Arctic charr farming
Production of juveniles; a manual

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Arctic charr farming

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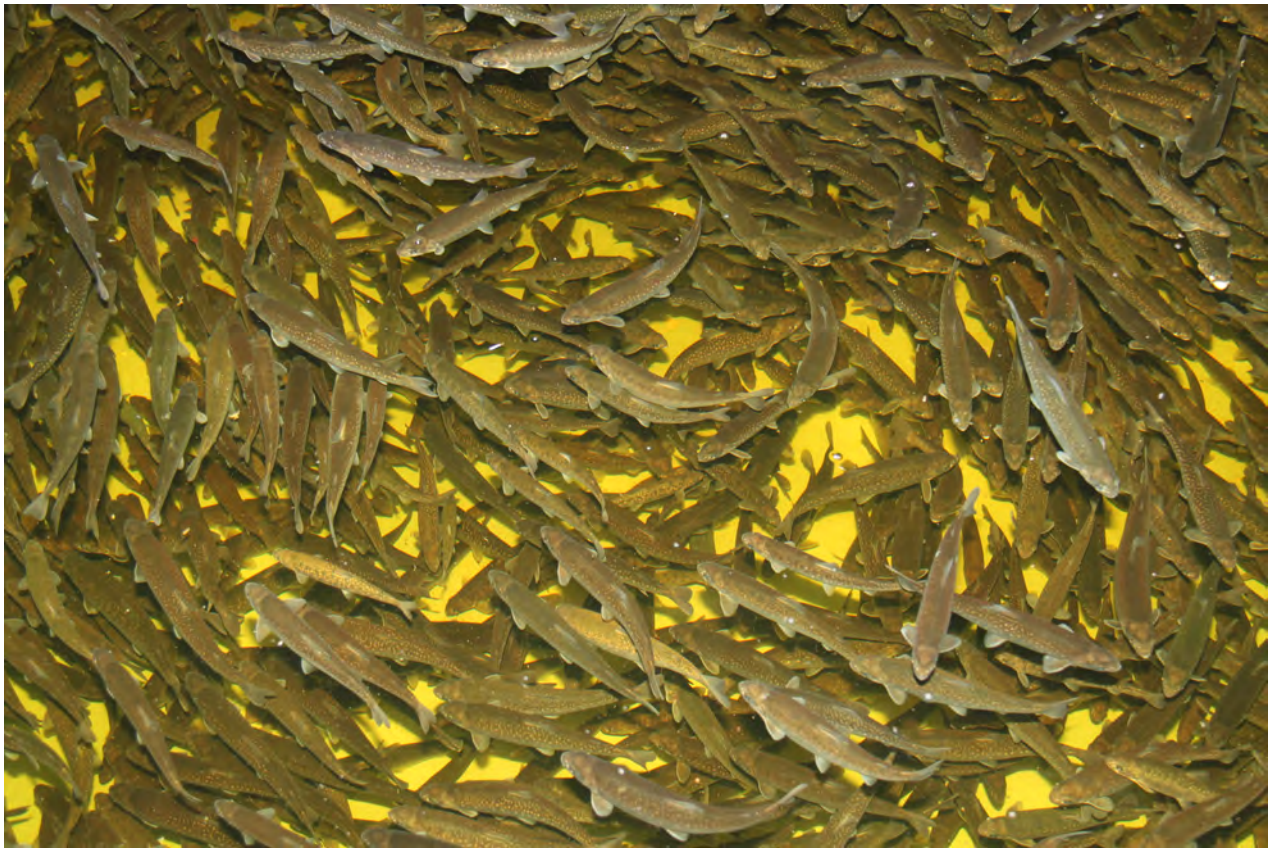

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The Arctic charr (*Salvelinus alpinus* L.) is a holarctic salmonid fish species with both landlocked and anadromous populations. In Scandinavia it is mainly found in the mountain area, but it also appears in deep and large lake further south, i.e. in the Alps. It is the northernmost freshwater fish and A. charr is generally regarded as the most cold-adapted freshwater fish. A. charr has been commercially farmed since the early 90^{ths} and today, the total production is 3000, 2300 and 700 tonnes/year in Iceland, Sweden and Norway, respectively. Both in Sweden and Iceland, there are selective breeding programs in effect and the bulk of the farming production is conducted using offspring from the national breeding programs in each country. A. charr is renowned for its ability of high growth rate at low temperature and is therefore especially suitable for farming at high latitudes and altitudes. Moreover, due to the success of the breeding programs, the charr used in farms today grow faster and mature at a larger size and age than the original wild charr. Hence, although being a fairly small industry at present, A. charr farming is predicted to grow in all three countries.

This booklet summarises up-to-date knowledge on A. charr farming production cycle, from brood stock to juvenile on-growing stage (Fig. 1). It is intended to be useful for people taking their first steps in A. charr culture but also to serve as a farming manual for more experienced farm staff. Therefore, the booklet is divided in to two chapters. The first chapter (*Arctic charr farming in practice*) provides a straight forward introduction to each production step from brood stock to juveniles. The second chapter (*theory and background*) presents some details and research data on the background of specific farming practices and procedures.

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Arctic charr farming in practice

1. The brood stock

Although sometimes overlooked, the quality and origin of a brood stock is one of the most important components in fish farming. The success may depend to a large extent on the quality and health of the eggs and fry produced. It will affect the mortality and growth rate in the ongrowing production cycle, from start feeding fry, fingerling to fish at harvesting size. Hence, the cost-effectiveness of the enterprises can be affected by the brood stock and the egg quality produced. In Sweden (Arctic Superior®, 7th generation) and Iceland (5th generation), there are selective breeding programs ongoing to improve important traits which affect the production economy i.e. growth (read more about selective breeding in chapter 5). In Norway, selective breeding of Arctic charr is prohibited due to national legislations and new practice from the authority only allow usage of local charr population in the new charr farms. Most of the charr produced in Sweden originate from the Arctic Superior© selective breeding program and in Iceland from a strain at Holar.

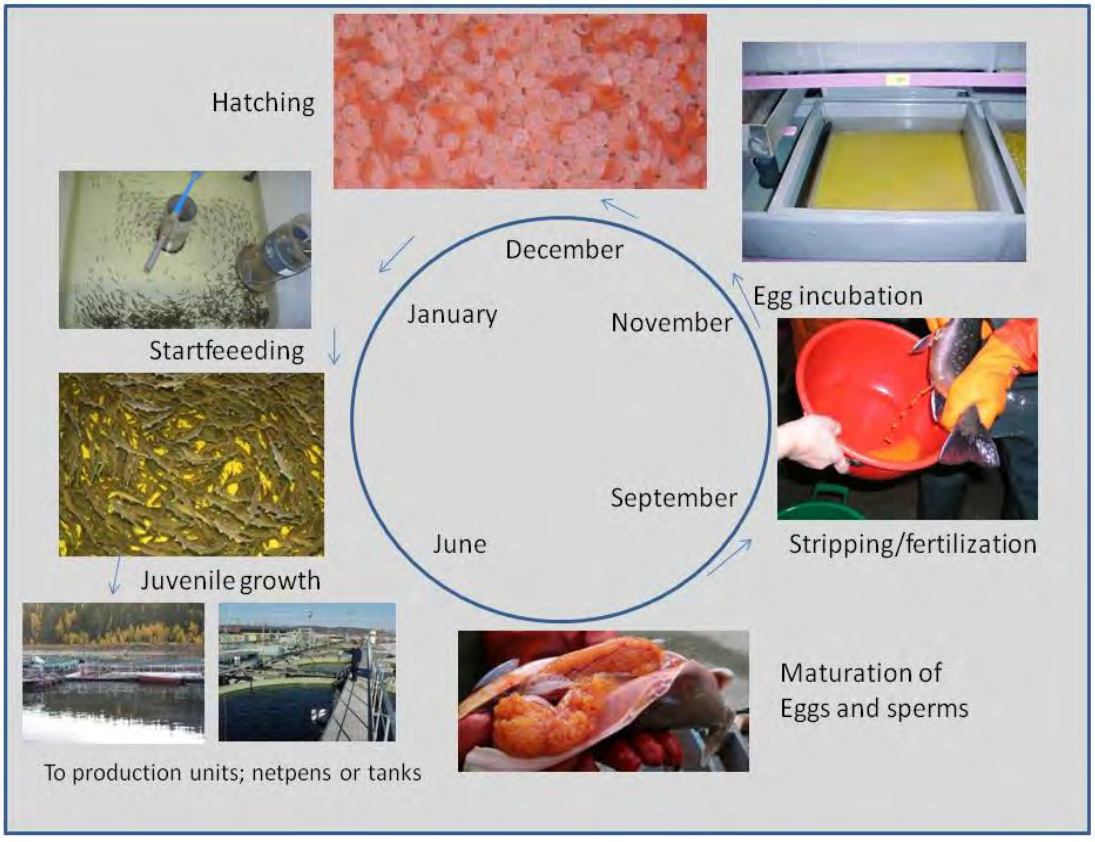

Fig. 1. A general overview of a production cycle from brood fish to juvenile Arctic charr. Juveniles can be stocked into different production units at different size and time of the year.

Most of the Norwegian production of Arctic charr is in the Northern part of Norway. The production is mainly based on the "original populations" best suited for farming, e.g. the domesticated "Hammerfest" strain. Most Icelandic A. charr farms base their production of harvesting sized fish on eggs from a central breeding station at Holar. In Sweden, most companies have their own breeding stock based on the breeding stock at Aquaculture Center North located in Kälarne. Maintaining a brood stocks at each individual farms is not cost-effective but there are at the moment no hatchery with the capacity to supply all farms with eggs. It is however, of outmost importance to keep reserves of the breeding material at more than on farm site in case of a disease outbreak or mortalities caused by other hazards.

This chapter gives information on best practice when keeping brood stock in a modern Arctic charr farm. The ambient conditions such as temperature, water quality and photoperiod as well as the feed formula are very important for the brood stock to ensure a high survival and quality of the offspring. The brood stock can be reared in circular tanks, raceways, net cages or earthen ponds, ensuring good control and maintenance of key environmental requirements. The growth of the brood stock can fluctuate during the year. The growth rate is normally low in winter and early autumn but maximum growth rate occurs in spring and early summer. These fluctuations can be affected in more controlled and stable culture conditions. Prior to spawning, the fish stop feeding and the growth is negligible. Hence, it is important to adjust the feed supply according to seasonal changes in growth potential. Nutritional requirements of spawners are different from immature fish. Specified brood fish diet for Arctic charr is slightly lower in fat/protein ratio than for ongrowing fish aimed for the table market. The ingredients in the brood stock feed are a majority of animal origin (fish meal and oil) and the diet usually contain additional amount of vitamins, minerals and astaxantin.

Ideally, the fish reach marketable size prior maturation. The maturation process stops the somatic growth and deteriorates the flesh quality. In Sweden, A. charr are slaughtered as 1+-2+ year old when they reach 0.7-1 kg in weight. In Iceland the harvesting size can vary between 0,4 – 2 kg, depending on the consumers request. The incidence of maturation will increase with prolonged production time, related to the age of the fish. The most efficient harvesting size for a fish farm might therefore be connected to the culture and growth condition at each farm. Early maturation is a character selected against in selective breeding programs. During the maturation the fish invest its energy into eggs and sperm instead of muscles (meat). When candidate strains of Arctic charr for fish farming were tested in Sweden, in the "best" strain; Hornavan charr 40-100% matured at 500 g which is below the common harvesting size. At present, in the Swedish Arctic superior, the maturation ratio in a group of slaughter size fish (0.7-1 kg) is below 1%. Similarly the maturation frequency of 1+ charr cultured in Iceland is around 5%.

Before the success of preventing early maturation by selective breeding, a treatment of eggs and alevins was common, resulting in a sterile or all-female off-spring (see pages 34-35).

In charr, the production of milt and eggs (gametogenesis) starts in spring and accelerates in late summer when the day length becomes shorter. The spawning period is usually in late fall. The seasonal timing of the spawning can also be manipulated by changing the length of the photoperiod. By shortening or prolonging the day length it is possible to either advance or postpone the spawning period by 3 and 2 month, respectively. Such manipulation is of advantage if the temperature conditions give a better egg quality before or after the "natural" spawning time. Advancing the ovulation by light manipulation can result in smaller eggs. If the hatchery has a common problem with males and females not synchronized as they are kept separated it is possible to manipulate one of the sexes. Ovulation can also be induced and synchronised by hormonal treatments (see page 28). Photomanipulation is used in the Norwegian charr farms to reduce sexual mature fish by a prolonged day length as Norwegian legislations don´t permit farmers to use strains selected for early sexual maturation.

Development of gonads (milt and eggs) and maturation of charr is possible within a large water temperature range (4-16 °C). However, high summer and fall water temperatures can reduce egg viability and should therefore be avoided. The most sensitive stages of egg development in the female body during the summer are not investigated yet. Knowledge from other salmonid fishes suggests that late July and August, late in the ovulation process, is the most critical period for the negative effect of high water temperature on egg quality (see page 29-30). Therefore, temperature conditions are crucial for egg quality during egg and sperm development in the brood stock as well as during spawning and egg treatment in hatcheries (see below).

The effect of different broodstock rearing temperatures on egg survival has been compared in Sweden. A brood stock from the same genetic families of the Swedish breeding stock "Arctic superior" was raised at two different sites. At one site, the water temperature never exceeded 15 ˚C while at the other the temperature was close to 20˚C in late summer. The mean survival of the offspring were 70 % when the brood stock were kept at 15°C compared to only 30 % survival at the warmer site (20°C). This illustrates how important optimum temperature conditions for broodfish are and its effect on egg quality and survival.

Arctic charr spawners are sensitive to diseases (such as furunculosis and bacterial kidney disease, BKD), especially at water temperatures above 10°C. Spawners can transmit BKD to their offsprings and it is therefore very important to ensure that the spawners are healthy. Samples are commonly taken from the kidney and the ovarian fluid to check if the broodfish is infected. BKD is a serious threat in A. charr culture and usages of wilde broodfish is risky since BKD infections are frequent in wild stocks.

Maturing Arctic charrs, particularly the males, are sensitive to fungal infections (Saprolegniosis). This fungus exists in almost all waterbodies. Fungal infections spread very rapidly at temperature around 8°C or above but its development is reduced at lower temperatures. Several antiseptic products (e.g. hydrogen peroxide, salt and formalin) are used for prevention at present. Hydrogen peroxide was found to be less effective than formalin which is the most commonly used antiseptic today. The recommended dosage is 100 ppm for formalin and 500 ml /m3for hydrogen peroxide.

Sometimes the broodstock are sorted into males and females to before the spawning season when the sexes can easily be separated visually. In other cases, the broodstock are not sorted by sex because separation of gender beyond the spawning period might be unfortunate since the maturation process is presumed to be synchronized between fishes by pheromones. Commonly the males are mature prior to the females and do keep their fertility over several weeks during the breeding perio

Figure 2. Sexually mature female (left) and Male (right) Arctic charr.

At the expected spawning period, the brood stock has to be checked at least once a week if kept at 5 °C, but more frequently at higher temperatures for the most suitable time for stripping males (Fig. 3). Ideally, the spawning is synchronized between and within sexes and the handling of the fish can be minimized. As the length of the spawning period of a group of broodfish is dependent on natural cues such as a combination of water temperature and day length (see page 26) the degree of synchronization can vary between years. When females ovulate, the eggs are released into the body cavity (prior to this, the eggs are encapsulated in the gonads). The females are now called ripe. The females will generally not voluntary release the mature eggs but have to be stripped manually. It takes experience and practice

to distinguish between fully ripe female and unripe ones. Stripping of female at sub-optimal times (i.e. when fish is overripe or underripe) will inevitably lead to low fertilization success.

2. Stripping fish and fertilising eggs

One time consuming duties in an Arctic charr hatchery during the spawning season is the checking of ripeness of the brood stock and the subsequent sorting of mature fish. It is important to recognize that this procedure is stressful not only for the staff but also for the fish. Stress can significantly affect the egg and milt quality and thus, subsequently the fertilization rates. Hence, minimising the stress of the brood stock while checking for ripeness should be of high priority in a charr farm.

The timing between female ovulation and stripping is crucial for the fertilisation success and is temperature dependent. About 4 days after the females ovulated, they can be stripped for eggs and the milt can be collected from the males. The subsequent fertilisation involves mixing collected eggs and milt from the fish. If necessary, both collected eggs and milt can be stored for at least 3 days without mixing, providing the gametes are not exposed to water and properly stored at low temperature. The density of sperm cells as well as the viability and mobility of the sperms can be checked in a microscope by mixing a drop of sperms with a drop of water and count the number of swimming sperm cells and estimate the swimming speed.

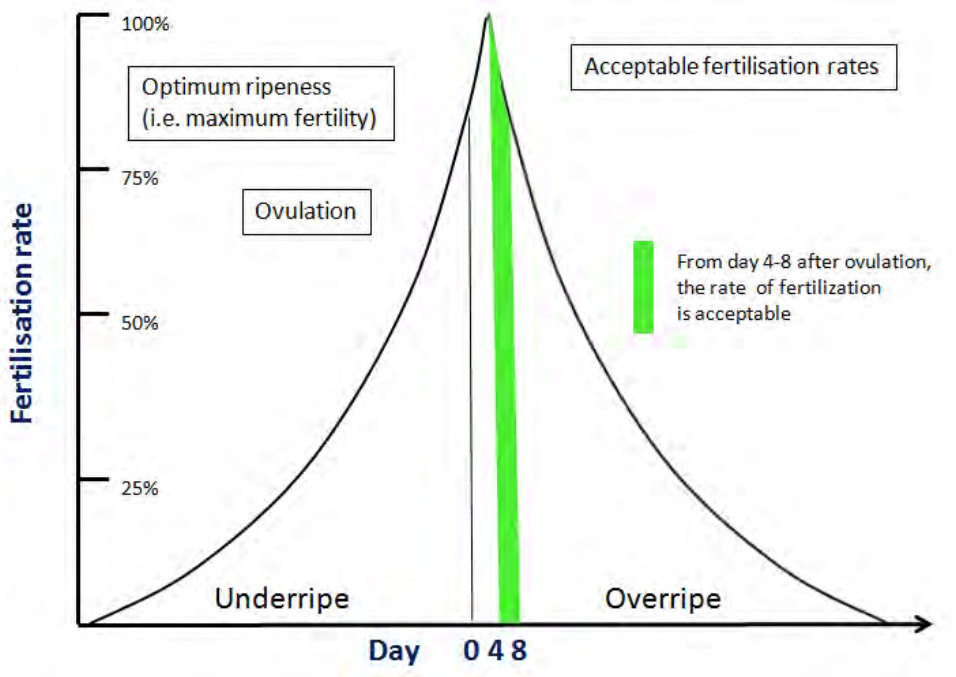

Figure 3. A schematic diagram of the time effect on female ripeness and subsequent egg fertilisation rate.

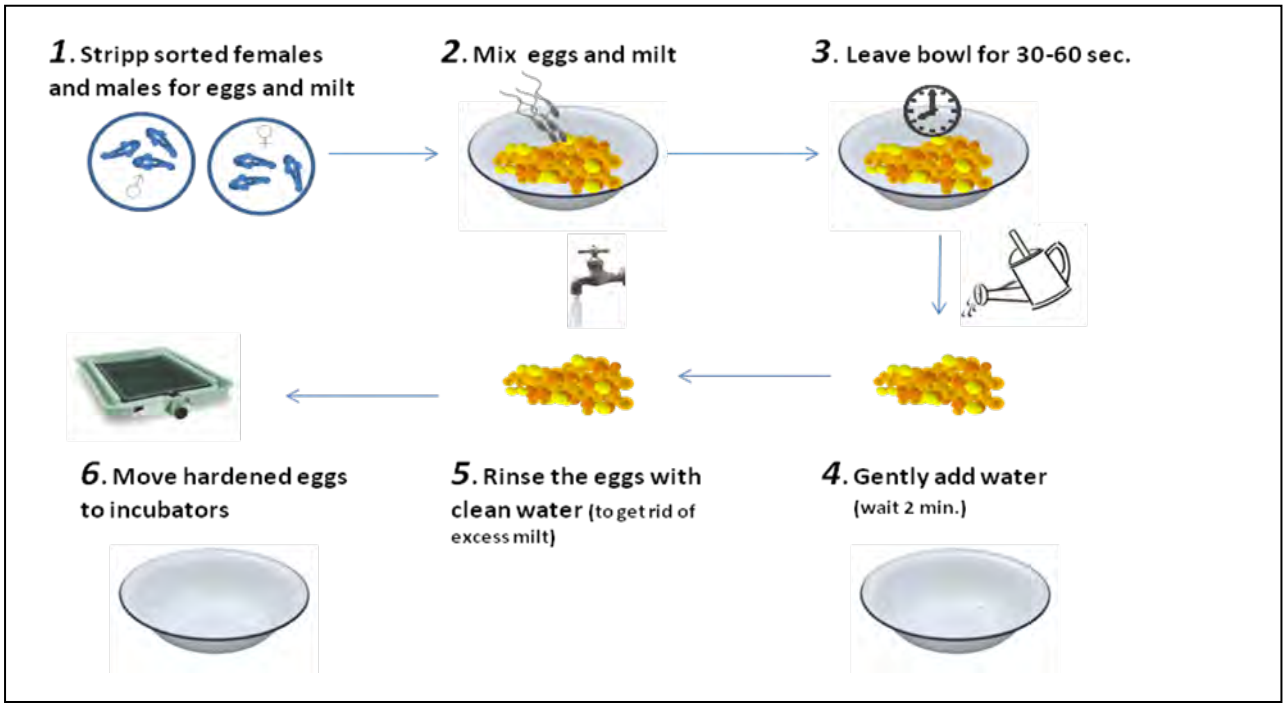

Figure 4. A schematic drawing on the fertilizing procedure of salmonid eggs.

Below is a schematic schedule on how to fertilize charr eggs:

1. Anaesthetize the fish. Benzocaine (50ppm for 3-4 minutes) or MS-222 can be used. Find a comfortable (sitting) position and grab the caudal peduncle with one hand and hold the fish under the belly with the other. Make sure not to compress the operculum. Use a soft cloth and dry off surface water from the fish and try not to damage the mucus layer. Bend the relaxed fish gently and slightly backwards and the egg will be released without touching the belly. Finish by gently striking the fish from behind the pectoral fins backwards to the vent (anus). This needs to be repeated several times. Do not use to much force, as this will damage the internal organs. Males are stripped according to the same procedure as for females, although the striking motion should start further back. Males are more susceptible of contracting fungal infections than females, so be cautious not to damage their mucus layer.

2. Collect the eggs in a clean and dry bucket and in Swedish hatcheries (not in Iceland) disinfect these with a 1% solution of Natrium hydrogen carbonate. Mix the milt gently with the eggs. Make sure the milt is evenly distributed in the egg batch.

3. Wait 30-60 seconds. The ovarian fluids will reduce the concentration of the inhibiting potassium in the milt so the sperm cells become active and fertilisation begins.

Figure 5. Comfortable position of stripping a female fish.

4. Add water to the egg batch, mix it gently and leave for another 2 minutes. Use water with the same temperature as the holding water of the brood stock. The water will dilute potassium even more, and sperm cell activity gets a second burst. This improves the fertilisation rate.

5. Rinse the eggs with clean water to remove excess milt, egg shells and dirt.

6. Leave the eggs to harden their shell for 2-4 hours. They can also be moved directly to the incubators for hardening. The entering of a sperm cell into the egg activates a series of actions (cortical reaction). The eggs absorb water and increases in size (approx. 30%).

3. Egg incubation

The period when the eggs have been placed in the incubators (egg trays) until the eyed stage, the eggs are sensitive and should be handled as little as possible. Successful egg incubation depends on:

- Temperature
- High freshwater quality
- Protecting the eggs from light
- Minimal handling of the eggs
- Controlling pathogenic and fungal infections
- Removing eggs of low quality

The water hardening process starts immediately when water is mixed with the eggs. This happen independent of fertilization and actually the hardening process will prevent fertilization since the micophylum will close. Therefore the milt is mixed with the eggs before adding water. The water hardening takes 1-3 hours. Mechanical disturbances during this period is unfortunate and may effect survival. The eggs should therefore be transferred to the incubators either before or after the water hardening process. During this time the eggs must be kept cold. After the water hardening is complete, the eggs are hardy for a period of approximately 48 hours. During this period, the eggs can be handled and transferred to other farms. It is important to handle the eggs with great care during transfer. After the water hardening the eggs are disinfected, commonly with a buffiodine solution (10ml/liter for 10 minutes) to remove bacteria from the surface of the eggs.

During embryonic development, the egg needs shelter from light and clean water. Many different types of egg incubators are available (Fig. 6). When possible, the eggs should be incubated in one layer only. This facilitates dead egg removal and improves general inspections of the eggs.

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, goldfish, cod and salmon. Arctic charr are probably not an exception and malformation has been documented for this species. Malformations appear at temperatures both above and below optimum depending on species.

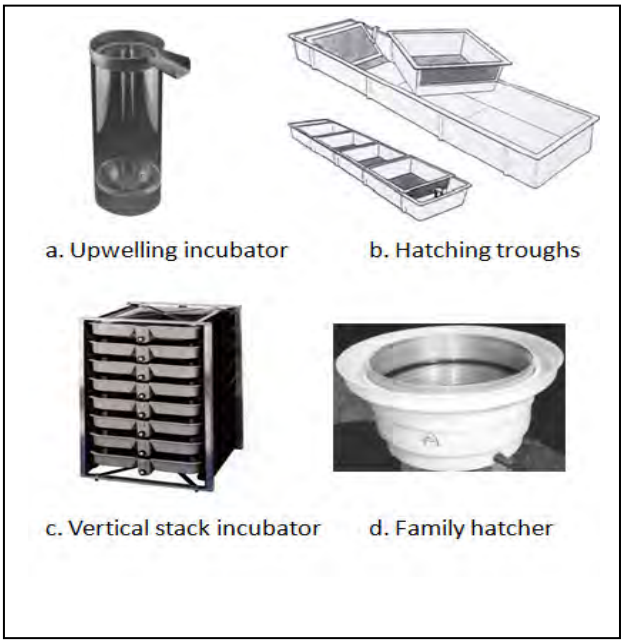

Fig. 6. Different types of egg incubators.

The basic needs of the eggs during incubation are oxygen and ability to get rid of metabolites by flow through water. It is therefore useful to know the amount of eggs in each incubator. For this, there are racks that count a high number of eggs at a time (i.e. 200 or 500). Alternatively, the number of eggs that fits a specific length side by side (Fig. 7) together with total egg volume can then be used for back calculation of number of eggs on a number to volume ratio as well as egg size. The oxygen consumption of charr eggs is low in general, but dependent on temperature. The water flow should not disturb the eggs, but flow gently past them. The oxygen concentration of the in-flowing water should be kept close to 100% and should not drop below 95% in the outlet water.

Fig 7. The number of eggs that fits into a specific length and the volume of the total eggs batch are used to calculate the total number of eggs.

Direct sunlight (UV-radiation) is detrimental to charr eggs as is strong indoor illumination. Therefore, the eggs should be covered and kept in darkness throughout the whole embryonic development. When working with the eggs, a soft red light is preferable.

Temperature affects the embryonic development time (Fig. 8). It is common to express temperature dependent development in terms of accumulated temperature units (ATU) or degree days (d°C). ATU (d°C) is calculated by multiply the number of days with the average temperature during the time period. The egg development rates at different incubation temperatures are somewhat different then when temperature is expressed as ATU (see In depth section). Arctic charr eggs are sensetive to high temperatures. In general, temperature should be between 2 and 8 °C. The eggs are extra sensitive during the first period of development. After organogenesis (approx. 100 ATU), the temperature may be increased to 8 °C, but at no time exceed 10 °C.

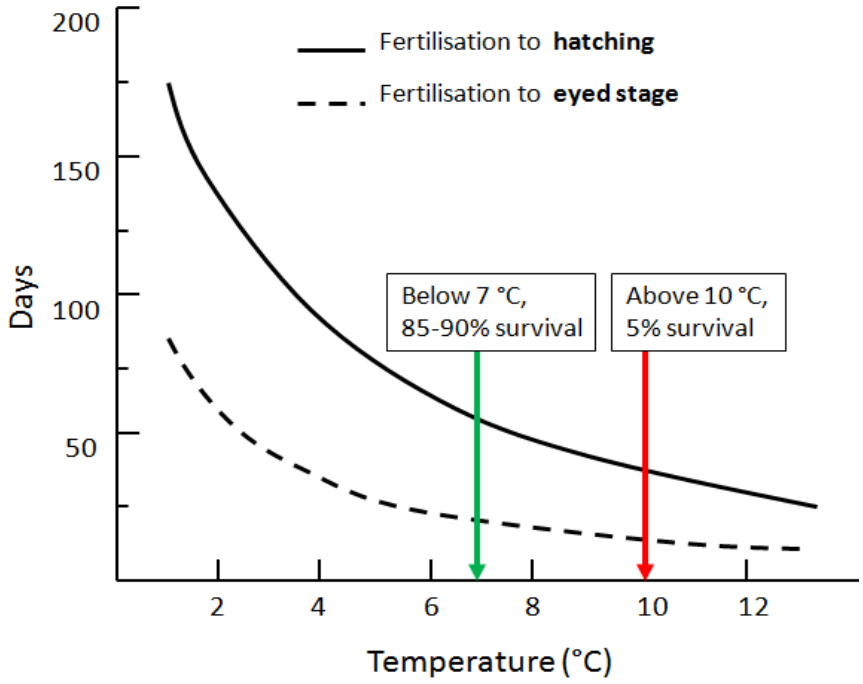

Figure 8. Effect of incubation temperature on the rate of Arctic charr egg development from spawning until the eyed stage and hatching. Note that survival rates have been found to be 85 and 90 % at temperatures below 7 °C, but only 5 % or less at temperatures above 10°C.

Dead eggs should be removed as soon as possible from the incubators to avoid problems with bacterial and fungal infections. During the period from fertilization to the eyed stage (Fig. 9), eggs are vulnerable to light and handling. Removal of dead eggs during this period is unadvised or should be done very carefully. After the organogenesis has taken place and the eyes are visible (hence the term “eyed eggs”), eggs are relatively hardy and can be handled more easily without risking increased mortality. From the eyed stage and onwards, unfertilized eggs should be removed and the incubators rinsed thoroughly. The eggs can even be moved between incubators during cleaning.

Figure 9. Eyed eggs

There are several ways of removing the dead eggs. One could use forceps and pick one egg at a time. This is effective if there are few dead eggs. A siphon is more effective when there are many dead eggs. To ease identification of dead and unfertilized eggs, the eggs can be “shocked”. From a height of 50 - 100 cm, the eggs and water are poured from one container to another. Unfertilized eggs or eggs containing dead embryos will break and turn white as the yolk coagulates. The dead and viable eggs can then be separated. This can be done automatically (fig 10). In some cases fungal infections have already established and then it is better to remove the whole lump of infected eggs, for instance with a spoon. If the infection is severe, treatment might be necessary. All equipment used must be changed or disinfected between incubators to avoid potential infections to spread. After dead eggs have been removed it is advised that all equipment used, together with clothing and working areas are cleaned and disinfected. To keep track of the number of remaining eggs and to evaluate the quality and health status of each egg batch, number of dead eggs should be carefully recorded in each incubator tray.

Figure 10. From above: automatic sorting and counting dead (chocked) eggs to the right and viable eggs to the left.

4. Juvenile rearing

When hatching, the muscular activity of fully developed embryo (referred to as an alevin) increases, causing the eggshell to break open. The alevin will immediately move downwards and seek for shelter. For some weeks (ca 250-300 d°C), the alevin uses only energy from the yolk sac. To ensure that as much of the energy as possible is utilized for growth and development, alevins should be provided with some sort of shelter and support (e.g. biomaths). This minimizes the amount of energy spent on movement and holding an "upright" position. The alevins stay in this shelter until 70-80 % of the yolk is utilized. Then they start moving toward the surface (the swim-up stage) to snap a small amount of air for

their swimbladder which helps the alevins to keep their buoyancy in the water. They are now ready to take external feed and are now called fry. The first feeding period is often referred to as the “on-growing” stage. The swim-up is not synchronized in the group but the fry progressively start to swim in the waterbody and spread evenly in the tank. Normally, alevins/fry are moved to juvenile rearing tanks prior to start feeding and it is vice to put the biomaths as a shelter in the startfeeding tank until the swim up start. It is important to adjust the water current preferable to the svimming speed and spreading of the fry as well as to ensure that the self-cleaning of the tank works. To high water velocity and water inflow might draw the alevins/fry into the water outlet grid.

Figure11. Yolksac alevins

The farmer should keep record of data on biological and environmental factors, such as fish weight, water temperature, oxygen etc. which is of crucial importance. The resulting database forms a unique farm specific knowledge, necessary for good production planning, quality control and farming practice.

Water quality

Good water quality is of high importance for the success of a fish farm. Not only the quality of the incoming water is central, but also practices within the farm, such as feeding routines, removal of dead fish, cleaning of tanks etc., which might effect the water quality. There are several important water quality parameters to keep track on which are described in various handbooks on fish farming listed in the back of this report.

Oxygen (O2)

Fish consume oxygen from the water. The total oxygen demand is related to the metabolic rate of the fish. The metabolic rate is affected by the water temperature but is also size

related. The metabolic rate decreases with increasing fish size and increases with higher water temperature. Hence, 1 Kg of 10 gram fish needs more oxygen than 1 Kg of 100 gram fish. The oxygen consumption is also affected by factors such as svimming activity, stress and feeding. Mean oxygen consumption of 100 g Arctic charr during a 24 hour period is about 100-200 mg per kilo fish per hour (kg^{-1} h^{-1}). During periods of fast growth, the oxygen demand is typically above 150 mg kg^{-1} h^{-1} .Following feeding, the demand may increase 40-50 % above the average consumption. Oxygen concentration below 80 % saturation in the outlet water will affect the growth rate, although the lethal limit is much lower. The oxygen concentration of 100 % saturated freshwater is affected by the temperature (and salinity) of the water. (Fig. 12). For example, at 5 °C and 100 % saturation the oxygen concentration is 13 mg/L. Consequently, 6.5 mg/l at 5 °C equal 50 % saturation, which is below optimum and might affect feeding and growth, specially for alevins and fry. Therefore it is recommend at all times to maintain the oxygen saturation above 80 % in the culture unit. Aeration of the inlet water is recommended, not only to saturate the water with oxygen but also to prevent supersaturation of nitrogen in the on-growing tank. If atmospheric air solve under pressure in the water, for example if air is sucked in to a pipeline, the water can be nitrogen supersaturated. Supersaturation will cause air bubble disease in the fish, leading to “pop”-eyes, gill damages and high mortality.

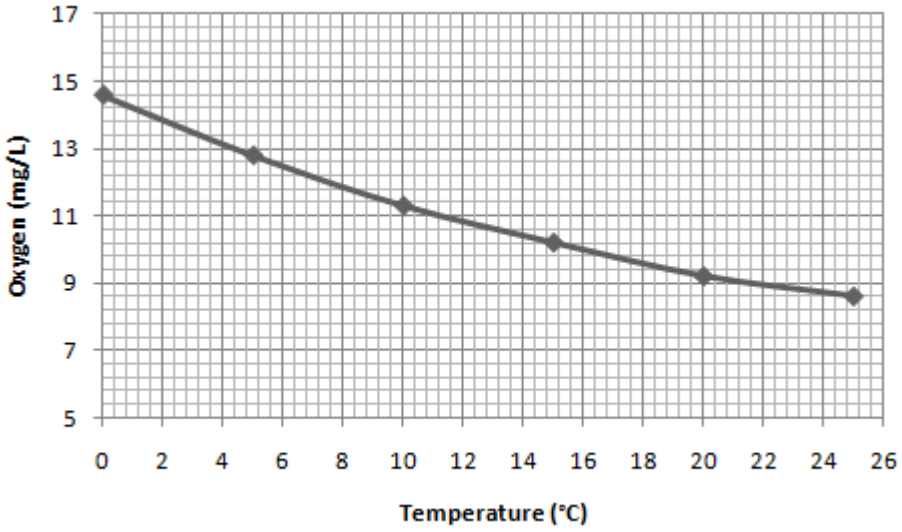

Figure 12. Relation of oxygen concentration (mg/L) and temperature in freshwater at normal atmospheric pressure (1 bar) and 100 % saturation.

Carbon dioxide (CO_2)

During respiration, fish excrete carbon dioxide (CO_2). Increased CO_2 concentrations results in higher concentration of hydrogen protons (H^+) in the water, and thereby the pH value of the water is reduced. This affects fish adversely and must be avoided. Recommended levels

under farming conditions are below 10 mg CO_2 L^{-1} at an alkalinity lower than 100 mg L^{-1} and less than 15 mg L^{-1} at higher alkalinities. If the CO_2 concentration is high the water can be degassed by aeration. There are several technical solutions for this, all with the purpose of increasing the water-air surface in order to strip the CO_2 into the air.

pH

pH is a measure of free hydrogen protons (H^+) in a solution, given on a logarithmic scale from 1 to 14. A pH value of 7 is neutral and water with pH below 7 (high in H+) is acidic, whereas water above 7 is termed alkaline. The pH balance is essential for fish metabolism and affects for instance oxygen uptake, salt-water balance and acid-base regulation. Although salmonids can tolerate pH values within the range of 5 to 9, optimal growth conditions area pH values between 6.5 and 8.5.

Ammonia (NH_3)

Ammonia (NH_3) is an excretory product of fish which can be toxic to charr. The toxicity strongly depends on the pH level of the water, water temperature, CO_2 concentration and salinity. The concentration of ammonia should be kept below 0.01 mg L^{-1} during the first feeding and juvenile stages.

Water temperature

In fish, preference and tolerance for temperature changes with life stage. Maturing females probably having the narrowest tolerance limits. With increasing temperatures, growth increases up to an optimum temperature for maximum growth. At higher temperatures, growth rapidly decreases. The temperature that results in highest growth rate does not only change with age and size in charr, but there also seems to be differences between populations. Most studies report on maximum growth rate of Arctic charr at temperatures between 12 to 16 °C. It is important to note that these temperatures are only valid when growth is not limited by feed supply. When feed is restricted, the optimum temperature for growth is lower. Optimal feed utilisation (Feed Conversion Ratio, FCR) is obtained at a lower temperature than optimum temperature for growth and is reported to be at approximately 9° C. High-density intensive farming imposes practical limits on rearing temperatures. Fish oxygen demands and ammonia excretion increases dramatically with temperature and fungus and bacterial diseases often become a problem at temperatures above 15° C. Hence, 12 °C seems to be a reasonable compromise, allowing high growth rate, good feed utilization and reduced risk of diseases and fungus infections.

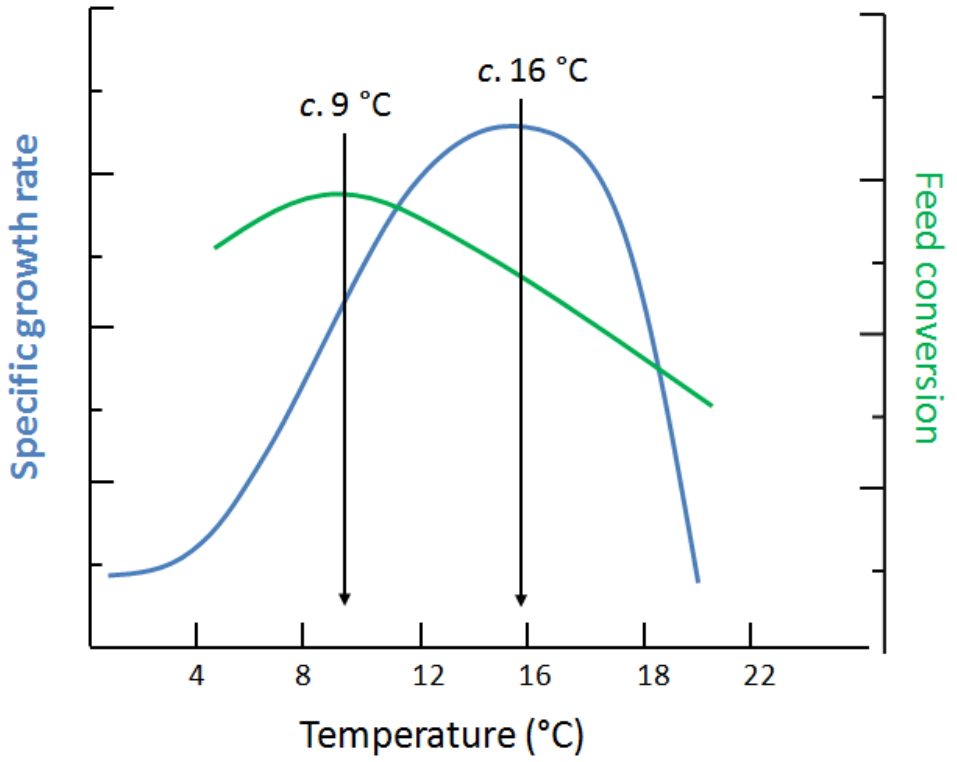

Figure 13. Generalised pattern of Arctic charr growth rate and feed conversion as an effect of temperature. Note that these relationships are under assumption of feed in excess.

Salinity

The highly variable life strategies of Arctic charr affect the salinity tolerance in this species. Anadromous charr tolerate full strength seawater (33-35 ppt.) during the about 2 month long seaward migration in summer. During the rest of the year the tolerance for sea water decreases but charr seem to cope well with brackish water up to 20 ppt, but this is very dependent on fish size and water temperature, with increasing problems at low temperatures. There is a small farming industry of cultured strain of anadromous Arctic charr (Hammerfest strain) in Northen Norway. In Iceland, Arctic charr is grown from ca 100 g to harvesting size in 26-30 ppt saline water at constant 6-8°C, without any osmotic problems recognized.

Start feeding

Start feeding of salmonids usually begins at the first sign of "swimming-up" behaviour, when the alevins/fry leave the bottom and swim up to the surface. This happens either in the hatchery trays or the first feeding tanks. Small particle dry feed is delivered frequently usually by automatic feeders. The start-feeding phase is very delicate and it is of most importance that the timing, feeding schedule as well as feed formulation and size is optimized. The procedure of start-feeding Arctic charr varies between farms and countries. At least in some farms in Sweden it is common to transfer the charr alevins to tanks and

start the feeding when about 50-35 % of the yolk-sac remains. That is before the alevins show any sign of start-feeding behaviour but this early start feeding improves the survival rate. A common problem in the beginning of the charr farming was a high proportion of alevins/fry that did not start to eat but quickly lost weight and died. Once the alevins/fry start losing weight they seem unable to recover in spite of access to food. At that time, the start feeding started at the first sign of swim up behaviour as for other salmonids. The difference may be the that the A.charr incubates mainly on the bottom of a lake whereas most other salmonids use the stream bed as spawning grounds resulting in a more defined swim-ups and start-feeding phase.

When alevins/fry are moved to the start-feeding tank, it is vice to add the astroturf or other type of artificial hatching substrate in the tank to create shelter for the alevins/fry.

Feed quality

The ingredients in fish feed for intensive farming are treated by high temperature and pressure (extruded) and the fat is added by vacuum coating. In the modern start feed diets the ingredients are treated milder. A new extruding process is made at a lower temperature and pressure where the fat are added already at the beginning and not coated after the pellets have been extruded. This new processes (SAS) reduces the risk that the fat separates from the pellets and creates an oily film on the water and are so far mainly used for start feed. Feeds used for juvenile charr are generally the same as salmon and trout feed, and in many cases identical. The requirement for macronutrients is similar as for other salmonids but in Sweden a diet with a lower fat content than used for salmon and trout is used for on-growing charr. The reason is that charr is farmed in extremely low temperatures (<2°C) for more than six months and high fat contents are suspected to result in a very fatty liver and anemia (Swedish fish health). However, nutritional requirement changes as the fish grow, with a decreased protein demand as fish weight increases. By including more fat in diets to larger fish, proteins can be saved which is a common practice for salmon and trout. There is an increasing proportion of plant material used in the diet for Arctic charr (Skretting) and other salmonids after start feeding (Table 1). Fishmeal and fish oil has been the major protein and fat sources in salmonid diets but increased demand has raised the price dramatically last years. Therefore, and also toward more sustainable and economically feasible aquaculture, the industry has looked for other sources for substitution. Various plant materials, mainly protein rich oil-seed cakes, have been tested in A. charr diet, with varying results. With a careful feed formulation A. charr can be grown on feed where the major protein part is of plant origin. Substitution of fish oil with different plant oils normally does not have any effect on the fish growth but it can affect the fatty acid composition of the fish flesh by increase the n-6/n-3 fatty acid ratio. That must be taken into consideration when substituting fish oil with plant oils in the diet. Pre-harvest feeding with fish oil diet can also recover the “normal” fatty acid ratio in the product.

Feeding practice

The gastrointestinal system in charr is short, with limited storage capacity in the stomach. It is therefore recommended to feed the fish on a daily basis. The feeding interval and feeding intensity is related to fish size and water temperature (metabolic rate). Smaller fishes need to be fed more frequently and more intensively when temperature rise. Although charr is able to feed from the bottom of the tank and in darkness it prefer to take feed from the water body during the daylight. Highest growth rates are obtained under long day length, but this is probably more closely connected to season. It is natural for an arctic species to grow intensively during the summer time.

Table 1. Ingredients in diets for Arctic char 2007 and 2008

Ingredient	**2007**	**2008**
Fishmeal	42.3 %	43.4 %
Fish oil	15.9 %	15.5 %
Soya protein concentrate	12.2 %	12.5 %
Rapeseed oil	1.8 %	1.8 %
Fish protein concentrate	6.7 %	5.2 %
Wheat	11.2 %	11.6 %
Wheat gluten	2.9 %	3.5 %
Corn gluten	0.5 %	
Sunflower meal	3.8 %	6.4 %
Soy meal	2.6 %	0.1 %
Total	100 %	100 %
% marine ingredients	65 %	64%
% plant ingredients	35 %	36%

In intensive fish farming, automatic feeding systems are used to spread the feed over the surface of the tank or a net pen during the day, often at short intervals. The feed intake commonly peaks at dawn or dusk when the activity of the fish is highest. Spreading the feed properly is also important to diminish the feeding activity of each fish and improve their access to the feed. There has been a tremendous development of computerised feeding equipment that calculate the daily portions based on number and size of fish, temperature day-length and other parameters. Appetite based systems that recognize pellet waste by IR sensors or video image analysis are also available on the market. Compared to salmon, charr have a smaller mouth and it is advised to feed charr with one smaller pellet size than what is used for salmon. As the charr grow, pellets size should be increased. For start-feeding, experience in Norway has shown best results with a granulated start-feed as small as 0.4 mm. In Iceland it is common practice to mince small particles to almost a dust for the initial one or two weeks feeding, for improve the accessibility for the small fry. During the on-growing stage, a pellet size (diameter) of about 2-2,5% of the fish's fork length results in the shortest consumption time and the lowest number of missed pellets. As fish grows it is advised to mix the small and large pellets for some time to ensure that all sizes of the fish group can find the “right” size of pellets.

Competition for feed is often seen in groups of Arctic charr. The most aggressive and competitive fish (social dominants) gains access to feed at the expense of others (social subordinates). By forming such hierarchies, social dominants grow at a higher rate, eventually leading to large size variability in the group. Subsequently, the size differences escalate as large fish seems more likely to gain access to feed. Under extreme conditions and even if the feed resource is not limited, dominant fish controls the feed to such an extent that subordinate fish cease feeding. By presenting the feed over the whole surface reduces the opportunity of dominants to monopolise the feed and hierarchies decreases. Aggression and stressful conditions reduces the growth potential in the group, depressing the immune system and compromises fish welfare. For inexperienced staff it is important to know to what extent fish are forming hierarchies in a tank. Regular measurements (or video observations) of the size distribution should be conducted and the fish might be size graded to ensure more even sized groups. Variable fish sizes and injuries on the fins in a group of fish are an indication of social hierarchies and counteracting measures should be applied. Later when the fish are held in full-scale farming conditions with thousands of fish held at high densities in net-pens or fish tanks, the social hierarchies are less pronounced. Instead the fish are swimming around in schools.

Water depth

Low water depth may cause swim bladder stress syndrome. In juvenile charr, critical water depth is 15 cm, but already at a 24 cm water depth increased mortality has been observed. Thus, recommended minimum water depth is >30 cm.

Rearing density

Arctic charr tolerate high densities without negative effects on feed intake or growth. In fact, low density should be avoided as it increases social interactions. Sixty kilo fish per m^3 of water is by some researchers considered the minimum density under growing conditions in smaller systems. The upper limit is uncertain, but at least 120 kg per m^{-3} is possible without affecting the production success, as long as the water quality and growth conditions are optimal. In commercial scale fish tanks (>10 m^3), densities may be reduced to approximately 20-30 kg fish per m^3 without inducing more aggression and hierarchy formation. Lower densities might be favourable when rearing small fish at high temperatures and limited oxygen concentrations during the summer.

Photoperiod

One of the most rigid environmental cues affecting Arctic charr is the photoperiod (day length). Salmonids (including charr) use changes in day length to time important seasonal events, such as parr-smolt transformation and sexual maturation. Such changes are complex events, involving morphological, behavioural and physiological alteration and their exact timing are of crucial importance. This can be utilised in fish farming since the time of smoltification or spawning can be shifted in a controlled manner by manipulating changes in day length. Salmonids are able to conceive light at very low intensities (below 1 lux). Highest growth rate of Arctic charr has been observed at 50 lux which is the light intensity recommended for charr rearing. Light of 50 lux is similar to the light in the average living room. Full day light (not direct sunlight) is 10.000 – 25.000 lux and 0.27 lux corresponds to a full moon on a clear night. For the fish's perception of day and night, the difference between dark and light is more important than the absolute light intensity. Hence it is important to keep the night as dark as possible. The photoperiod manipulation can be an important tool in enhance the growth of charr and prevent or delay early maturation.

5. Selective breeding programs

No farmer would consider raising wild boars and jungle fowls for producing ham and eggs. Instead, they grow the domesticated pigs and hens which have been bred for centuries to increase the production and level of domestication. As for farmed animals like chicken, pigs and cows, selective breeding programs of farmed fish for human consumption are a necessity in order to increase production (Fig. 14).

In Norway, the first major national breeding program on salmonid fish started in the late 1970: th to support the increasing aquaculture industry of salmon and rainbow trout. The first selective breeding program on Arctic charr was initiated in 1982 in Sweden and followed the steps outlined by the national Norwegian selection program on Atlantic salmon. In both

programs, the traits most important for the economy and quality of the farming industry are:

- Growth rate
- Feed conversion ratio (FCR)
- Survival rate
- Size and age at sexual maturation
- Meat quality

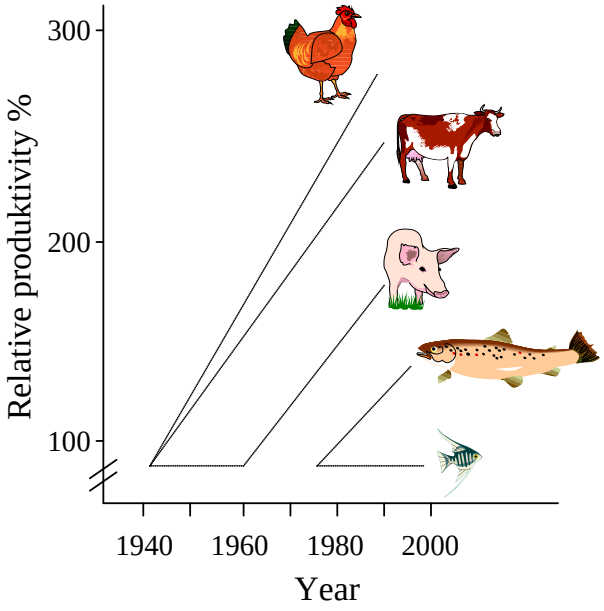

Fig. 14. Example of increased productivity by breeding of domesticated animals, including salmon. Tropic aquaria fish are used as a comparison (modified after Gjedrem 1995).

In the Swedish national Arctic charr program, Hornavan charr was found to be the strain with the best performance for a successful selection program. In 1984, ninety-six full-sib families were formed by stripping and mating randomly chosen males and females. Genetic parameters were estimated and the heritability was significant for growth characters, age at first sexual maturation and resistance to fungal infection. Each selected generation started by selecting the best individuals and crossing these with non-siblings. Since the start of the program, the 7th generation of the national breeding program has been stripped and new families incubated. The program has been a success and for each generation, the growth rate has increased by 10 %. Today most Arctic charr farms in Sweden are using off-spring from this selective breeding program. Their fish are sold under the trade name "Arctic superior®". A comparison of the farming performance of the "original" Hornavan charr and the selected Hornavan strain (Arctic superior®) is summarized in table 2 and Figure 15.

Table 2. Comparison of important farming traits in the original Hornavan charr and the selectively bred Arctic superior®.

Trait	Arctic charr farming in 1985	Arctic charr farming in 2009
Production cycle	3-4 years	1.5-2 years
Growth rate	Slow	3 times as fast
FCR*	Poor	Efficient
Maturation	70-100% before 500 g	< 1 % before 800 g
Survival	Low	High
Meat quality	Variable	Improved
Production cost	4.3-5.6 € kg^{-1}	3.3 € kg^{-1}

*Feed Conversion Ratio

In Iceland. breeding programme started in 1992 after a comparative study of growth and early maturation on several wild A. charr stocks under culture conditions. The improvement of growth rate has been calculated 3-4% per year (ca 10% per generation) and early maturation incidence prior harvesting size is negliable. Production time from start-feeding to 1500 g size is close to 20 months at 9°C with production cost close to 3€ kg^{-1}.

The concept of using local fish population for Arctic charr farming in Norwegian freshwater aquaculture gives a big variation in production cost of charr in Norway. The production cycle can take from 2 to 3 years and the average production cost is between 5.5-7 € kg^{-1}.

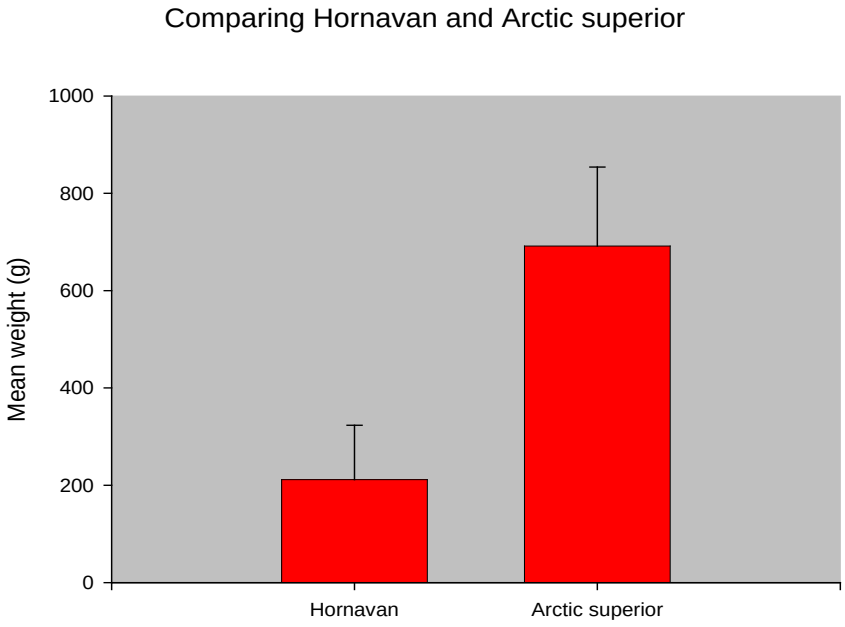

Figure 15. Average mean weight at 1 + of the selected Arctic superior and the original unselected strain farmed under identical conditions and time.

6. Theory and background

Brood stock maturation, egg fertilisation and hatching

Sex determination of brood stock fish at an early age is desirable by fish farmers. As one male can fertilize several females it is an economical advantage to keep fewer males than females in the brood stock, preferably a ratio close to 1:3. It is not possible to separate sexes in Arctic charr by visual cues until they become sexually mature close to spawning. A genetic identification of sex gene markers has so far not been successful and it will probably be expensive if and when a reliable method is developed, at least in the beginning. Instead sex identification by ultrasonic scanning is a promising method. It is more reliable and easy to use ultrasound scanners available on the market which is used for sex determination of fish. However, the technique requires skills and training before it can be used, especially to identify the sex of fish with small undeveloped sex organs.

High fertilization and hatching rate is crucially important for the success of a fish farm. There are several factors affecting these rates and they are known to vary considerably between and within farms. In Arctic charr farming, the fertilization and hatching rate has been reported to be low in many cases. That is although not a common problem in Icelandic charr culture. Today, there is work in progress in order to produce a common protocol and to increase the knowledge among farmers about factors that are central for high fertilization and hatching success.

Gametogenesis in fish

During gametogenesis, eggs and sperm are produced from just a few germ stem cells. This process is controlled by several factors; starting with daylength as the main environmental stimuli in species inhabiting Nordic conditions, followed by temperature, social interactions and nutritional status. After an environmental stimuli, the hypothalamus in the brain synthesis and excretes a releasing hormone (Gonadotropin Releasing Hormone, GnRH) which simulates the pituitary gland to produce gonadotropin hormones (GtH). GtH are the main hormones involved in the gametogenesis. In fish, there are two forms of gonadotropin, GtH I and GtH II. GtH I is involved in gametogenesis and steroidogenesis, whilst GtH II is functional during the last stages of maturation. The GtH hormones are released into the blood of the fish where they both stimulate germ cell development and steroidogenesis (i.e. production of sex steroids such as androgens, estrogens and progestins). The sex steroids then stimulate the gonads to produce eggs or sperm. This cascade of events is known as the “hypothalamus-pituitary-gonadal axis” (Fig. 16). In addition, hypothalamus-pituitary-gonadal axis is controlled by a feedback loop. For example, depending on the physiological and reproductive status of the fish, different estrogens concentrations (produced by the ovary in

the female fish) can have either a positive or negative effect on the hypothalamus-pituitary-gonadal axis and thus, the reproductive process can be advanced or held back. While the eggs are growing, vitellogenesis, yolk is deposited and stored in the eggs. In most fish, vitellogenin (VTG) is produced by the liver. VTG is selectively taken up by the egg and subsequently processed to yolk proteins (lipovitellines and phosphoproteins) in the egg.

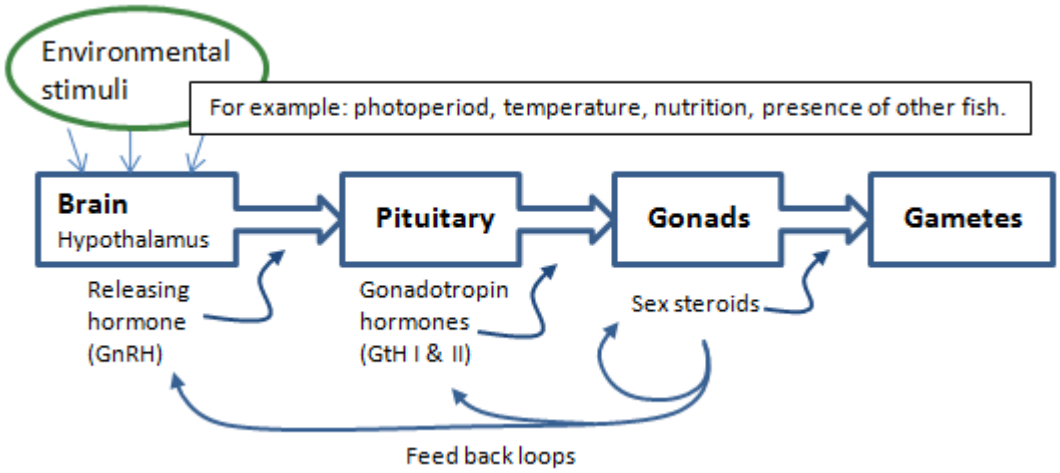

Figure 16. Regulatory mechanisms of reproduction in fish.

Egg and sperm quality is one of the most important components of a fish hatchery but can be highly variable. Good quality eggs are a prerequisite for low mortality at fertilization, eyeing, hatching and first feeding. The quality and quantity of the nutrients the egg is carrying will strongly influence the quality of the offspring. If the quality of eggs and sperm produced in a fish farm are low, this has to be compensated for by maintenance of a large brood stock. However, this will result in poor utilization of the hatchery facilities and will not be cost-effective. It is known that improvements in brood stock nutrition, feeding and husbandry can greatly enhance the quality of both eggs and sperm. Hence, understanding of the factors involved in the gamtogenesis and how good brood stock care will positively affect the hatching success and subsequent juvenile growth is essential for the success of a fish farm.

Daylength

Most salmonids including Arctic charr spawn in the fall or early winter and it is a short day that completes the ovary and testes development. There are several practical examples on how the spawning period can be delayed or postponed by manipulation of the daylength. If the brood stock is exposed to short days from midsummer the spawning is advanced while prolonging long day conditions in fall will postpone the ovulation (females) and spermination (males).

Both treatments have been tested on Arctic charr: Advancing the spawning has been done in Norway by inducing short day in May or late June. The ovulation time was advanced 10 weeks in both treatments and resulted in low egg survival but an increased synchronization of the ovulation period. A problem by advancing the ovulation for Arctic charr is higher water temperatures in the summer and early fall at least in some locations. In the Norwegian example the water temperature was between 6 and 10 °C which may have affected the quality of the eggs (see below).

In Iceland, photoperiod manipulation is used to induce spawning of Arctic charr twice a year. Once at the "normal" time in October by keeping the brood stock in a natural day length. The other by prolonging long light period (14L:10D) when the natural day length decreases in late summer. In December, the light period is then decreased to 6 hours per day and the brood stock will start the spawning period in January. Photoperiod manipulation can also be used to avoid unfavourable temperature conditions by postsponing the spawning a couple of weeks if the temperature conditions are too high in fall. This can be a problem if the hatchery is supplied by water from a huge lake where the water cools down more slowly than in a river during autumn.
Photoperiod manipulation of the brood stock is commonly used in rainbow framing industry to produce eggs at different times of the year Fig 17. The advantage is a more continuous annual production of fish at slaughter size.

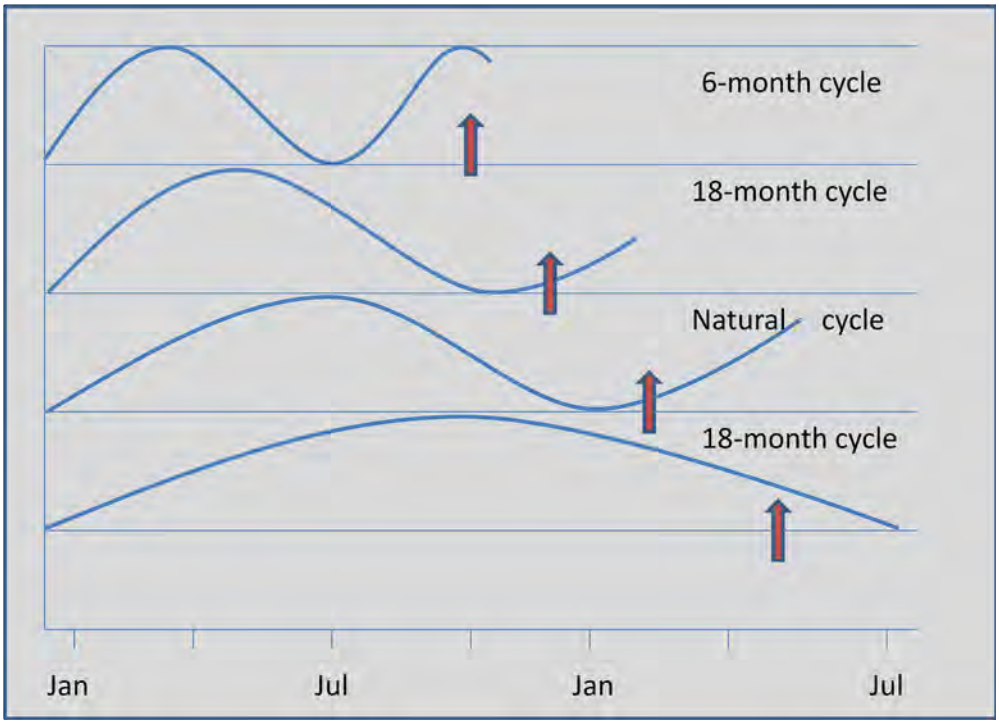

Figure 17. Alteration of spawning time in rainbow trout using day length manipulation. Spawning are indicated by red arrows (from Intensive Fish Farming 1992).

Stripping females and males synchronously, at an optimum time and thus enabling a mixing of high quality eggs with sperm of high mobility, velocity and longevity results in viable offspring. This can be a challenge since all brood fishesdoes not ripe simultaneously. The ovulation may carry on for 2 months or longer which means that the brood stock has to be handled during a long period of time. The males are often more synchronised and if they don´t mature later than the females and there sperm is viable, they are easier to handle. It is a recommendation to strip the males separately as the milt can be stored for weeks and the quality can be checked (see below).

Hormone-induced spawning

As described earlier, the gonadotropic hormones stimulate the gonads to release gonad hormones (steroids and prostaglandins), which alone or in concert with the gonadotropic hormones control gonad development and growth (Fig. 16). Thus, it is possible to induce and synchronize ovulation and spermiation in Arctic charr using hormone injections, such as gonadotropin releasing hormone analogues (GnRHa) alone or in combination with an antidopamine product: pimozide metoclopramide or domperidone. To prevent contamination and preserve the effectiveness of the hormones they must be mixed and stored properly. For each fish to be injected, the proper dosage must be calculated. The hormone is injected into the fish with a sterile syringe and hypodermic needle, either into a muscle (intramuscular) or into the body cavity (intraperitoneal). Usually, intramuscular injections are preferred since they are less harmful to the fish and result in a more constant hormone delivery.

Note that hormone-induced spawning of fish do not stimulate the development of eggs and sperm or improve the quality and viability, it only trigger the release of the gametes. A test trial was done on Arctic charr females at VBCN in Sweden which have had problems with too high water temperatures in the summer and therefore poor egg quality as well as a prolonged period of ovulation among the females. The ovulation was more synchronized among the hormone treated females compared to the control groups but there was no difference in the quality of the eggs. Thus, the fish must be in the advanced stage of sexual development for the induced spawning to work and preferably kept in temperature conditions that doesn´t result in poor egg quality. Today, there are commercial induced spawning kits available but in Arctic charr farming it is not a routine treatment for this species. However, as mentioned earlier, checking of ripeness of the charr brood stock is time consuming and costly. Thus, synchronisation of the ovulation by hormone injections could reduce work and costs.

Effect of temperature on ovulation and spermiation

As Arctic charr generally spawn in late fall to early winter, much of the egg growth (vitellogenesis) takes place during summer. High summer water temperatures are suggested

to negatively affect the vitellogeneisis in charr but this has not been thoroughly investigated. It is, however, known that ovulation in charr is inhibited at temperatures above about 10 °C, and problems with over-ripening escalate at temperatures above 5 °C. Over-ripe eggs are of much lower quality and the hatching success of such eggs is generally low. At 10 °C, males produce a lower quantity of milt and fewer spermatozoa per ml of milt (spermatocrite) than at 5°C. Temperature act on both the hypothalamus-pituitary-gonadal axis and on the gonads to control ovulation. At 10°C, a dopaminergic inhibition of gonadotropin secretion occurs and the ovary secretes a small amount of maturation inducing steroid (MSI). At transfer of the fish from 10 to 5°C, the plasma level of MSI quickly increases and the dopaminergic inhibition is spontaneously suppressed. Therefore, the temperature should preferably be below 7°C some weeks before the onset of ovulation and 5°C or less at stripping. Conclusively, high summer temperature during gonad development most likely induces decreased egg quality in Arctic charr. The mechanisms behind this are not fully known as well as which periods during the gamete growth (vitellogenesis) might be the most sensitive. Studies on Atlantic salmon in Tasmania have shown short periods of high temperatures can be as detrimental to reproductive success as exposure of the brood stock to prolonged periods of warm water (Fig. 18). For this specific stock of salmon, eggs from fish held at a low temperature during the full period of vitellogenesis showed high fertilisation rate and survival to the eyed stage. In contrast, low fertilisation rate and survival to the eyed stage was observed among fish that were held at high temperatures during the egg growing stage but only if the fish where exposed in late February and early March (corresponding to July early August here). Other fish that were exposed to high temperatures during vitellogeneis but to lower temperatures during the critical period showed as high fertilisation rate and survival to eyeing as fish kept at low temperatures during the full study period. The salmon in this study ovulated in late May/early June and hence, the critical period occurred about 3 months prior to ovulation.

There are no corresponding studies on Arctic charr but it is reasonable to assume that a similar critical period is present in charr. Charr generally ovulate in October-November and consequently, the critical period of high temperatures on subsequent fertilisation rate and egg survival to eyeing should then be in July-August when the water temperature is highest. Studies on the effect of short periods of high temperatures during vitellogenensis in Arctic charr, aiming at identifying possible critical periods, are needed. Knowing when brood fish are most sensitive would help farmers to take action during exceptionally warm summer conditions by, if possible, supplying the hatchery with colder water at the most “sensitive” period, rather than throughout the entire period of vitellogenesis.

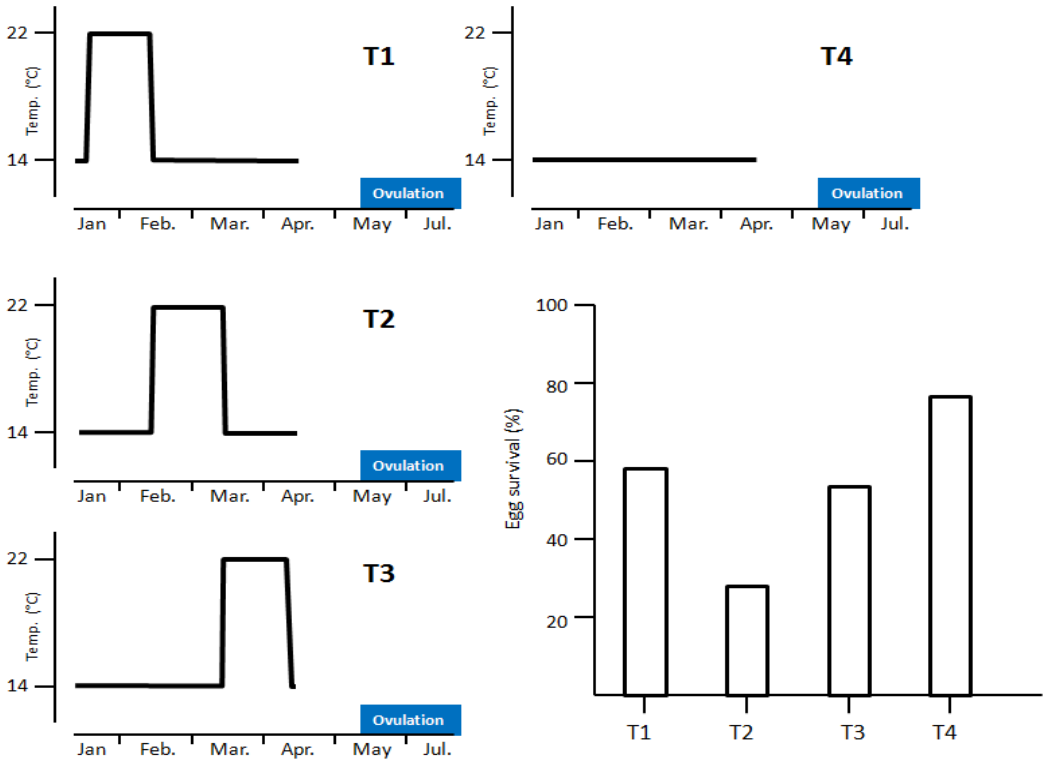

Figure 18. Survival (%) to the eyed staged of Atlantic salmon eggs in Tasmania after four temperature treatments during the period of egg development (vitellogenesis).

As discussed above, charr held at high temperatures may produce over-ripe eggs of poorer quality. In other fish species, temperature is known to influences the composition of egg yolk deposition. Fatty acids (omega-3) are essential for salmonid fish and are, for example, important for the production of the lipoproteins involved in vitellogenesis. In a study of the effect of reproductive development in charr, it was found that the eggs of females held at 16 °C from mid-June until late September contained less fatty acids than eggs from females kept at lower temperatures.

Fertilisation

Before release, the eggs are stored in the ovarian fluid in the body cavity of the female fish. In salmonids, the ovarian fluid is about 10-30% of the total egg volume. The ovarian fluid is known to prolong the period of mobility and the velocity of the sperms. In Arctic charr, sperm velocity has been found to be dependent on individual female ovarian fluid i.e. sperm have higher velocity in some females' ovarian fluids and lower in others. Similar, some charr males have faster sperm than others. Furthermore, it seems to matter which female is fertilized with which male, in that variation in sperm velocity also depends on individual female-male interactions.

At fertilisation, the male and female gametes interact to form a diploid zygote. The unfertilized egg is enclosed by membranes. The thick outer membrane (chorion) has a small

funnel-shaped opening (micropyle) where a sperm can enter to fertilise the egg. Prior to fertilization, the chorion is rather soft. Only one sperm at a time can pass the micropyle (Fig. 19). Once a sperm has entered the egg, chorion and the ooplasm separate and a plug is formed in the micropyle. Further sperm are now prevented to enter the egg and the chorion starts hardening. Hence, sperm with high velocity/mobility will have higher fertilization probability than sperm of low quality and overall, high fertilization rate is known to correspond positively to sperm velocity and motility. In addition, it has been shown that genes of the major histocompatibility complex (MHC) can have effect on Arctic charr fertilization success. Studies suggest that MHC-heterozygous males have significantly higher fertilization success than MHC-homozygous males and it plausible that this difference is due to that the egg can discriminate between MHC-heterozygous and MHC-homozygous MHC sperm.

However, the chorion is still water permeable and water continues to diffuse into the egg, separating the chorion from the ooplasm. The hardening of the egg protects the egg from mechanical damage and microbes. In order for fertilization to occur, small quantities of Ca2+ and Mg2+ ions has to be present.

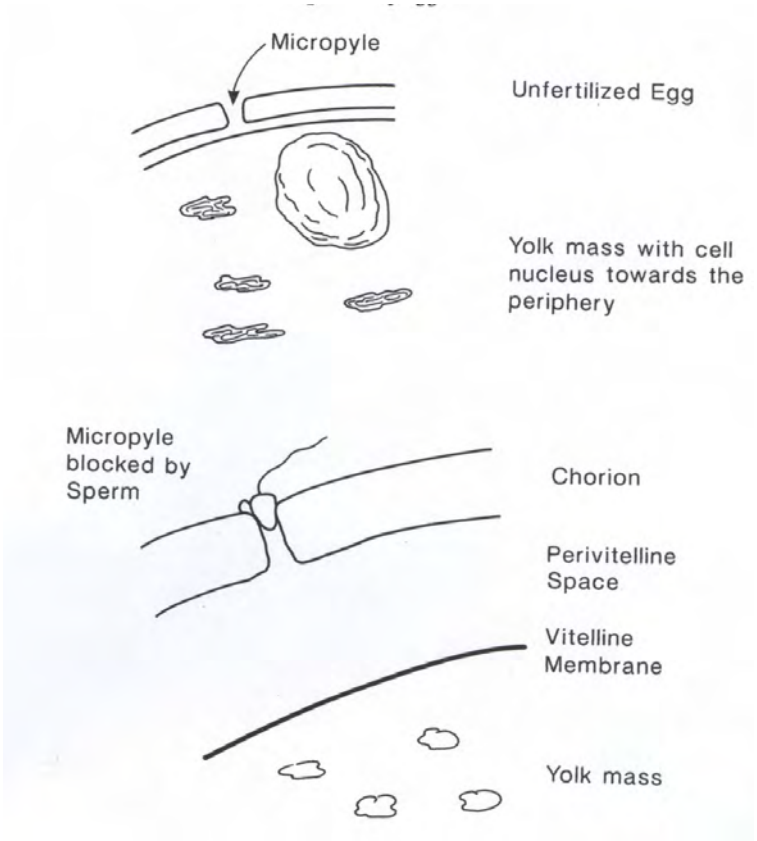

Fig. 19 A schematic drawing of the microphyle in an unfertilized egg (above) and in a fertilized egg (below). From Fish Physiology 11 a (eds WS Hoar & PJ Randall)

Egg development

Egg development constitutes three distinct stages: Cleavage, Epiboly and Organogenesis.

Cleavage

The embryo starts developing after fertilization. First the cytoplasm, a colourless cell fluid, moves over the surface of the yolk. It concentrates at the animal pole where it rounds up and rises slightly to form a hemispherical dome. This is the first cell of the embryo, known as the blastodisc. Cell division starts with the first cleavage of the blastodisc to form two cells (the two cell stage – Fig. 20). Each of the two new cells will divide and form four cells (the four cell stage), and this will continue with these four dividing into 8 cells and further to 16 - 32 - 64 - 128 and so on. It is advised to check fertilization rate early at this stage (at the four to eight cell stages). Later, the cells will be too small for detection under low magnification. As cleavage goes on the cells become smaller and smaller. After the 32 cell stage, the morula stage is formed (Fig 21). The individual cells may still be seen in the granular appearance of the morula, but they are difficult to count.

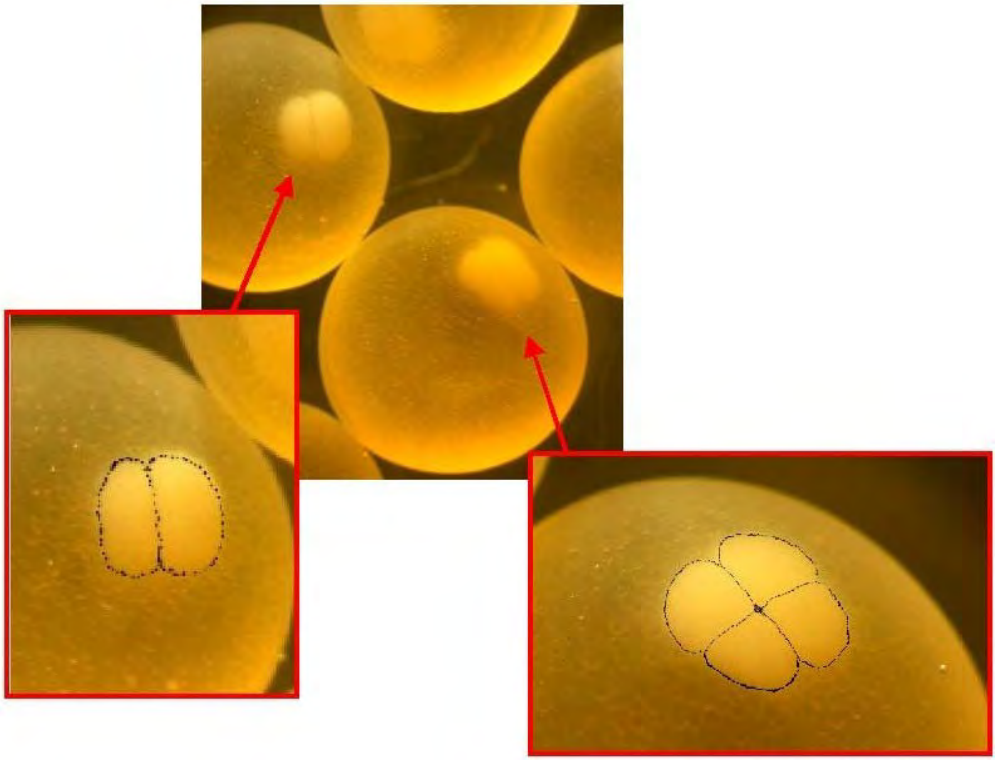

Fig.20: Arctic charr eggs in early cleavage stages. The pictures in red frames show the two-cell stage (left) and the four-cell stage (right) with the cells outlined. (Photo: Marianne Frantzen, NFH, University of Tromsø).

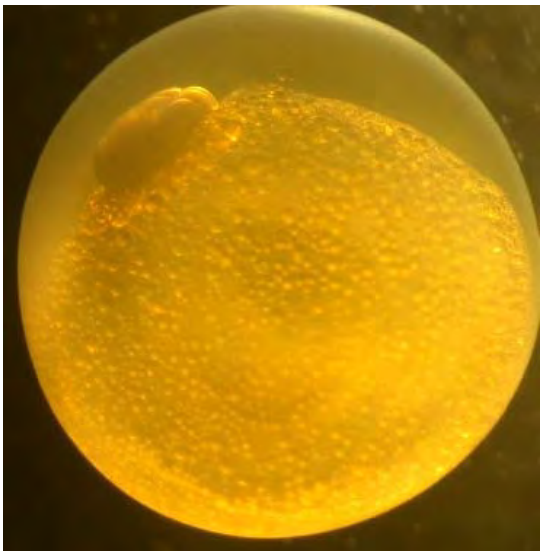

Fig.21. Arctic charr egg in the 32 cell stage. The cluster of cells is on the upper left side in the egg. The small round shapes in the yolk are oil droplets. (Photo: Marianne Frantzen, NFH,University of Tromsø).

Epiboly

The formation of the early embryo initiates the second stage. During this stage the cells formed in the cleavage phase starts to specialise to form tissues. The edge of the blastodisc expands and grows down, covering the surface of the yolk. The overgrowing edge, sometimes called the germ ring, eventually envelopes the yolk - this process is the actual epiboly (=growth of one part over another). The tissue formed by this germ ring will become the yolk sac and later the tissues enclosing the body cavity. During this process, the organisation of the cells becomes clearer. The head of the developing embryo is at the original animal pole, and the beginning of the tail is near the border of the germ ring.

The head can be seen when 1/3 of the yolk is overgrown by the germinal layer. Future muscle tissues are formed in the body region on either side of the spinal cord. Optic vesicles - the developing eyes ¬are visible at 1/2 epiboly and the eyes are formed at 2/3 epiboly. Dependent on temperature, epiboly is completed within a few days when the edges meet at the opposite side of where it started - enclosing of blastopore. A new membrane of cellular epithelium now envelops the yolk. Handling of eggs during epiboly must be avoided. Even the slightest movement of eggs may cause the membrane to leak, leading to loss of salts and denaturation of cytoplasma proteins. The end result is reduced survival of eggs. At the end of this stage the cells formed during cleavage have turned into tissues which form the basic structures of the embryo.

Organogenesis

Internal organs occur during organogenesis. When the epiboly is complete the posterior end of the embryo extends and lifts free from the surface of the yolk. This is the beginning of formation of the caudal fin. Brain, eyes, neural arch, muscles (myomeres), heart, circulatory

system, intestines and liver are developing. Blood vessels are covering the surface of the yolk, a development called vascularisation. When 3/4 of the yolk is vascularised the head is free, and the eyes are fully pigmented. This stage is easily recognised by the two dark spots within the eggs - the eyes - hence eyed stage. The heart starts to beat, and later during this stage the muscle activity increases. The embryo is now fully pigmented and complete.

Cryopreservation

Cryopreservation is a process where cells are preserved by cooling the cells to low temperatures (typically -196 °C, the boiling point of liquid nitrogen). At these low temperatures, all biological activity is stopped, and thereby Arctic charr sperm cells for instance can be stored for later use. Preferably, the sperm should be checked for quality (e.g. mobility, longevity and numbers) before the cryopreservation. There are several benefits of sperm cryopreservation that could promote the development of Arctic charr farming:

- synchronization of gamete availability of both sexes
- provides a year-round supply of sperm
- sperm economy (all available sperm of good quality can be used)
- simplification of brood stock management
- facilitate transport of gametes between different fish farms
- storage for genetic selection programs or conservation of species.

However, due to formation of ice crystals both within the cells and in the external medium, cryopreservation can damage the cells, which affects sperm quality. A number of studies have been conducted in order to produce common cryopreservation protocols for fish sperm, but there are still room for further improvement. A few studies have been conducted on cryopreservation of Arctic charr sperm. For instance, the effect of three cryoprotectants (dimethyl sulphoxide DMSO, dimethyl acetamide DMA and glycerol) on charr sperm viability post freezing and thawing has been evaluated. In the study, the sperm motility was found to be higher in the DMSO and DMA treatments than in the glycerol treatment. In conclusion, cryopreservation of fish sperm has potential already today to benefit the aquaculture industry and likely even more in the future.

Triploidy

In diploid fish, each cell has two homologous copies of each chromosome, one from the female and one from the male parent. By subjecting fertilised eggs to a pressure chock (e.g. 650 bar for 5 minutes), triploidy can be induced in Arctic charr (Fig. 22). Instead of two

chromosomes of each pair, a triploid fish carry three homologous sets of each chromosome. In triploid fish, sexual maturation is inhibited and pressure shocking fertilised eggs can thus be used to intentionally produce sterile off-spring. Early sexual maturation (i.e. prior to harvesting) which is a problem in unselected stains as energy is diverted from somatic growth into gonadal growth, which also negatively affects flesh quality. Hence, sexual maturation gives decreased marketability and smaller fish. This, and in combination with concerns about genetic interactions between escaped farm fish and wild populations, could be counteracted by production of sterile triploid fish. To date, only a few studies have focused on the effect of triploidy on growth and maturation in charr. These studies have demonstrated that it is feasible to produce triploid charr but the expected positive effect on the somatic growth has not been convincing. In fact, during the juvenile phase, diploid fish have shown higher growth rates. However, during the spawning period the growth of diploids ceases and the weight of triploids become similar to the diploids. Triploid fish do not mature and can continue to use energy in somatic growth, while maturation fish are putting their energy into gonad growth. Still, more research is needed to fully explore the possible positive effects of triploidy in charr farming, especially when considering interactions with wild and farmed fish.

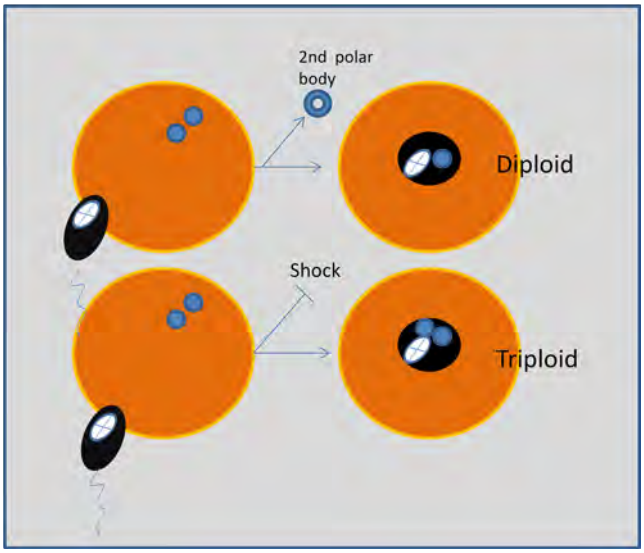

Figure 22. Production of triploid embryos by shocking fertilised eggs (modified from Intensive Fish farming).

Production of all-females

Sexual differentiation occurs early in development for salmonids, shortly after hatching, and is controlled by the chromosomal (genetic) configuration of the nucleus in the fertilised eggs. If the fertilize eggs have an Y and X chromosome it will be a male and if it have two X chromosomes it will be a female. While the genetic sex characteristics are set from fertilization the morphological sex characteristics are set by hormones released in the alevins after hatching, which hormones depends on the genetic set up. As the embryonic

development including morphological and functional sex differentiations take place outside the female´s body in most fish species compared to vertebrates, fish can easily be morphological sex reversed. In fish farming, it may be desirable to produce all females, either to produce fish eggs or to avoid sexually mature males in the production.

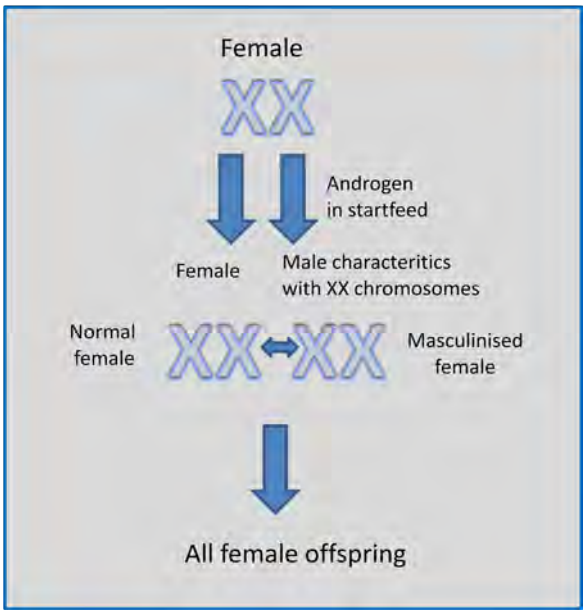

Figure 23. Two stage production of all-female salmonids. Stage 1 (upper figure) involves the morphological masculinisation of genetically female fish. Stage 2 (lower figure) involves using the sperm from these to fertilize eggs from a normal female.

It is possible to administrate sexual hormones, oestrogens or androgens in the start feed during about 2 month to salmonid alevins to produce fish with either female or male characteristics, respectively. Half of these fish will, however, still be genetically males or females irrespective of morphological characteristics. Hormone additions in feeds aimed for human consumption is however forbidden in EU but the production of sex reversed fish can be done in two-stages where the all-female offspring have not been fed with hormones (Fig.23). In such production, a masculinisation is done to produce a brood stock as a first stage. Half of these males are genetically females and can usually be separated from the true males because they don´t release their milt although they appear to be sexually mature and ready to spawn. Instead these “males” have to be sacrificed and the milt removed after opening the abdomen. This X-milt is then used to fertilize X-eggs from normal untreated female. In the Nordic countries sex-reversal is mainly done on rainbow trout for the table-market in Finland. There is still consumer scepticism towards sex reversal in fish although there are to direct hormonal administration to the fish delivered to the consumers.

Egg quality – the importance of brood stock diet

For most fish species, maturing fish has higher demand for protein and amino acids, since protein is the main component in vitellogenin. Furthermore, the feed should contain species-specific amounts and proportions of the essential fatty acids (i.e. omega-3 and omega-6). The ratio of the polyunsaturated fatty (omega-3/omega-6) acid differs in the

natural diet of fish whether it origins from the fresh or marine environment. In the freshwater nutrient system, the proportion of omega-6 is higher than in the marine environment. Although most charr farmed today is of freshwater origin, feed of marine origin (used for other salmonid species) is still used in farming of Arctic charr. Recent studies have shown that hatching rates of farmed charr are significantly lower in comparison to eggs from wild charr. Furthermore, the fatty acid composition of the eggs differed significantly between the farmed and the wild fish, especially the omega-6 fatty acids arachidonic, which concentration was 15 times higher in the eggs of the wild fish. It is well known that arachidonic acid improves both stress tolerance and survival during larval metamorphosis in marine fish eggs and larvae. The reason for the different fatty acid composition is the difference in proportions of the essential fatty acids in the commercial fish feed of marine origin fed to the farmed fish and the food of freshwater origin consumed by the wild fish. Most likely, future development of Arctic charr farming would benefit from using a brood stock feed specifically formulated for charr.

Juvenile rearing – the on-growing phase

Intensive fish farming means rearing the fish in tanks and/or net-pens at high densities and achieving as high growth rate as possible. To farm fish in land based tank systems until slaughter is much more expensive than rearing them in net pens. The most common rearing routine is to rear the young more sensitive stages in tanks and then move the fish into net-pens at sizes between 20-100 g. Some farms in Sweden move the juveniles already at 5 g to avoid too high temperature at the hatchery in the summer. The result is very variable with successful transfers some years and high death rate during other years. It is difficult for very small fish to catch pellets in a big net pen if the feed are not distributed very well and some probably die of starvation. It would be a better strategy to increase growth rates shortly after start feeding by increasing the water temperature and thereby the growth rate. This can be achieved by partly recirculating and/or heating the water and using heating exchangers. As the water needed for the juvenile stages are in relatively small amount this is probably economically feasible. However, the speed of production is improved and a marked sized charr (800g-1 kg) can be achieved in 2 years from start feeding with a feed conversion factor close to 1. An increased awareness of fish behaviour resulting in an increased farming skill, together with selection programs, has made the good results possible.

Social groupings in Arctic charr

Most studies on the behaviour of fish when confined in higher densities are done in experimental setups due to the difficulty of studying fish in full rearing conditions. This is especially true when it comes to separate individual behaviour among thousands of other fish in net-pens or tanks. Most of these laboratory experiments are aimed to scale the

situations down from farming condition. When groups of fish are observed in aquarium, they typically develop dominance hierarchies.

Individual fish that are successful competitors have higher feed intakes and consequently higher growth rates than less competitive ones. This is particularly pronounced in small scale experimental conditions were less competitive fish (subordinates) often are excluded from the feeding area or physically loose feed to more aggressive and dominant individuals. The subordinates suffer from stress and have increased levels of stress hormones. These fish often keep a very low profile and cease eating even with excess feeding.

However, groups of fish that are kept in aquarium without refuges and not giving subordinate individuals a possibility to hide or escape from dominant fish, is indeed an artificial condition. In full scaling condition with thousands of fish reared in high densities in net-pens and tanks individual variation in growth is probably more related to competitive ability, higher or lower feeding motivation or a different level of aggression rather than by dominance hierarchies, where high ranked fish are more aggressive or competitive during food release. For fish in general, it has been shown that the significance of social dominance is reduced when group sizes increase. The reason is that neither repeated attacks nor defense of a favorable area or food resource can be sustained by dominant individuals under such conditions (Fig 24).

Behaviour problems when farming Arctic charr

In fish farms, no matter how skilful the farmer is, there will always be size discrepancies between individuals. The reason for this may be both genetically determined and/or an effect of competition between individuals governed by their social status. Evidence for this is found in more or less all fish farms today, where a varying proportion of the fish exhibits higher growth rates. Several problems related to the behaviour of fish can be identified in farming conditions. The causes for these problems are often linked to undesirable social behaviour caused by aggression between individuals. Visible signs of behavioural problems are often a reduced or skew grow rate, stress, fin damages, mortality and abnormal behaviour. Some of these problems are fairly easy to detect early and therefore possible to solve, whereas others are more difficult.

It is important that fish farmers are aware of the behaviour problems aggression can cause and have some form of tool to detect it. It is suggested that the social environment could be assessed by observations of weight gain, size variation and feed conversion ratios. Three scenarios were given:

1. A high growth rate and little variation in body size (i.e. low coefficient of variation for growth rate), indicates good rearing conditions in which there is little or no depression of growth due to aggressive behaviour.

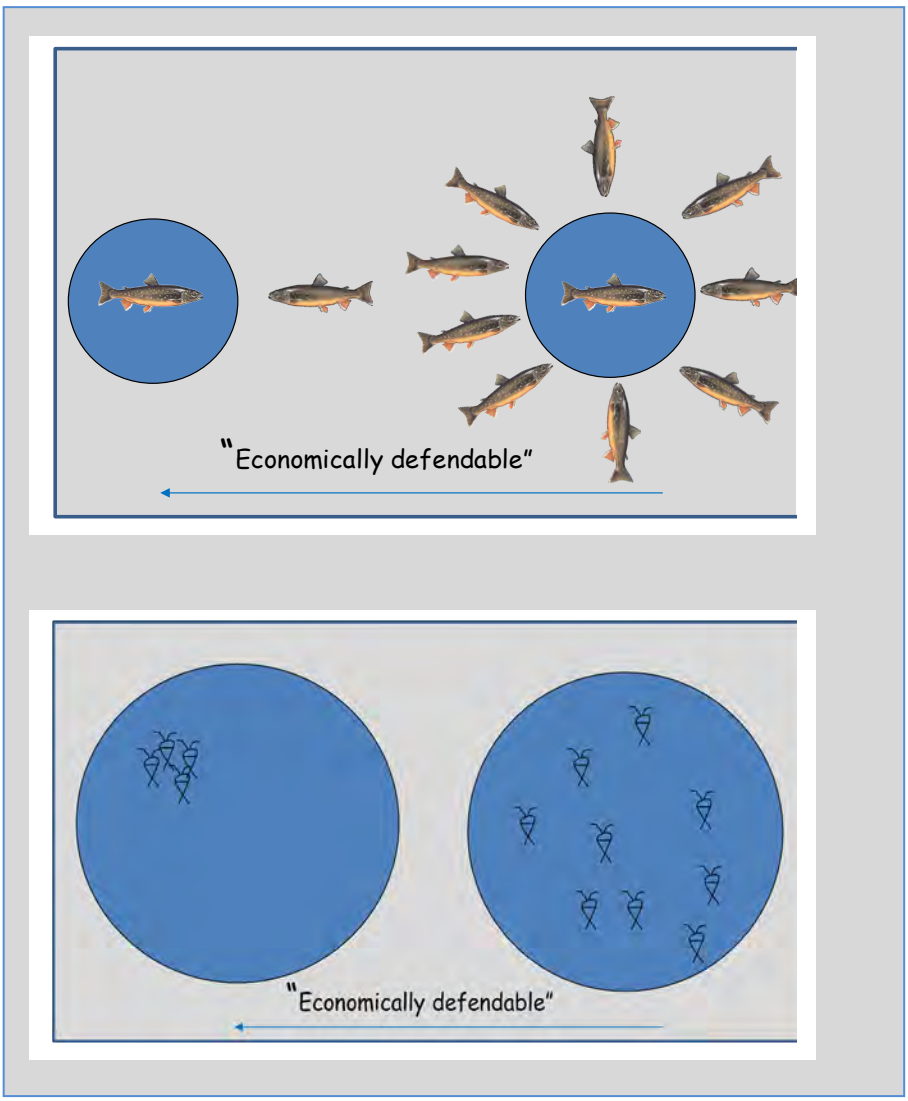

Figure 24. Illustrations of the effect of density (above) and feed distribution (below) on if a resource (space or feed) are worth defending.

2 Sub-optimal and disparate growth coupled to poor feed conversion ratios reflects a poor social environment, which may be the result of competition due to underfeeding or because of bad temporal or spatial distribution of feed.

3 Poor growth with little variation indicates a general growth depression resulting from a poor environment, and a poor feed conversion ratio would reflect feed waste.

Thus, a fish farmer can, by keeping precise track of fish growth and feed consumption, easily evaluate the social situation in his or hers rearing environment and thereby avoid behavioural problems that may lead to severe growth variation. There are systems that

enable the farmer to estimate the size of fish without stressful handing. Image analyses of video images or IR (Vaki) measurements are available on the market.
It is also important to know that growth reduction can also reflect a seasonal change in growth potential, sometimes referred to as “autumn depression”. Interestingly, there is evidence that the seasonal changes in the growth has decreased in selected Arctic charr. The seasonal cycling allows the fish to adapt to seasonal changes in food abundance in nature, a trait that may have been lost during brood stock selection in the hatchery. Temporal cycling in food intake and body condition have been described of charr held under controlled conditions, and it is those species that live at high latitudes that appear to exhibit the greatest seasonal fluctuations.

In order to reduce the formation of social hierarchies and the resulting aggressive behaviour, fish can be sorted by size. Size grading fish displaying reduced growth give an opportunity to increase their growth rate, since larger and more competitive fish are removed. Size grading is also done for managing purpose, to have fish reaching slaughtering size during all year. The amount of feed to be delivered is estimated by feeding rate tables for Arctic charr. These tables estimate growth reasonably well over a long period of time (i.e. a year) but can result in major over or under feeding at particular times. Indeed, charr growth potential changes with external factors, such as temperature, but is also controlled by endogenous factors. The growth also seems to be season dependent. Most feeding and growth tables do not consider these fluctuations in feed intake and they should therefore be used with great caution. Best feeding practice seems to be the one controlled by the appetite of the fish.

Besides being necessary in self-cleaning tank systems (circular tanks require 4-6 cm $s^{-}1$), aggression and social dominancy can be decreased by creating a stable and moderate water current in the rearing tank. Without water current, fish swims less organised and seems to engage more time in aggressive interactions. At higher water current, fish are more evenly distributed and swim in the same direction, starting to school in the tank. Fish that are evenly distributed are less occupied by social interactions, which are reflected in more uniform growth among individuals as well as a reduction of fin damages (Fig. 23). The threshold current speed for schooling is known to be somewhere between 0.5 and 1 body length s^{-1}. In addition, high water current distribute the feed across the whole rearing tank, reducing the opportunity for aggressive fish to defend the feed. A current speed up to 1.5-1.75 body lengths $s^{-}1$, the exercise effect of increased swimming speed also results in improved growth and feed utilisation.

Fish density has also an effect on aggression and social interactions (see below)

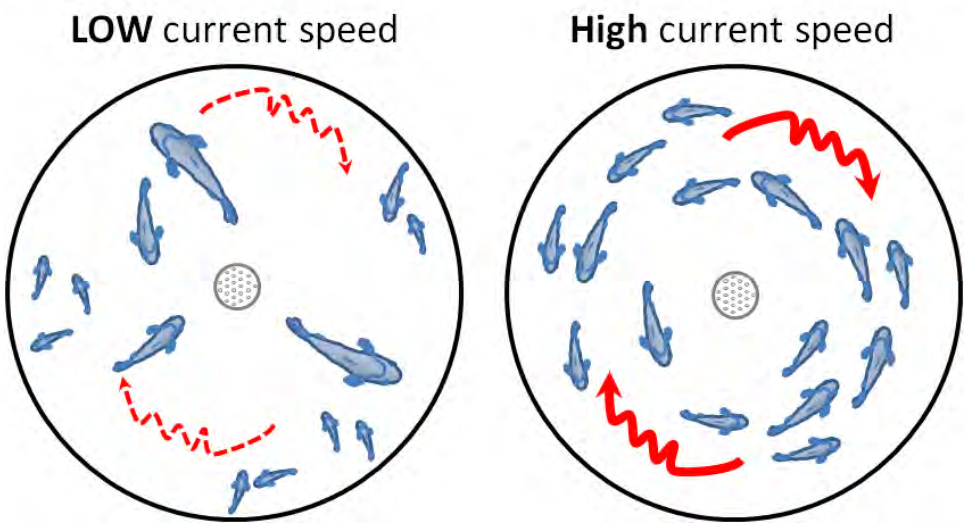

Figure 23. Schematic figure of the effect of water current speed on charr swimming orientation, schooling and growth variability in a fish rearing tank.

Stocking density

Opposed to terrestrially farmed animals on land, fish are reared in a three-dimensional space and voluntarily form tight schools. However, the schools of fish need space so they can alter their swimming depth to preferable environmental conditions, i.e. access to feed, light intensity, temperature or salinity.

Growth rates in Arctic charr seem to be positively correlated to stocking density when feed supplies are adequate. Even at densities over 100 kg m-3 in tanks, the growth rate doesn't seem to be suppressed. In Swedish and Norwegian fish farms were fish is reared in net-pens, charr are generally kept at densities between 50-60 kg m^{-3} compared to 30-40 kg m^{-3} in rainbow trout farming.

Feeding regime and feed distribution

Fish-farmers attend to feed the fish to satiation or near satiation and therefore a behavioural problem like aggression that is related restricted feed access is of minor importance. More important is the spatial and temporal distribution of the feed. If feed is delivered to a single location in the rearing unit, dominant fish is likely to defend and hold position, giving them greater opportunities than lower ranked fish. Aggression is less frequent if the feed is dispersed rather than concentrated in a restricted area.

A fish farmer have to balance carefully between the size of the feed portion and the number of pellets the fish is able to catch before the feed pass out of the rearing system. To avoid feed waste, the best strategy would be to deliver very small portions, which ensures that the fish are able to catch all. There are however differences between species in how they react to the feeding regime. Arctic charr are much slower at catching pellets in the water column

than rainbow trout, and doesn´t create as much boiling appearance when the pellets hit the water surface. Studies have shown if the daily meal is given in few portions rainbow trout are favored whereas Arctic charr grow better and with less size differences between individuals with frequent daily meals.

Breeding and behaviour

In most farmed animals, domestication has a considerable effect on the behaviour and an increase or decrease in aggression level between individuals may be an undesired or desired, respectively, goal in breeding programs. In fish, there is a lack of knowledge on how aggression and correlated behaviours are effected by domestication. Aggression and schooling are alternative behaviours that fish apply depending on the situation. It is therefore likely that a selection for lower aggression, i.e. an increase in the threshold that induces an aggressive behaviour, will increase the tendency to school.

The scarce studies on social behaviour in fish at full scale rearing condition reveal that most fish species that are reared in net cages gather in schools as ring structures with few fish near the centre or close to the cage wall rather than dispersing within the entire cage volume. Atlantic salmon and rainbow trout form circular polarised schools during daytime. At night the schooling groups have been observed (by IR cameras!) to disperse. It is likely that Arctic charr show the same response.

Cannibalism

Cannibalism in farmed fish is mainly a problem in the early life stages of predatory species that have large mouth relative to body size. Salmonid larvae have a smaller mouth relative to body size and, consequently, cannibalism is rare unless the size difference between fish is very large. In addition to size-variation, the potential for cannibalism is increased by low food availability, high densities and the absence of hiding places. Since conspecifics constitute an optimal combination of nutrients needed, growth rates are often high in cannibals. Thus, the individuals that become cannibals probably have a great advantage over others due to a higher growth rate and a higher chance to survive.

Health and welfare

Fish in all environments, including culture and in the wild, sometimes suffers from stressful conditions. One of the first signs of stress that is related to behavioural problems in farmed fish is the occurrence of fin damages. These are to large extent a cause of social interactions and commonly considered a sign of unsuitable rearing conditions. Stress have negative effects on the immunocompetence of fish. Fish may seem healthy before, during and immediately after a period of stress, but disease problems may occur later on. They may be

asymptomatic carriers of pathogens that under normal conditions are held back by the immune system. When that system is impaired or suppressed by stress, the disease-causing agent may start to grow, gain control and kill the fish. In addition, stress will suppress the immune system and increase the vulnerability of the fish to invading pathogens.

Surface activity is an important behaviour of fish held in culture, and situations with over activity may indicate that something is wrong in the rearing environment. Leaping (fish jumping with their whole body breaking the water surface) and rolling (the dorsal part of the body breaking the surface) are well-known behaviours in salmonids The cause for leaping seems to be related to infections of ectoparasites, exposure to acute stress and the presence of predators. No seasonal variation in rolling has been observed, and high rolling activity may be explained by buoyancy compensation (gas bladder filling) as an effect of stress exposure. Fish often loose air from the swimbladder during stress exposure and it is important that the neutral buoyancy is restored within a relative short period of time.

Measuring stress on fish health in culture is difficult as described above but there are, however, signs of stress and bad environment that are possible to detect in culture conditions:

- Abnormal swimming behaviour (e.g. leaping, rolling).
- Low locomotor activity
- Low and variable growth
- Fin damages, wounds and scale loss
- High number of external parasites

Since many immune assays are becoming available in kits, farmers can perform quick and sensitive tests directly at their farm. Many of these tests do not require the fish to be killed but a blood sample is enough.
Successful rearing methods in intensive systems really deal with making the fish apply a schooling behaviour. A schooling behaviour is achieved by taking advantage of the plastistity the fish have in their behaviour repertoire and rearing them in conditions where it doesn´t pay to be territorial but where it is more advantageous to swim in groups.

Suggested reading:

Intensive Fish framing J. Shepherd and N. Bromage, Wiley. ISBN: 978-0-632-03467-3.

Fish Welfare, ed. EJ. Bransson Blackwell publishing. ISBN 978-1 4051-4629-6.