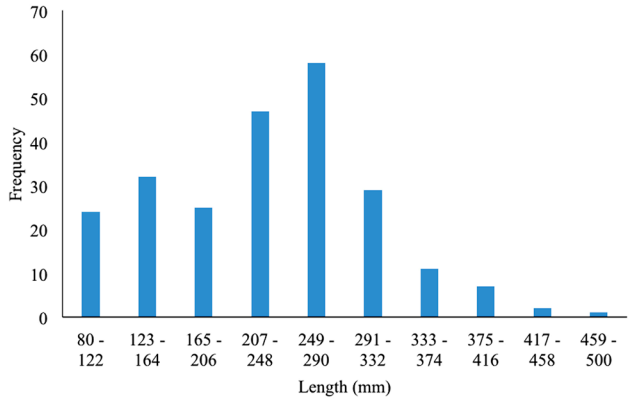

Figure A2-5. Length distribution of charr caught.

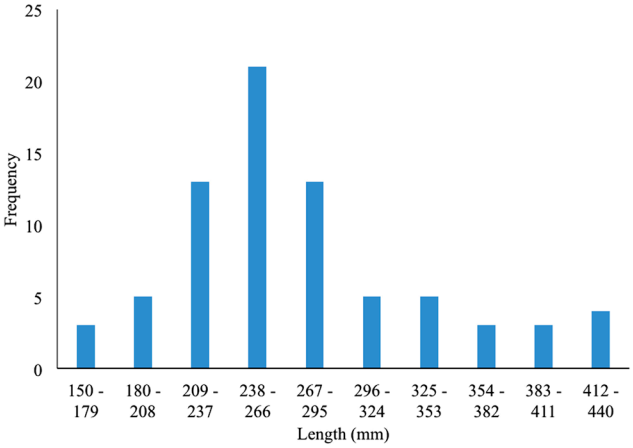

Figure A2-6. Length distribution of charr selected for parasite sampling.

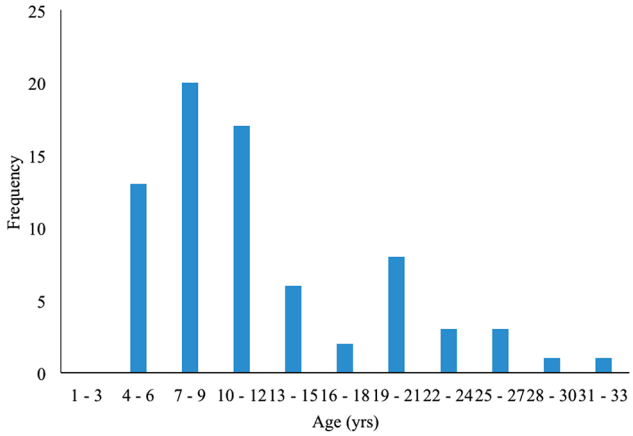

Figure A2-7. Age distribution of charr selected for parasite sampling.

Appendix 2 continued.

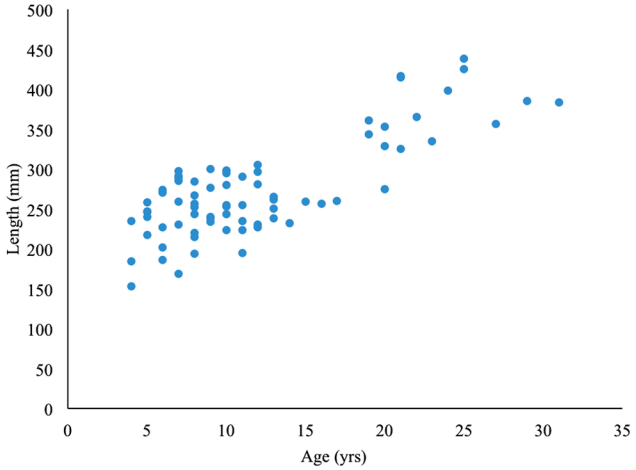

Figure A2-8. Length of Arctic charr selected for parasite analysis in relation to age.

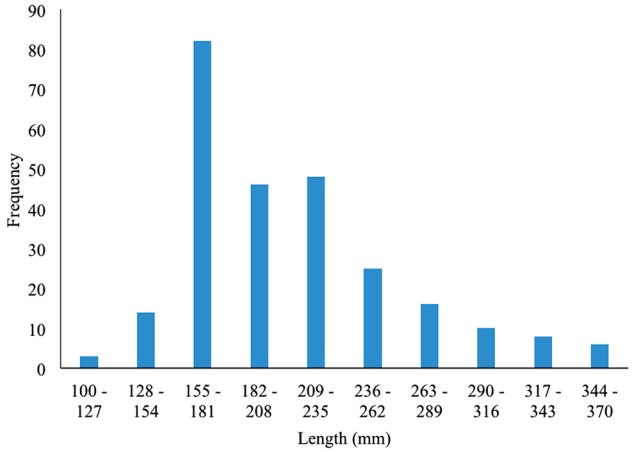

Figure A2- 9. Length distribution of perch caught

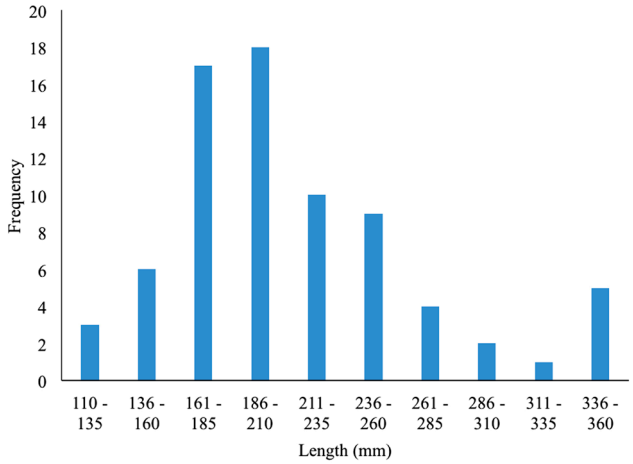

Figure A2-10. Length distribution of perch selected for parasite sampling.

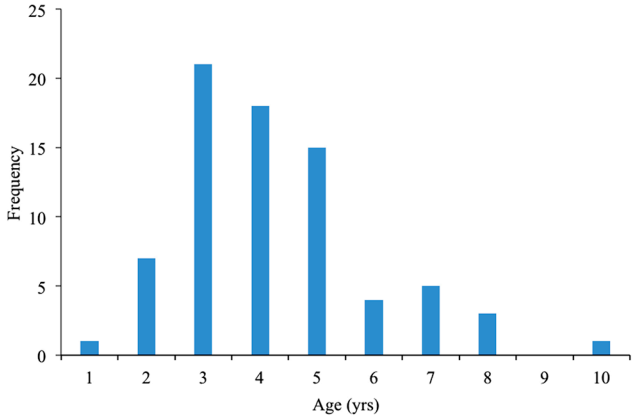

Figure A2-11. Age distribution of perch selected for parasite sampling.

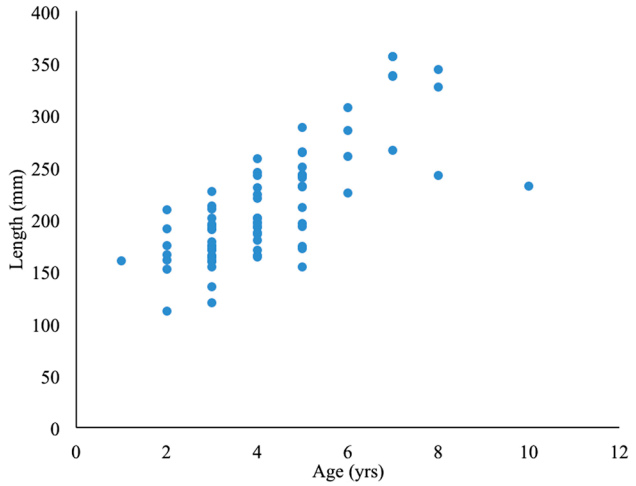

Figure A2-12. Length of Perch selected for further analysis in relation to age.

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