Reproductive Performance of Farmed Arctic Charr

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Cover: Arctic charr (*Salvelinus alpinus*) eggs at the eyed stage
(photo: H. Jeuthe)

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Abstract

The production of farmed Arctic charr (*Salvelinus alpinus)* has increased rapidly over the last decade, and the industry is predicted to continue to grow in the coming years. One major bottleneck for this future expansion concerns the supply of viable eggs and juveniles. Hatching rates of Arctic charr eggs in aquaculture are generally much lower than for other farmed salmonids, and exhibit a large variation between individuals and years. The aim of this thesis is to evaluate current hatchery conditions and to deepen the understanding on how environmental and biological factors affect the reproductive performance of Arctic charr in routine farming. The thesis comprises four separate studies.

In **paper I**, records of biological and environmental variables and individual egg survival data from an Arctic charr hatchery, covering a period of 12 years, were analysed in an attempt to find single and combined factors that can explain some of the variation in egg survival. Rearing temperature during summer was identified as the most critical factor for reproductive success of the current broodstock. The study also revealed strong positive relationships between female age, egg size, and egg viability, most likely enhanced by the thermal stress experienced by the broodstock.

Paper II assessed broodstock rearing temperature and egg viability over a period of 28 years with focus on thermal stress in a changing climate. Mean summer water temperatures in the hatchery increased by approximately 2°C from 1986 to 2010. The temperature increase was most evident in July but was also apparent in May, August, and September. Egg survival was most closely linked to September temperatures, which indicates that it is not necessarily the warmest days that are the most detrimental.

Paper III is a study on egg incubation temperature, and the first, to my knowledge, to show that the initial stages of embryogenesis in Arctic charr are much more cold-sensitive than later in the incubation period. Incubations initiated at low temperature (2.3°C to 2.8°C) resulted in significantly higher mortality and deformity rates.

Paper IV is a case study which assessed hormonal status and gamete quality of male and female Arctic charr during routine artificial fertilisation. Female plasma levels of maturation inducing hormone during stripping indicated that post-ovulation aging of oocytes can explain part of the egg loss. The results also suggest that the paternal effect on reproductive performance of the current broodstock is considerable.

Keywords: Salmonidae, aquaculture, breeding, environmental factors, egg quality, sperm quality, fertilisation, embryogenesis

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Dedication

To my family

Pappa, är du beredd?
Arild Jeuthe

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Jeuthe, H., Brännäs, E., Nilsson, J. (2013). Effects of egg size, maternal age and temperature on egg viability of farmed Arctic charr. *Aquaculture* 408-409, 70-77.

II Jeuthe, H., Brännäs, E., Nilsson, J. (2015) Thermal stress in Arctic charr *Salvelinus alpinus* broodstock: a 28-year case study. *Journal of Fish Biology* 83, 1139-1152.

III Jeuthe, H., Brännäs, E., Nilsson, J. Effects of variable egg incubation temperatures on the embryonic development in Arctic charr *Salvelinus alpinus* (submitted manuscript).

IV Jeuthe, H., Schmitz, M., Brännäs, E. A case study on hormonal status and gamete quality of Arctic charr *Salvelinus alpinus* broodstock at stripping (manuscript).

Papers I and II are reproduced with the permission of the publishers.

The contribution of Henrik Jeuthe to the papers included in this thesis was as follows:

I Designed jointly with co-authors; Jeuthe performed the majority of data analysis and writing.

II Designed jointly with co-authors; Jeuthe analysed the data and had main responsibility for the writing.

III Majority of experiment design and execution, and manuscript writing, as well as all data analysis by Jeuthe.

IV Study design, planning and coordination of sampling, parts of laboratory analyses, and majority of data analysis and manuscript writing by Jeuthe.

1 Introduction

One of the major steps in establishing a sustainable aquaculture production of any new species is to close the reproductive cycle in captivity. The hatchery production is thereby made independent of wild stock and in extension a breeding programme can be developed. Control of the reproduction cycle requires a deep understanding of the species' behaviour, physiology, environmental requirements, etc. In captivity, the fish are unable to choose food, habitat, and other environmental conditions for themselves, and hence, these factors must be controlled by the culturist to mimic natural conditions that induce sexual maturation and spawning. Alternatively, sexual maturation and spawning may be induced by hormonal manipulation of the broodstock. With the increasing insight into the endocrine systems of fish, hormonal treatment has become more commonly used to achieve reproduction in novel species introduced to aquaculture (Mylonas *et al.*, 2010).

The Arctic charr (*Salvelinus alpinus*) can hardly be considered a new aquaculture species in Sweden today, after 30 years of selective breeding. Yet, the species still exhibits poor and erratic reproductive success similar to what is found in developing aquaculture of new species. The issue of unreliable egg and juvenile production has been identified as one of the major restraints of a continued expansion of the Arctic charr farming industry (Eriksson *et al.*, 2010; Jobling *et al.*, 1998).

The aim of this thesis is to evaluate current hatchery conditions of Arctic charr broodstock and to deepen the understanding on how environmental and biological factors affect the reproductive performance in routine farming.

1.1 The Arctic charr

The Arctic charr is, as the name suggests, adapted to a cold environment. The species is mainly found in Arctic and subarctic regions around the globe; in Europe, natural populations of Arctic charr are found as far south as the British

Isles and in the Alps. The southern populations are exclusively landlocked, while the anadromous lifestyle becomes more common towards the north. Spawning typically occurs in the autumn, from September in Arctic waters to December in the far south. In the south, spawning may also occur in the spring. One well-studied example of this is Lake Windermere in England, where two separate Arctic charr populations are found within the lake. One population spawns in shallow waters in autumn and the other one in deep water in the spring. Generally, spawning takes place over gravel or rocky bottoms in both rivers and lakes. The depths of the spawning sites vary greatly from near shore locations of just one or a few meter depth to mid lake locations at 100 meters depth. In Scandinavian river-spawning populations, spawning sites are often restricted to the uppermost parts of the system where there is no competition with the brown trout (*Salmo trutta*). Fertilisation and deposition of the eggs in the redd occur at a temperature of approximately 4°C (Scott & Crossman, 1973). The eggs are then incubated in the gravel over winter at temperatures between approximately 0°C and 3°C (Johnson, 1980; Schindler *et al.*, 1974; Scott & Crossman, 1973). The upper incipient lethal temperature for eggs is approximately 8°C (Janhunen *et al.*, 2010; De March, 1995; Jungwirth & Winkler, 1984). For the juvenile stages as well as feeding and growth in adults the limit is approximately 20°C (Elliott & Klemetsen, 2002; Thyrel *et al.*, 1999; Lyytikainen *et al.*, 1997).

The Swedish Arctic charr breeding programme (described in Eriksson *et al.*, 2010; Nilsson *et al.*, 2010) is based on a population found in Lake Hornavan, northern Sweden (66°13'N 17°34'E). Lake Hornavan is one of the largest lakes in Sweden and contains several Arctic charr spawning locations at a variety of depths. Spawning takes place in October to November at an estimated water temperature of 4°C to 5°C. Following the spawning event, the temperature at the redd sites decreases and reaches a steady level of approximately 1.5°C in early January, and remains stable at this level until spring warming takes effect in early May (fig. 1), at which point the eggs are expected to start hatching.

1.2 Egg quality

Reproductive success and egg quality are sometimes used synonymously in discussions on juvenile production in fish. Indeed, egg quality is a major determinant of reproductive success and offspring viability, and the female's contribution to her progeny is greater than that of the male. However, the paternal effects on both fertilisation and embryonic development should not be neglected. In a general sense, egg quality can be defined as the potential of an egg to be fertilised and develop into a viable hatchling (reviewed in e.g.,

Migaud *et al.*, 2013; Bobe & Labbé, 2010; Lubzens *et al.*, 2010; Brooks *et al.*, 1997; Kjørsvik *et al.*, 1990). By this definition, egg quality is determined by both environmental conditions during fertilisation and incubation and intrinsic properties of the oocyte itself. These intrinsic properties, in turn, are products of the genetic qualities of the female as well as the conditions she has been subjected to. The most common way to quantify egg quality is to measure survival rates at different stages of development, i.e. fertilisation rate and survival rate to the eyed stage, hatching, first feeding, etc. This is the truest, as well as the most practical measure of egg quality; in a farming situation it is synonymous with the hatchery production rate. However, it provides no information on the factors behind the apparent variations in egg quality.

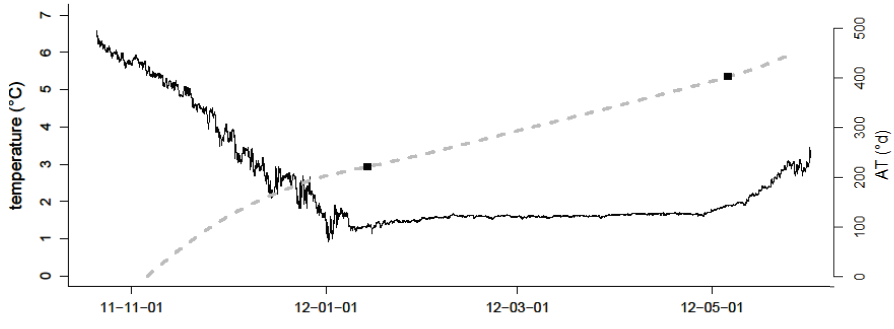

Figure 1. Temperature at natural Arctic charr redd sites in Lake Hornavan (solid line; mean from three sites), and a hypothetical egg incubation period, from November 5 to May 5 (dashed line) including estimated time (points) and AT (accumulated temperature) of the eyed stage (220°d) and hatching (400°d).

1.2.1 Assessment of egg quality

Early indicators of egg quality that enable predictions of juvenile production are highly desirable to farmers and breeders. Several such indicators have been proposed and are applied in the industry; most of them, however, have questionable accuracy and they are often species specific (reviewed in Kjørsvik *et al.*, 1990). Historically, there has been popular belief in a general, positive relationship between egg size and quality. It is true that egg size can be correlated with both larval size and yolk storage, which, under natural conditions of limited food availability and predator presence, may be advantageous for growth and survival (Einum & Fleming, 1999). In aquaculture, however, where the risk of starvation and predation is diminished, the advantage of a large endogenous energy reserve is minimal. There are several studies that indicate no direct connection between egg size and survival under favourable conditions in salmonids (Leblanc *et al.*, 2014; Springate &

Bromage, 1985; Thorpe *et al.*, 1984). Under inadequate farming conditions, however, small quality deficiencies may become evident, making e.g., egg size a valid quality indicator. Egg size may act as an indicator of a quality parameter without being part of the causality, e.g. egg size increases during the post-ovulatory aging process (Aegerter & Jalabert, 2004). Other egg quality indicators that have been used for salmonids include lipid droplet distribution (Mansour *et al.*, 2008; Mansour *et al.*, 2007), transparency (Escaffre & Billard, 1979; Nomura et al., 1974 cited in Kjørsvik *et al.*, 1990), weight gain during hardening (Mansour *et al.*, 2008; Mansour *et al.*, 2007), and ovarian fluid composition (Aegerter & Jalabert, 2004; Lahnsteiner *et al.*, 1999).

1.3 Sperm quality

Historically, sperm quality has been defined as the ability of a sperm to successfully fertilise an egg (reviewed in Bobe & Labbé, 2010; Rurangwa *et al.*, 2004). However, sperm quality is now known to have a major influence on embryonic development as well (reviewed in Cabrita *et al.*, 2014). The issue of variable sperm quality has received relatively little attention in aquaculture. The strategy in commercial salmonid farming is usually to use an excessive amount of sperm and a minimal volume of water in the fertilisation vessel, thereby maximising the likelihood of the sperm to localise and fertilise each of the oocytes in the batch. In addition, milt from a number of males are often mixed and used to fertilise the eggs of several females. In this way, the influence of potentially bad milt is reduced and high fertilisation rates can be maintained. But of course, when eggs are fertilised by milt of mixed paternity, it is difficult to evaluate the relative sperm quality of different males. In addition, differences in sperm quality between males under these circumstances will affect the genetic diversity and population size of the offspring generation and this method is not sustainable in a breeding situation.

1.3.1 Assessment of sperm quality

It is desirable to be able to assess and predict sperm quality prior to fertilisation. And there are several methods to do this, none of which are universally applicable or individually reliable. Therefore, sperm quality is often assessed by combining measurements of several characteristics. Traditional and simple measures of sperm quality are milt volume and density (number of sperm per volume), which can be determined either manually, using a light microscope and counting chamber, or by automated methods, e.g., with the NucleoCounter® SP-100™ (Chemometech, Denmark) (Nynca & Ciereszko, 2009). Sperm density may also be estimated indirectly by determining

spermatocrit (ratio of matter to total volume in a centrifuged sperm sample) or by spectrophotometry (Rurangwa *et al.*, 2004). Spermatocrit and optical density values can then be converted into sperm density using species specific formulas (Ciereszko & Dabrowski, 1993; Piironen, 1985; Bouck & Jacobson, 1976). Another commonly used indicator of sperm quality is motility. Traditionally, motility is a measure of the proportion of sperm within a batch that are actively swimming, but motility measurements can also include more detailed information on the swimming patterns of individual spermatozoa. With video recordings of active sperm, quantitative swimming characteristics such as speed, linearity, and stamina can be determined either manually or with the help of CASA (Computer Assisted Sperm Analysis) software (reviewed in e.g. Fauvel *et al.*, 2010; Rurangwa *et al.*, 2004). Ultimately, volume, density, and motility parameters affect fertilisation by means of probability. However, variations in these parameters may result from several different biological and environmental factors and be accompanied by, and thereby act as indicators for, other quality issues (Fauvel *et al.*, 2010). Other sperm quality indicators that are directly related to fertilisation rates include e.g., morphology and ultra-structure as well as seminal plasma composition (e.g., enzymes, sugars, vitamins, osmolarity and pH) (Migaud *et al.*, 2013; Rurangwa *et al.*, 2004).

The paternal effects on embryonic viability and development have received increasing attention during recent years and the definition of sperm quality is no longer limited to fertilisation potential. The paternal influence on embryo viability, and offspring viability in general, was previously believed to be limited to the actual genes transferred from father to progeny. It has now been suggested that embryo viability is directly affected by epigenetic and transcriptional impairments in the sperm (Cabrita *et al.*, 2014). Remnant mRNA from spermatogenesis, although inactive within the spermatozoa (Lalancette *et al.*, 2008), can play key role in early embryonic development in mammals (Johnson *et al.*, 2011; Lalancette *et al.*, 2008). However, to my knowledge, analyses of RNA have not yet been used to predict sperm quality, at least not in fish. Chromatin fragmentation may affect both fertilisation and embryonic development. Many of the factors that affect chromatin integrity also affect other cell structures, and a selection for spermatozoa with intact chromatins is therefore applied during fertilisation (Hourcade *et al.*, 2010). However, sperm with damaged DNA may still fertilise oocytes. In rainbow trout (*Oncorhynchus mykiss*), sperm carrying 10% fragmented DNA has been shown to successfully fertilise eggs, but with significantly elevated embryo mortality (Pérez-Cerezales *et al.*, 2010). Other studies on rainbow trout, brown trout, and Arctic charr have reported on embryo mortality and deformities that have resulted from DNA-damage in the sperm (Devaux *et al.*, 2011; Dietrich *et*

al., 2005). The most common method for assessing chromatin integrity in fish is the comet assay or SCGE (single cell gel electrophoresis) (Cabrita *et al.*, 2014; Devaux *et al.*, 2011; Cabrita *et al.*, 2005). The method is based on differences in migration patterns of different sized DNA-fragments, where fragmentation results in a comet-like distribution, with intact DNA in the head and small fragments in the tail of the comet.

1.4 Hypothalamic-pituitary-gonadal axis

Sexual maturation in fish is controlled through the hypothalamic-pituitary-gonadal (HPG) axis (reviewed in Taranger *et al.*, 2010). This endocrine system, in turn, is influenced by a range of environmental and biological factors (fig. 2); the most important factors for salmonid reproduction are briefly presented in the next section.

Very simplified, the hormonal cascade along the HPG axis starts with the release of GnRH (gonadotropin releasing hormone) and dopamine by hypothalamus, which stimulate (or inhibit) the release of GTHs (gonadotropins) from the pituitary (reviewed in e.g., Peter & Yu, 1997). There are two distinct GTHs, FSH (follicle-stimulating hormone) and LH (luteinising hormone). Both stimulate growth and development of the gonads, but during different stages of the sexual maturation process. Generally, plasma levels of FSH are high during vitellogenesis and spermatogenesis followed by a drop in connection to spawning, while LH levels are low throughout the major parts of vitellogenesis and spermatogenesis and peak during spawning (Levavi-Sivan *et al.*, 2010; Yaron *et al.*, 2003; Schulz & Miura, 2002; Nagahama, 1994).

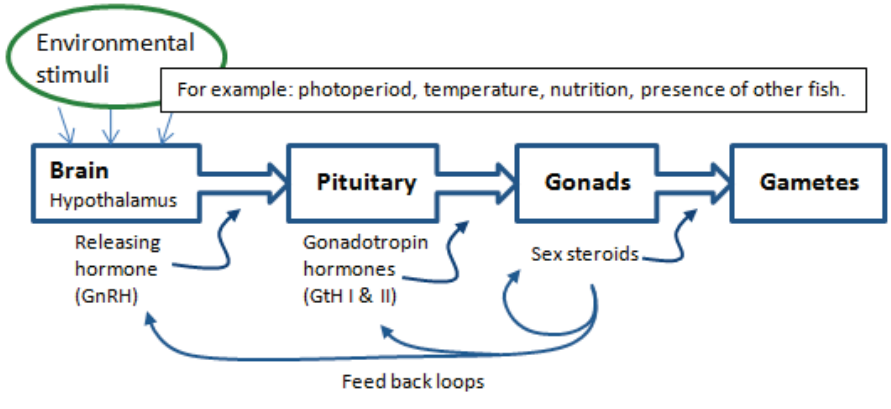

Figure 2. Schematic description of the hypothalamic-pituitary-gonadal (HPG) axis, that controls sexual maturation in fish.(from Brännäs et al., 2011)

In females, FSH stimulates the secretion of testosterone (T) in the gonads during vitellogenesis. T is aromatised to 17β-estradiol (E_2), which in turn feeds back to the vitellogenetic process. During the final maturation of the oocytes, there is a fall in plasma levels of first E_2 and then T. Simultaneously, increased levels of LH cause the ovarian follicle cells to produce maturation-inducing-hormone (MIH); in salmonids this is the 17α,20β-dihydroxy-4-pregnene-3-one (17,20β-P). MIH then acts directly on the oocytes, promoting final maturation and ovulation (Nagahama, 1997).

In males, production of androgens, T and its derivative 11-ketotestosterone (11KT), is stimulated by both FSH and LH (Schulz *et al.*, 2010; Schulz & Miura, 2002). The androgens drive the spermatogenetic process in the testis and gives feedback to the brain and pituitary, thereby stimulating the development of secondary sexual characters as well as sexual behaviour (Borg, 1994; Nagahama, 1994). 17,20β-P is also produced in the testis during the spawning season and acts on the final stages of sperm maturation and activation (Schulz & Miura, 2002).

1.5 Determinants of gamete quality

1.5.1 Nutrition

One of the most fundamental requirements for successful reproduction is a generally high health status of the broodstock; feeding and nutrition plays a major role in achieving this. Insufficient feeding may by itself have serious implications for gonadal development and maturation, including fecundity and individual investment in the gametes (reviewed in Izquierdo *et al.*, 2001). Feed restrictions, even during relatively short periods, have been reported to inhibit or delay sexual maturation in salmon (*Salmo salar*) (Norrgård *et al.*, 2014; Rowe & Thorpe, 1990). However, the quantity of food is seldom an issue in aquaculture. What is more likely to be a problem is the nutritional composition of the feed. The most widely studied feed components, with regard to fish reproduction, are lipids, essential fatty acids in particular, and vitamins (reviewed in e.g., Migaud *et al.*, 2013; Bobe & Labbé, 2010; Izquierdo *et al.*, 2001; Kjørsvik *et al.*, 1990). In order to assess the effects of inadequate nutrition on reproductive performance, first, the specific requirements of the particular species have to be determined. This can be done by comparing the nutritional composition of eggs from wild and farmed fish. Such comparisons for Arctic charr and salmon revealed significant differences in fatty acid (FA) composition accompanied by differences in egg survival (Pickova *et al.*, 2007; Pickova *et al.*, 1999). However, it could not be concluded, in either case, that

the lower egg survival of the farmed fish was caused by imbalanced FA composition of the broodstock diet.

1.5.2 Light

Seasonally breeding animals rely on environmental cues to synchronise spawning within a population. Sexual maturation and spawning in salmonids is influenced by a range of environmental factors, however, changes in photo period is regarded as the main external cue (Bromage *et al.*, 2001). Photoperiod manipulations can be used to alter the time of spawning in Arctic charr, thereby enabling off-season egg production (Gillet & Breton, 2009; Frantzen *et al.*, 1997; Gillet, 1994). Exposure to changes in day length acts on the timing of maturation and spawning through the hypothalamic-pituitary-gonadal axis (fig. 2). However, the exact mechanism through which photoperiod exerts control on the endocrine system is, to my knowledge, still unknown. Also the required intensity and spectral compositions of photo cues that will affect reproduction are largely unknown. Frantzen et al. (1997) used a setup with a light intensity of 100 lux at the water surface for their study on sexual maturation in Arctic charr. Oppdal et al. (1997) used a range of different light intensities, 5, 17, 56, and 594 lux at 10 m depth, the highest value representing natural day light, and concluded that salmon have different light intensity requirements for effects on growth and sexual maturation. Only the fish subjected to natural light underwent sexual maturation while all artificial light regimes affected growth. In Arctic charr hatcheries, the broodstock are often moved to indoor rearing tanks prior to the spawning season. One reason for this is to avoid exposing the fish to sub-zero temperatures during stripping. It is uncertain whether the light conditions in such indoor broodstock facilities have ever been evaluated with regard to effects on sexual maturation of the broodstock. In addition, the effects of disrupted photoperiod cues during the late stages of vitellogenesis and final oocyte maturation are, to my knowledge, undocumented.

Poor synchronisation of spawning can have severe implications for reproductive success. If ovulation is triggered too early in autumn spawning species, like the Arctic charr, high water temperature will accelerate egg quality deterioration (Gillet, 1994). Poor synchronisation among the broodfish, i.e., a prolonged spawning season, will also lead to more examinations to find ovulating and spermiating individuals and thereby more handling of the fish which increases the stress level, a factor known to have negative impact on gamete quality and offspring viability (Li & Leatherland, 2012; Li *et al.*, 2010; Campbell *et al.*, 1994; Campbell *et al.*, 1992).

1.5.3 Temperature

Temperature has major impact on reproductive performance in fish and may act on egg quality through many different pathways. It is not always easy to sort out the causality between environmental factor, egg quality traits, and effects on fertilisation and survival. Temperature may act as both an ultimate factor affecting physiological processes directly, and as a proximate factor, co-varying seasonally with other factors (reviewed in e.g., Pankhurst & King, 2010). In addition to the influence of photoperiod in salmonids, temperature changes during both spring and autumn have been shown to affect timing of the reproductive cycle (King *et al.*, 2007; Taranger *et al.*, 2003; Taranger & Hansen, 1993). Studies on salmon have shown that elevated temperature during vitellogenesis, in summer, causes reduction in maternal investment into the oocytes, which results in smaller eggs with impaired viability (King *et al.*, 2003). A short period, approximately one month, of elevated temperature was sufficient to reduce fertilisation and survival rates (King *et al.*, 2007). In addition, first time spawning female salmon seem to be more susceptible to thermal stress during this period than repeat spawners (Pankhurst *et al.*, 2011). Elevated temperature during vitellogenesis has been shown to affect FA composition in the eggs of Arctic charr, which in turn may have implications for egg quality (Jobling *et al.*, 1995). In addition, temperature elevations during vitellogenesis and oocyte maturation can delay and even inhibit ovulation in Arctic charr, as well as other salmonids (Pankhurst & Porter, 2003; Jobling *et al.*, 1995; Gillet, 1991). Gillet (1991) reported that ovulation in Arctic charr was significantly delayed at 8°C and inhibited at 11°C. Further, if the ovulated eggs are not fertilised within a given time their quality will start to deteriorate. Arctic charr eggs retain their full potential for approximately five days post-ovulation at 5°C (Pankhurst & Porter, 2003; Jobling *et al.*, 1995; Gillet, 1991), but this period becomes shorter with increasing temperature (Aegerter & Jalabert, 2004; Bromage *et al.*, 1994).

Embryonic development is also strictly limited by temperature. For Arctic charr, the upper limit for unimpaired embryonic survival is approximately 6°C to 8°C (Janhunen *et al.*, 2010; De March, 1995; Jungwirth & Winkler, 1984). The lower limit has not been determined for the species; in nature Arctic charr eggs may experience temperatures at least as low as 0.5°C (Johnson, 1980; Schindler *et al.*, 1974). However, studies on other salmonids have shown that the initial stages of embryogenesis are more sensitive to low temperature than later during egg incubation; e.g., eggs from chinook salmon (*Oncorhynchus tshawytscha)*, pink salmon (*Oncorhynchus gorbuscha)*, and sockeye salmon (*Oncorhynchus nerka)* exhibit elevated mortalities if initial incubation

temperature is below 4.5°C (Bailey & Evans, 1971; Combs, 1965; Combs & Burrows, 1957; Seymour, 1956).

2 Objectives

The aim of this thesis was to evaluate past and present Arctic charr hatchery conditions in an attempt to identify inadequacies that need to be amended to reach the full potential of Arctic charr broodstock in the future. While doing this, the ambition was to extend the knowledge on the effects of environmental and biological factors on fish reproduction. The individual objectives of the four papers included in this thesis were as follows.

I Records of biological and environmental variables and individual egg survival data from an Arctic charr hatchery, covering a period of 12 years, were analysed in an attempt to find single and combined factors that can explain some of the variation in egg survival of farmed Arctic charr. The specific objectives of paper I were to:

- Quantify individual egg viability variation between years and broodfish.
- Determine which stages of the reproductive cycle are impaired by thermal stress under standard rearing conditions?
- Quantify the effects of maternal age and egg size on egg viability in a thermally stressed broodstock.

II Records of annual egg production and daily water temperatures from an Arctic charr hatchery, covering a period of 28 years, were used to assess thermal stress in a changing climate. The specific objectives of paper II were to:

- Quantify the long term change in water temperature at the main Arctic charr breeding facility in Sweden.
- Further (in extension to paper I) evaluate the effect of annual temperature variation on egg viability.

- Document the impacts of thermal stress under routine farming conditions.

III Different temperature profiles, constant and variable, for Arctic charr egg incubation were evaluated. The paper consists of two main experiments with the following objectives:

- Quantify the effects of commonly used incubation programmes on embryonic survival and development status at hatching.
- Assess embryo survival and development of spinal deformities in response to the timing of a shift from autumn to winter temperature.

IV This case study assessed hormonal status and gamete quality of male and female Arctic charr during routine artificial fertilisation, with the following objectives:

- Determine specific egg loss in terms of non-viable, fertilisation failure and mortality under standard hatchery conditions.
- Approximate the time of ovulation and spermiation in relation to stripping, using plasma levels of sex steroids.
- Investigate the relationships between hormonal status of broodfish at stripping and subsequent gamete quality and offspring viability.

3 Materials and Methods

3.1 Hatchery facilities

3.1.1 Aquaculture Centre North

Aquaculture Centre North (ACN) is situated in Kälarne, central Sweden (62° 59' N; 16° 6'E) and is a combined research station and commercial hatchery (fig. 3). It was established in 1909, and Arctic charr were introduced to the station in the late 1970s. Since the mid-1980s, the station has been the main facility of the Swedish Arctic charr breeding program (described in Eriksson *et al.*, 2010; Nilsson *et al.*, 2010). It is a land-based, flow through facility that takes water from a nearby lake. Until the year 2010, the water inlet was situated near the surface of the lake, which resulted in high water temperatures during warm summers. In April 2011, a deeper inlet was installed to provide cooler water during the summer. The temperature of the incoming water is measured daily. The Arctic charr broodstock is reared indoors in concrete tanks with depths of 1 to 3 m and volumes of 7.5 to 14 m^2. Rearing follows standard farming routines with automatic feeders, artificial lighting that follows the natural ambient day length, and a maximum rearing density of 25 kg/m^3. The hatchery is subjected to strict disease control routines, and no disease outbreaks have occurred during the study period.

3.1.2 Umlax in Vilhelmina and Slussfors

Umlax is a commercial aquaculture company that is used for back-up protection of the broodstock from the Arctic charr breeding programme. The fish are kept at a net-pen facility for on-growth and maturation. This facility is situated near Slussfors, northern Sweden (65° 25' N; 16° 10'E), in a river regulated by hydroelectric power stations (fig. 3). Once sexually mature, the fish are moved to a hatchery in Vilhelmina, 100 km to the south (64° 37' N;

16° 39' E), where they are reared indoors in 2 m deep circular 25 m^3 tanks at a maximum density of 25 kg/m^3.

In comparison, the two main differences between ACN and the Umlax facilities, with regard to environmental factors affecting reproduction, are the temperature and light conditions. Summer water temperatures were generally lower at the Slussfors site. At ACN, the broodstock is kept indoors under artificial lighting while the Slussfors broodstock is outside in natural light until September. The ACN-facility is supported by water from a lake and the Vilhelmina facility is supported with water from a small river. As a result, the autumn temperature decreases faster in Vilhelmina than at the ACN.

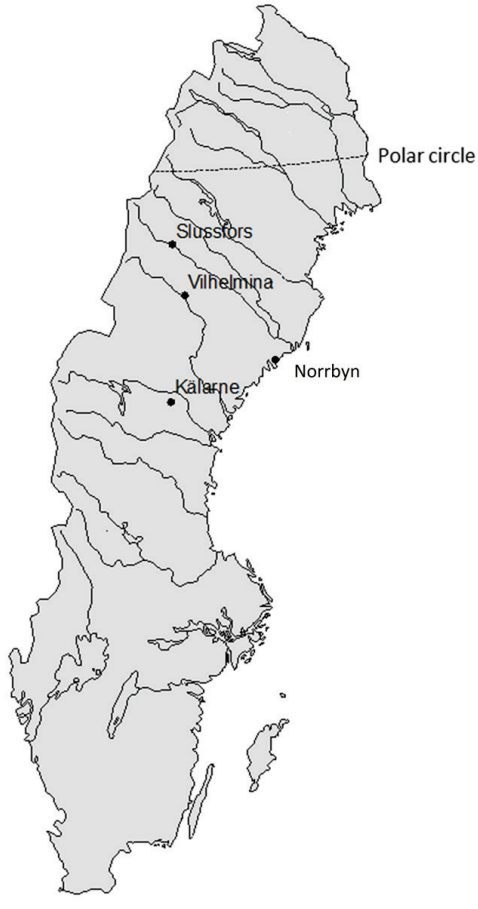

Figure 3. Locations of facilities involved in the thesis: Aquaculture Centre North in Kälarne is the main hatchery for the Swedish Arctic charr breeding programme (all papers). Umlax in Slussfors (net pens) and Vilhelmina (hatchery) were involved the site comparison study (paper II) and provided eggs for incubation experiments (paper III). The egg incubation experiments were performed at a field station/laboratory in Norrbyn (modified from Jeuthe et al., 2015).

3.2 Broodstock

The broodstock consisted of Arctic charr, mainly the 'Arctic superior', which is the selectively bred strain of the Swedish Arctic charr breeding programme. The Arctic superior descend from a pelagic predatory population found in Lake Hornavan, northern Sweden (66° 10' N; 17° 40' E), and have been selectively bred for growth, flesh pigmentation, and age at sexual maturity since the mid-1980s (Nilsson *et al.*, 2010). Paper I involved Arctic superior generations four to six and papers III and IV used broodstock of the seventh generation. Paper II included data from all seven generations of the Arctic superior, as well as three populations from Lakes Ottsjön, Rensjön and Hornavan that were bred at ACN until the 1990s, with no intended selection, for restocking in the wild.

3.3 Study design

Here follow a brief overview of the set-ups of the separate studies. Please see respective paper for more detailed descriptions.

3.3.1 Long-term hatchery evaluations

Papers I and II are both based on hatchery data from the archives of ACN. At ACN, daily logs are kept on the temperature of incoming water and are available for the entire duration of the Arctic charr breeding programme, i.e., since 1986. The average water temperature in the years 1986 to 2010 was 2.1°C (0.5 to 3.2°C) from December to February and 15.7°C (11.2 to 20.8°C) in July and August.

Paper I included data on temperature conditions for the broodfish, female age, time and temperature of egg fertilization, average egg size (per batch), incubation temperature of eggs (daily), and proportion of eyed eggs. All incubated egg batches from single dam/sire fertilizations produced with the Arctic Superior strain from 2000 to 2011 (n=540) were included in the analyses. The study involved three generations of the Arctic superior, year classes 1996, 2001, and 2005, covering broodfish ages three to nine years. Many of the females were used during more than one spawning season. Individual tagging made it possible to follow the reproductive performance of these individuals during several years. Nine females were followed for four consecutive spawning seasons, from 2004 to 2007. Six of these individuals were followed for an additional season, providing unique insight into the development of reproductive performance for individual dams over a considerable part of their lives. The data were analysed for effects on egg viability from single and combined factors (logistic regression and exploratory factor analysis).

Paper II included daily temperature data and egg viability data, pooled from individual and mixed egg batches, covering a period of 28 years (1986 to 2013). Mean monthly temperatures for the entire study period were presented and analysed for changes over time (linear regression). Annual egg viability and monthly temperatures were tested for correlations (Pearson's). Egg viability data containing reliable information on broodfish age (Arctic superior from 1994 to 2013) were used to evaluate the importance of the different temperature variables combined with female age in predicting egg viability (multiple linear regression). In addition, a comparative study of egg viability and size was performed using duplicate broodstock of equal family distribution, reared at different farming sites, Kälarne and Slussfors/Vilhelmina (described in section 3.1).

3.3.2 Egg incubation

Paper III was based on experiments on egg survival and development that were performed during three consecutive egg incubation seasons (2011 to 2013) and focused on (A) commonly used temperature profiles in hatcheries, (B) the timing of a shift to low winter temperatures, and (C) the rapidly fluctuating temperatures after the eyed stage. The purpose of last experiment was solely to test whether a technical issue that arose during the previous experiment season could have affected the results.

All incubation experiments were performed in a laboratory/field station in Norrbyn (63° 34' N; 19° 50' E), 45 km south of Umeå, north-eastern Sweden. Eggs were incubated in 100-litre glass tanks, one or two per treatment, placed in a cold room with an air temperature of approximately 2°C. The tanks were filled with non-chlorinated tap water and equipped with filtration pumps and electric aquarium heaters connected to separate digital thermostats.

In experiments A, egg batches of mixed parentage were used to study the effects of commonly used incubation temperature profiles on embryonic survival (proportion test) and development status at hatching (alevin and yolk size, t-test).

In experiment B, several full-sibling egg batches were split and incubated in parallel, in a range of treatments that shifted from autumn temperature (6°C) to winter temperature (2.5°C) at different times. Treatment effects on embryo survival and the occurrence of spinal deformities were evaluated (Cochran-Mantel-Haenszel test and logistic regression).

One of the treatment units in experiment B was unintentionally subjected to a period of rapidly fluctuating temperatures after the eggs had reached the eyed stage In consequence, a third experiment (C) was designed to test whether temperature fluctuations occurring after the eyed stage could have a negative

effect on survival and spinal development, thereby obscuring the results of the intended treatment in experiment B. Treatments are described in more detail in table 1.

Table 1. *Temperature profiles for the incubation of Arctic charr eggs used in paper III.*

exp	treatment	mean temp ± s.d. (°C)	profile description (time and AT to temperature shift)
A	TA1	2.7 ± 0.2	constant low
	TA2	2.8 ± 0.3 to 7.0 ± 0.1	temperature increase before eyed stage (day 62, 180°d)
	TA3	5.0 ± 0.3	constant intermediate
	TA4	6.8 ± 0.5 to 2.6 ± 0.2	temperature decrease before eyed stage (day 35, 220°d)
	TA5	7.0 ± 0.3 to 2.8 ± 0.6	temperature decrease after eyed stage (day 61, 410°d)
	TA6	7.0 ± 0.3	constant high
B	TB1	5.8 ± 0.02 to 2.3 ± 0.2	decrease from day one (3°d)
	TB2	5.8 ± 0.1 to 2.3 ± 0.1	decrease after one week (45°d)
	TB3	5.7 ± 0.2 to 2.2 ± 0.1	decrease after two weeks (90°d)
	TB4	6.2 ± 0.1 to 2.6 ± 0.2	decrease after three weeks (137°d)
	TB5	6.0 ± 0.2 to 2.6 ± 0.3	decrease after four weeks (183°d)
	TB6	6.1 ± 0.1	constant high
C	TC1	3.6 ± 0.2	constant
	TC2	3.6 ± 0.2 to 4.6 ± 0.8	fluctuations between 3.5°C and 6°C (day 70, 250°d)

3.3.3 Hormones and gamete quality

Paper IV was based on an assessment of broodstock hormonal status during routine artificial fertilisation and subsequent gamete quality of both male and female. In connection to stripping, blood samples were taken from 45 females and 23 males and for analyses of sex steroids (T, 11KT, E_2, and 17,20β-P). Milt was collected for volume measurement and subsamples were extracted for density (NucleoCounter® SP-100™) and motility measurements (CASA). Subsamples of eggs were used to estimate individual mean egg size and fertilisation rate. Embryo survival was measured at the eyed stage. The data were analyses for relationships between different variables using Pearson's correlation and linear regression.

4 Main findings

4.1 Long-term hatchery evaluations (papers I & II)

4.1.1 Temperature

Paper I identified summer water temperature as the most critical factor for reproductive success of the Arctic charr broodstock at the main hatchery of the Swedish Arctic charr breeding programme in Kälarne (ACN). Higher temperatures resulted in fewer eyed eggs, most likely caused by temperature stress during major oocyte development. This finding is in agreement with previous studies by King et al. (2007; 2003) on Atlantic salmon in Tasmania. They determined that high temperatures during summer, 3 months before fertilization, cause disruptions in gonadal development by hormonal disturbance.

Autumn temperature was also negatively correlated with reproductive success. Although this relationship was not as strong as that for summer temperatures, it was statistically significant. Temperature stress during summer and autumn were unrelated according to the exploratory factor analysis (EFA), suggesting that stress in different seasons disrupts reproduction through different mechanisms and/or stages in reproduction.

The relationships between egg viability and summer and autumn temperatures were verified, and somewhat clarified in paper II. The results in the more extensive analysis of rearing temperature in paper II showed that egg survival was most closely linked to September temperatures (fig. 4), which indicates that it is not necessarily the warmest days that are the most detrimental. Results of paper II also suggest that the correlation found between egg survival and autumn temperature conditions reflect a positive effect of a distinct autumn cooling on egg viability, likely through synchronisation of spawning. This would reduce the handling stress on the broodstock, i.e., the number of examinations before the females reach ovulation, a factor known to affect egg quality (Campbell *et al.*, 1994; Li and Leatherland, 2012). In

addition, exposure to elevated temperatures (above 8°C) close to and during spawning speeds up the maturation of the oocytes and causes over-ripening of the eggs (Gillet, 1991; Jobling *et al.*, 1995) and may delay or even inhibit ovulation (reviewed in Pankhurst and Porter, 2003). Fertilisations in the ACN hatchery occurred at temperatures between 8°C and 10°C on several occasions during the present study period.

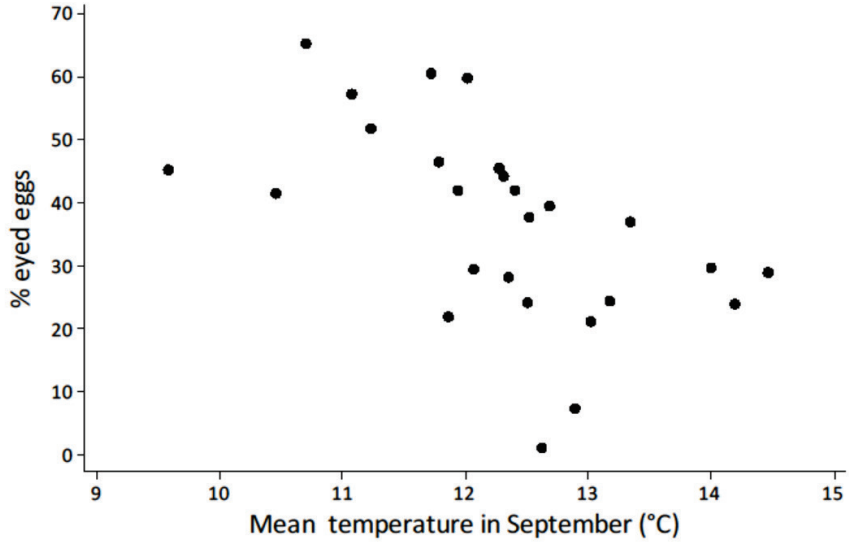

Figure 4. Annual egg survival (% eyed eggs) in relation to mean temperature in September of the same year. Significant correlations were also found for May, July, and August (from Jeuthe et al., 2015).

Mean summer temperatures of incoming water at the main hatchery of the Swedish Arctic charr breeding programme in Kälarne (ACN) increased by approximately 2°C from 1986 to 2010. The temperature increase was most evident in July but was also apparent in May, August, and September, as well as in the number of days per year exceeding 15°C (fig. 5). The installation of a new water inlet reduced the temperature substantially during the warmest months (July and August) and thereby the overall maximum temperatures. However, its effect on September temperatures was not as evident and non-existing for later autumn months, which could explain the lack of a clear shift in egg survival in connection to the inlet installation in 2011.

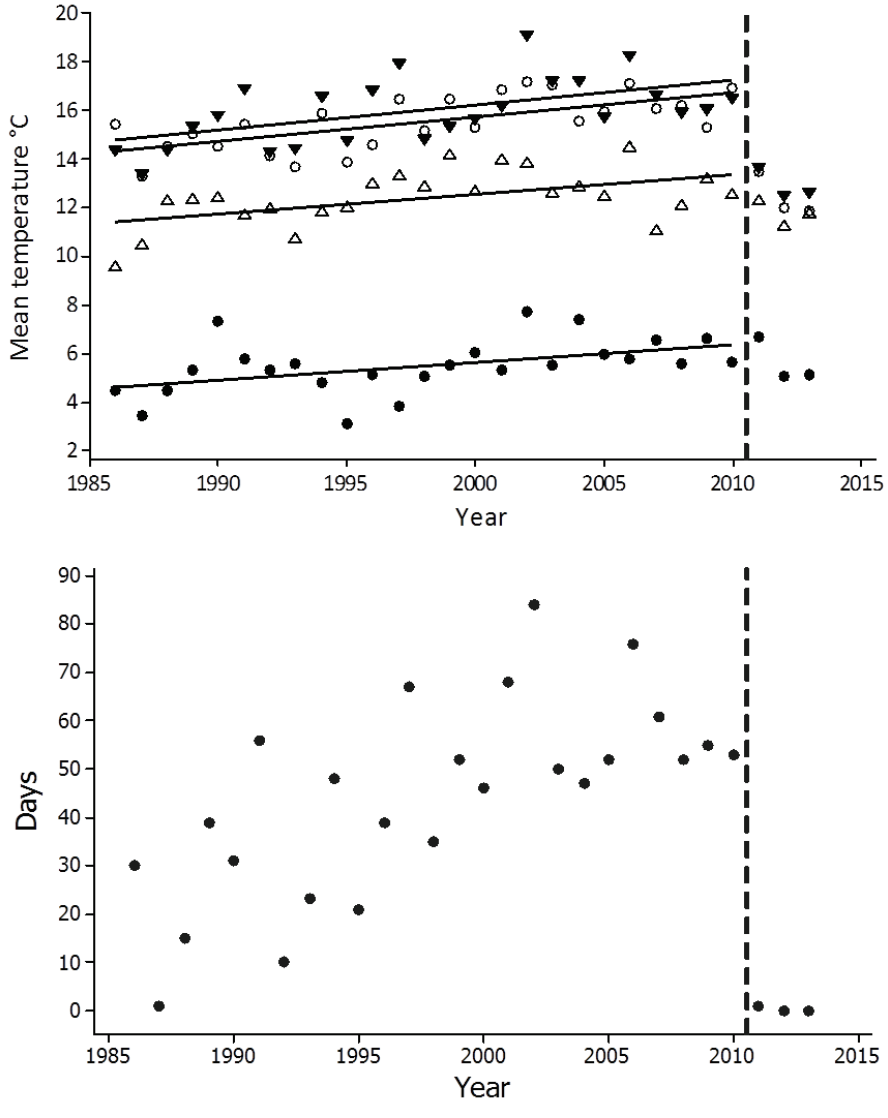

Figure 5. The temperature conditions in a S. alpinus hatchery located in central Sweden (fig. 3) from 1986 to 2013. The vertical line indicates the installation of a new deeper water inlet providing colder water to the facility during summer. Upper panel: The mean monthly temperatures (points) from 1986 to 2013 and linear regressions (solid lines) showing the general temperature change from 1986 to 2010 for the four months with significant increase. From the bottom, the order of the presented months (points and lines) are May ●, September △, July ○, and August ▼. Lower panel: The number of days with water temperatures exceeding 15°C from 1986 to 2013 (from Jeuthe et al., 2015).

The EFA in paper I suggested that incubation temperatures could explain some of the variation in egg survival at ACN. However, the nature of this relationship was not very clear. Regression analysis did show a negative trend between mean incubation temperature and egg survival, but with variable temperature profiles, the relationship was likely to be more complex. Earlier studies to determine the critical upper incubation temperatures for Arctic charr have been performed with constant temperatures from fertilization to hatching (De March, 1995; Janhunen et al., 2010; Jungwirth and Winkler, 1984). There were also reports that pink salmon and chinook salmon eggs require an initial incubation temperature above 4.5°C for normal embryonic development (Bailey and Evans, 1971; Combs and Burrows, 1957). It seems likely that sensitivity to low temperature during early incubation is a common trait for late summer and autumn spawners, as their eggs are naturally subjected to decreasing temperature from fertilization to the eyed stage. These results from previous studies together with those of paper I lead to the development of the experiments in paper III.

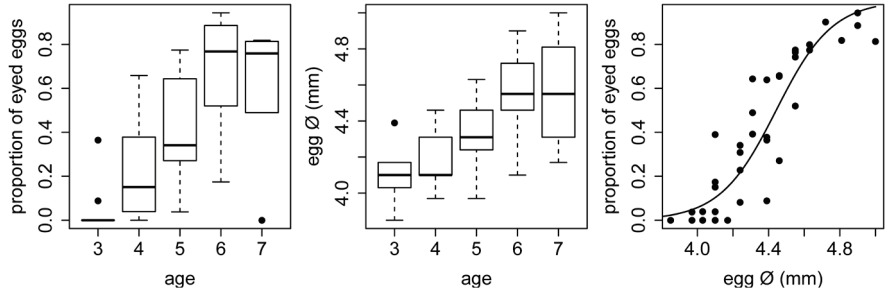

Figure 6. Egg characteristics of repeat-spawning female Arctic charr followed for 5 consecutive spawning seasons (n=9 for age 3 to 6, n=6 for age 7). Box plots present median (bold line), quartiles (box), and total range of values (whiskers and points).Left panel: Proportion of eyed eggs in relation to female age. Centre panel: Egg size in relation to female age. Right panel: Proportion of eyed eggs in relation to average egg size in each egg batch. Line shows fitted logistic regression model (z=3.204, p<0.01) (from Jeuthe et al., 2013).

4.1.2 Female age and egg size

In paper I, strong relationships were found between female age, egg size, and egg viability (fig. 6). Egg size and survival both increased with female age up to 6 years, after which there was no further improvement. Egg size was also more closely linked to egg survival than female age. The relationship between egg size and survival in this study was stronger than have been described in earlier publications on Arctic charr as well as other salmonids. In particular, the data on successive spawning seasons of the same individuals provided a

strong model for predicting egg viability from egg size. Egg size can also be affected by the temperature conditions under which the females are kept. In this study we found a negative correlation between average August temperature and egg size during the following spawning season. It is therefore likely that the relationships that were observed between female age and egg survival, as well as between egg size and survival, were strengthened by thermal stress.

4.2 Egg incubation (paper III)

Paper III is the first study, to my knowledge, to show that the initial stages of embryogenesis in Arctic charr are much more sensitive to low temperature than later in the incubation period. Furthermore, this lower temperature limit for unimpaired survival and development during the initial incubation period is relatively high (≥2.8°C) for such a pronounced cold-water species. Incubations initiated at 2.8°C (experiment A) resulted in significantly higher mortality rates both before and after the eyed stage. In experiment B, a general positive correlation was found between the duration of the initial warmer period (6°) and survival to both the eyed stage and hatching and even more clearly with the number of live normally shaped alevins. The total absence of the initial warmer period (constant 2.3°C) was particularly detrimental to development and survival, supporting the findings from experiment A. Deformity frequencies were several times higher at constant 2.3°C compared to incubations that were initiated with one or more weeks of 6°C. Hence, the most pronounced period of elevated sensitivity to low temperature is restricted to the first week of incubation; and it seems that a suboptimal temperature during this early stage of embryogenesis causes disturbances in development that result in severe deformities (examples in fig. 7), which reduces the viability of the embryos.

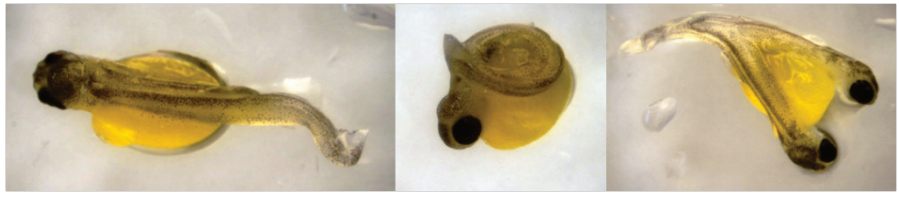

Figure 7. Examples of deformities found in live newly hatched Arctic charr alevins in paper III, left and centre panels showing curvature of the spine to different extent, right panel showing conjoined twins.

Temperatures as low as 0.5°C have been reported for natural Arctic charr redd sites during spawning in Char Lake, Canada, in September (Johnson, 1980; Schindler *et al.*, 1974). Hence, one would expect the Arctic charr eggs to be quite insensitive to low temperature. This is the case during the major part of incubation. Recommendations on the lower temperature limit for Arctic charr egg incubations are as low as 0°C (Rombough, 1997; Scott & Crossman, 1973). However, it seems as if temperature conditions during spawning and initial incubation are seldom taken into account in reports on successful incubations at low temperatures (e.g. in Pakkasmaa *et al.*, 2006, see paper III for details). In the case of the Char Lake example above, to my knowledge, there are no evaluations of the outcome of the fertilisations that occurred at 0.5°C. Given the results of the present study, significant inter-annual variations in reproductive success connected to temperature conditions during spawning can be expected in extreme high latitude populations of Arctic charr.

4.3 Hormones and gamete quality (paper IV)

4.3.1 Sexual steroid in females

Generally, females exhibited low plasma levels of 17,20β-P and only few females had elevated 17,20β-P indicating recent ovulation. In the present study circulating steroid levels were measured only at a single time point at stripping, which makes it difficult to draw conclusions on the hormone profile during maturation. Previous studies on Arctic charr and related species report rapid declines in 17,20β-P levels post ovulation (e.g., Frantzen *et al.*, 1997; Goetz *et al.*, 1987), which could suggest that the low levels of 17,20β-P levels measured at the time of stripping in the present study indicate that females had already past ovulation and post-ovulation aging of oocyte had started to effect egg quality and hence fertilisation rates. The period after ovulation of unimpaired egg viability is relatively long in salmonids (Bromage *et al.*, 1994), especially at low temperatures (Aegerter & Jalabert, 2004) and Arctic charr eggs retain their full potential for approximately five days post ovulation at 5°C, (Gillet, 1991). In the present study, fertilisations occurred at 4°C and 2.5°C, giving a period of optimal fertilisation potential of at least five days.

Despite the large number of previous studies on salmonids that describe the relationships between steroid levels and ovulation as well as ovulation time and egg viability, to our knowledge, none include both steps to connect steroid levels and egg viability.) No significant correlation between plasma levels of 17,20β-P in the females and fertilisation rates were observed in the present study and high fertilisation rates were achieved by females covering the whole range of 17,20β-P levels. However, low fertilisation rates were found only in

egg batches from females with very low levels of 17,20β-P (fig. 8). Variation in egg viability has been reported to increase with post-ovulation aging of oocytes in rainbow trout (Aegerter & Jalabert, 2004). Thus it seems likely that parts of the egg loss, failed fertilisation in particular, in the present study could be explained by post-ovulation aging of the oocytes.

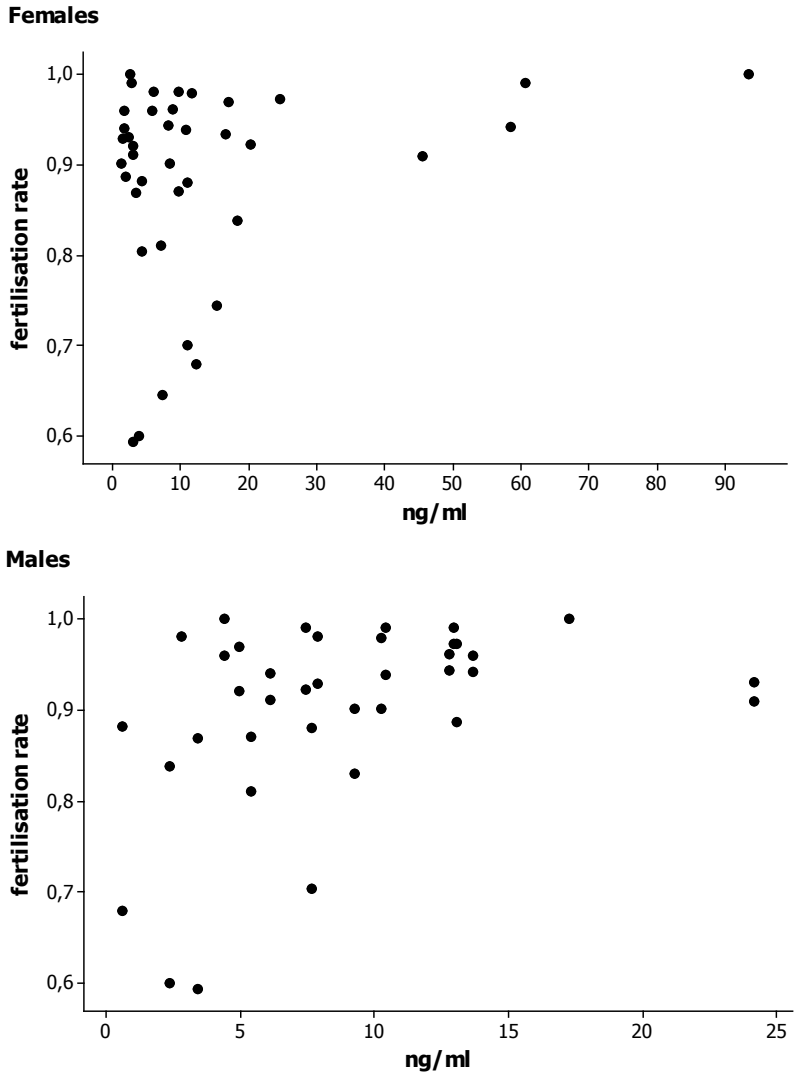

Figure 8. Plasma levels of 17,20β-P in female (upper panel) and male (lower panel) Arctic charr, and the estimated proportion of fertilised eggs in the individual egg batches fertilised by the different males (from paper IV).

4.3.2 Sexual steroid in males

17,20β-P levels were in agreement with spawning levels previously reported for the species by Mayer et al. (1992), and highest levels (mean 15 ng/ml) were found towards the end of the spawning season. As for the females, there were individual differences in steroid levels between the males. 17,20β-P levels were positively correlated with fertilisation rates (fig. 8). Most likely, the differences in 17,20β-P levels as well as fertilisation rate and milt volume were reflections of when during the spermiation period stripping occurred. Though, social hierarchy is also known to be associated with individual differences in steroid levels, and in turn differences in sperm quality parameters in Arctic charr (Haugland *et al.*, 2009; Rudolfsen *et al.*, 2006). Plasma levels of T and 17,20β-P are higher in dominant than subordinate rainbow trout, 11-KT is higher in dominant brown trout (Cardwell *et al.*, 1996) and the same differences are seen in Arctic charr (Rudolfsen *et al.*, 2006).

4.3.3 Egg quality

Paper IV revealed large individual variations in fertilisation and egg survival rates within the broodstock, although all fish had experienced the exact same rearing conditions. Fertilisation rates ranged from 59% to 100% and egg survival rates from 9% to 98%. Hence, the majority of egg loss occurred during embryogenesis, but there was also a significant egg loss due to failed fertilisation. Springate (1984) reported strong correlation between fertilisation and survival to eyeing, hatching, and swim-up, in a study on egg viability in relation to post-ovulatory aging (over-ripening) of oocytes in rainbow trout. The present results showed no correlation between fertilisation and survival rates, which could indicate that there are several different factors causing the egg loss. Over-ripe oocytes are likely to explain some of the loss, as well as sperm quality parameters. The water temperature at the hatchery during summer and early autumn were high enough to be considered stressful, in particular to first time spawners, which were used in the present study. First time spawning salmonids are generally more sensitive to thermal stress during vitellogenesis than repeat spawners (Pankhurst *et al.*, 2011; King *et al.*, 2003). One consequence of such thermal stress is a reduced maternal investment into the oocytes, resulting in smaller and less viable eggs. We found no correlation between egg size and viability in the present study. However, the range of egg sizes (4.1±0.17 mm) belong to the lower end of the size range for eggs attained from the current broodstock, and has been associated with poor viability (Jeuthe *et al.*, 2013). It is therefore likely that a significant part of the egg mortality presented in the present study can be explained by supra-optimal

rearing temperature, which has been identified as a chronic problem at the current hatchery (Jeuthe *et al.*, 2015; Jeuthe *et al.*, 2013).

4.3.4 Sperm quality

Sperm swimming velocity (VAP, VSL, and VCL) showed positive correlation with fertilisation rate. In conditions of limited sperm to egg ratio, this relationship would most likely be explained be changes in the probability of a sperm making its way to the micropyle of the oocyte. The recommendations for minimum sperm to egg ratio in salmonids range from 20,000 to 200,000 (Rurangwa *et al.*, 1998; Erdahl & Graham, 1987; Billard, 1975). In the present study, the sperm to egg ratio was estimated to be above 500.000 in all fertilisations, not taking motility into account, leading to the assumption that maximum fertilisation potential was reached. Thus the positive correlation between sperm swimming speed and fertilisation rate is more likely to reflect an actual quality issue.

In recent years, an increasing number of studies have emerged on the paternal effects on embryonic survival and development (reviewed in Cabrita *et al.*, 2014). If DNA and RNA in the spermatozoa are damaged, often due to oxidative stress during spermatogenesis (Aitken *et al.*, 2012), the sperm may still be able to fertilise an oocyte. After fertilisation however, paternal mRNA plays a key role in the early development of the embryo, and damages to these molecules, as well as the paternal chromatins, will disturb early embryonic development (Johnson *et al.*, 2011). In the present study, both milt density and the CASA-parameter beat cross frequency (BCF) were correlated with egg mortality (r_p=-0.54 and r_p=0.67, respectively), but not with fertilisation rate. It could be that these two sperm quality traits act as indicators of another, possibly epigenetic, quality parameter affecting embryonic development. The relationship between BCF and egg survival was the strongest one among our results (fig. 9). Linear regression using the highest fertilisation rates from each male showed that BCF and the plasma levels of 17,20β-P in the males, together explained 65% of variation of egg survival to the eyed stage. Evidently, there are considerable paternal effects on the variable reproductive success of the current broodstock, acting through impairment of both fertilisation and embryonic development. However, the mechanisms and causes of these paternal effects on offspring viability are yet to be determined.

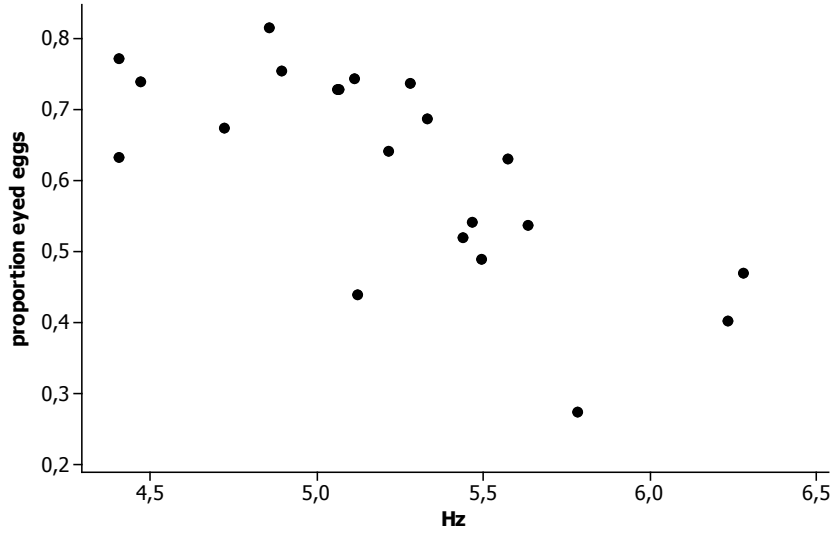

Figure 9. The CASA-parameter beat cross frequency (BCF, see paper IV for description) of sperm from individual males and proportion of eyed eggs in egg batches fertilised by the different males (from paper IV).

5 Conclusions and future perspectives

When the work with the present thesis began, the general conception was that high rearing temperature during summer was largely to blame for the poor reproductive performance of Arctic charr broodstock. However, there were also indications that the issue might be more complex than that. For instance, there was suspicion that inadequate FA-composition in the feed was affecting egg quality. Five years later there are still many unanswered question; in fact, as is often the case, many new questions have arisen as well. But still, the results within this thesis have provided some clarity and new knowledge on the matter. We now have a better understanding of the extent of the thermal stress experienced by the broodstock during summer and autumn and the effects this has on the reproductive performance (papers I & II). If the temperature development of the last three decades continues, the issues of poor and erratic juvenile production will persist, and probably increase, unless proper measures are taken. The studies on rearing temperature within this thesis are mainly focused on one hatchery, ACN in Kälarne, and the validity of extrapolating the results to other hatchery sites may be questioned. More 'real world' studies on the topic would be beneficial. However, we have no reason to believe that the temperature development over time is unique to this location, and temperature trends need to be taken into consideration in sustainable site selection and management of aquaculture hatcheries.

Further, on the topic of temperature, the present thesis has provided new and valuable insight on the requirements of Arctic charr eggs during incubation (paper III). The early stages of embryogenesis prove to have a narrower temperature tolerance interval than what has previously been reported. The new information on the cold sensitivity during initial incubation is of great use to the industry. However, more detailed studies are required to deepen the understanding of the effects of temperature on the developmental processes of early embryogenesis.

The case study on broodstock status during stripping (paper IV) provided few conclusive results on the causes behind the variable reproductive performance of farmed Arctic charr. It does, however, open up for further discussion and speculation on the topic, and inspire to plan future studies.

One concrete result is the quantification of egg loss. The distinction between failed fertilisation and mortality is often ignored in studies on gamete quality. In order to determine the causes of reproductive impairment, the mediating mechanisms need to be understood. A first, but major, step towards this goal is to determine at which stage the potential offspring are lost.

The results in paper IV suggest that the paternal effect on reproductive performance of the current broodstock is considerable. One topic that ought to be revisited in this aspect is the effect of feed composition on reproductive performance of Arctic charr, with emphasis on the dietary effects on sperm quality. But more importantly, further basic knowledge needs to be obtained on the effects of environmental and biological factors, and the mechanisms through which they act on sperm quality, and in extension, how offspring viability, post-fertilisation in particular, is affected.

With each study in this thesis, it has become more apparent that the issue of poor reproductive performance in farmed Arctic charr, to major extent, has to do with the actual spawning period and the timing of ovulation and gamete stripping. Post-ovulation ageing of oocytes is an important factor causing poor egg quality. Spawning is often stretched out over time, partly due to thermal stress during summer and early autumn. However, there may also be inadequacies in the environmental cues triggering final maturation and ovulation. One common trait of Arctic charr hatcheries in the north is that broodstock are moved indoor prior to spawning. In connection to this, the male and female fish are separated, natural day light is eliminated, and the temperature profile during the spawning period may differ from natural conditions. These are all factors that play crucial roles in the process of sexual maturation of fish, but their effects in ‘real world’ aquaculture are thus far poorly documented.

6 Sammanfattning på svenska

6.1 Bakgrund

Röding har odlats i Sverige åtminstone sedan 1970-talet. I början var syftet stödutsättning och återetablering i naturliga miljöer, men på senare tid har det kommersiella intresset för odlad rödingen ökat och branschen växer. Ett av de viktigaste momenten man ska igenom när man påbörjar arbetet med en ny art för vattenbruk, är att kunna reproducera den i fångenskap. Med den erfarenhet som fanns från tidigare arbete med t.ex. lax, var detta inget större hinder då man började odla röding. Redan i mitten av 1980-talet hade arbetet med att etablera ett avelsprogram för röding påbörjats. Idag har avelsarbetet pågått i trettio år och åtta generationer och resultatet är en snabbväxande röding med god köttkvalitet och som lämpar sig väl för odling. Det finns dock ett stort bekymmer för en fortsatt utveckling av branschen. Äggöverlevnaden hos odlad röding är låg och uppvisar stora variationer mellan både föräldraindivider och år. Denna osäkerhet i kläckeriproduktionen innebär att onödigt stora stamfiskbesättningar krävs för att tillgodose efterfrågan från odlare. Orsakerna bakom den sviktande yngelproduktionen ligger med största sannolikhet i bristande odlingsförhållanden. För att säkerställa produktionen för framtiden måste dessa brister utredas och åtgärdas.

6.2 Syfte och utförande

Syftet med denna avhandling är att utvärdera nuvarande odlingsförhållanden och hur dessa påverkar reproduktionsframgången hos röding. Avhandlingen består av fyra delarbeten. Två långtidsstudier (artikel I & I) utvärderar den fysiska och biologiska miljön vid huvudanläggningen för svenska avelsprogrammet för röding, Vattenbrukscentrum Norr (VBCN) i Kälarne, för att fastställa hur dessa faktorer, individuellt eller i kombination,

har påverkat variationen i äggöverlevnad genom åren. Artikel III ägnas åt att studera effekter av olika temperaturprofiler för ägginkubation på embryonalutveckling och -överlevnad och omfattar i huvudsak två experiment. Experiment A är en utvärdering av en rad förekommande temperaturprofiler, konstanta och variabla, och deras effekt på överlevnad och utvecklingsstatus vid kläckning. Experiment B testar hur tidpunkten för ett skifte mellan höst- (6°C) och vintertemperatur (2,5°C) påverkar överlevnad och förekomst av missbildningar hos ägg och yngel. Artikel IV är en fallstudie som utvärderar hormonstatus hos föräldrafiskar vid reproduktionstillfället (kramning), diverse ägg- och spermiekvalitetsparametrar samt resulterande befruktningsgrad och embryonal överlevnad.

6.3 Resultat

En av de viktigaste faktorerna för att förklara de variationer som finns i äggöverlevnad vid VBCN visade sig vara temperaturen som stamfisken utsätts för från juli till september (artikel II & II). Men även temperaturförhållanden under lekperioden, vilka är oberoende av sommartemperatur, korrelerade med äggöverlevnad. Detta framstod som ett positivt samband mellan en distinkt temperatursänkning under hösten och äggöverlevnad. Det är troligt att detta samband speglar en mer synkroniserad lek och ägglossning under år med mer distinkt kylningsperiod (artikel II).

Utöver effekterna av odlingstemperatur visade resultaten från artikel I ett svagt samband mellan inkubationstemperatur och äggöverlevnad. Detta samband var dock inte helt enkelt att tolka, då äggen ofta utsatts för varierande temperaturer i kläckeriet. Detta ledde till utformandet av inkubations-experimenten som presenteras i artikel III.

Analyserna i artikel I visade även ett även ett starkt samband mellan honornas ålder, äggstorlek och äggöverlevnad. Både äggstorlek och äggöverlevnad ökade generellt tills honorna nått 6 års ålder, varefter utvecklingen stagnerade. Det finns inga tidigare studier som visar ett så tydligt samband mellan dessa variabler. Troligen har sambandet i de aktuella resultaten förstärkts av den temperaturstress som stamfisken utsatts för.

Inkubationsstudien (artikel III) är, så vitt vi vet, den första att visa att de tidiga stadierna av embryogenesen hos röding är känsligare för låga temperaturer än senare stadier. I tillägg kan denna nedre gräns för normal utveckling och överlevnad anses relativt hög (≥2,8°C) för en så uttalad kallvattenart som rödingen. Ägg som utsattes för temperaturer under 2,8°C i början av inkubationen visade högre dödlighet och framför allt flera gånger högre andel missbildade yngel.

Resultaten i artikel IV visade att det huvudsakliga bortfallet av avkomma sker i form av dödlighet under embryonalstadiet. Men en ansenlig del förloras även p.g.a. misslyckad befruktning. Analyserna visade ingen korrelation mellan dessa två variabler vilket kan antyda att olika orsaker ligger bakom respektive bortfall.

Blodprovsanalyser visade generellt låga nivåer av det hormon (17α,20β-dihydroxy-4-pregnene-3-one) som är starkast knutet till ägglossning hos honorna. Detta kan betyda att äggen kramas och befruktas för sent efter ägglossning, vilket skulle medföra en kraftig nedgång i äggkvalitet, vilket i sin tur kan förklara en stor del av bortfallet. Analyser av hannarnas hormonnivåer samt diverse spermiekvalitetsparametrar visade även en tydlig effekt från fadern på både befruktningsgrad och embryonalöverlevnad. Faderns inverkan på avkommans överlevnad har under lång tid förbisetts i avelssammanhang, men detta är ett ämne som behöver utforskas vidare, båda genom grundforskning och tillämpad forsking med riktning mot vattenbruk.

Sammanfattningsvis kan konstateras att resultaten i denna avhandling fört oss en liten bit närmare förklaringen bakom den låga, men kraftigt varierande, reproduktionsframgången som finns hos odlad röding. Men det finns fortfarande många frågor som behöver utredas innan målet med en god och stabil yngelproduktion kan nås.

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