Introductory Research Essay

Reproductive success of farmed Arctic charr (*Salvelinus alpinus*)

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Abstract

The Swedish Arctic charr breeding programme was established in the early 1980's. Over 25 years of selective breeding has resulted in a fast-growing, late-maturing strain of charr called the Arctic Superior. However, the reproductive success of this strain is far from satisfactory. Egg hatching rates are erratic and often very low compared to other salmonid species in Scandinavian aquaculture. Securing a reliable supply of eggs and stocking fish is imperative to a continued expansion of the Swedish Arctic charr farming industry. The aim of this essay is to look into the biological and physical factors affecting gamete quality and offspring survival in fish and elucidate the most crucial of these factors concerning the Arctic Superior.

I believe that physical factors regarding the holding conditions are the cause of the problem, rather than biological traits attained as by-products of the breeding process. I suggest high temperature to be the main factor behind the problems of poor reproductive success. However, it is unlikely the only explanation to the problem.

Timing of spawning is an important part of the problem. Spawning period is often prolonged and poorly synchronised between sires and dams. This leads to increased handling and stress of the fish, over-ripening of eggs and difficulties with fertilisation, all contributing to lower reproductive success. Spawning period can be manipulated through altered photoperiod or hormonal treatment. Hormone implants can efficiently induce ovulation and spermiation, but may have detrimental effects on gamete quality. If the environmental holding conditions are right synchronised spawning and improved gamete quality should be achieved and the use of hormones should not be needed.

Introduction

Biology and life history

Arctic charr (*Salvelinus alpinus*) is the most pronounced coldwater species among freshwater and anadromous fish. Also among charrs (genus *Salvelinus*), it is the northernmost species and the only one with a circumpolar distribution. In Europe, Arctic charr is found from the Pyrenees (introduced) and Alps (natural) in the south to Svalbard in the north. Many populations in the north are anadromous, In Europe these are mainly found on Iceland and in Norway (northern mainland and Svalbard). Stationary freshwater populations are found throughout the distribution area in cold or cool lakes and river, often at high altitudes. In many of the northern and alpine lakes it is the only fish species present. All of Sweden's estimated 13 000 Arctic charr population (Maitland, 1995) from Lake Mycklaflon in the south to Kilpisjärvi in the north live exclusively in freshwater. The closest anadromous populations are found in northern Norway.

As a species the Arctic charr is a broad generalist in habitat and prey choice. It exhibits great ecological and phenotypical variation, which has led to some confusion regarding speciation. In specific populations variation in habitat and prey choice is often manifested as polymorphism. Morphologically different phenotypes, morphs, specialise in their own separate niche and this is reflected in their physical appearance. The classic example is Tingvallavatn, Iceland, where you find four clearly distinct morphs, large and small benthivores, planktivores and piscivores (Malmquist *et al.*, 1992). Great variation is also found between populations in traits like growth, maturation and spawning season.

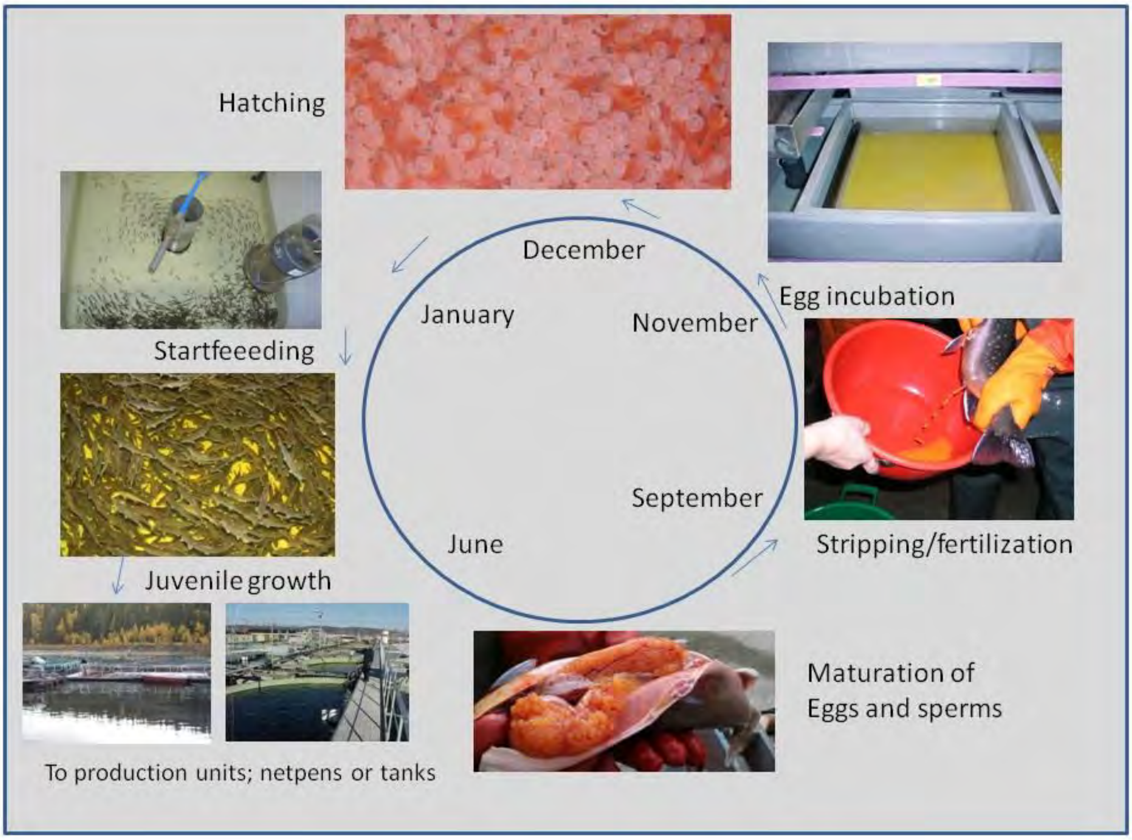

Figure 1. A general overview of the Arctic charr aquaculture production cycle (Brännäs et al., 2011a).

Sexually mature females have been found in the range from 3g to at least 12kg. The smallest ones are found among the smaller morphs in polymorphic populations or in dwarf populations of size restricted habitats (e.g. rock crevices), while the largest ones are anadromous fish and landlocked predatory forms. There seems to be a minimum size of about 70mm total length for females to become sexually mature. These small females produce less than 20 eggs each, which can be compared to large anadromous females with a total fecundity well over 10 000. Spawning occurs sometime between September and March depending on population. There are spring spawners and autumn spawners, the latter being more common. Scandinavian populations spawn in autumn, typically October to November (figure 1). In Windermere, England, two populations with different spawning cycles are found in the same lake. Spawning of these two populations is separated in both time and space. One population spawn in shallow water (1-3m) in the fall (October-November) and the other in deep water (20.30m) in the spring (February-March). Females that spawn exceptionally late generally produce very small eggs (ca. 3.5mm). This is thought to be due to selection driven by the shorter period available for development of the eggs of late spawners. Generally, the size range of Arctic charr eggs is said to be 3.6-5.0mm (Johnson, 1980).

A very good and detailed description of the Arctic charr and other charr species is found in the book "Charrs" by Eugene Balon (1980).

The Swedish Arctic charr breeding programme

The Swedish breeding programme for Arctic charr was started in the early 1980's. A native strain of charr from Lake Hornavan in northern Sweden was chosen to form the basis of this breeding programme. The choice was made after comparison and evaluation of several charr populations from different Swedish high latitude lakes. The Hornavan-strain was found superior in growth performance and later onset of sexual maturation (Eriksson *et al.*, 2010). Since the wild founders of the breeding programme were collected in 1979 and farming commenced, no fish of other origin have been included in the strain. The result of over 25 years of selective breeding is a fast-growing, late-maturing charr with high pigment retention in the fillet (figure 2, table I), a fish much better suited for aquaculture than the wild relative from whom it descends (Nilsson *et al.*, 2010). This strain of Arctic charr created through the breeding programme is called "Arctic Superior" and is much appreciated by fish farmers and consumers alike and it is used by the majority of Sweden's charr farmers.

Table I. Comparison of important farming traits in the original Hornavan charr and the selectively bred Arctic superior (Eriksson et al., 2010; Brännäs et al., 2011b).

Arctic charr farming 1985	**Arctic charr farming 2011**
Farming cycle 3–4 years	Farming cycle 1.5–2 years
Relatively slow growth, poor feed utilization	Fast growth, efficient feed utilization
Early maturation (70–100% before 500 g)	Late maturation (0.02% before 800 g)
Variable fillet coloration and quality	Improved flesh coloration and quality
Production costs 50–60 SEK (4.3–5.6 Euro)	Production costs 25-35 SEK (3.3 Euro)

In the shadows of the success achieved in the breeding programme there are still some challenges. The reproductive success of the Arctic Superior is far from satisfactory. Gamete quality is generally low, with high frequency of opaque eggs and watery sperm. Spawning period is often prolonged and poorly synchronised between sires and dams, leading to increased handling and hence stress to the fish as well as a more difficult and laborious stripping procedure. Fertilisation and hatching are erratic and on average very low, with hatching rates typically between 30 and 70 percent. Other salmonids in Scandinavian aquaculture such as Atlantic salmon (*Salmo salar*), brown/sea trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) exhibit high egg survival rates, rarely below 90 percent. Both the Atlantic salmon and rainbow trout often have survival rates of eggs close to 100 percent.

Figure 2. Arctic charr of the selectively bred Arctic Superior-strain (left). In the early days of charr farming fillet coloration was poor and variable, this is no longer the case (right).

Charr farming in Sweden

Most of Sweden's Arctic charr farming takes place in the north, mainly due to the species' requirement for cold water. Hatcheries and stocking fish facilities are typically land based flow-through systems. After hatching the fish are kept here for on-growth, generally until early summer (figure 1). Once the fish have reached sufficient size they are moved to net pens in lakes and reservoirs of river systems exploited for hydroelectric power production. Here they are kept until reaching slaughter size, at around 1.5 years of age. Typical slaughter size of farmed Swedish charr is 600-800 grams, enough for two portion-sized fillets. The fish are mostly sold fresh by fishmongers or well-stocked supermarkets, whole and gutted. The use for a fish of this format is somewhat limited, in my opinion. However, with the growth rates and late maturation exhibited by the Arctic superior today it would be possible to grow the fish to ca 2 kg before fillet quality is compromised by sexual maturation. This would open up for a range of new products, e.g. fresh or frozen fillets and sushi/sashimi, not the least.

When fish are farmed in open water net pens (figure 3), nutrients from faeces and feed are released to the environment, unlike land-based facilities where it is possible to filter the outgoing water. In many areas addition of nutrients to aquatic systems is considered a problem as it causes eutrophication, which in turn may lead to harmful algal blooms and anoxic environments etc. However, regulated lakes and reservoirs in northern Sweden are nutrient depleted as a result of the power companies' water management. Draining of reservoirs flushes out nutrients and highly variable water levels inhibit biological communities to establish along the shorelines (Stockner *et al.*, 2000). In these locations nutrients provided by fish farms may boost biological production and counteract further depletion of the river system. This is, along with the species cold water preference two of the arguments promoting establishment of charr farms in the Swedish northern inland. Another strong incentive is creating job opportunities in rural areas.

Figure 3. Arctic charr net pen in Lake Landö in January. In the background, recreational anglers taking advantage of increased fish production of the lake due to the presence of the fish farm.

From the start of commercial fish farming in the 1980's until just a few years ago the development of the Swedish charr farming industry was very slow, basically status quo. Production remained at a low but steady level of 500 to 800 tonnes annually. However, around 2008 things started to happen in the charr farming industry and production suddenly increased. In 2010 the Swedish production was 2300 tonnes and the expansion seems to continue.

A reliable supply of eggs and stocking fish is imperative to future expansion of the Arctic charr farming industry. Because of the unpredictable and often poor egg survival of the Arctic Superior today, unreasonably large broodstocks are needed to produce enough eggs. If egg survival is improved to resemble those of other salmonids in culture, half of the broodstock kept today would be sufficient to produce the same number of viable offspring. The erratic nature of Arctic charr egg quality today increases the number of brood fish needed even further as a safety margin is needed in case of a bad spawning season. In order to improve egg survival of the Arctic Superior-strain, an in depth analysis is needed of the biological and environmental factors affecting their reproductive success. Is poor egg survival a biological trait resulting from the breeding process, or is it a consequence of suboptimal rearing conditions? In this essay I present the most important factors affecting reproduction in fish, and discuss the most likely explanations to the problems regarding the Swedish Arctic charr reproductive success. Some conclusions can be drawn from the data present today, but further studies are needed to solve the problem. I will present my suggestions for future studies and which factors I believe to be most crucial for the Arctic Superior.

Factors influencing reproductive success

Temperature

Arctic charr, as the name suggests, is one of the most pronounced cold water freshwater fishes, and among salmonids it is the species with the northernmost distribution. It has a very strong preference for cold water and can grow at temperatures close to 0°C (Brännäs and

Wiklund, 1992). Compared to other salmonids like Atlantic salmon (*Salmo salar*) and brown/sea trout (*Salmo trutta*) Arctic charr exhibits much more efficient growth below 10°C (Elliott and Elliott, 2010). However, this comes at the expense of a lower tolerance to warm water. For parr and later life stages, the temperature range for optimal growth is quite similar for trout and Arctic charr, extending around ca 15°C (figure 4) (Larsson and Berglund, 1998). However, the preferred temperatures for the two species differ significantly. Trout favour a water temperature of 16°C, coinciding with their optimal growth, while charr prefer 11°C, which is significantly lower than their optimum for fast growth (Larsson, 2005). The preferred temperature of charr is closer to the optimum for energy efficient growth which is found at ca 9°C (Larsson and Berglund, 1998). The upper temperature limit for feeding and growth of 0+ charr is about 20°C (Thyrel *et al.*, 1999).

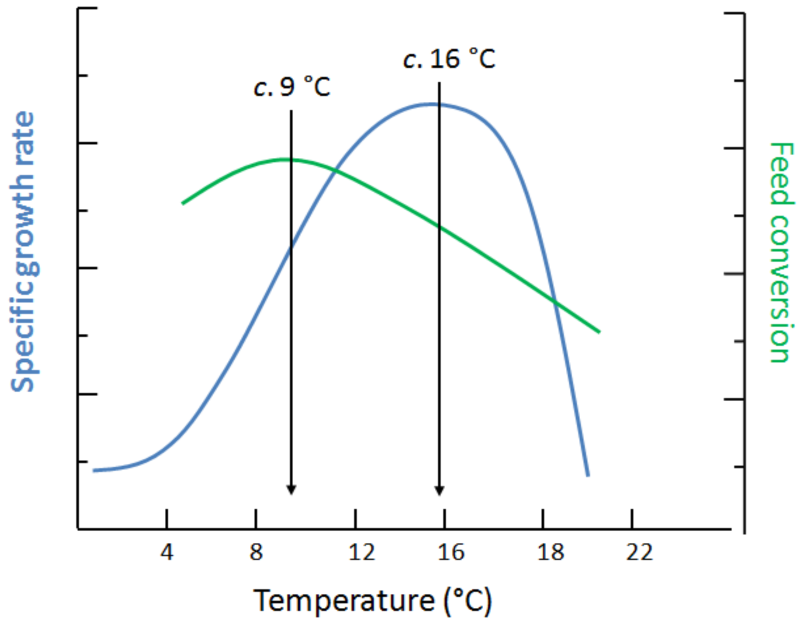

Figure 4. Generalised pattern of charr growth rate and feed conversion as an effect of temperature. Note that these relationships are under the assumption that feed is available in excess, modified from Larsson and Berglund, 1998.

For early life stages the difference in temperature tolerance is considerable for charr and trout. Charr eggs are said to have an upper critical limit for survival at 8°C (table II), compared to 13 and 16°C for trout and salmon (Elliott and Elliott, 2010). For alevins, fry and parr the upper incipient lethal temperature (50% mortality after ca 7-day exposure) is 19-23°C, and the tolerance increases with age (Lyytikainen *et al.*, 1997; Elliott and Klemetsen, 2002). In a study by Jungwirth and Winkler (1984) over 80% mortality was found in charr eggs at 11°C and 100% at 12-13°C. Already at 6°C there is a significant reduction in hatching success (De March, 1995; Janhunen *et al.*, 2010). Incubation of eggs at sub- and supra-optimal temperatures has also been proven to cause malformations of jaw and vertebral column in several different fish species, e.g. halibut (Bolla and Holmefjord, 1988), goldfish (Wiegand *et al.*, 1989), cod (Fitzsimmons and Perutz, 2006) and salmon (Eriksen *et al.*, 2006). Malformations appear at temperatures both above and below optimum depending on species. There are, to my knowledge, no studies done on temperature induced malformation in Arctic charr. However, being a coldwater species closely related to salmon, one might expect elevated incubation temperatures to result in malformations.

*Table II. Examples of approximate critical temperatures during different stages of the Arctic charr farming cycle (*from fertilisation to hatch).*

Temp. °C	**Effect**	**Reference**
9	efficient growth	(Larsson and Berglund, 1998)
11	preferred temperature	(Larsson, 2005)
15	optimal growth	(Larsson and Berglund, 1998)
20	limit feeding and growth	(Thyrel *et al.*, 1999)
8	ovulation delayed	(Gillet, 1991)
11	ovulation inhibited	(Gillet, 1991)
6	reduced hatching success	(De March, 1995; Janhunen *et al.*, 2010)
11	80% egg mortality*	(Jungwirth and Winkler, 1984)
13	100% egg mortality*	(Jungwirth and Winkler, 1984)
19-23	Incipient lethal temp. for alevins, fry and parr	(Lyytikainen *et al.*, 1997; Elliott and Klemetsen, 2002)

Salmonid eggs are large compared to most other fish species and need a relatively long time to hatch. For Arctic charr, hatching occurs after approximately 450 degree-days at 4.5°C (Jungwirth and Winkler, 1984; Atse *et al.*, 2002). Incubation time, both days and degree-days, increases with decreasing temperature (figure 5). Fish farmers may be tempted to incubate eggs in water warmer than the ambient winter temperature in order to shorten the production time. As fish are ectothermic this effect will be achieved but will also likely reduce survival of the eggs. However, it is a question of balancing production costs. Incubating at higher temperatures means shorter production time, but one would probably have to compensate for the increased egg mortality by keeping a larger brood stock. Hence, shortening egg incubation time may not necessarily result in lower total production costs.

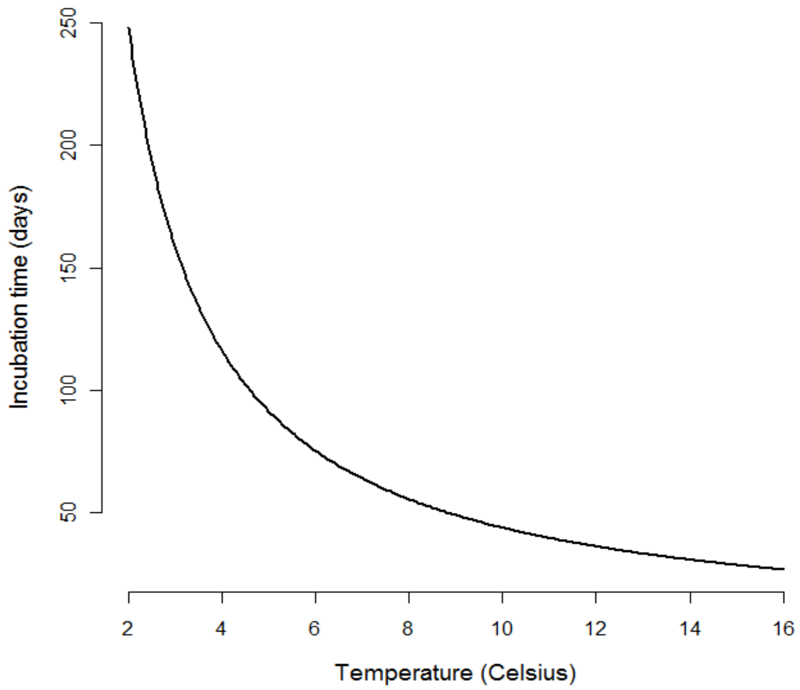

Figure 5. Effect of incubation temperature on the rate of Arctic charr egg development from fertilisation until hatching, modified from Jungwirth and Winkler 1984.

In the wild, Arctic charr spawn in autumn at temperatures around 4°C. Most charr hatcheries in Sweden have no problem maintaining such low temperatures during winter. Facilities that take their water from rivers and lakes typically have winter temperatures down to 2°C, or even less and heat the water to varying extent in order to shorten the incubation time. With this set-up it would be a rather simple task to find the optimal temperature for the specific strain of charr used at the hatchery in question. Too high temperatures coinciding with the spawning period may, during some years and on some locations, be a problem in autumn. This is the case especially in hatcheries that are supplied with water from a big lake as these conserve the summer temperature longer.

The egg incubation period is not, however, the only temperature sensitive phase of reproduction. The holding conditions for broodstock also have a strong influence on development of gametes, and hence egg quality. Elevated water temperatures have been shown to affect and interrupt several processes during oogenesis and oocyte maturation. It is not quite clear which stages of oocyte development are more temperature sensitive than others. However, studies on Atlantic salmon performed in Tasmania have shown that elevated temperatures during the summer months reduce spawning success (King *et al.*, 2003), and that shorter temperature spikes during this period are enough for a significant effect (King *et al.*, 2007). When salmon dams were held at constant high temperatures (18 or 22°C) for three months prior to spawning, both fertility and survival of the eggs were reduced compared to the control (14°C). At the highest temperature, fertilization and survival rates were 69 and 42% respectively, this compared to 93 and 86% in the control group. King et al. (2007) found indications that the most critical period for exposure to high temperatures is late February to early March, which in the northern hemisphere would equal late July (figure 6). In other words the reproductive success may be the most sensitive to warm water during the period when the fish are most likely to encounter it. High summer temperature is a common problem among Swedish freshwater fish farms, especially for those holding Arctic charr. Other species commonly cultured in Sweden such as rainbow trout, brown/sea trout and salmon generally have a better tolerance for high water temperatures. In fish farms taking water from lakes and streams it is not uncommon to reach temperatures above 15, even 20°C during the summer. Sites with access to groundwater, however, can maintain temperatures below 10°C year round.

Few studies have evaluated the holding temperature of Arctic charr broodstock and its effect on reproductive success and egg quality. Deposition of proteins and lipids into the oocyte during vitellogenesis is affected by the thermal environment. Jobling et al. (1995) found that the triacylglycerol/phospholipid ratio increases with temperature. Also, at high rearing temperatures the composition of the ovulated egg's phospholipids has a lower proportion of the essential fatty acids DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid). High summer temperatures also affect the rate of oocyte development, timing of spawning and even result in inhibition of ovulation (Gillet, 1991; Gillet and Breton, 1992; Jobling *et al.*, 1995). Christian Gillet has done a lot of important work on a French strain of Arctic charr broodstock. In his paper from 1991 he reveals several effects of high rearing temperatures on reproduction. Ovulation of females was delayed at 8°C compared to those at 5°C. At 11°C ovulation was inhibited. The delaying effect of temperature on ovulation has also been confirmed by Jobling et al. (1995). In Jobling's study an increase in temperature from 4 to 12 and 16°C during the summer months caused a delay of ovulation by 3-4 weeks. If the elevated temperatures occur closer to spawning it speeds up the maturation of the oocytes, causing over-ripening of the eggs (figure 7). This topic will be covered in more detail in the "timing of spawning" section.

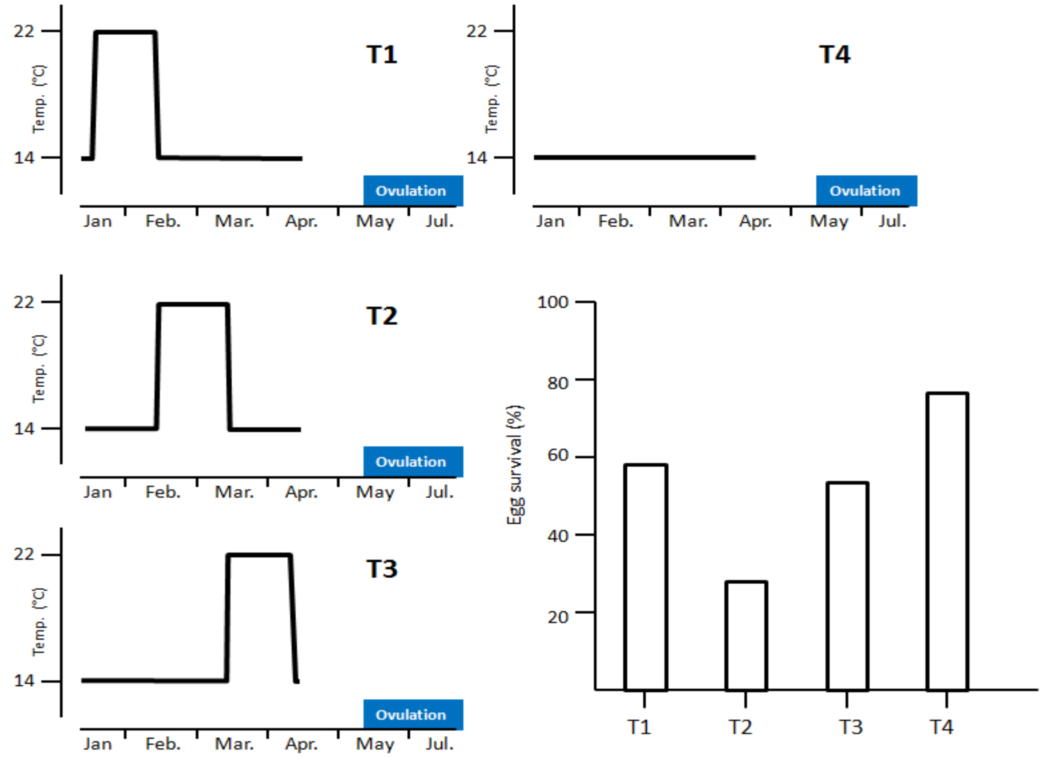

Figure 6. Survival (%) to eyed staged of Atlantic salmon eggs as a result of different temperature treatments (T1-4) during the period of egg development (vitellogenesis), modified from King et al. 2007.

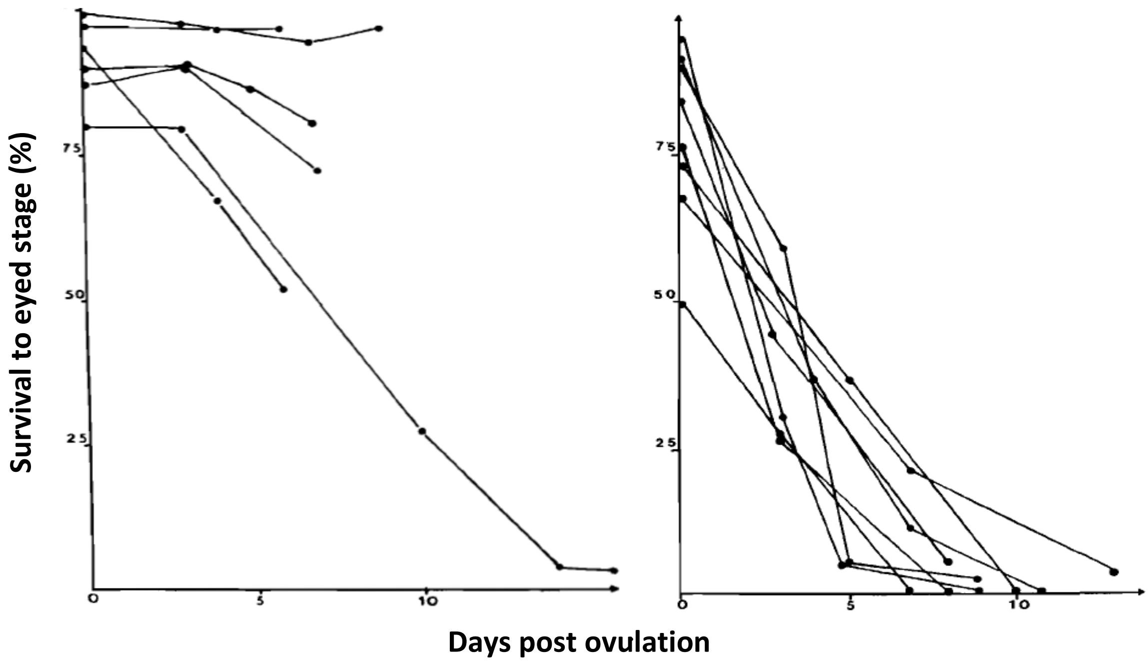

Figure 7. Effect of over-ripening on egg survival to eyed stage in farmed Arctic charr, females reared at 5°C (left) and 8°C (right). Each line represents data for one female (Gillet, 1991).

Nutrition

Nutritional requirements of Arctic charr are generally very similar to other salmonid species in culture. Few studies have been made on the specific requirements for charr, mainly because early trails showed that the feed already commercially available gave sufficient growth results. Tabachek (1986) found that charr grew fastest with feed containing 54% protein and 20% lipids. However, the most cost efficient growth was achieved with 44% protein and 20% lipids. These proportions of protein and lipids are covered in feed produced for salmon and trout, and hence for a long time these were used for charr as well. Today, feed producers sell dry feed specially designed for Arctic charr. Even specialised feed for specific life stages and production purposes, e.g. breeding or table market, are manufactured, differing mainly in contents of some nutrients and vitamins. Generally broodstock feed have higher levels of protein, n-3 PUFA (Polyunsaturated fatty acids), vitamins C and E, astaxanthin and beta-glucan, the last one strengthening the immune system.

As with other animals, fish are known to be unable to produce certain amino and fatty acids *de novo*. Looking at fish in general, they require 10 essential amino acids through food (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine). The specific quantitative intake requirements of these for Arctic charr have, to my knowledge, not yet been determined. In the wild we find differences in feeding pattern and prey preference between anadromous trout and charr (Rikardsen *et al.*, 2007). Generally, it seems that the crustacean/fish ratio of the diet is higher for charr. Crustaceans also make an important part of the diet for young Arctic charr (Cavalli *et al.*, 2002). One could speculate whether a stronger preference for crustaceans is a reflection of a higher astaxanthin requirement in charr or an indication to other differences in nutrient needs compared to other salmonids. In the early days of charr farming, uptake and muscle retention of astaxanthin was a problem, and most farmed charr were poorly pigmented in the beginning. Although today this problem has been more or less eliminated, Arctic charr feed still contain more astaxanthin than those of other farmed salmonids, like salmon and trout (rainbow and brown). A comparison of muscle tissue composition with regard to essential amino acids in different salmonids has been presented by Jobling *et al*. (1993). The authors conclude that the composition of the Arctic charr muscle tissue is similar to the other species in the study and that it differs only slightly from the requirements found in growth trials. It is not made clear in the paper whether the small differences that can be seen in the comparison are statistically significant.

All feeds on the market today, designed or used for Arctic charr are based on marine lipid sources. However, Arctic charr is the species among salmonids that is the most adapted to freshwater. Farming of charr in Norway is based on anadromous stocks, but in Sweden there are no naturally occurring anadromous charr. Here farming and breeding is based on landlocked charr. Nutrient content of marine and freshwater diets differ significantly, especially in lipid composition (Kaitaranta and Linko, 1984; Pickova and Brännäs, 2006). Typically the ratio of n-6/n-3 FA is higher in a freshwater based diet. It has been suggested that this, especially the low levels of Arachidonic acid (20:4 n-6) in the marine diet, is one of the factors contributing to poor egg quality among farmed arctic charr. The basis for this argument is that there may be specific differences in ability to de-saturate and elongate fatty acids between marine and freshwater species. This ability does generally vary between different species of fish (Torstensen *et al.*, 2001). N-3 and n-6 PUFA, especially DHA (docosahexaeneoic acid) and EPA (eicosapentaenoic acid) are essential for normal development and growth (Jobling *et al.*, 1993; Pickova *et al.*, 1997; Rønnestad and Hamre, 2001; Czesny *et al.*, 2009). No PUFA can be synthesized *de novo* by fish or mammals. However, provided they receive alpha-linolenic (18:3 n-3) and linoleic acid (18:2 n-6) through the diet they can to varying extent, as mentioned above, use these to produce HUFA (highly unsaturated fatty acids).

The importance of Arachidonic acid in fish diet, particularly in association with broodstock diet and egg quality has been emphasized in several papers by Pickova et al. (Pickova *et al.*, 1997; Pickova and Brännäs, 2006; Pickova *et al.*, 2007) as well as in a review article by Bell and Sargent (2003), in which the authors predict an increased focus on Arachidonic acid in aquaculture research. It has been shown for gilthead sea bream (*Sparus aurata*) that increased content of n-3 FA in the feed results in higher percentage of normal eggs, and increased EPA content improves fertilization rate (Fernandez-Palacios *et al.*, 1995). A recent study on Arctic charr showed correlations between gamete quality and FA composition. Surprisingly, correlations were stronger for sperm than for eggs. High fertility spermatozoa contained less short-chained saturated FA, more n-3 and n-6 FA with higher n-3/n-6 ratio compared to the low fertility group (Mansour *et al.*, 2011).The content of Arachidonic acid was also significantly higher in the high fertility spermatozoa, along with several other specific FA's (Figure 8). No correlations were found between FA-composition and egg fertility. It should be noted that all the fish in this study were given the same feed. Hence, variation in FA-composition found in the spermatozoa was not derived from differences in FA-supply to the individual males. FA-composition of eggs and sperm is however closely related to FAs in the parents' diet. This was shown for Rainbow trout by Vassallo Agius *et al.* (2001) in a study that, in concurrence with Mansour *et al.*, (2011) showed correlations between low levels of n-3 FA and low sperm motility and fertility.

Vitamins are, of course, also important in the broodstock diet. Vitamin E (α-, β-, γ- and δ-tocopherol) plays a vital role in reproduction. Deficiency of this vitamin has detrimental effects on fertility. This has to do with its stabilizing effect on the cell membranes of embryos, as a structural component and by being the most important antioxidant in living cells. Levels of vitamin E in feed affect both hatching rates of eggs and survival during the early stages post hatching (Izquierdo *et al.*, 2001; Rønnestad and Waagbø, 2001). During the early stages of gonad development, while the broodstock fish are still feeding, vitamin E from the feed is partly still being deposited in the muscle tissue, but it is also transported straight to the oocytes after uptake in the gut. Once the pre-spawning starvation period commences (September-December for salmon) the majority of vitamin E in the muscle, along with other nutrients, is transported to the maturing eggs (Lie *et al.*, 1994; Izquierdo *et al.*, 2001; Rønnestad and Waagbø, 2001). It is therefore crucial for broodstock to receive a vitamin E rich diet already at an early stage prior to spawning.

Vitamin C (ascorbic acid) is important to fish during sexual maturation as it is crucial for production of steroid hormones such as estrogen, which in turn e.g. stimulates vitellogenesis (Rønnestad and Waagbø, 2001). Vitamin C is also important for collagen production in the embryo as well as being an antioxidant both in broodstock fish and their eggs. Both hatching success and early survival is strongly correlated with vitamin C intake (Sandnes *et al.*, 1984). Eggs and yolk-sac larvae are dependent on the vitamins received from their mother, but it is also important that sufficient amounts of vitamin C are attained when the larvae reach start-feeding.

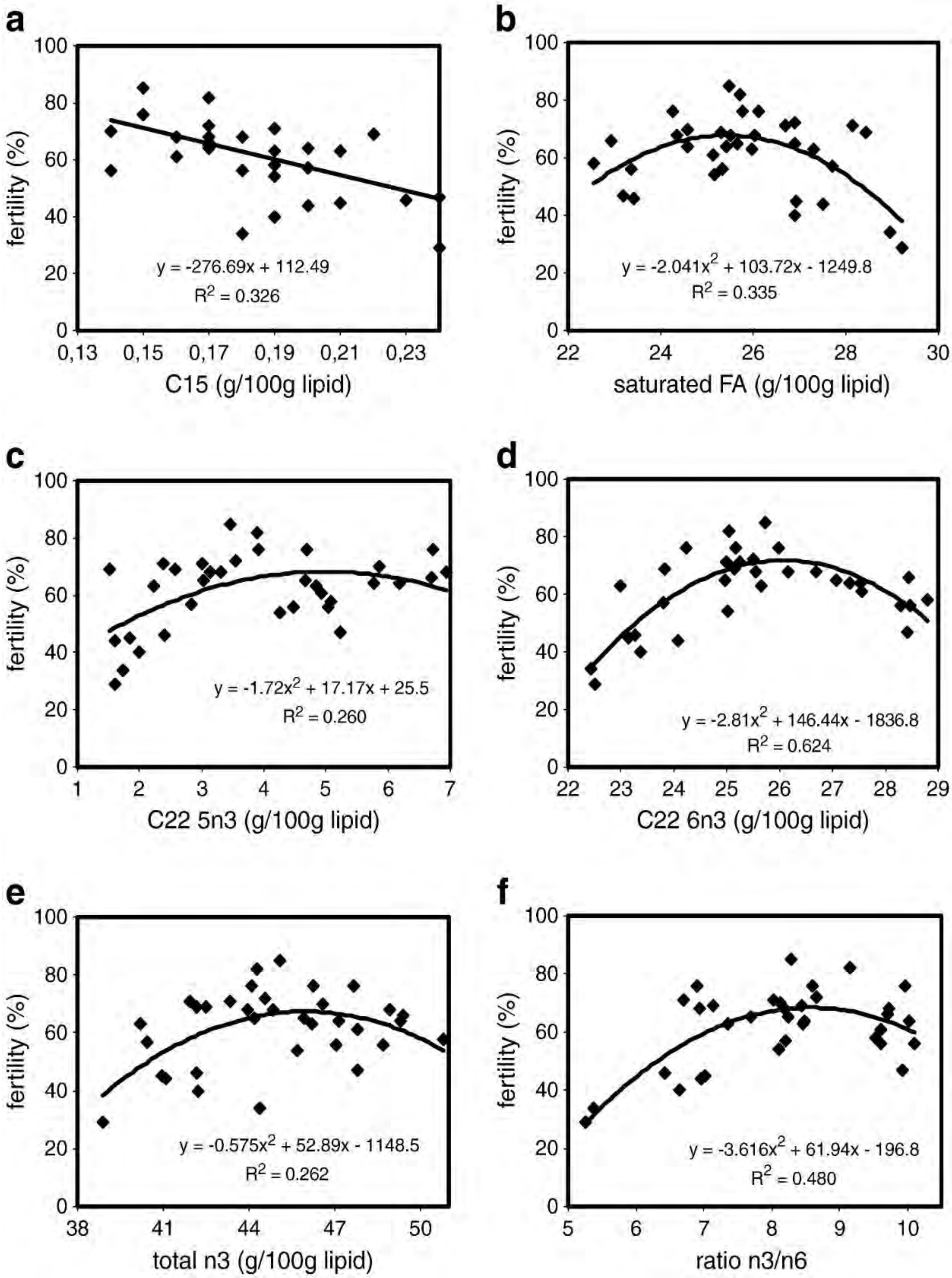

Figure 8. Relationships between fatty acid profile and fertility in spermatozoa of Arctic charr (a) C15, (b) total saturated fatty acids, (c) C22 5n3, (d) C22 6n3, (e) total n3 fatty acids, (f) ratio of n3 to n6 (n = 34) (Mansour et al., 2011).

Several B vitamins are important during reproduction and early life stages of fish. Strong correlations have been found between thiamin (B_1) levels in eggs and early mortality syndrome (EMS) as well as early growth rate in lake trout (*Salvelinus namaycush*) from the great lakes in North America. The lake trout is a species closely related to Arctic charr. Fitzsimons et al. (2009) found that larvae and fry of wild lake trout exhibited a significant decrease in standard growth rate (SGR), when thiamin concentrations of the eggs were 10nmol/g or less. Another study on the same species by Czesny et al. (2009) showed high EMS mortality for egg containing 1nmol/g thiamin or less. Above this value EMS was more or less eliminated. Sometimes salmonid eggs are soaked in a thiamin solution during the late stages of incubation to provide them with sufficient thiamin. It is theorized that thiamin deficiency in salmonids (which is quite common in wild stocks) is related to naturally high occurrence of thiaminase in their prey, e.g small crustaceans and sprat (*Sprattus sprattus*). In aquaculture such enzymatic activity in the feed poses a potential problem with species that require live organisms at start feeding, and also if a formulated feed is based on raw fish or other non-processed marine products (Rønnestad and Waagbø, 2001). Vitamin B6 can also be a critical factor when using live feed, e.g. *Artemia* sp., as sufficient enrichment of this vitamin to live feed has proven difficult. Large amounts of B6 are consumed both by the dam and later in the egg, during embryonic development. It is therefore important to provide the broodstock fish with sufficient amounts of B6 to last until the fry is ready for formulated feed.

Timing of spawning

The two major environmental factors controlling timing of spawning are photoperiod (day length) and water temperature, with emphasis on photoperiod. High temperatures during the pre-spawning period will delay ovulation. However, during the spawning period elevated temperatures will accelerate the final maturation of eggs and there is greater risk of over ripening, leading to poor egg quality (Bromage *et al.*, 1994; Gaudemar and Beall, 1998). Arctic charr in Sweden spawn in late autumn when water temperatures are decreasing. Hence, later spawning means lower temperature for the eggs. The delaying effect of pre-spawning high temperatures will therefore work in a self-regulatory way enabling spawning to occur when the temperatures have dropped. This works at least to some extent, but if the temperatures are too high, ovulation will be inhibited altogether. If the broodfish have experienced pre-spawning temperatures high enough to inhibit ovulation, ovulation may still be induced by transferring the fish to cold water some time before spawning. However the effect is limited and the egg quality is probably low compared to fish held at optimal temperatures year round.

Photoperiod manipulation is an efficient method to advance, delay or synchronize ovulation and spawning (Davies and Bromage, 2002; Frantzen *et al.*, 2004; Gillet and Breton, 2009). Photoperiod manipulation is used to alter the reproductive cycle of several species of fish in order to provide the market with eggs outside the natural spawning season. For instance long day length in spring and switching to short day length in summer will accelerate the maturation process, resulting in early ovulation (Gillet, 1994; Frantzen *et al.*, 2004). Advancing ovulation will however only be successful if the water temperature in sufficiently low (Gillet, 1994; Gillet and Breton, 2009). Keeping the fish at summer day length or 24 hour light cycle into autumn will delay maturation. Even when the temperature drops during this period the delaying effect of the extended photoperiod will prevail. As previously mentioned some charr hatcheries struggle with high water temperatures at stripping and egg incubation. Postponing spawning by photoperiod manipulation, until the water has reached temperatures suitable for the eggs, could overcome such problems.

Very few studies have been done on the influence of light intensity and spectral composition on the effectiveness of photo manipulation of fish. Full-spectrum lights, which mimic day light in spectral composition, are generally recommended. But I have not found supporting scientific evidence for this. Recommendations for minimum light intensity are found around 100 lux (Hansen *et al.*, 1992; Frantzen *et al.*, 2004), although there are indications that growth and sexual maturation have different thresholds for light intensity (Oppedal *et al.*, 1997).

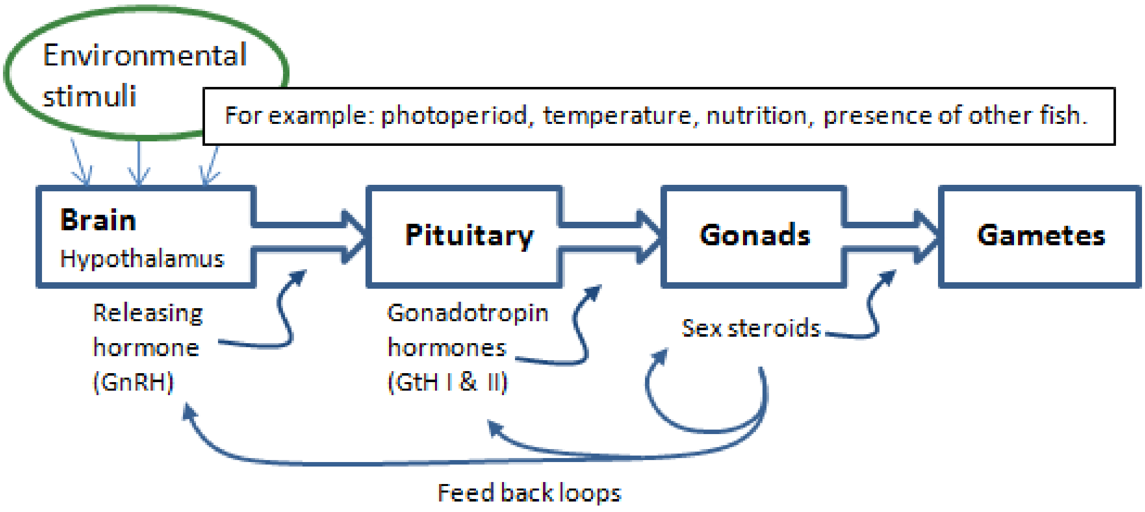

Figure 9. Schematic of hormonal regulatory mechanisms of reproduction in fish (Brännäs et al., 2011a).

Timing of spawning can also be manipulated with hormonal treatment. Releasing hormones are produced by the hypothalamus (figure 9) and stimulate the production of gonadotropic hormones in the pituitary gland. These induce release of androgens and estrogens in the gonads, thus affecting sexual development and maturation (Goetz, 1983; Bromage *et al.*, 1992). In farmed charr (and other fish) this process can be manipulated by introducing artificial releasing hormone analogues (Jansen, 1993). They are given to the fish either as single intramuscular injections or by surgical implants of pelleted hormone analogues, commonly GnRH (Gonadotropin Releasing Hormone) and GnRH agonists and analogues such as LHRHa (Luteinizing Releasing Hormone analog) (Crim *et al.*, 1988; Mylonas and Zohar, 2000). Administration of these releasing hormones to broodstock fish prior to spawning will advance and synchronize ovulation (Jansen, 1993; Olito *et al.*, 2001; Vikingstad *et al.*, 2008). The method is efficient for short term and acute manipulation of spawning, not for long term alteration of spawning season, for which photoperiod manipulation is better suited (Davies and Bromage, 2002; Frantzen *et al.*, 2004). In cases where the spawning period of a broodstock is prolonged due to high water temperatures, ovulation can be initiated which hormonal treatment. The result is a short, synchronized stripping period which helps to minimize handling and stress of the fish as well as the risk of overripe eggs. However, such an artificially initiated ovulation will not result in higher egg quality and survival in itself. In fact, high doses of LHRHa have been shown to decrease survival of eggs (Crim *et al.*, 1983; Haraldsson *et al.*, 1993; Olito *et al.*, 2001). Hence, the method is not an adequate replacement for optimal temperature conditions of the brood stock during summer and autumn but can act as a last resort to salvage egg batches that would otherwise be lost because of unexpected periods with high water temperatures (Vikingstad *et al.*, 2008). Sufficiently cold water is still necessary for charr farming, specifically in combination with a sharp autumn drop in temperature. Under such conditions hormone treatment should not be required. In combination with transfer to cold water, LHRHa treatment prior to spawning is an efficient way to save and increase the quality of eggs from temperature stressed fish (Gillet *et al.*, 1996; King and Pankhurst, 2004). Hormones can be administered both to sires and dams. LHRHa treatment to male Chinook salmon has been shown to increase milt production without compromising sperm quality (Olito *et al.*, 2001).

Genetics

The importance of a direct heritability factor of egg quality in farmed Arctic charr is not quite clear. Traits closely linked to offspring survival show very little variation within species or populations in the wild, according to basic evolutionary theories. I.e. genetic strains exhibiting significantly lower survival of their young will be eliminated quickly in the wild, through natural selection. But in aquaculture the selection pressure due to limited resources is not as strong as in the wild. And in a selective breeding programme the selective pressure is determined by man and limited to certain desirable traits. The Swedish Breeding programme for Arctic charr has had to cope with a very limited size of the available broodstock. This has resulted in a very generous acceptance towards egg batches of poor quality, a necessity to keep the programme going through generations. Maybe this loosening of "natural" selection pressure has lead to the poor and highly variable egg quality exhibited by the strain today. In other breeding programmes with more extensive resources, e.g. the Norwegian national cod breeding programme, egg quality is one of the selection traits. Only the best egg batches are accepted for inclusion in the programme, i.e. minimum 80% fertilisation and 80% normal cell cleavage.

Estimates of heritability on a range of commercially important physiological traits in cultured fish are presented in a review by Gjedrem (1983). The article includes traits that can be interpreted as measurements of egg quality, such as survival of eggs and alevins. From the presented heritability estimates it is conclusive that there is a heritability factor, although quite limited. There is however a strong genetic parental influence on fertility and egg quality. A study on human ova showed chromosomal abnormalities to be the major cause for poor egg quality (Almeida and Bolton, 1993). Several studies on fish also suggest effects of maternal DNA on egg quality (Withler, 1987; Brooks *et al.*, 1997). Brooks et al. (1997) claim to have (unpublished) data showing that female trout exhibiting good egg quality at first spawning will also produce good eggs at second spawning. This suggests a maternal genetic factor to egg quality.

According to the "genetic compatibility hypothesis" some mates are more compatible than others for producing viable offspring due to genetic interactions between the maternal and paternal haplotypes (Nordeide, 2007). In many species of fish the female chooses which of the courting males will fertilize her eggs, and in many cases the eggs of one female are fertilized by several males. Both these factors have shown to have a positive effect on egg quality compared to forced mate selection, also in cultivated fish (Withler, 1988; Kekäläinen *et al.*, 2010). For practical reasons spontaneous mate choice and spawning is usually not an option in aquaculture. Arctic charr males and females are commonly held in separate rearing tanks prior to and during spawning in order to avoid aggressive behaviour and injuries amongst the fish. Also, spontaneous spawning in the tank is undesirable as it would lead to loss of eggs and difficulties in collection and transfer to incubation. This is, however, applied on some species, e.g. cod, where the neutrally buoyant eggs can be collected from the water through filtration. The advantage to such a system is that handling of the fish is minimized. This is, of course, not an option for breeding, as you cannot control who is mated with whom.

Using mixed milt from several males for egg fertilization is quite common in aquaculture. Usually both milt and eggs from several individuals of each sex are mixed, both for practical reasons and to achieve higher fertilization rates. But, as for spontaneous spawning this is mainly suited for fish production. For selective breeding purposes there is little to gain from fertilizing one female's eggs with a multi-male milt mix. Then the offspring would have to be identified with genetic markers to establish their pedigree. However, splitting the egg batch from one female and fertilizing the splits with milt from different males is often applied in fish breeding, resulting in half sibling families. Mixing of milt does not only affect fertilization and survival according to the "genetic compatibility hypothesis". Introducing inter-male sperm competition may also improve fertilization rate and viability of the fertilized eggs. It has been shown for Arctic charr that high sperm velocity is associated with low offspring mortality (Kekäläinen *et al.*, 2010). Sperm quality is also associated with social hierarchy. Once social hierarchy has been established in a group of Arctic charr there will be a differentiation in sperm production and sperm velocity between dominant and subordinate fish (Liljedal and Folstad, 2003; Vaz Serrano *et al.*, 2006; Haugland *et al.*, 2009). The smaller subordinate charr will take on the role of sneak spawners, and as an adaptation to this behaviour they have a larger production of milt, with higher sperm density and velocity. The cost of being a larger mate guarding dominant male is said to results in lower sperm quality in these individuals. Whether this has any implications for charr hatcheries is doubtful. If the subordinates' sperm outcompete that of the dominants in a milt mix, and hierarchy is a heritable trait, maybe this would have a dampening effect on hierarchal differentiation. On the other hand, as growth is a strongly heritable trait (Gjedrem, 1983; Nilsson, 1990; 1992a) it could have a negative effect on growth rates in the stock, as the sperm of the largest fish are outcompeted. However, in my opinion such effects are likely to be negligible, and heterogeneity of the milt mix is more likely to affect fertilization rate accredited the different males. Especially if the milt is not premixed, but added from one male at the time, the sperm first added to the eggs probably gain an advantage. Although, according to R.E. Withler (1988) there is differences in potency between males, which shows when milt is mixed prior to egg fertilization. Whether this is a genotypic or phenotypic trait is unclear. There are also theories saying that MHCs (major histocompatibility complexes) influence reproductive success. The idea is that males and females of different MHC-genotypes are more successful in reproduction and produce better quality offspring. Mate selection is likely, at least partly, based on MHC. Skarstein et al. (2005) found that MHC-heterozygous males had greater fertilization success than homozygous ones, however they found no correlation between mate MHC-similarities and fertilization success.

Pathogens

In aquaculture there is an ever-present threat of infections. It may be in the form of parasites, fungi, bacteria, or viruses (Barker *et al.*, 1989; Nilsson, 1992b; Brock and Bullis, 2001; Pylkko *et al.*, 2003; Madsen *et al.*, 2005; Yuasa *et al.*, 2007; Overton *et al.*, 2010; Tveiten *et al.*, 2010). Arctic charr is often infected by fungi, e.g. *Saprolegnia* spp., typically during late summer when the water temperature is high, and into the spawning season in autumn (Nilsson, 1992b). This can lead to direct mortality losses of fish and may weaken survivors, potentially affecting the reproductive development and success. Fungal infections can be treated. In the past this has been done with malachite green, which is quite effective. But since this compound was banned for use in EU, salt, hydrogen peroxide and formaldehyde are most commonly used today. However, the effectiveness of these treatments is somewhat limited. The fish is fungus-free after treatment, but is soon re-infected. Charr and other salmonids are also targets for bacterial pathogens such as *Aeromonas* sp., *Pseudomonas* sp., *Cytophaga* sp. and *Flavobacterium psychrophilum* (Barker *et al.*, 1989; Madsen *et al.*, 2005). If the eggs are subjected to bacterial infections this may result in increased mortalities during egg incubation and post hatching. Barker et al. (1989) found correlation between number of egg surface bacteria and hatching rate. Infections can be transferred to the eggs both horizontally from the water during fertilization and incubation or vertically from the parents. Vertical transfer may occur onto the egg surface via ovarian fluid and sperm, to the eggs internally either in the gonads before spawning or by sperms at fertilization. It has been shown that sperm effectively adsorb Rhabdoviruses from the surrounding water, the viruses may then be transferred by sperm to the egg (Mulcahy and Pascho, 1984).

In seawater, Arctic charr are subjected to infection by the parasitic sea louse, *Lepeophtheirus salmonis*. Extensive infections may result in delay of reproductive maturation as well as reduced growth and health condition (Tveiten *et al.*, 2010). Of course, this example is not an issue for freshwater based aquaculture such as the Swedish charr-farming. But there are many parasites in freshwater systems as well. The eye-fluke (e.g. *Diplostomum* sp.) for instance, causing cataract and eventually blindness can be a major concern in freshwater farming of Arctic charr (Voutilainen *et al.*, 2009). Parasites in general will affect the wellbeing of its host, which in turn may have implications for reproductive success.

The most important matter concerning pathogens is prevention and control. The first and maybe most basic measure is minimizing the risk for infectious agents to enter the aquaculture facility. Infections can enter mainly through transport of fish to the facility and inflowing water as well as by people and equipment that have been in contact with fish at other facilities or in the wild. In outdoor facilities there is the additional risk of animals, feral or domestic, introducing disease or parasites to the fish. Generally, infections can be prevented by good hygiene, disinfection of equipment, adequate handling of deceased fish and screening of inflowing water. This applies to all facilities, food production, breeding or hatchery. However, some stages of the production cycle are more critical and sensitive than others.

The health of the broodstock fish is crucial, as they form the foundation of all fish recruitment. They should be checked regularly for common diseases to prevent major infectious outbreaks. Good health in the broodstock reduces the risk of infection during egg production, as disease can be transferred to the next generation during gamete maturation as well as stripping and fertilization. Good hygiene is important during stripping, which is one of the most critical times for infection. In addition to the eggs being vulnerable, the parents may be extra sensitive to pathogens due to pre-spawn starvation as well as handling which has caused both stress and damage to their protective mucus layers. During stripping, eggs are collected and mixed with milt in clean, disinfected containers and the gametes are activated by adding fresh clean water to the mix. After fertilization, preferably before the eggs are transferred to incubation they should be disinfected. For practical reasons the disinfectant can be pre-added to water which is poured into the egg-milt mix. There are several disinfectants for aquaculture-use available on the market. They may be based on iodine, glutaraldehyde or ozone (Grotmol and Totland, 2000; Overton *et al.*, 2010). For charr eggs an iodine based disinfectant like Buffodine is recommended, as glutaraldehyde is carcinogenic and overexposure to ozonated water may delay or reduce hatching (Grotmol *et al.*, 2003; Overton *et al.*, 2010). Sodium bicarbonate is also used as an egg disinfectant. During incubation eggs should be checked regularly and dead ones should be removed (figure 10). These are easily identified as they turn milky white. If left in the incubator, dead eggs provide growing grounds for bacteria which are then spread to the live ones, reducing survival (Barker *et al.*, 1989; Madsen *et al.*, 2005).

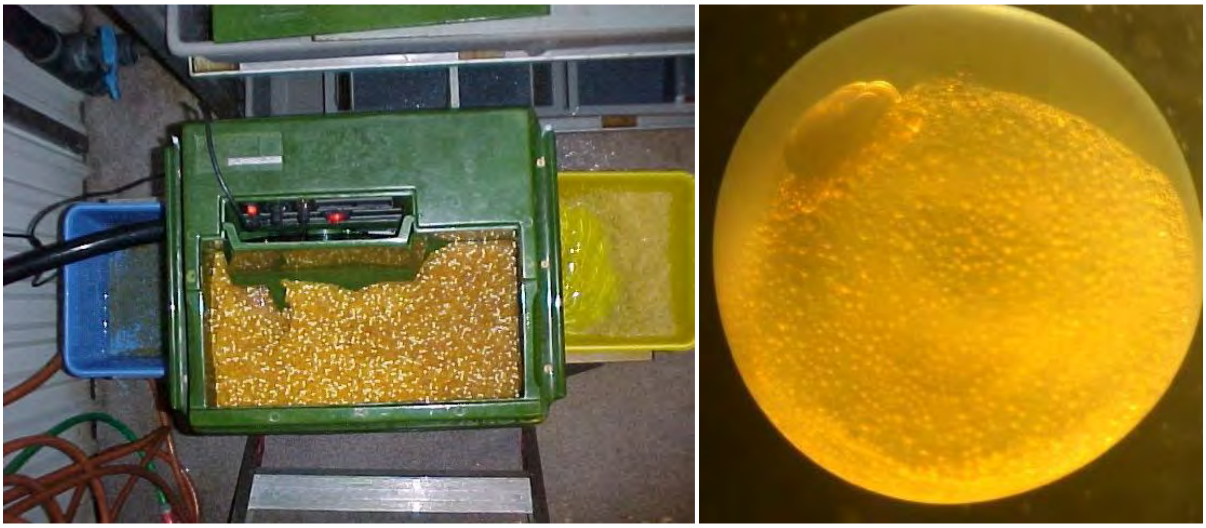

Figure 10. Automatic sorting of viable, orange eggs and dead, white eggs (left) and a fertilised Arctic charr egg at the 32-cell stage (right). (Photo: Marianne Frantzen, NFH,University of Tromsø).

Husbandry related stress

Fish are subjected to many types of environmental stress, both in the wild and in culture. Stress can result in a range of physiological responses and is likely to affect reproduction (reviewed by e.g. Leatherland, 1999; Schreck *et al.*, 2001; Schreck, 2010). Previous chapters have dealt with environmental factors that when they are sub-optimal can be perceived as stressors for the fish. Stress can be defined as "…physiological cascade of events that occurs when the organism is attempting to resist death or re-establish homeostatic norms in the face of insult" (Schreck, 2010). There are other definitions, but a common thread is energy expenditure in face of (extraordinary) changes in environmental factors. In this context repartitioning of energy is a key factor in how stressors may affect fish reproduction. Maybe the most obvious and profound example of environmental stressors in fish farms is lack of food, but may also be sub- or supra-optimal temperatures, unfavourable levels of gases and metabolites (O_2, CO_2, NO_X, NH_4 etc.) as well as presence of pathogens and parasites. In well managed aquaculture facilities the extent of these stressors is minimized. There are however additional stressors associated with husbandry. Keeping fish in tanks or net pens may lead to stress through crowding and confinement. If there is room for hierarchical development, subordinate individuals will have no means of escaping their tormentors, leading to high levels of stress. All types of handling will also induce stress in varying extent to the fish, e.g. netting, sorting, transport, anesthetization and stripping.

When fish are stressed through handling or restraint they produce cortisol (Pickering *et al.*, 1987; Stratholt *et al.*, 1997). In addition to the long term effects of stress through energy repartitioning, acute stress is said to have an effect on fish reproduction through elevated cortisol levels (Pankhurst and Van der Kraak, 2000). Several studies suggest that both handling mediated stress and cortisol itself have negative effects on maternal fitness, egg size, sperm count, hatching rates and progeny survival and behaviour (Campbell *et al.*, 1992; 1994; Contreras-Sanchez *et al.*, 1998; Clements *et al.*, 2002; Li *et al.*, 2010; Sloman, 2010; Mileva *et al.*, 2011). Subjecting brood fish to stressful conditions may cause an advance or delay in ovulation depending on when during gonad maturation they are stressed (Campbell *et al.*, 1992; Contreras-Sanchez *et al.*, 1998). High cortisol plasma levels seem to affect the gonad development in pubertal and adult fish. Cortisol produced by a stressed female can also be passed on to her eggs, potentially with detrimental effects on egg viability (Stratholt *et al.*, 1997). The latter was shown in a study by Li et al. (2010) where rainbow trout eggs incubated in cortisol enriched ovarian fluid exhibited lower hatching rates. Early exposure of trout eggs to high levels of cortisol has also been shown to decrease sensitivity to stress later in life, i.e. 5 months after hatching (Auperin and Geslin, 2008). Whether this can be considered a positive or negative trait in an aquaculture situation is not clear. If this decreased sensitivity is synonymous with higher stress tolerance I suppose it may be advantageous. I any case, it is clear that stripping of stressed brood fish may result in egg batches containing high levels of cortisol, which in turn can affect the viability of the offspring.

In 1999, John F. Leatherland published a very critical review paper concerning the relation between stress, cortisol and reproductive dysfunction in salmonids. He claimed that, at the time of writing his paper, there was no scientific proof that either husbandry caused stress was severe enough to have detrimental effects on reproduction, or that cortisol had negative effects on progeny survival. According to Leatherland stressors in the few existing studies were highly exaggerated and could not be considered fish farm husbandry, e.g. total drainage of rearing tanks. Studies of cortisols effect on progeny survival were all inconclusive. He further argued that cortisol plasma levels of many salmonid species are often naturally elevated during the period of late gonad maturation. Hence, sensitivity to cortisol in these species would have detrimental effects on gamete quality, for which there is no evidence. However, later research provide a plausibly explanation to this dilemma. It seems fish have a biphasic reaction to cortisol. Low levels of cortisol have no negative effect. It may even have a positive effect on growth rate through feed conversion efficiency or growth hormone gene expression (Yada *et al.*, 2005; Li *et al.*, 2010). At higher levels of cortisol there is no effect on GH gene expression, but detrimental effects such as reduced hatching rates have been demonstrated (Li *et al.*, 2010). Eriksen *et al.* (2006; 2007) found profound negative effects of high pre-natal cortisol levels in salmon, especially in combination with mild hyperthermia during egg incubation. Administration of cortisol to female salmon, 6 days prior to stripping, resulted in severe impacts on offspring survival, growth and morphological abnormalities.

After examining the literature on stress and reproduction in fish there is no doubt, to me, that stress from handling can have negative effect on reproductive success. Whether this effect is through hormonal processes, e.g. from direct effects of cortisol itself, decreased food intake or something else is interesting research but not crucial knowledge for the fish farmer. The fish should be treated carefully, and handling kept at a minimum.

Synthesis

Before I started writing this essay and digging into the literature presented herein, I did make some speculations on what may cause the poor survival rates of the Arctic Superior. At that point I theorised that high water temperature was the single most important factor. Now, after having read most of the key literature on biological and environmental factors affecting reproduction and gamete quality, I am still convinced temperature is the most critical aspect for the current broodstock. Temperature has substantial impact on various phases of reproductive process, from gonad maturation (Jobling *et al.*, 1995) to timing of spawning (Davies and Bromage, 2002; Gillet and Breton, 2009), as well as embryonic development (Jungwirth and Winkler, 1984), growth (Bebak *et al.*, 2000) and survival (De March, 1995). We also know that the Arctic charr is particularly sensitive to high temperatures throughout its lifecycle, not only during the reproductive period, compared to other related species (Elliott and Elliott, 2010). Several conclusive studies have been made on the detrimental effect of high temperatures on the reproductive success of Arctic charr, covering rearing of broodstock (King *et al.*, 2007), stripping and fertilisation (Gillet, 1994) as well as incubation of eggs (Jungwirth and Winkler, 1984). Temperatures reported as having devastating effects on offspring survival are commonly occurring in many broodstock and hatchery facilities in Sweden. In my opinion Arctic charr broodstock should never under any circumstances be exposed to temperatures above 15°C. I would even set the upper limit to 12°C. At Aquaculture Centre North (ACN) in Kälarne, where most of the fish from the Swedish Arctic charr breeding programme is kept, water temperatures have reached above 15°C every summer since at least 1986 to 2010. Occasionally temperatures have even reached 20°C. When farming fish for the table market these temperatures are rarely problematic. With maximum somatic growth at 15°C, such rearing conditions are fairly good for rapid production of biomass. My point is: It is important to differentiate between fish kept for breeding and food production purposes when evaluating or managing rearing conditions. From this year (2011) ACN has a new water inlet that should enable access to colder water during the summer. Thus far (end of July), reports say that the water temperature at the station has not exceeded 15°C. The new inlet is also likely to affect winter temperatures, but it is still uncertain how and to what extent. It will be very interesting to see whether these new conditions have any effect on the coming spawning season.

Autumn temperatures, specifically at stripping and fertilisation, are just as important as, or possibly even more important than summer temperatures to egg quality. High temperatures at this stage will speed up maturation of the eggs, increasing the risk of over-maturation. As the maturation process continues past optimum, fertilisation rates decrease very rapidly (Bromage *et al.*, 1994). At least some years this is a problem in several Swedish Arctic charr farms. Often the temperature has not dropped enough in the water reservoir by the time the fish are ready to spawn.

One way to lower summer temperatures is to use groundwater. This is for instance done at the Omegalax hatchery/stocking fish facility in Timrå, one of two backup facilities for the Arctic charr breeding programme. Here temperatures are kept at 5-7°C year round, well below harmful summer temperatures. For egg fertilisation and incubation however, I would say these temperatures are too high. In the wild Arctic charr spawn at ca 4°C in autumn. The eggs develop during the winter, often at even lower temperatures. We know that incubation temperatures above 5°C can result in significantly reduced hatching rates (De March, 1995; Janhunen *et al.*, 2010). Cooling of water is very costly and therefore in most cases not considered an option. In my opinion, the best set-up would be combining river/lake water and groundwater. In a facility with access to both, it is possible to manipulate the temperature to an optimum year round and different sections of the facility can have separate temperature regimes. So, not only are fish of different stages or production purposes kept at their separate optimal temperature, but growth and development rates can be manipulated to e.g. provide continuous supply of stocking fish all year.

In addition to the direct effects on gamete development in the parent fish, over-maturation of eggs and embryonic development during incubation, I think there are other, more indirect consequences of supra-optimal temperatures on reproductive success. For instance, if ovulation becomes unsynchronised and unpredictable due to unfavourable temperature conditions, this in turn will lead to more handling and disturbance to the fish. The fish has to be checked for maturity more regularly and during a longer time period, causing stress levels to rise. Stress itself will affect reproductive success negatively (Pankhurst and Van der Kraak, 2000; Mileva *et al.*, 2011), as well as direct physical handling that may injure the fish. But, although handling and stress are the direct causes in this example, they may not be the ultimate source of the problem. It is important to try and see the whole picture to get to the bottom of such problems. Of course I think it is important to minimise the amount of stress and handling of the fish. But we cannot expect to eliminate handling altogether, because then it wouldn't be aquaculture. Call me naive, but I believe fish farmers treat their fish well, without unnecessarily rough handling. The exception would be during stripping, when rather brutal treatment may be implemented if the fish are "uncooperative", i.e. not ovulating. Such rough treatment, I'm sure is not intentional but due to ignorance and must be avoided through education. And again, it can be minimised by optimising rearing conditions of the broodstock to enable synchronous spawning under the right environmental conditions.

Unsynchronised spawning between sexes makes the fertilisation process more difficult. If the maturation of sperm and eggs peak at different times, one of them, typically sperm, may have to be stored until the females are ovulating. There are several cryo-preservation protocols for fish sperm that work rather well, but fresh is always better. Hopefully, optimised holding conditions will eliminate such problems. If not, time has to be spent evaluating stripping procedures and maybe sperm preservation methods. Synchronisation and general improvement of spermiation may also be achieved through hormonal treatment of males.

I the case of unsynchronised spawning period, one explanation may also be found in light conditions. Light, or photoperiod more specifically, is a stronger tool than temperature for manipulation of sexual maturation and spawning (Davies and Bromage, 2002; Frantzen *et al.*, 2004; Gillet and Breton, 2009). There are few studies made on the requirements of light intensity and spectral composition for triggering physiological responses in Arctic charr, or other salmonids for that matter. In studies made on photoperiod manipulation of spawning and ovulation of Arctic charr a range of light source have been used, e.g. traditional tungsten light bulbs, fluorescent light tube and full spectrum lights. I have found recommendations in the literature to use light sources with a spectrum resembling that of natural day light at a minimum of 100 lux (Hansen *et al.*, 1992; Frantzen *et al.*, 2004), but no conclusive studies to validate these recommendations. Indoor broodstock facilities in Sweden commonly have few if any windows and use traditional fluorescent lights in the ceiling for lighting. I have a theory that this type of lighting is inadequate as an efficient zeitgeber for the reproductive cycle, either in intensity or spectral composition. There are indications that the light intensity threshold differs for promoting growth and sexual maturation in Atlantic salmon (Oppedal *et al.*, 1997). Unpublished data from a study where fish from the Arctic Superior-strain where reared parallel at an indoor and outdoor facility during the sexual maturation period show much more synchronised ovulation in the outdoor facility. Offspring survival rates were also significantly higher in the outdoor facility. It should be said that the two facilities differed in more aspects than light conditions. For instance, the outdoor facility also had colder water. But it shows, yet again, the great impact broodstock holding conditions have on reproductive success.

Dietary intake of the brood fish certainly has impact on gamete quality. Surprisingly, the effect on sperm seems to be even greater than on eggs. Personally, I think minor adjustments of the FA composition of broodstock feed from what is used today will have small if any effect on gamete quality. Even though a marine feed is given to a (semi-obligate) freshwater fish, I think the composition of this feed is well suited to the Arctic charr. And with today's variable and suboptimal holding conditions at the research facility I think it will be difficult to conclusively evaluate these parameters, and little is to gain from this in comparison to optimising holding conditions. Firstly, we should focus on the rough stuff, like adequate temperature and light conditions, then we can start with the fine-tuning of diet, stress etc.

Suggested study topics

As I am sure can be deduced from de discussion section I am quite convinced holding conditions, particularly high temperature is one of the most crucial factors regarding the reproductive success of the Arctic Superior. However, I am that convinced the explanation is not that simple and will not dismiss other factors as completely irrelevant. Working chronologically through the production cycle, here are some issues I would like to study.

1. Nutrition. First of all the broodstock needs to be in top shape when entering the sexual maturation process. Their diet plays an important part in securing this. Quite a few studies have been made on the effect of diet on egg quality, but lately attention has also turned to its effect on sperm quality. It has been concluded that FA composition has substantial impact on sperm fertility. Now, more detailed studies on factors (dietary and other) effecting sperm quality are needed.

2. Timing of spawning. This is an important and complex part of the problem. I would like to look at the effect of light intensity and spectral composition on sexual maturation and timing of spawning and ovulation. The question behind this study is whether today's light conditions in indoor broodstock facilities are sufficient for effective steering of these processes. Solving the problems with synchronisation (inter and intra gender) and prolonging of spawning period will facilitate much of the following stripping and fertilisation procedure. If we cannot come to terms with high temperatures during stripping, effort should be made to delay ovulation until adequate temperatures are reached. This is best done with photoperiod manipulation and quantification of its effect on egg quality.

Sires and dams are separated prior to spawning, mainly in order to inhibit aggression and spontaneous spawning. I theorise that this absence of the opposite sex will have negative effects on final maturation and ovulation/spermiation. The idea is that sensory cues, e.g. hormones, physical appearance or behaviour will stimulate maturation in individuals of the opposite sex. Studies on the effects of inter-gender contact (visual or olfactory) on reproductive success would be interesting to do.

3. Stripping and fertilisation. There are several interesting aspects of the stripping procedure. If unsynchronised spawning persists, changes need to be made to the stripping protocol. Cryo-preservation and short term storage protocols for sperm need to be evaluated. The effects of injuries and stress related to handling need to be quantified. Use of hormone treatment may also be considered and evaluated for inclusion in stripping protocol, at least for particularly problematic spawning seasons.

4. Egg incubation temperature regimes. From what I hear, today incubation starts at rather high temperatures and is lowered along the incubation period. Without going into too much detail, I would like to evaluate alternative temperature regimes. It would also be interesting to determine the exact temperatures experienced by eggs of the original strain in the wild as a comparison

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