Effect of accelerated or delayed maturation on growth and quality of cod (*Gadus morhua*) farmed 67° N at a commercial scale

Master thesis in Aquaculture

Faculty of Biosciences and Aquaculture

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Foreword

The Master of Science (MSc) thesis was completed as the final part of the MSc degree in Aquaculture with the Faculty of Biosciences and Aquaculture (FBA) at Bodø University College. The thesis is supervised, independent work which represents 60 from a total of 120 credits, collected throughout a two year study period. This thesis has been performed as a topic in seafood quality.

The main objective of this thesis was to investigate the effect of different light treatment regimes on the growth and quality of farmed cod under full-scale commercial production. The work was performed in collaboration with Codfarmers ASA.

I would especially like to thank my very helpful professor and main supervisor Dr. Christel Solberg, for outstanding guidance throughout the course of this program, including experimental work, statistical analysis, and the writing of the master thesis.

I would also like to thank my co-supervisors, Stian Amble at Codfarmers, for all his assistance with the delivery of the samples as well as work in the lab and Dr. Jarle Tryti Nordeide for taking time to complete the gonad assessments.

Many thanks to the lab technicians at FBA, Marion Skog Nilsen, Kevin Klingan and Kjell-Arne Størseth, for all the time and energy they put into helping me during the sampling and chemical analysis.

Much gratitude is expressed to Maricom, Norway for providing financial support to allow me to attend the Sats på torsk conference in 2008 in Tromsø.

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Abstract

Atlantic cod (*Gadus morhua*) farming has received considerable attention in recent years, and is now becoming a rapid growing industry in Norway. This industry has shown approximately 2,000 tons harvested in 2003 compared with approximately 13,500 tons in 2008. The harvest quantity is expected to increase by another 50% in 2009. The major bottleneck for economical cod farming is with early maturation. In Atlantic salmon (*Salmo salar*), light manipulation has been a successful method for avoiding early maturation, but this has not been as effective in cod farming to date.

Two different light manipulation regimes were tried in commercial-scale cod production in the north of Norway. The first regime (delayed group) had additional lights installed in the netpens from August 17, 2007 until June 1, 2008. This treatment postpones the maturation by 2-3 months, with a peak spawning in June. In the second regime (accelerated group), additional light was installed in the netpens from November 23, 2007 until June 1, 2008. This treatment resulted in an accelerated spawning with a peak in February approximately 2 months before the untreated group. The maturation peak occurs approximately 4-6 weeks earlier in males than in females. The impact on growth and quality is also substantially larger among female cod.

The accelerated maturation resulted in a decreased gutted weight in February and an enhanced increase in gutted weight from April. The lowest protein content (17.7%) in the muscle tissue was also found in female cod in February. The mean HSI in females increased from ~17% in December/February to ~20% in June. In female cod, the delayed maturation showed an increase in gutted weight during the winter, with no further growth from the start of maturation in April. The delayed group did not experience a decrease in muscle protein content during maturation (19.7% in June), but instead a significant decrease in the HSI content from ~17% in December/February to ~15% in June.

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1.0 Introduction

Commercial aquaculture in Norway began around 1970, and since that time has developed into a major industry in coastal areas. Intensive farming of Atlantic salmon (*Salmo salar*) is considered to be the most important sector, accounting for more than 80% of the total Norwegian aquaculture production (FAO, 2005). However, other species are now also in the process of becoming commercialized, one of these species being the Atlantic cod (*Gadus morhua).*

This industry has shown approximately 2,000 tons harvested in 2003 compared with approximately 13,500 tons in 2008. The harvest quantity is expected to increase by another 50% in 2009 (Directorate of Fisheries, Kantail Analyse AS). It has also been predicted that the production of farmed cod will reach a similar level as that of Atlantic salmon within the next 15–20 years (Rosenlund et al., 2006)

The increased interest in cod farming was brought about mainly when wild cod stocks began to drop to almost extinct levels in the 1980's and 1990's (Norberg et al., 2004). Because of the increasing market demands and the declination of wild cod stocks, cod aquaculture is increasing rapidly in Norway. Cod farming may help reverse this decline by reducing the pressure on wild cod stocks. There are however many issues to be resolved to make cod farming as successful as the salmon farming industry. Some issues facing this industry include cannibalization (among cod <100 g), low survival rates and most of all premature sexual maturation (Ridgeway et al., 2007). Low survival rates during early the life stages has brought about much research interest over the past few years, and with this, a lack of focus and research on the grow-out stage has

been seen. This has led to several flesh quality problems (e.g. soft texture and gaping), especially during the spring and summer (Hagen et al., 2009).

Because of the continuous feed supply and rapid growth, farmed cod reaches maturity at a lower age than their wild counterpart. When cod mature, there is a loss of somatic growth, condition and a reduction in weight (Braaten, 1984), along with the development of large gonads at the expense of myotomal growth. Light manipulation has successfully been used as a means to suppress gonad development and maturation in Atlantic salmon; however this technology has not been that easily transferrable to cod farming (Kjesbu et al., 2006).

Because of the potential impact light has on sexual maturation, it is very important to understand how to regulate it to ensure optimum quality of farmed cod.

The main purpose of this thesis was to determine the effect of different light treatment regimes on the growth and quality of farmed cod in full scale commercial production.

1.1 Cod biology

The cod fish (*Gadus morhua*) is a relatively large and solid bodied fish with a protruding upper jaw and long chin barbel. It has three dorsal fins and two ventral fins without spines and a truncated caudal fin. It has a prominent white lateral line, which is used to detect movement and vibration in the surrounding water.

It is a cold-water species, and in nature is distributed along the continental shelves and in the coastal waters of the northern North Atlantic (Cavendish, 2000). In the wild, adult can be found in temperatures from below 0 °C to 20 °C, although spawning sites (located 70° N off the coast of Norway) tend to range from -1.5 °C to 9.2 °C (Galloway et al., 1998).

Generally, cod are demersal, meaning they prefer to live on or near the sea bottom, and tend to inhabit various areas from rocky bottoms to muddy or sandy seabeds. Adult cod are omnivorous carnivores, eating various species such as mussels, squids, capelin and herring. They are known to be cannibalistic, consuming smaller cod if the need arises (Cavendish, 2000).

The hepato somatic index (HSI) is measured as the size of the cod liver in relation to the cod's body mass, and is a good indicator of the liver's energy content (Dahle et al., 2003). Typical wild cod have a hepato somatic index (HSI) of 2%- 8%. Typical farmed cod may have an HSI of approximately 12% (calculated against total weight) due to the high energy and digestibility of commercial diets (Karlsen et al., 2006). HSI calculated against gutted weight to avoid the influence of the variable gonad weight has been found to range between 11-16% (Solberg and Willumsen, 2008). A low HSI is an indication of insufficient feeding (Lambert and Dûtil, 1997).

1.2 Cod chemistry

The lipid content and distribution in fish varies from species to species. The major lipid storage site for fish is the liver, muscle, mesenteric tissue and sub-dermal fat layers. In lean fish species like the Atlantic cod, there tends to be relatively little lipid in the muscle (less than 1%) (Shengying et al., 1995). The lipids which are present in the muscle tissue are found in the cell membranes as phospholipids (Huss, 1995).

Lipids and water together make up about 80% of the muscle tissue, but tend to vary depending on the spawning status of the fish. As the lipid content decreases, the water content in the muscle increases ('fat-water line'). In non-fatty fish, like cod there is instead a 'water-protein line'. In wild Atlantic cod this 'water-protein line' is so sensitive that, while a water content of 80.9% or less can be regarded as normal, 81%, or more may indicate depletion (seen during maturation or

starvation) (Love, 1988). Farmed cod normally have a water content below 80%. An increased water content has been associated with maturing female cod (Solberg and Willumsen, 2008). The amount of fat content in the liver tends to vary with the seasons, depending on the stage of spawning in the female cod (Kjesbu et al., 1991), whereas the stage of spawning in males may show a variation in liver size. (Solberg and Willumsen, 2008).

1.3 Maturation and spawning

The cod along Norway and Iceland both spawn from February through April. In the North Sea spawning continues through April while Baltic cod start spawning late in March but continue through October (Jørstad et al., 2006). Under favorable growing conditions, farmed Atlantic cod of both sexes reach sexual maturity or puberty and spawn at two years of age, before reaching commercial harvest size (Braaten, 1984; Godø and Moksness, 1987; Karlsen et al., 1995), although males may mature one year after hatching, are typically smaller in size than females, and continue to spawn over a longer time frame (Karlsen et al., 1995). In the wild however, Atlantic cod typically reach puberty at ages between 4-6 and 6-8 years, respectively (Godø and Moksness, 1987, Berg and Albert, 2003).

In maturing cod, the appetite is reduced approximately one month prior to spawning and during the first three-quarters of the spawning period (Kjesbu et al., 1991). Because Atlantic cod release approximately15-20 egg batches in one spawning season and distribute large amounts of energy to the gonads throughout the spawning period (Kjesbu et al., 1996), the energy requirements for gonad growth and spawning are mainly taken from stored reserves located in the liver and muscle (Kjesbu et al., 1991). This can make the spawning result in a significant weight loss for the fish. Large well-fed female cod tend to lose upwards of 50% of its normal

weight during one spawning season (Kjesbu et al., 1991), while 30-35% weight loss is usually observed for recruit spawning farmed cod (Karlsen et al., 1995). Compared with wild stocks, early maturation in farmed cod appears to be linked to increased growth and energy deposition (Karlsen et al., 2006).

1.4 Photoperiod manipulation

One of the big challenges in developing cod farming in Nordic countries as well as others is trying to prevent the early sexual maturation of farmed cod before they have reached the optimum marketable size (Björnsson et al., 2005).

There have been many experiments and research projects conducted to try and delay puberty in farmed cod by implementing periods of restricted feeding or starvation. However, these have been shown to have limited effect on age during maturation and tend to result in dramatically reduced growth (Karlsen et al., 1995).

Photoperiod manipulation has been proving to be an effective means to control the timing of puberty and spawning in a number of farmed fish species. These species include the Atlantic halibut (*Hippoglossus hippoglossus*; Norberg et al., 2001), the Atlantic salmon (*Salmo salar*; Oppedal et al., 1997, Porter et al., 1999; Taranger et al., 1999), and the brook trout (*Salvelinus fontinalis*; Holcombe et al., 2000), to name a few.

As is the case with salmon, the use of light can postpone first maturation and increase weight in cod. Various experiments showed that it was possible to control spawning using a photoperiod treatment with Atlantic cod kept in indoor tanks (Hansen et al., 2001; Norberg et al., 2004;

Skjæraasen et al., 2005). Overall it has been shown that the sexual maturation of cod can be postponed to at least three years of age by controlling light conditions (Svåsand et al., 2007).

Other experiments have shown more variety with the results when continuous light treatment was applied to cod kept in sea cages under natural light. For example, experiments conducted (Taranger et al., 2006), have shown it to be possible to postpone maturation by four to six months, so that it takes place in the summer instead of during the natural spawning season between February and April.

Photoperiod treatment appears to be more effective when applied to Atlantic cod in indoor tanks than in sea cages (Taranger et al., 2006). One of the main differences between sea cages and tanks is that the cod swim at a rather high velocity in circular tanks, whereas their swimming behavior in sea cages tends to be more varied. Therefore the increased swimming activity and exercise levels in tanks, (which may affect energy deposition and utilization in the liver), may be one reason for the differences in response to photoperiod treatment in cod observed between tanks and sea cages (Björnsson et al., 2005).

The pineal gland is a light sensitive neuro-endocrine structure that lies in the anterior brain and is a well-vascularized organ. This gland secretes melatonin that may play a role in controlling reproduction, growth, and migration (Moeller, 2008).

In many teleosts, such as the Atlantic cod, it appears as though the pineal gland (or epiphysis) also plays an important role in spawning behavior. It is located on the dorsal surface of the brain and is the principle photoperiod interpreting organ. It receives photoperiod signals and transfers a signal via photoreceptor cells to the brain/gonad axis which determines the onset of maturation (Bromage et al., 2001).

1.5 Geographic locations

In Norway, the Institute of Marine Research (IMR) has played an important role in the development of cod farming. In the early 1990's, scientists at the Matre Research Station showed that sexual maturation in cod can be halted or delayed by controlling light conditions. This became the basis for the further development of cod as a farmed species, since all fish normally become sexually mature two years after they hatch (Taranger et al., 2006).

Most of the experiments conducted in sea cages in Norway are conducted by the IMR in the south-western part of Norway (60° N). At that latitude daylight cycles vary less and water temperatures are higher than in the northern part of Norway (67°N). Summer water temperatures in SW Norway can be as high as 20° C, whereas the summer sea temperature in northern Norway in the trial area is very rarely above 14° C (Solberg et al., 2006). This means that growth conditions differ between locations (Amble, 2007).

These temperature variations along with different fish sizes and blood type (Amble, 2007) shows the period from May to November being the optimal growing period for cod in northern Norway (Solberg et al., 2006).

As noted by Amble (2007) sea temperatures are very reliant on location, and may change according to the salinity levels, freshwater and glacier outflows, depth, local and global current systems resulting from tidal and wind systems.

2.0 Materials and Methods

2.1 Fish husbandry and sampling

The cod used in this research came from a Codfarmers ASA site located in Sketneset, which is on the inside of Straumøya (an island outside of Bodø, Norway). This location is situated 67º north, where daylight hours fluctuate, depending on the season. In the winter, daylight is extremely limited or non-existent for a period of 1 month. Also, during the summertime an opposite effect occurs, with up to 24 hours of sunlight available.

In this experiment, the water temperature showed a fluctuation throughout the seasons. In December, the water temperature was recorded as 7ºC (Figure 1). This temperature showed a steady decline to 5.5ºC in February and continued to 4.5ºC in April.

The period between February and April showed the lowest water temperatures throughout the entire experimental period. Towards the end of April there was a steady increase in water temperature until the final sampling date in June, when the water temperature was 7.5ºC. The water temperature continued to rise to 10ºC by the end of July.

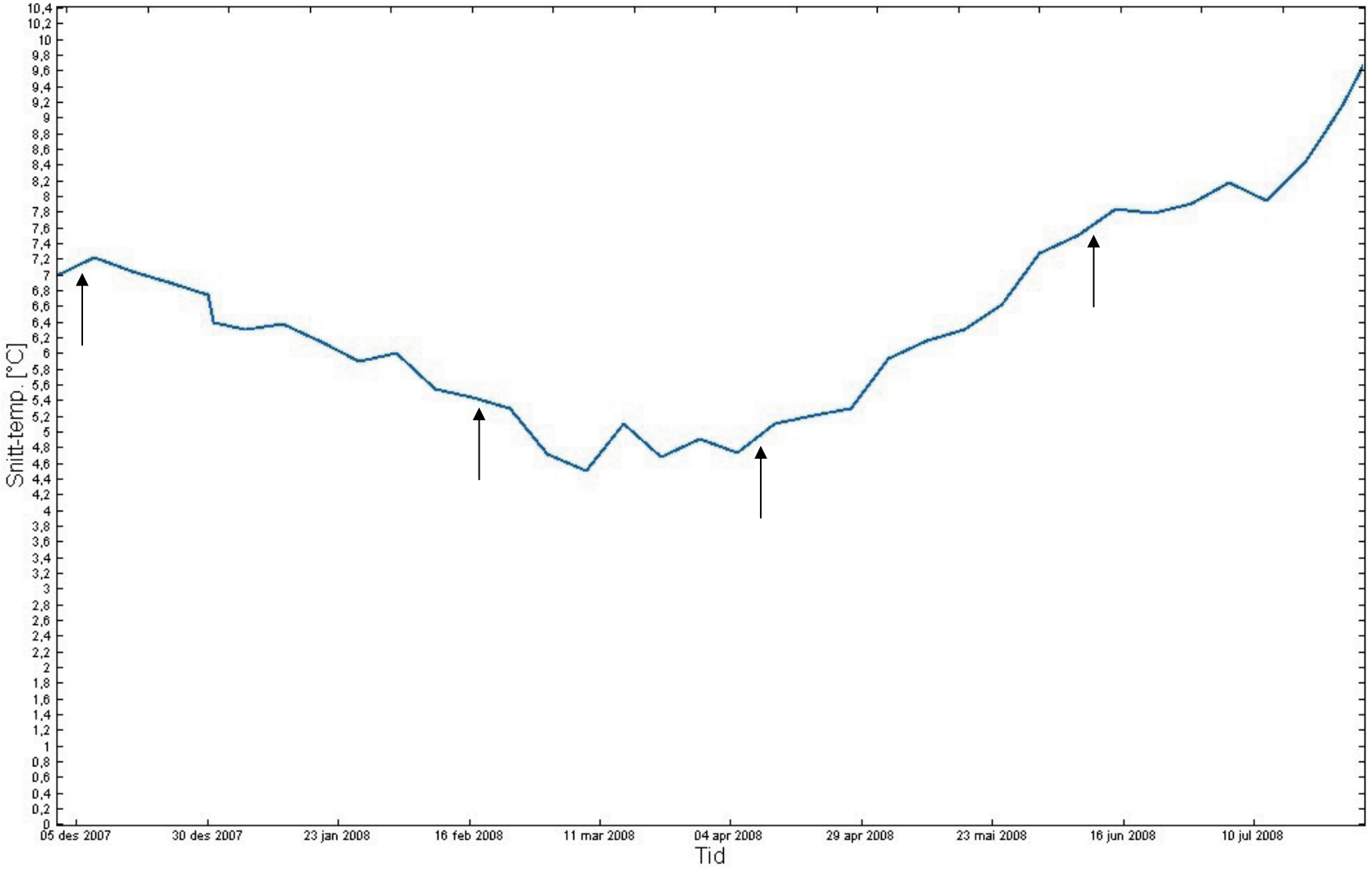

Figure 1: Line graph showing the water temperature profile as a week average temperature in Celsius. Arrows indicate the approximate dates in which sampling occurred.

The fish were contained in commercial-scale netpens. For this experiments purpose, there were three types of light treatment in the netpens; delayed and accelerated, as well as a control netpen. There were two delayed groups, with nine 1kW lights in each and the lights turned on from August 2007 until June 2008. There were two accelerated groups, six 1kW lights in each, with the lights turned on from November 2007 until June 2008. There was one "control" group, consisting of the out-graded smaller cod, and were collected in one netpen without any additional light.

Table 1: Dates, sample sizes and sex ratios for each sampling from the accelerated (acc) and delayed (del) groups throughout the experimental period.

	Total sampled		Total females sampled		Total males sampled	
Sampling Date	***Acc***	***Del***	***Acc***	***Del***	***Acc***	***Del***
December 4-5, 2007	60	61	27	30	33	31
February 19-20, 2008	60	60	35	28	25	32
April 15-16, 2008	60	61	31	34	29	27
June 5-6 & 12-13, 2008	60	62	25	30	35	32

The fish were reared in 90m circular netpens which were 20m deep with 60,000 cod in each netpen. All fish were fed with commercial feed (Classic Marine 1600) from Biomar, containing 20% fat and 48% protein (proximate content given by Biomar).

The fish were chosen at random from the netpens, bled out, and transported on ice to the laboratory at the Faculty of Biosciences and Aquaculture (FBA). All sampling took place December 2007, February, April and June 2008 (Table 1). During each sampling there were approximately 60 cod sampled from each the delayed and accelerated netpens, and 30 from the control netpen, with a total of 600 cod sampled altogether.

2.2 Biological measurements

All biological measurements were taken at the basement laboratory located on campus at FBA. Each fish was measured for length, weight (whole, gutted and headless), and tagged with an individual sample number. All gonad and liver samples were weighed, stored in a -40ºC freezer and kept until analysis could be conducted by trained personnel.

2.3 Maturity assessment

All gonads sampled throughout the experimental period, December 2007 to June 2008 were observed to designate a stage of maturation. The maturation scale used was developed by the Institute of Marine Research, Norway (Fotland et al., 2000), and which was similar to the one published by Katsiadaki et al., (1999). Maturity levels were designated by qualified assessment personnel (J.T. Nordeide, Bodø University College). The maturation scale is found in Appendix 3: Maturation scale for cod. The maturation scale values range from stage 1 (immature) to stage 6 (spent).

2.4 Calculations

The Gonado Somatic Index (GSI %) was calculated as (Gonad Weight / Gutted Weight) * 100

The Hepato Somatic Index (HSI %) was calculated as (Liver Weight / Gutted Weight) * 100

The Condition Factor (C-factor) was calculated as (Gutted Weight / Length^3) * 100

2.5 Muscle sampling

At the same time as the biological measurements, muscle fillets were taken from every fish. After the fillet was removed, they were stored in a - 40ºC freezer and kept there until analysis could be conducted.

2.6 Chemical analysis

Prior to any chemical analysis, all fillets were skinned and made into a homogenous mince using a general food processor with blades. Protein analysis was performed on randomly chosen samples (approximately one in five) by weighing 0.8 – 1.2 g of the minced sample and placing it in a nitrogen free weighing cup. Each sample was placed into individual Kjeldahl reagent tubes. Two Cu-Kjeltabs (Foss Analytical AB, Sweden) and 15 ml of 96% sulphuric acid were added to each of the tubes, placed on a holding rack and transferred to a hot plate for digestion; approximately 45 minutes. Prior to this, the hot plate was turned on and heated to 420ºC. After the digestion process was complete, the holding rack was removed from the hot plate and the samples left to cool for 20-30 minutes. Once the samples were sufficiently cooled, 75ml of water was added to each of the tubes, and individually analyzed using the Kjeltec 2300 (Foss Analytical AB, Sweden). The average of the parallels for each sample was used in the results. The water analysis was performed by weighing approximately 5 g of the minced samples (in parallel) into separate aluminum foil cups. Each sample was then placed into a drying oven at 104ºC for 16-18 hours, and then re-weighed. The water content was retrieved by subtracting the dried weight from the initial sample weight. The average of the parallels for each sample was used in the results.

2.7 Near-infrared analysis

Near infrared measurements (NIT) were then taken using the Infratec 1255, Food and Feed Analyzer (Foss Analytical AB, Sweden). Every sample taken was analyzed using the Infratec 1255. Approximately one in every five samples was analyzed for protein and water content, and a parallel of each sample was completed.

A NIT analysis was performed to determine the water and protein levels of each sample. A NIT analysis is done by using the minced sample along with the Infratec 1255 Food & Feed Analyzer (Foss Analytical AB, Sweden). The mince from each sample is placed into a cup holder using five, 23 mm thick rings (red). It is very important to ensure that while placing the mince into the cup holder rings that no air pockets are in the sample. This is to prevent diffraction of the light, which would cause inaccurate measurements. The NIT works by passing a near infrared light through each of the samples and recording the amount of light that passes through. The data was then transferred to the analytical software Unscrambler, in order to pair the NIR absorbance results with the results from the conventional reference analysis using a Partial Least Square regression analysis. This analysis was used to predict the values for all of the samples in the trial.

2.8 Statistical analysis

Statistical analysis was performed using SigmaPlot v. 11(Systat Software Inc., 2008). Data was analyzed to check the variation between seasons and were performed using one-way ANOVAs. The tests were run separately for each sex and each light treatment. P-values of less than 0.05 were considered significant. When the data did not show a normal distribution, a Kruskall-Wallis one-way ANOVA was used, along with Dunn's Method for pairwise comparisons.

For all two-group comparisons, Student's t-test was used, or a Mann-Whitney U test when the equal variance assumption was not met. This was done to indicate any potential differences between treatments and sexes.

2.9 Principal component analysis

Principal component analysis was performed with Uncrambler (Ver. 9, Camo A/S) and used to analyze different variables. This was performed to help determine which variations contributed the most with the differences between the samples at each of the sample dates. The samples are presented in a score plot and the variables are presented in a loading plot.

3.0 Results

3.1 Length

Figure 2 shows the length for female cod with the different light treatments from December to June. There was a significant difference between the treatments during the whole season (Appendix 2: Table 4).

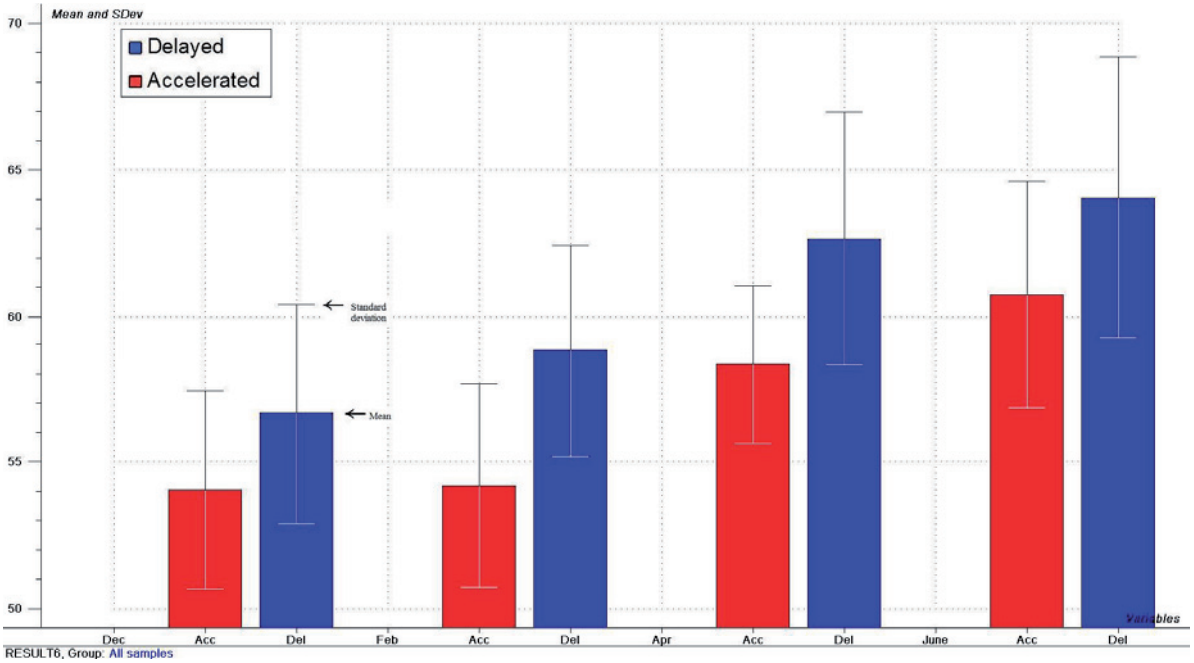

Figure 2: Female lengths (cm) during the sampling period, for the accelerated (red) and delayed (blue) groups. The Bar Chart shows the average value and the standard deviation together. The vertical bar is the average value, and the standard deviation is shown as an error bar around the average. A description of significance levels can be found in appendix 2.

From December to June, the length increase from 54 cm to 60 cm in the accelerated group. A one-way ANOVA shows significant increases between the different samplings. Similarly, the length increased from 57 cm to 64 cm in the delayed group with significant differences.

Figure 3 shows the length for male cod with the different light treatments from December to June. There was a significant difference between the treatments from December through April. In June, there were no significant differences between treatments (Appendix 2: Table 5).

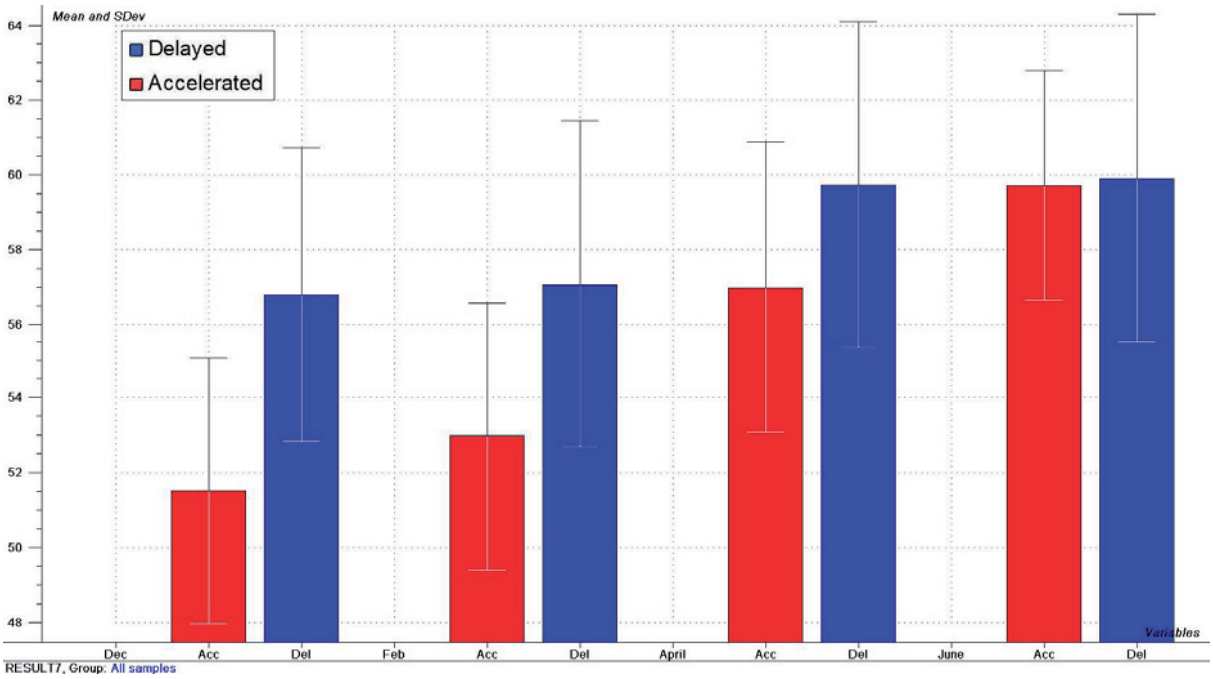

Figure 3: Male lengths (cm) during the sampling period, for the accelerated (red) and delayed (blue) groups. The Bar Chart shows the average value and the standard deviation together. The vertical bar is the average value, and the standard deviation is shown as an error bar around the average. A description of significance levels can be found in appendix 2.

From December to June, the length increase from 51 cm to 59 cm in the accelerated group. A one-way ANOVA shows significant increases between the different samplings.

Similarly, the length increased from 57 cm to 61 cm in the delayed group with significant differences from December to April, and no significant differences between April and June.

3.2 Gutted weight

Figure 4 shows the gutted weight for female cod with the different light treatments from December to June. There was a significant difference between the treatments from December through June (Appendix 2: Table 4).

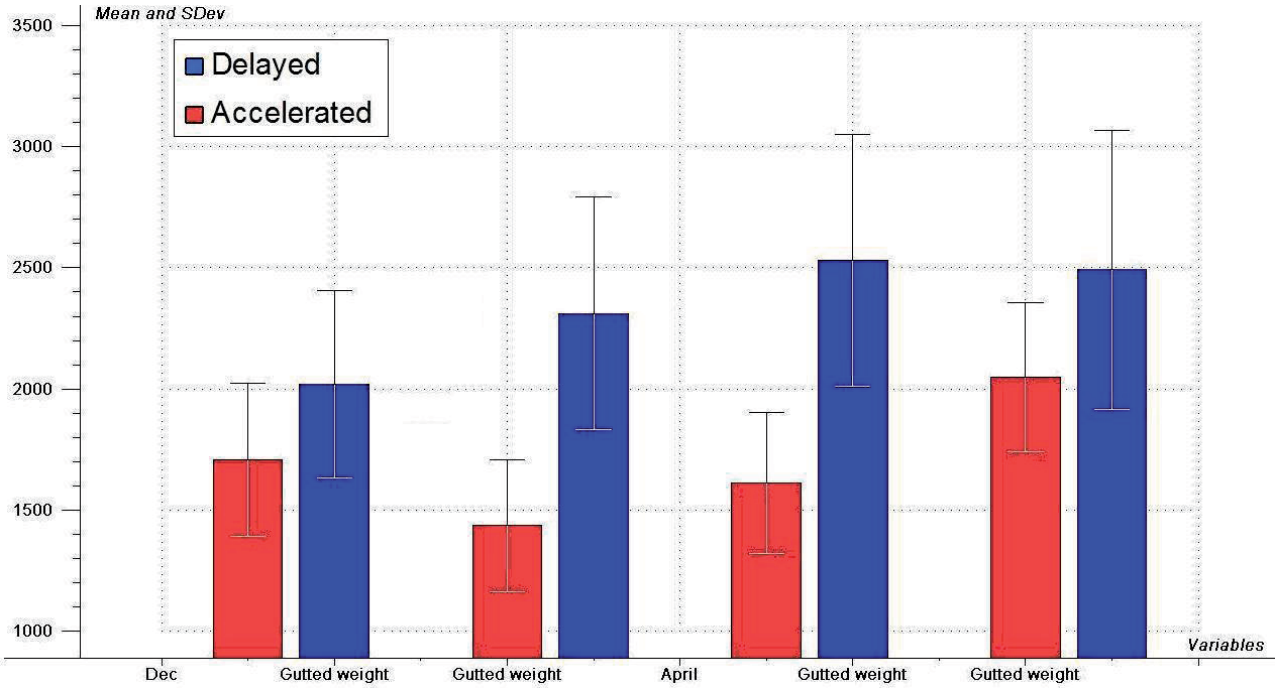

Figure 4: Female gutted weight (kg) during the sampling period, for the accelerated (red) and delayed (blue) groups. The Bar Chart shows the average value and the standard deviation together. The vertical bar is the average value, and the standard deviation is shown as an error bar around the average. A description of significance levels can be found in appendix 2.

In the accelerated group there was 1.71 kg in December. One-way ANOVA showed a significant decrease to 1.44 kg in February. From February to June, there was a significant increase in gutted weight to 2.05 kg.

In December, the delayed group was 2.01 kg and had a significant increase to 2.31 kg in February. There were no significant increases from February to June (2.49 kg).

Figure 5 shows the gutted weight for male cod with the different light treatments from December to June. There was a significant difference between the treatments from December through April, with no significant differences in June (Appendix 2: Table 5).

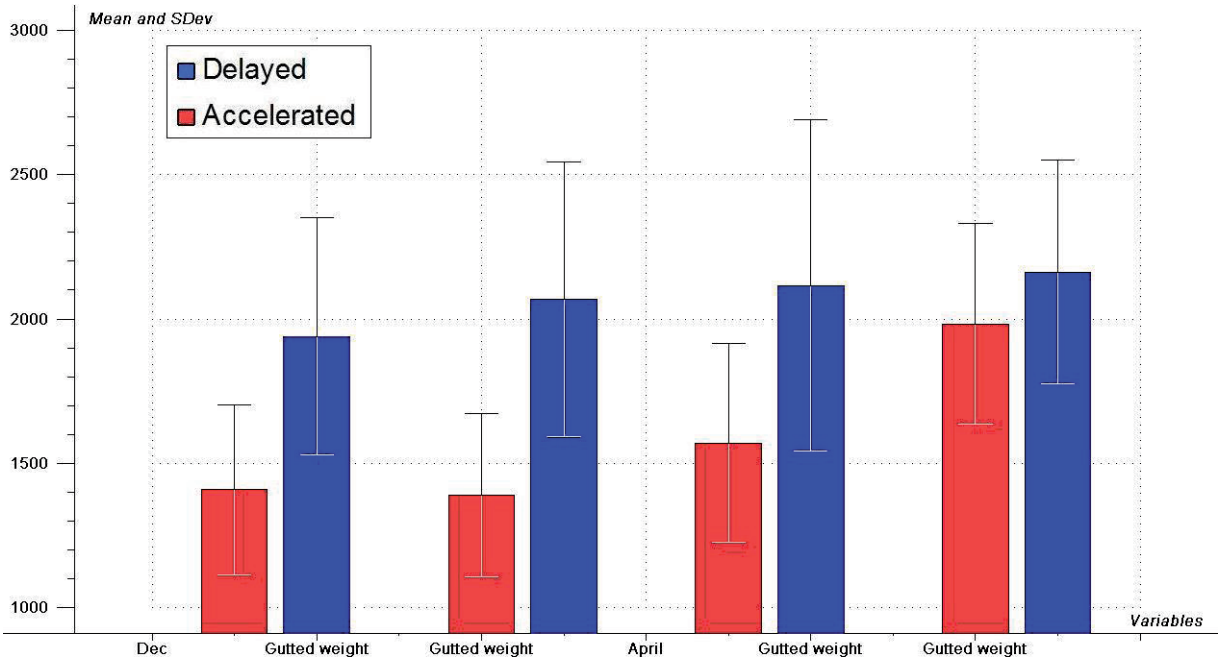

Figure 5: Male gutted weight (Kg) during the sampling period, for the accelerated (red) and delayed (blue) groups. The Bar Chart shows the average value and the standard deviation together. The vertical bar is the average value, and the standard deviation is shown as an error bar around the average. A description of significance levels can be found in appendix 2.

The accelerated group had a gutted weight of 1.41 kg in December. One-way ANOVA showed there was no significant difference between December and February. From February to June, there were significant increases in gutted weight, up to 1.98 kg.

The delayed group had a gutted weight of 1.94 kg in December. One-way ANOVA showed there was no significant difference between December and June, with a final mean weight of 2.16 kg.

3.3 Condition factor

Figure 6 shows the Condition factor for female cod with the different light treatments from December to June. There was no significant difference between the treatments in December. In February and April there was a significant difference between the treatments, and in June the C-factor was 0.92, with no difference between the groups (Appendix 2: Table 4).

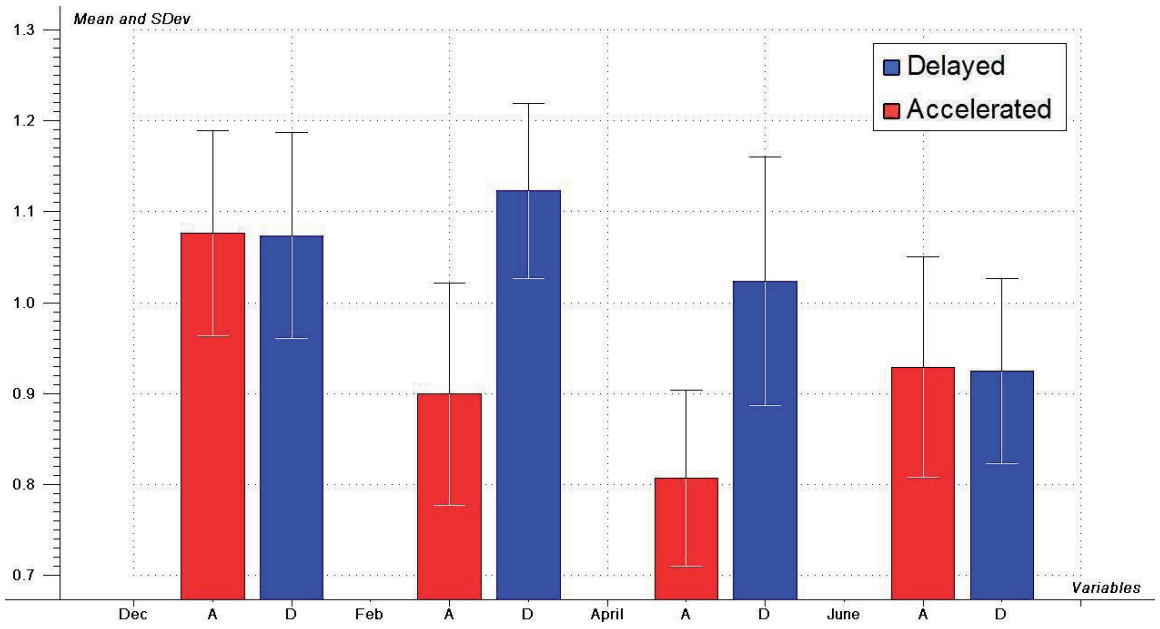

Figure 6: Female gutted C-factor during the sampling period, for the accelerated (red) and delayed (blue) groups. The bar chart shows the average value and the standard deviation together. The vertical bar is the average value, and the standard deviation is shown as an error bar around the average. A description of significance levels can be found in appendix 2.

In the accelerated group, a one-way ANOVA showed a significant decrease in the C-factor between and February and April, followed by a significant increase in June.

In the delayed group, from December to February, there was a non-significant increase to 1.12, followed by a significant decrease in April and June (0.92).

Figure 7 shows the C-factor for male cod with the different light treatments from December to June. There was no significant difference between the treatments in December. In February and April there was a significant difference between the treatments, and in June there was no difference between the groups (Appendix 2: Table 5).

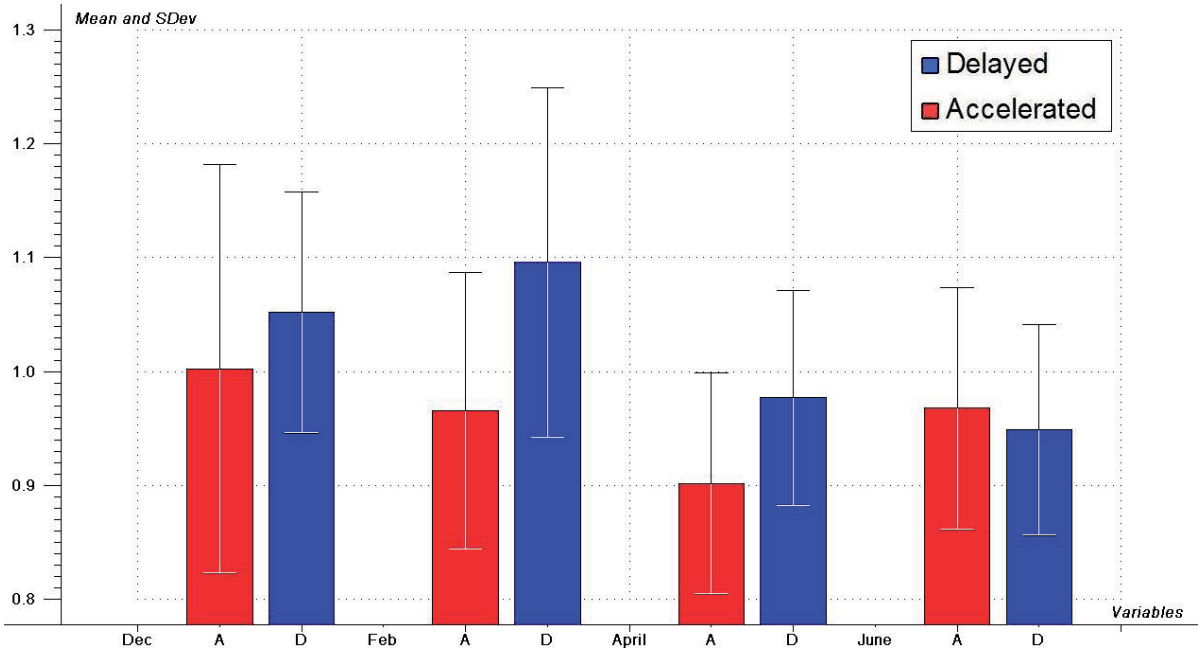

Figure 7: Male gutted C-factor during the sampling period, for the accelerated (red) and delayed (blue) groups. The bar chart shows the average value and the standard deviation together. The vertical bar is the average value, and the standard deviation is shown as an error bar around the average. A description of significance levels can be found in appendix 2.

In the accelerated group, a one-way ANOVA showed there was no significant difference between December and February, followed by a significant decrease to April, and a significant increase in the C-factor to June (0.96).

In the delayed group, there were no significant change from December to February (1.09) followed by a significant decrease to April and further non-significant decrease to June (0.94). However, there were significant differences between the December/February and the June samplings.

3.4 Gonado somatic index

Figure 8 shows the GSI for female cod with the different light treatments from December to June. There was a significant difference between the treatments from December through June (Appendix 2: Table 4).

The GSI had a significant increase from 4.7% to 22.4% between December and February in the accelerated group. There was a significant decrease to 3.0% between February and April, with no further changes between April and June.

A one-way ANOVA showed a significant increase from December (1.9%) to February (4.1%) in the delayed group, a further significant increase to April (6.2%), and a strong increase to 13.7% in June.

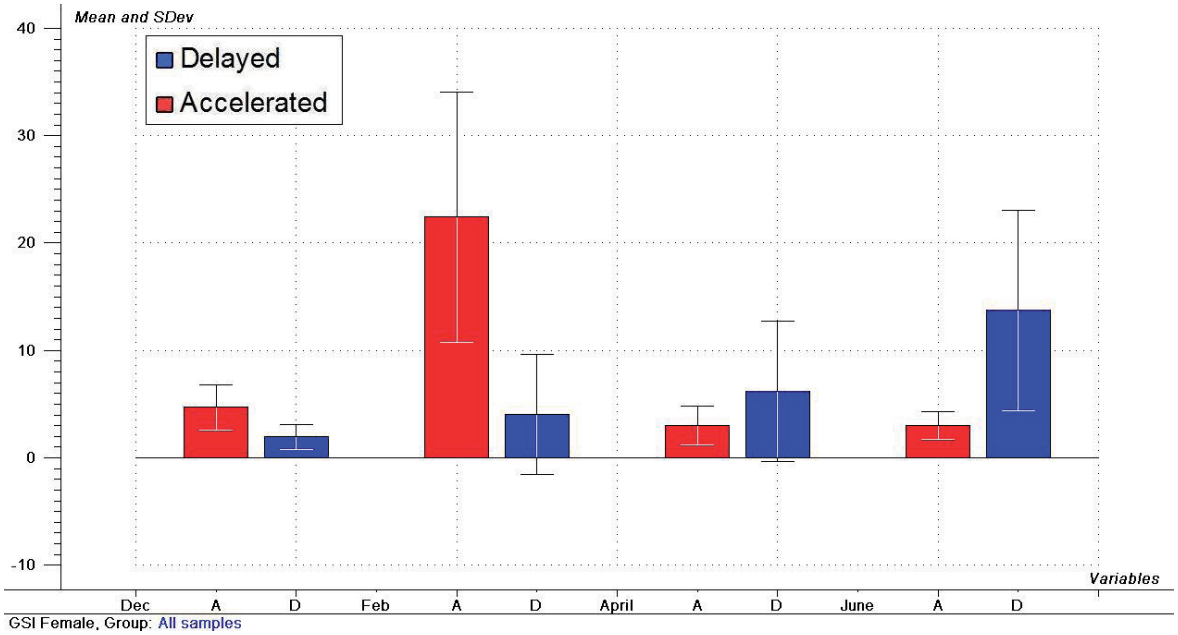

Figure 8: The female gonado somatic index (GSI %) is calculated as a percentage of the gutted weight during the sampling period, for the accelerated (red) and the delayed (blue) light treatments. The GSI is shown here using a bar chart, which shows the average value and the standard deviation together. A description of significance levels can be found in appendix 2.

Figure 9 shows the GSI for male cod with the different light treatments from December to June. There was a significant difference between the treatments from December through June with the exception of February, where no significant differences were found (Appendix 2: Table 5).

A one-way ANOVA showed the accelerated group had a significant decrease in the GSI from December (9.5%), February (2.8%) and April (1.3%). A significant increase to 2.3% was shown in June.

The delayed group had a significant increase from December (0.8%), February (4.5%) and April (7.8%), and a non-significant increase in June (8.9%).

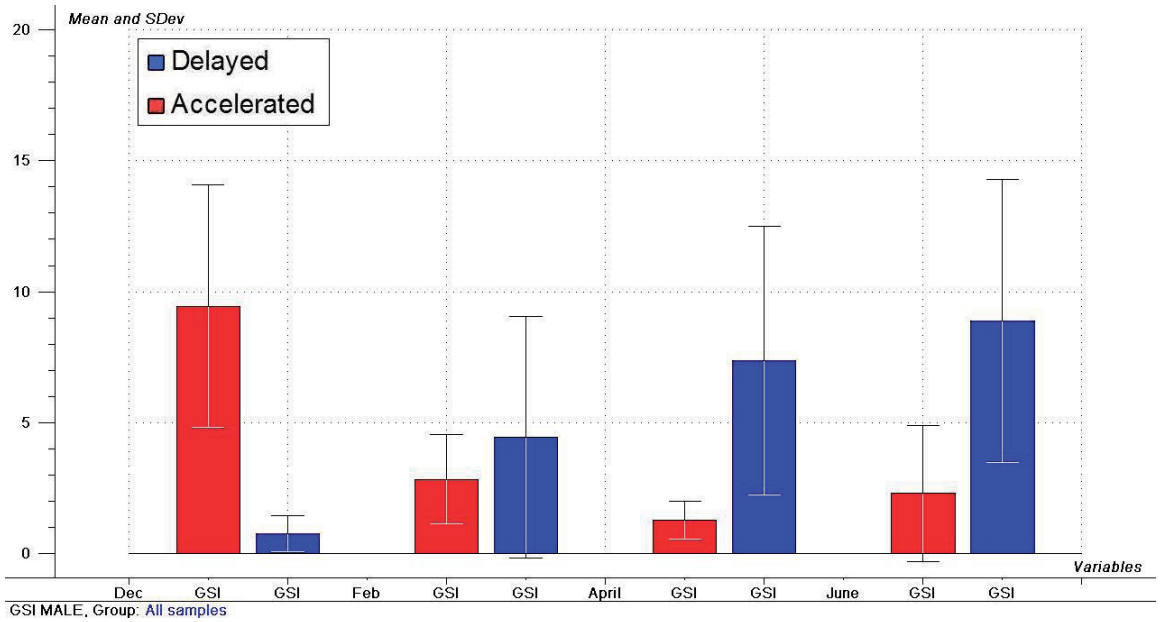

Figure 9: The male gonado somatic index (GSI %) is calculated as a percentage of the gutted weight during the sampling period, for the accelerated (red) and the delayed (blue) light treatments. The GSI is shown here using a bar chart, which shows the average value and the standard deviation together. A description of significance levels can be found in appendix 2.

3.4.1 Gonad index assessment

The average maturity levels for females in December, at the beginning of the experiment, were assessed at stage 2 for 100% of the samples in both the accelerated and delayed groups. In February, the accelerated group saw 8% of the females to be stage 2, 29% in stage 3 and 63% being assessed as being in stage 4 of maturity (Table 2).

Table 2: Average maturity levels for female codfish throughout the experimental sampling period, December to June. All values are given in percentage within each maturity stage and light treatment group.

Stage	1		2		3		4		5		6	
Group	**Acc**	**Del**	**Acc**	**Del**	**Acc**	**Del**	**Acc**	**Del**	**Acc**	**Del**	**Acc**	**Del**
December	*0*	*0*	*100*	*100*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
February	*0*	*0*	*8*	*89*	*29*	*7*	*63*	*4*	*0*	*0*	*0*	*0*
April	*0*	*0*	*94*	*94*	*6*	*6*	*0*	*0*	*0*	*0*	*0*	*0*
June	*0*	*0*	*0*	*63*	*0*	*10*	*0*	*17*	*0*	*3*	*100*	*7*

The delayed group however, saw 89% of the females to be in stage 2, with 7% in stage 3, and 4% of the females in stage 4 of maturity.

In April, 94% of the females in both the accelerated and delayed groups were assessed to be in stage 2, with 6% of the females at stage 3. During the final month of sampling, in June, the accelerated group showed 100% of the females sampled to be in stage 6 (spent).

The delayed group, still showed the majority (63%) of the sampled fish to be in stage 2, with 10% in stage 3, 17% in stage 4, 3% in stage 5 and finally, 7% were assessed to be in stage 6.

Table 3: Average maturity levels for male codfish throughout the experimental sampling period, December to June. All values are given in percentage within each maturity stage and light treatment group.

Stage	1		2		3		4		5		6	
Group	**Acc**	**Del**	**Acc**	**Del**	**Acc**	**Del**	**Acc**	**Del**	**Acc**	**Del**	**Acc**	**Del**
December	*0*	*0*	*3*	*87*	*0*	*13*	*97*	*0*	*0*	*0*	*0*	*0*
February	*0*	*0*	*0*	*0*	*76*	*69*	*24*	*28*	*0*	*0*	*0*	*3*
April	*0*	*0*	*3*	*7*	*97*	*11*	*0*	*82*	*0*	*0*	*0*	*0*
June	*0*	*0*	*0*	*0*	*0*	*6*	*0*	*91*	*0*	*0*	*100*	*3*

The average maturity level for males in December, at the beginning of the experiment, for the accelerated group were assessed as 3% being in stage 2, with 97% being in stage 3 of maturity (Table 3). The delayed group showed 87% being in stage 2, with 13% being in stage 3. In February, the accelerated group showed 76% of its males to be in stage 3, with 24% being in stage 4. The delayed group showed 69% to be in stage 3, with 24% being at stage 4 of maturity. In April, the accelerated group assessed 97% of its males to be in stage 3 and 3% were in stage 2. The delayed group had the majority of its males in stage 4, with 82%, 11% in stage 3, and 7% of the males were assessed as being in stage 2.

At the final stage of sampling, in June, the accelerated group for males was assessed as being 100% in stage 6 (spent).

The delayed group had 91% assessed to be in stage 4, with 6% in stage 3 and 3% were found to be in stage 6 (spent).

3.5 Hepato somatic index

Figure 10 shows the HSI for female cod with the different light treatments from December to June. There was a no significant difference between the treatments in December and February, but a decrease in the delayed group created a significant difference between the groups in April and June (Appendix 2: Table 4).

A one-way ANOVA showed there were no significant differences in the accelerated group between December (17.2%), February (17.2%) and April (17.8%). A significant increase occurred between April and June (20.4%).

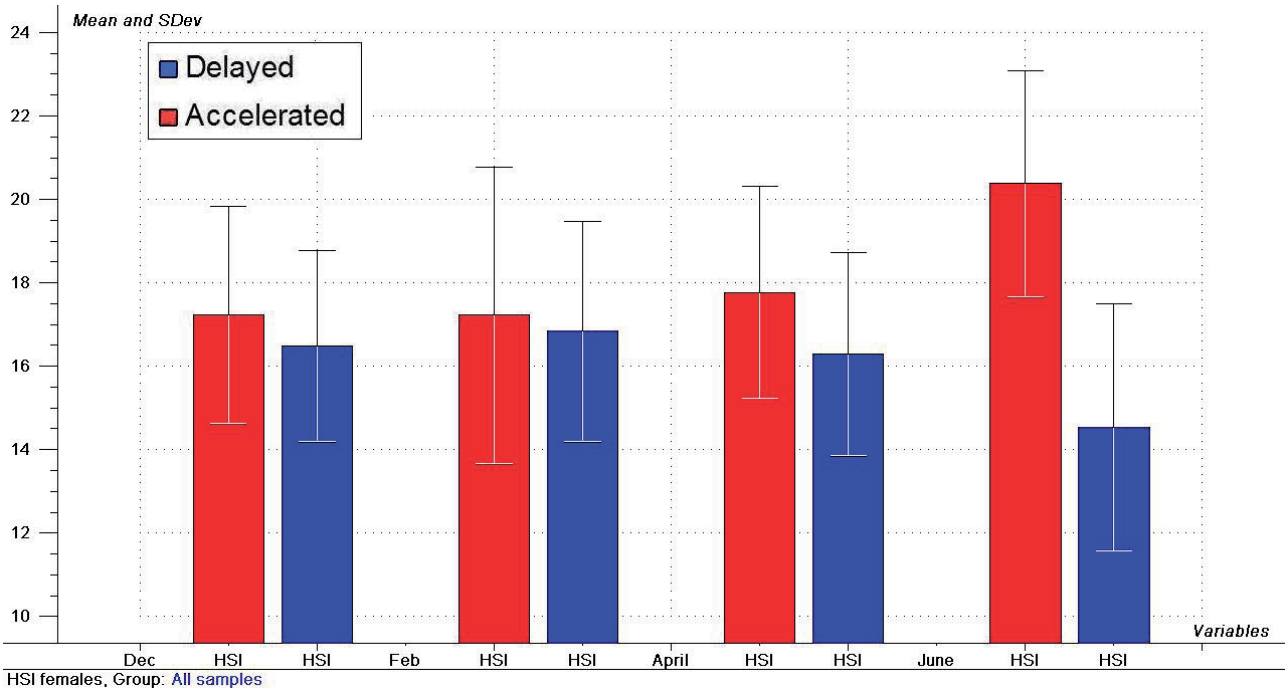

Figure 10: The female hepato somatic index (HSI %) is the liver weight calculated as a percentage of the whole body weight during the sampling period, for the accelerated (red) and the delayed (blue). The HSI is shown here using a bar chart, which shows the average value and the standard deviation together. A description of significance levels can be found in appendix 2.

In the delayed group, a one-way ANOVA showed there were also no significant differences between December (16.5%), February (16.8%), and April (16.3). A significant decrease was shown between April and June (14.5%).

Figure 11 shows the HSI for male cod with the different light treatments from December to June. There was a no significant difference between the treatments in December, but a significant difference between the groups for the remainder of the samplings. (Appendix 2: Table 5).

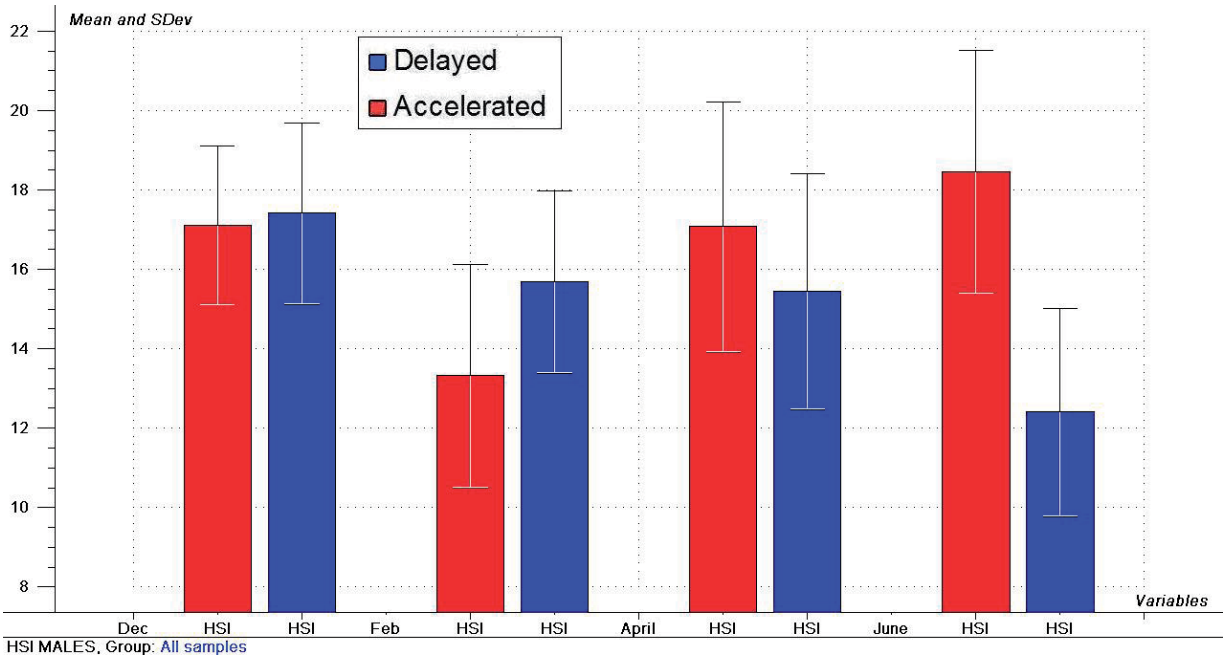

Figure 11: The male hepato somatic index (HSI %) is the liver weight calculated as a percentage of the whole body weight during the sampling period, for the accelerated (red) and the delayed (blue) light treatments. The HSI is shown here using a bar chart, which shows the average value and the standard deviation together. A description of significance levels can be found in appendix 2.

In the accelerated group, a one-way ANOVA showed a significant decrease between December (17.1%) and February (13.3%), a significant increase to April (17.1%), with a further non-significant decrease between April and June (18.5%).

In the delayed group, there was a significant decrease between December (17.4%) and February (15.7%), with no significant change to April (15.4%). A significant decrease between April and June (12.4%) was shown.

3.6 Muscle water content

Figure 12 shows the muscle water content for female cod with the different light treatments from December to June. There were significant differences between the treatments from December to June (Appendix 2: Table 4).

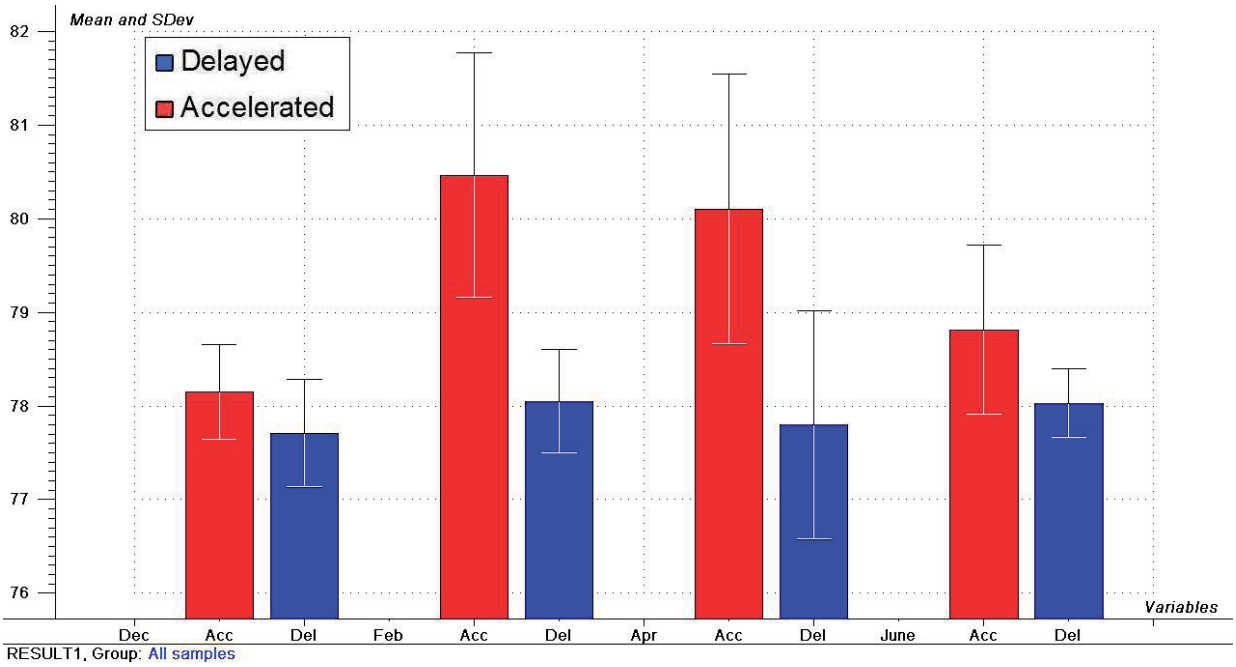

Figure 12: The percentage of muscle water content for female cod throughout the sampling period, for the accelerated (red) and delayed (blue) groups. Each is represented by using a bar chart, which shows the average value and the standard deviation together. A description of significance levels can be found in appendix 2.

In the accelerated group, a one-way ANOVA showed there was a significant increase between December (78.2%) to February (78.5%), with no significant decrease between February and April (78.1%), but a further significant decrease to 78.8% in June.

In the delayed group, there was a significant increase from 77.7% in December to 78.1% in February, followed by a significant decrease to 77.8% in April, and a significant increase to 78.0% in June.

Figure 13 shows the muscle water content for male cod with the different light treatments from December to June. There were significant differences between the treatments from December to June (Appendix 2: Table 5).

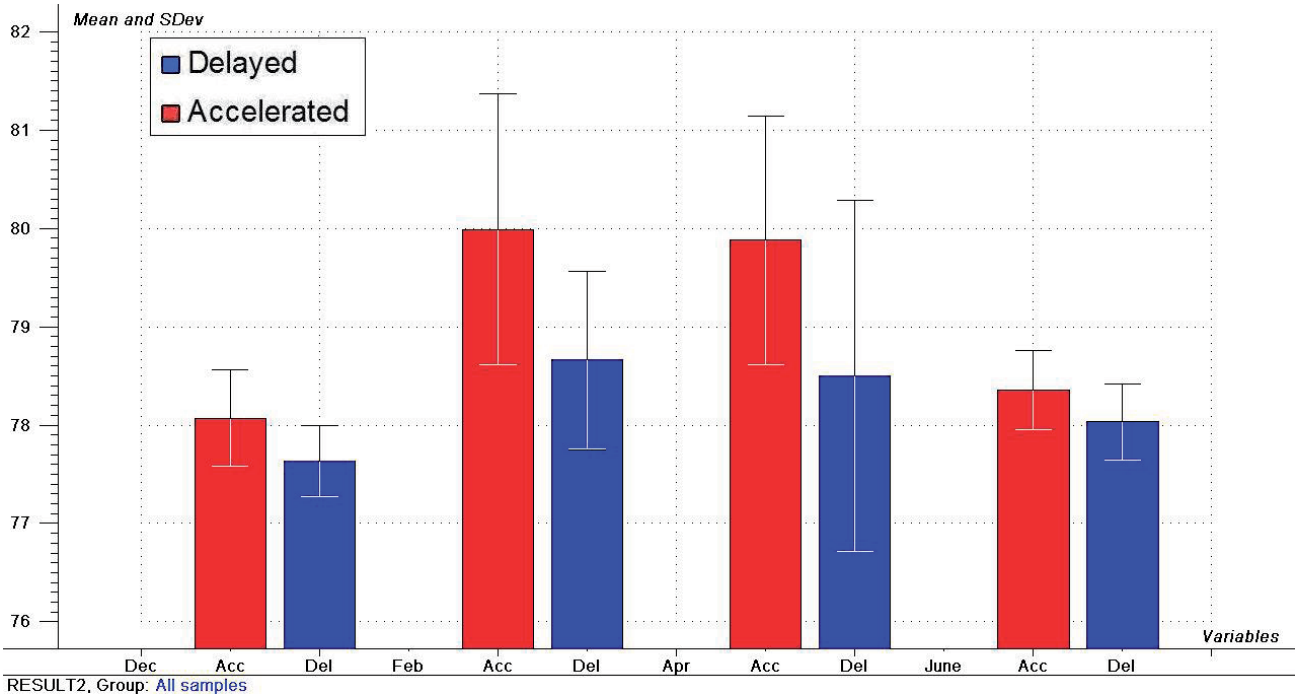

Figure 13: The percentage of muscle water content for male cod throughout the sampling period, for the accelerated (red) and delayed (blue) groups. Each is represented by using a bar chart, which shows the average value and the standard deviation together. A description of significance levels can be found in appendix 2.

One-way ANOVA showed that the accelerated group had a significant increase from 78.1% in December to 80.0% in February, a non-significant decrease in April to 79.9% and a significant decrease to 78.4% in June.

The delayed group had a significant increase from 77.6% in December to 78.7% in February, with a non-significant decrease to 78.5% in April, and a further non-significant reduction to 78.0% in June.

3.7 Protein content

Figure 14 shows the muscle water content for female cod with the different light treatments from December to June. There were no significant differences between the treatments in December and June, whereas significant differences were shown in February and April (Appendix 2: Table 4).

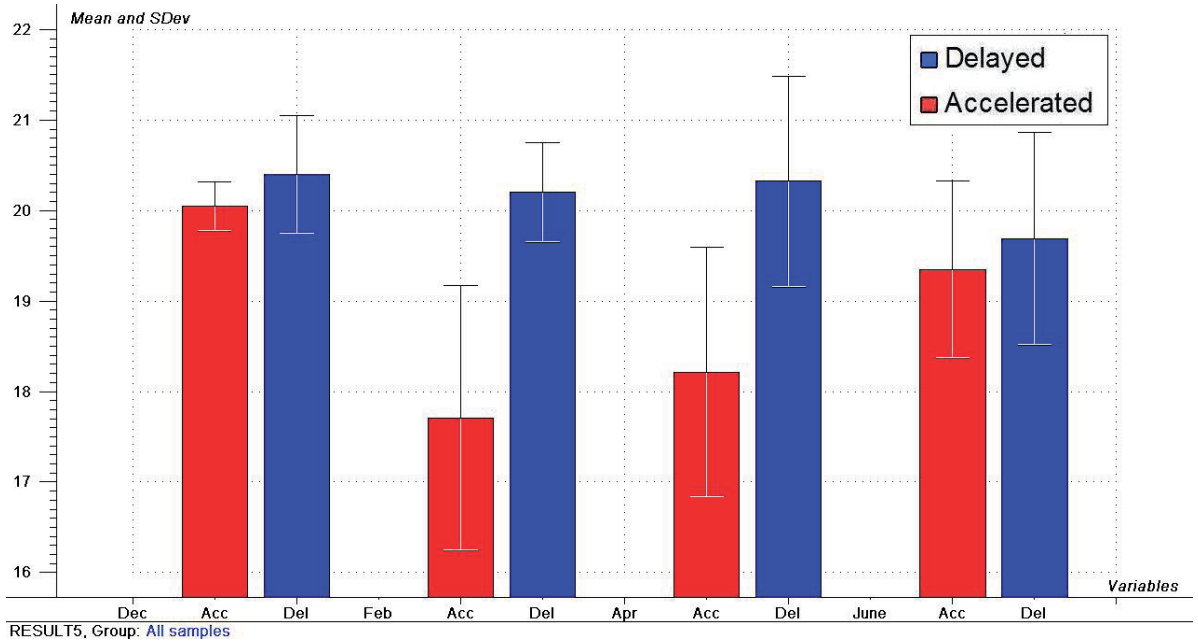

Figure 14: The percentage of protein content in females throughout the sampling period, for the accelerated (red) and delayed (blue) groups. A description of significance levels can be found in appendix 2.

A one-way ANOVA in the accelerated group showed a significant decrease from 20.1% in December to 17.7% in February, a significant increase to 18.2% in April and a further significant increase to 19.4% in June.

The delayed group did not show any significant differences between December (20.4%), and February (20.2%). There was a significant (P = 0.03) decrease between February and April (20.3%), as well as a significant decrease was shown between April and June (19.7%).

Figure 15 shows the muscle water content for male cod with the different light treatments from December to June. There were no significant differences between the treatments from December to June. (Appendix 2: Table 5).

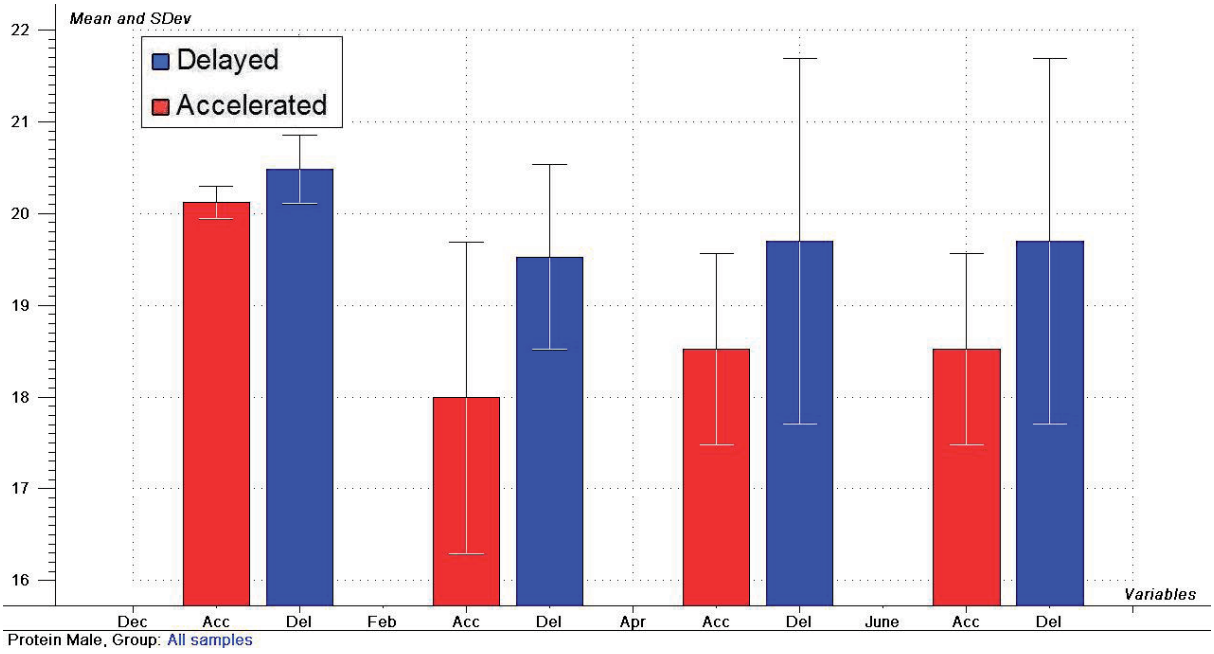

Figure 15: The percentage of protein content in males throughout the sampling period, for the accelerated (red) and delayed (blue) groups. A description of significance levels can be found in appendix 2.

A one-way ANOVA in the accelerated group showed a significant decrease from 20.1% in December to 18.0% in February, a significant increase to 18.5% April with no further increase in June (18.5%).

In the delayed group, there was a significant decrease from December (20.5%) to February (19.5%), with no further significant changes in April (19.7%) and June (19.7%).

A strong correlation (r^2=0.89) was found between the muscle water content and the protein content (Figure 16) between the males and females combined, in both the accelerated and delayed groups.

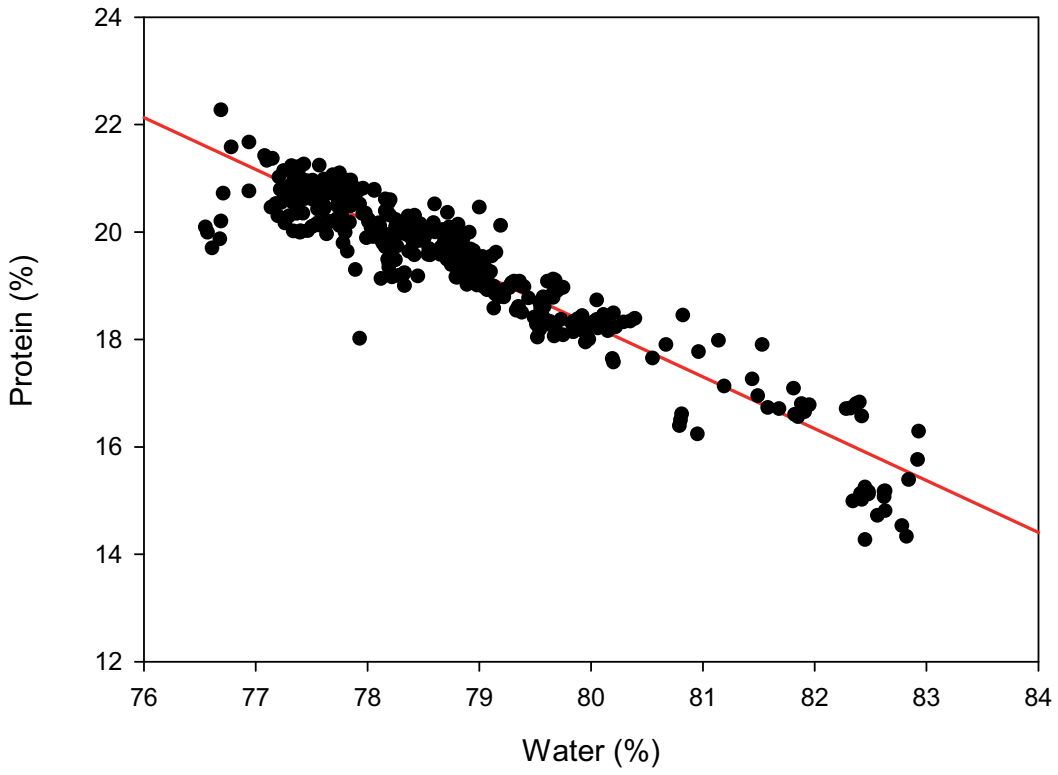

Figure 16: Showing a very good linear fit between protein and water (r^2=0.89).

The strong linear relationship between the water and protein indicates that the majority of protein lost is replaced with water.

3.8 Principal component analysis (PCA)

As can be seen in Figure 17, female and male fish in both groups had a seasonal variation. The female fish in the accelerated group appeared to have the largest variation, whereas the male fish in the delayed group appeared to have the smallest variation throughout the season.

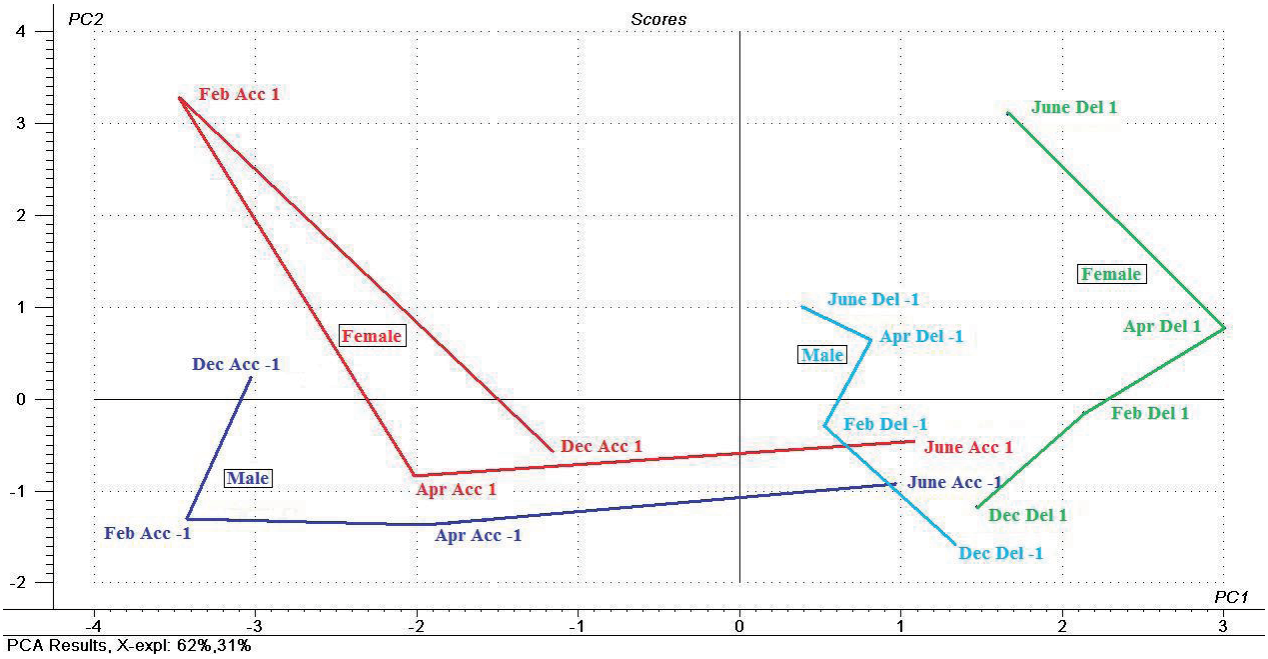

Figure 17: Score plot showing variation in PC1 and PC2. The female fish are varied throughout the plot, and are coloured red and green (accelerated and delayed, respectively). The male fish have also shown a wide variation, and are coloured dark and light blue (accelerated and delayed, respectively).

Multivariate analysis was conducted and revealed 93% of the variation between groups and sexes could be explained by the differences in: water, GSI, gonad, whole weight, gutted weight, liver and protein (Figure 18).

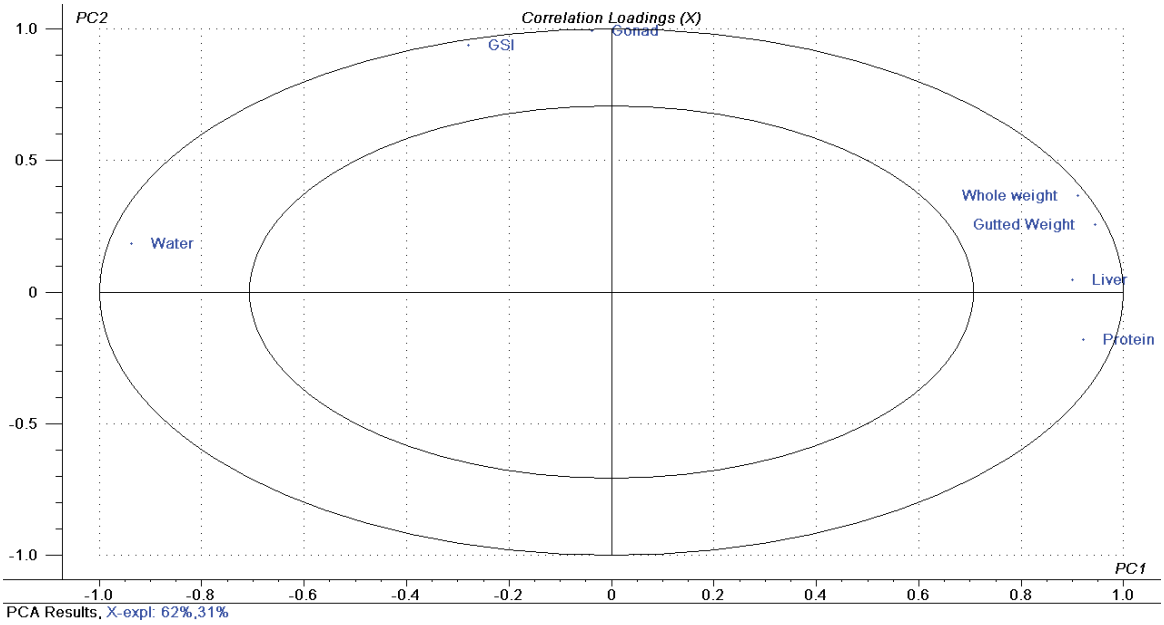

Figure 18: Loadings plot showing seven parameters which explain 93% of the variation between the sampling dates, groups and sexes

4.0 Discussion

This study looked at the effect of using light treatments as a means to manipulate the time for sexual maturation in farmed Atlantic cod. 480 cod were sampled to compare the effect of adding artificial light in August (delayed maturation), or in November (accelerated maturation).
An additional 120 cod from an "out-graded" group were analyzed as a control group. Maturation in this group occurred in April for the female cod, which were similar to small scale experiments that have been conducted (Amble, 2007; Solberg and Willumsen, 2008).
When looking at certain biological differences, it must be noted that while the ratio between groups (accelerated:delayed) stayed consistent, the sex ratio (male:female) was more varied throughout the entire trial period. Therefore, this must be considered when assessing the differences between the sexes.

4.1 Length

The length increased in all groups throughout the experiment, but to a different degree. The female fish showed a significant difference between the accelerated and delayed groups throughout the entire experiment, with the delayed group showing significantly larger lengths. The delayed male cod had a significantly larger length than the accelerated male cod throughout the experiment, with a small decrease towards the end of the experiment, which was not considered to be significant. Overall, length was not heavily influenced by the onset of maturation, as it continued to increase throughout the experiment, in both male and females with accelerated and delayed maturities.

4.2 Gutted weights

A comparison of the male and female gutted weights was completed for the experimental period between the accelerated and delayed groups.

Despite the water temperature decrease between February and April (Figure 1), a significant growth was encountered in the delayed gutted weights in the males and females. Throughout the experimental period, both the male and female cod in the delayed group experienced significant growth, although the slight increase in weight for males between April and June was not considered to be significant.

The female cod from the accelerated group had an overall lower gutted weight in February, and at this time, 63% of all females sampled from the accelerated group were assessed as being in stage 4 of maturity. A stage 4 maturity indicates that the female is at a pre-spawning level and therefore results in a significant weight loss for the fish (Taranger et al., 2006).

The female fish appeared to have more of a variation in gutted weight when compared with the male fish within the accelerated and delayed groups. This may indicate that female codfish experience more muscle loss during spawning than male codfish, which would agree with previous observations as seen by Solberg and Willumsen, 2008.

4.3 Condition factor

The C-factor indicates the degree of well-being of a fish species. Variations in the C-factor may be caused by sexual maturity, degree of nourishment, fish age and in many species, sex differences. An undernourished/thin fish will have a C-factor of less than 0.7 (Lambert and Dûtil, 1997), while an adequately fed or large fish will have a C-factor typically greater than 1.0. The C-factor tends to decrease when sexual maturation approaches. When calculating the C-factor, the gutted weight was used instead of the whole weight, as this method removes any variation related to gonad and/or liver size, thus giving a more precise depiction of the condition of the fish (Solberg et al., 2006).

There was a strong decrease in the C-factor for both sexes in the accelerated group between December and April, and showed the start of an increase during the final sampling in June, which was similar to earlier results by Amble (2007).
Both sexes in the delayed group had an increased C-factor during December and February, indicating a muscle growth (even at low temperatures) if the fish is not maturing. The C-factor started to decrease between April and June.

The female fish had a significantly lower C-factor in the accelerated group compared to the delayed group, in both February and April, indicating an accelerated maturation in that group. The male fish also experienced a significantly lower C-factor in the accelerated group than in the delayed group between February and April, which showed the indications of an accelerated maturation. There appeared to be more differences in the females of the accelerated and delayed

groups than there were in the males. This may be because female fish tend to lose more muscle mass during the development of eggs.

4.4 GSI

The GSI is defined as the proportion of reproductive tissue in the body of a fish to total body weight, and was used to help estimate the reproductive condition of the fish. An increase in GSI indicates the start of maturation, where a decline in GSI takes place after spawning has occurred.

The GSI in the delayed group was significantly lower than the accelerated group. The females in the accelerated group experienced a large growth of gonads in February, which produced a decrease in the gutted weight of the same group. However, an increase in the gonads with the females in the delayed group did not result in a significant decrease in gutted weight during the same period.

The females in the accelerated group showed a larger mean GSI in February (22.4%) during the peak of spawning, than the females from the delayed group, which showed a mean GSI of 13.7% during spawning in June, but the peak for GSI may be in July.

The GSI in males in the accelerated group was 9.5% in December and decreased to 2.8% in February, indicating that the GSI peak may be in January. The males in the delayed group had a mean GSI of 8.9% during spawning in June. The males in the delayed group showed a continual significant increase of GSI through the experimental period. This again indicates that the peak for maturation in the delayed group could be in July.

Both sexes (100%) in the accelerated group were assessed as being in stage 6 of maturation or spent (Tables 2-3) at the final sampling in June, whereas the delayed group showed 63% of the females were assessed as being in stage 2, and 91% of males were assessed as being in stage 4.

4.5 HSI

The hepatosomatic index (HSI) is the liver weight as a percentage of the whole body weight. It is associated with the energy content of liver, but is also linked to the lipid content of cod. According to a study by Lambert and Dutil (1997), as the muscle energy content reaches its maximum and the somatic energy content grows high, the energy storage in liver (HSI) will increase.

The mean HSI in females did not show a significant change in the accelerated or delayed groups during the experimental period. In June, however, significant increases were experienced in the accelerated light treatment for both sexes. During the peak of spawning, the mean HSI in the accelerated group did not show a significant change; instead the muscle protein content was reduced by approximately 2%. The delayed maturation, however, had a large significant reduction in the mean HSI, but no significant changes in the muscle protein content.

The mean HSI in males in the accelerated group showed a significant decrease in February and after maturation the HSI increases. In the male delayed group the HSI decreased from December (17.4%) to 12.4% in June.

During spawning, it has been suggested that male cod may have a lower HSI than the females (Solberg and Willumsen, 2007), which could indicate that males use more energy from their liver throughout spawning than that of the females. A similar study completed by Taranger et al., (2006) showed that a decrease in HSI was more pronounced in males than females, possibly suggesting that the reproductive cost was higher in males than females.

4.6 Chemical analyses

Chemical analyses was performed (approximately one in five) for both protein and muscle water content. All of the samples were analyzed using near infrared transmission (NIT) spectroscopy. When looking at the muscle water content, the results showed that there were significant differences between males and females in the accelerated and delayed groups throughout the trial period. The accelerated group had an increase in water content between February and April (throughout maturation) for both the male and females, with the females experiencing the highest mean water content of the sexes in February with 80.5%. A decrease in water content for both male and females was seen in June.

Male fish in the accelerated group had significantly higher water content than male fish in the delayed group between February and April. This would suggest that the male fish in the accelerated group had lost a percentage of its muscle protein during maturation. The muscle water content of the males did not increase as greatly as did the females in both of the groups. As noted by Amble (2007), this might be explained by the differences in sexes in relation to their needs for developing gonads during maturation and spawning.

Because there was a strong positive correlation (r^2=0.89) between the water and protein content (figure 13), the differences between the sexes and groups types can be effectively considered by focusing on only one of these variables.

5.0 Conclusions

The addition of artificial light in November 2007 (accelerated group) resulted in approximately a two month earlier maturation in cod, with a peak of spawning shown in February. This earlier maturation resulted in lower muscle water content, higher protein content and a higher C-factor of the fish in June, which are indications of a better fish quality. The addition of artificial light in August 2007 (delayed group) resulted in a postponement of maturation by 2-3 months, with a peak spawning in June.

Previous results from accelerated maturation in small net pens (Amble, 2007), coincided with the results from this experiment, which indicated similar results when it was performed in commercial scale (60,000 fish/netpen), as compared with the small scale (500 fish) experiment. The experiment concluded that it was possible to delay the maturation in Atlantic cod, although further delay of maturation is preferred.

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Appendix 1: Control group results

Table 4: Total fish sampled by sex, mean values and standard deviations (SD), in the control group for all dates throughout the sampling period.

Type	Month	Female	SD	Male	SD
Total Fish	December	10	n/a	19	n/a
Total Fish	February	10	n/a	19	n/a
Total Fish	April	14	n/a	18	n/a
Total Fish	June	13	n/a	18	n/a
GW (kg)	December	1.33	0.18	1.11	0.14
GW (kg)	February	1.48	0.26	1.21	0.23
GW (kg)	April	0.96	0.24	1.15	0.24
GW (kg)	June	1.40	0.23	1.46	0.29
L (cm)	December	49.70	3.92	48.60	2.18
L (cm)	February	52.10	2.87	50.24	3.60
L (cm)	April	51.04	3.16	51.94	1.77
L (cm)	June	55.96	3.16	54.58	4.36
Maturity	December	2.00	n/a	4.0	n/a
Maturity	February	2.40	n/a	3.5	n/a
Maturity	April	3.40	n/a	3.0	n/a
Maturity	June	6.0	n/a	6.0	n/a
C-factor	December	1.10	0.25	0.96	0.08
C-factor	February	1.05	0.18	0.95	0.12
C-factor	April	0.71	0.12	0.81	0.10
C-factor	June	0.79	0.07	0.90	0.13
GSI (%)	December	4.40	1.69	12.18	3.67
GSI (%)	February	15.58	7.74	13.51	3.75
GSI (%)	April	14.93	11.00	2.30	2.61
GSI (%)	June	1.75	0.26	0.65	0.22
HSI (%)	December	17.43	2.60	17.81	2.19
HSI (%)	February	16.74	2.44	13.33	2.22
HSI (%)	April	15.45	3.90	12.85	3.14
HSI (%)	June	17.20	4.97	15.84	2.79

Appendix 2: Mean values and test results

Table 4: Mean values, standard deviations (SD), and test results for female fish, between the accelerated and delayed groups for all dates throughout the sampling period.

		Accelerated		Delayed			
Type	Month	Mean	SD	Mean	SD	P-value	Test
GW (kg)	December	1.71	0.32	2.01	0.39	0.002	t-test
GW (kg)	February	1.44	0.27	2.31	0.48	<0.001	t-test
GW (kg)	April	1.61	0.29	2.53	0.52	<0.001	Mann-Whitney
GW (kg)	June	2.05	0.31	2.49	0.57	<0.001	Shapiro-Wilk
L (cm)	December	54.0	3.36	56.7	3.77	0.008	t-test
L (cm)	February	54.2	3.47	58.8	3.62	<0.001	t-test
L (cm)	April	58.3	2.72	62.6	4.34	<0.001	Mann-Whitney
L (cm)	June	60.4	3.89	63.8	4.83	0.006	t-test
C-factor	December	1.07	0.11	1.07	0.11	0.928	t-test
C-factor	February	0.89	0.12	1.12	0.09	<0.001	t-test
C-factor	April	0.81	0.09	1.02	0.14	<0.001	Mann-Whitney
C-factor	June	0.92	0.12	0.92	0.12	0.894	t-test
GSI (%)	December	4.69	0.21	1.94	0.11	<0.001	Mann-Whitney
GSI (%)	February	22.43	1.17	4.05	0.56	<0.001	Mann-Whitney
GSI (%)	April	3.00	0.18	6.21	0.65	0.007	Mann-Whitney
GSI (%)	June	3.01	0.13	13.73	0.94	<0.001	Mann-Whitney
HSI (%)	December	17.23	0.26	16.48	0.23	0.253	t-test
HSI (%)	February	17.22	0.36	16.84	0.26	0.638	t-test
HSI (%)	April	17.77	0.25	16.31	0.24	0.019	t-test
HSI (%)	June	20.38	0.27	14.54	0.30	<0.001	t-test
Water	December	78.15	0.50	77.71	0.57	0.012	Mann-Whitney
Water	February	80.47	1.31	78.05	0.55	<0.001	Mann-Whitney
Water	April	80.11	1.44	77.80	1.22	<0.001	Mann-Whitney
Water	June	78.82	0.91	78.03	0.37	0.002	Mann-Whitney
Protein	December	20.05	0.27	20.40	0.65	0.053	Mann-Whitney
Protein	February	17.71	1.46	20.20	0.55	<0.001	Mann-Whitney
Protein	April	18.22	1.38	20.33	1.16	<0.001	Mann-Whitney
Protein	June	19.35	0.97	19.69	1.17	0.242	Mann-Whitney

Table 5: Mean values, standard deviations (SD), and test results for male fish, between the accelerated and delayed groups for all dates throughout the sampling period.

		Accelerated		Delayed			
Type	**Month**	**Mean**	**SD**	**Mean**	**SD**	**P-value**	**Test**
GW (kg)	December	1.41	0.29	1.94	0.41	<0.001	t-test
GW (kg)	February	1.39	0.28	2.07	0.48	<0.001	Mann-Whitney
GW (kg)	April	1.57	0.34	2.12	0.57	<0.001	Mann-Whitney
GW (kg)	June	1.98	0.35	2.16	0.29	0.052	t-test
L (cm)	December	51.3	3.54	56.7	3.95	<0.001	t-test
L (cm)	February	52.3	3.58	57.2	4.38	<0.001	t-test
L (cm)	April	55.6	3.89	59.6	4.39	<0.001	t-test
L (cm)	June	58.9	3.08	60.5	4.04	0.081	t-test
C-factor	December	1.00	0.17	1.05	0.10	0.259	Mann-Whitney
C-factor	February	0.96	0.12	1.09	0.15	<0.001	Mann-Whitney
C-factor	April	0.90	0.09	0.97	0.09	0.005	t-test
C-factor	June	0.96	0.10	0.94	0.09	0.447	t-test
GSI (%)	December	9.45	0.46	0.77	0.67	<0.001	Mann-Whitney
GSI (%)	February	2.84	0.17	4.45	0.46	0.816	Mann-Whitney
GSI (%)	April	1.29	0.72	7.83	0.51	<0.001	Mann-Whitney
GSI (%)	June	2.31	0.26	8.89	0.54	<0.001	Mann-Whitney
HSI (%)	December	17.11	0.20	17.41	0.23	0.578	t-test
HSI (%)	February	13.32	0.28	15.68	0.23	<0.001	t-test
HSI (%)	April	17.08	0.31	15.44	0.30	0.050	t-test
HSI (%)	June	18.46	0.31	12.41	0.26	<0.001	t-test
Water	December	78.07	0.49	77.63	0.36	0.008	Mann-Whitney
Water	February	79.99	1.37	78.66	0.90	<0.001	Mann-Whitney
Water	April	79.88	1.27	78.50	1.78	<0.001	Mann-Whitney
Water	June	78.36	0.40	78.03	0.39	0.021	t-test
Protein	December	20.12	0.18	20.48	0.37	<0.001	Mann-Whitney
Protein	February	17.99	1.69	19.53	1.01	<0.001	Mann-Whitney
Protein	April	18.52	1.05	19.69	1.99	<0.001	Mann-Whitney
Protein	June	18.52	1.05	19.69	1.99	<0.001	Mann-Whitney

Appendix 3: Maturation scale for cod

Table 6: Maturation scale for female cod (Katsiadaki et al., 1999)

Stage	Description
1	Ovary small at beginning of stage, Colourless to pale red with slightly visible blood vessels. Weight is 0.5 – 5g and GSI (gonadosomatic index) ≤ 0.3 %. Sex distinguishable.
2	Ovary enlarges and takes up a bright rose-red colour. The weight is up to 15 g and the GSI is 0.5 to 0.8 %. The blood vessels are thickened. Stage 2 can occur either after spawning or in virgin fish.
3	Ovary red, pink, orange or cream in colour and opaque. Its dimensions increase, it occupies half of the body cavity and the GSI is 1-2 % at the beginning of the stage and 3 – 4% at the end. Oocytes are visible under membrane.
4	Ovary enlarges, filling two-thirds of the body cavity, and takes up an orange colour. The GSI is 5 % at the beginning and 10 % at the end of the stage. The differences in oocyte diameters are visible to the naked eye. Blood vessels distended
5	Ovulating ovaries are filling the body cavity, The GSI reaches 18 – 22%, whilst the presence of hyaline eggs give the ovary a marble appearance. Two types of eggs are visible through the membrane: large opaque and large transparent eggs. With the commencement of spawning actions, the GSI starts diminishing
6	Spent ovaries have a purple-red colour due to hyperaemia and haemorrhage. The dimensions and weight are very much reduced. The GSI falls to 0.75 %. The membrane thickens up and becomes opaque with a whitish cast thickens up and becomes opaque with a whitish cast.

Table 7: Maturation scale for male cod (Fotland et al., 2000)

Stage	Description
1	Immature. Thin string
2	Mature. Thick Milk. Whitish
3	Viscous thick milk, white.
4	More low fluid milk, white
5	Flowing milk, spawning, white
6	Spent, blue/red rugged string.