Early induced maturation in Cod (*Gadus morhua*) using Low Energy Light

-Effect on muscle quality

Master thesis in Aquaculture

Department of Fisheries and Aquaculture

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Foreword

This thesis was written as the final part of a Master study at Bodø University College. It represents 60 credit points of a total of 120 credit points collected over a two year duration of the Master study in Aquaculture. The work is performed as a topic in seafood quality.

The work was performed in collaboration with Gildeskål Research Station, Codfarmers ASA and the Department of Fisheries and Natural Sciences.

There was a great deal of work invested in the collecting of samples, analysis and the writing process, and this has no doubt left me better equipped to face the challenges awaiting in the aquaculture industry.

I would like to use this opportunity to give many thanks to all the people that have helped me during the course of this work, both with their helping hands, practical inputs and knowledge.

A special thanks to my very helpful professor and main supervisor Dr. Christel Solberg, for her priceless help in planning, executing, writing and editing the manuscript. To the project manager at GIFAS Lasse Willumsen for all the help with planning, sampling and editing the manuscript, as well as, nurturing the fish throughout the length of the study. To my co-supervisors Dr. Jarle Tryti Nordeide for gonad assessments and editing my manuscript, and co-supervisor Ørjan Hagen for help with muscle fibre analysis. And last but not least a warm and special thanks to my wonderful wife, Trish, for all her patience and help in editing the final manuscript.

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Sammendrag

Målet med prosjektet var å undersøke effekten av kunstig blått og grønt LED-lys tilført en oppdrettsmerd i perioden 1.november til medio mai på torsk som gjennomgikk kjønnsmodning. Det ble undersøkt effekten lyset hadde på utviklingen av kjønnsprodukter, leverstørrelse og effekter på kjemi, biologi og tekstur i fiskemuskelen, differensiert mellom kjønn.

LED-lyset tilførte relativt små mengder (6 W) blått og grønt lys i en not på 125 m^3. Dette viste seg å være tilstrekkelig til å akselerere kjønnsmodningsprosessen med ca. en måned for begge kjønn. Noe som førte til at GSI verdiene i hunn torsken i den lysmanipulerte gruppen (L-gruppen) nådde sitt maksimum i mars (35 ±11), en måned tidligere enn hunn fiskene i kontroll gruppen (K-gruppen) som nådde maksimum GSI verdier i april (45±12). Forskyvningen i modningen hos torsk i L-gruppen førte til at fisk i denne gruppen oppnådde lavere protein nivåer og hardere tekstur tidligere i sesongen, sammenlignet med fisk som gjennomgikk kjønnsmodning på det naturlige tidspunktet for torsk i dette området. Den tidligere modningen i L-gruppen førte til at fisk i denne gruppen nærmet seg kvaliteten den hadde før modningen tidligere på våren sammenlignet med fisk i K-gruppen, med høyere protein verdier (15,4±1,5) for hunn fisk i L-gruppen sammenlignet med hunn fisk i K-gruppen (14,0±1,3) i mai.

Kjønnsmodning hos torsk er en meget energikrevende prosess, hvor torsken stopper å vokse i flere måneder. I tillegg reduseres muskelmasse og leverstørrelse. Hunntorsken mister relativt mye mer protein fra muskel gjennom kjønnsmodning og gyting sammenlignet med hanntorsken.

Dette skjedde for både lysmanipulert og fisk i kontroll gruppen i dette forsøket, men lysmanipulert fisk hadde et mindre tap av muskelprotein enn fisk i kontroll gruppen. Målingene antyder også at de lysmanipulert hunn- og hanntorskene produserte mindre kjønnsprodukter enn fisken i kontroll gruppen.

Abstract

In this project, light emitting diodes (LED) where added to sea pens containing farmed cod in order to investigate whether additional light influenced the timing of fish maturation, and/or the chemical, biological, cellular and textural properties of the fish musculature. This project, which took place in northern Norway, commenced November 1, 2005 and ended at the end of June 2006. After analysis, the results showed that additional LED lights effected the timing of maturation in both male and female cod. The cod subjected to additional lighting (L-group), matured approximately a month earlier than the fish in the control (ambient) sea pen (C-group).

Light manipulation resulted in a peak in GSI values in the L-group female fish (35±11) in April, one month earlier than the peak in the C-group females in May (45±12). The alteration in spawning time for fish in the L-group fish resulted in lower protein values and a harder texture earlier in the season in this fish, in comparison to fish in the C-group. The earlier spawning in the L-group led to an earlier increase towards pre-spawning quality, in comparison to fish in the C-group. This is shown by higher protein values in the female L-group (15.4±1.5) compared to 14,0±1.3 in the C-group in May.

Undergoing maturation is a very energy consuming process, and cod stops growing for several months during this period. In addition the fish degrade existing muscle mass and liver tissue.

There were marked differences between the sexes in relation to the spawning cycle in both the L- and C- groups. In both groups the female cod lost significantly more muscle protein than the male fish, and had a harder texture (shear resistance). However, although the female fish lost more protein than male fish, the results suggest that the female fish in the L-group had less muscle protein loss than female cod in the C-group, thereby possible indicating that lights used to speed up maturation can reduce energy loss in females during spawning.

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Introduction

Cod aquaculture is still only in an early stage of its industrialisation. Although salmon farming is more advanced in both Norway and worldwide, cod aquaculture is growing in size. In addition to some salmon aquaculture companies which also produce cod, there are certain aquaculture companies that specifically produce cod. Kjesbu et al. (2006) list several good reasons why cod aquaculture is likely to become more successful than it has been in the past:

(i) "We are better prepared scientifically

(ii) Private industry is now involved in the form of large international companies, which use closed production cycles and control the entire value chain themselves, which should make them more independent and successful

(iii) Globally there is a steadily increasing demand for marine aquaculture products while, at the same time, the world landings of fish has levelled off

(iv) Wild fish landings of gadoids are unpredictable *per se*

(v) Most wild gadoid stocks are producing far below maximum sustainable yield." (p.187)

Over the years from 1968 until present, worldwide cod captures have been reduced from 3.9 million tonnes (t) to 0.8 million tonnes in 2004. This can be seen in relation to the reduction in the size of the world's cod stocks. In Norway the cod captures have decreased from 470 000 t in 1972, to 230 000 t in 2004. (www.fao.org)

The growth of cod aquaculture has fluctuated since 1980. At that time, most of the cod production was done in Canada where the total amount of cod produced weighed approximately 200 t. Since then, the production of cod worldwide has increased. In 2004, the total amount of cod produced weighed approximately 4000 t. Most of the cod aquaculture is now located in Norway; in fact, Norway was responsible for 3000 t of the 4000 t of produced cod in 2004. Iceland, which was the second largest cod producer in that year, produced approximately 600 t. The production numbers from Norway and Iceland in 2004 were based on both farmed fish as well as on catch-based aquaculture. (www.fao.org)

Developing cod aquaculture for the future requires finding possible solutions to a range of challenges related to breeding, fry rearing and fish on-growth. One of the challenges is clearly developing an understanding of how to manipulate the fish's sexual maturation cycles in order to increase the production yield.

This study aims to investigate the effect of manipulating the light environment, with a narrow spectrum light with a very low intensity (LED), on cod held in sea net pens. The working hypothesis is:

1. Additional light added to a sea net pen from the 1st of November at 67° north results in earlier than "normal" maturation of fish.
    a. The earlier maturation results in a better fish quality earlier in the spring
    b. The earlier maturation results in a reduced impact of maturation
    c. There is marked differences in the impact of seasonal variation in fish quality between sexes

Cod biology and chemical content

Cod (*Gadus morhua* L.) is a lean fish as it holds less than 1 % fat in the muscle tissue (Hemre et al., 2004), and all of the lipids in the muscle are found in cell membranes as phospholipids (Hemre et al., 2000). The main constituent of the muscle tissue is water, approx. 80 %, which varies somewhat with the fish's spawning status (Davie et al., 2007b). Water soluble globular storage proteins, fibrous insoluble proteins, actin and myosin function as active components together with the connective tissue to give structure, form and function to the muscle tissue (Luther et al., 1995). The total protein content in cod muscle is around 20 %, and negatively correlated to the water content. The total protein content is therefore also dependent on spawning status (Davie et al., 2007b).

The size of the cod liver in relation to the cod's body mass, measured as the hepato somatic index (HSI), is a good indicator of the liver's energy content (Dahle et al., 2003). The liver is found to have seasonal variations in the amount of fat content depending on the stage of spawning in female cod (Kjesbu et al., 1991), and a distinct size variation depending on the stage of the male spawning (Solberg and Willumsen, 2007). The liver size in farmed cod lies on average around 12 % (for cod > 1 kg). A low HSI is an indication of insufficient feeding (Lambert and Dûtil, 1997).

The post rigor pH status of fish varies in relation to the amount of muscle glycogen present at slaughter (Bjørnevik, 2003a). The amounts of muscle glycogen are naturally high in farmed cod and as a result of this, the pH level is rarely above 6.5 when measured post rigor (Solberg et al., 2001). The pH is normally very low in farmed cod compared to wild cod. This is believed to be the result of the high energy content in the farmed cod compared to the wild. However, there is a tendency towards higher post rigor pH in a starved or post spawned cod (Pers. Comm.Solberg, 2007).

Seasonal effects

Even though cod aquaculture attempts to produce fish of a stable quality year round, seasonal variations are hard to avoid or change. Even though artificial light can be introduced in sea farming operations, it has proven difficult to avoid the ambient fluctuations in natural light and temperature (Davie et al., 2007b). These fluctuations influence the fish and lead to seasonal variations in both behaviour and flesh quality. The seasonal variations are represented in fluctuations in pH, fat, protein and water content, and in the liver size and content. These fluctuations can influence the fish's processing and functional properties (Ingólfsdóttir et al., 1998; Lambert and Dûtil, 1997). Furthermore, these fluctuations are also strongly correlated to the spawning status of the fish (Davie et al., 2007b), as well as nutrient availability.

In northern Norway the ocean temperature can vary from 3 to 4°C in March, to 13 to 16° C at the end of the summer. This temperature variation follows the yearly winter and summer cycles of the north, although it is slight delayed as a result of the saltwater's heat capacity. The amount of daylight varies greatly between winter and summer in northern Norway. For a month from December 9th to January 9th, there is no direct sunlight as opposed to the 24 hours direct sunlight which occurs in the month from June 9th to July 9th. This variation in the natural light is believed to contribute/trigger maturation in cod (Taranger et al., 2006; Davie et al., 2007).

Sex differences

There are distinct differences between the sexes in relation to their growth patterns following the occurrence of maturation. These differences are measured by the gonado somatic index (GSI) and HSI. Solberg and Willumsen (2007) found that the GSI increased more rapidly in male cod, from 0.4 % in September to 8% in December, compared to 1.1 % to 4.6 % in the female fish. This suggests an earlier maturation in male cod.

Solberg and Willumsen (2007) also found a negative specific growth rate (SGR) in male cod (-0.08 %) undergoing maturation from November through February, compared to a positive growth rate for female cod (SGR = 0.05%) in the same period. The female GSI increased 3 times during that period of time, whereas the male GSI only increased twofold. However, the male GSI was still significantly higher than the female GSI at the end of February. From the end of February through April, the overall pattern of growth shifted to a positive growth rate for the male fish (SGR = 0.12 %) and to a negative growth rate for female fish (SGR = -0.2). The female GSI increased twofold from February through April, compared to an almost threefold decrease in male GSI in the same

period. The male HSI decreased more than female HSI during the spawning cycle, and was found to be 4 % less than female HSI in April.

Solberg and Willumsen (2007) also found that the protein content in female cod muscle decreased from April to June, whereas the male cod had no decrease in muscle protein during the same period. In September, three months after the fish finished spawning, significantly higher protein content in male muscle was found when compared to the female cod. Their results show clear differences between male and female spawning cycles of cod in aquaculture, and the effect sex has on cod growth patterns.

Although cases of male fish developing later than female fish have been observed in both wild and farmed fish (Pers. Comm. Nordeide, 2007b), the gross portion of male fish develop gonads earlier than the females. In the farmed cod this could be related to the effects that captivity have on the spawning activity of the fish, as well as their natural spawning strategy (Windle and Rose, 2007).

Maturation and photoperiod manipulation

Cod hatched and reared for aquaculture show a very early maturation pattern consistent with the phenotypic plasticity theory. This theory explains that increased food availability results in earlier spawning due to unnaturally high growth factors for fish fed to satiation in captivity (Olsen et al., 2004; Thorpe, 2004). Kjesbu (1989) found that fecundity was around 2.5 times that of wild cod, and that farmed female cod released on average 17 to 19 batches of eggs over a 50 to 60 day period during spawning. The appetite of maturing fish drops markedly up to 1 month prior to spawning and stays low during the first three quarters of the spawning period (Karlsen et al., 2006).

All farmed cod spawns within two years and a substantial amount of males mature one year after hatching (Braaten, 1984; Jobling, 1988; Svåsand et al., 1996). In addition to food availability, the photoperiod influences spawning time. In fact, its influence is greater than both that of food availability and water temperature (Hansen et al., 2001; Norberg et al., 2004). Davie at al. (2007a) demonstrated that it is the falling autumnal signal that causes fish to begin the maturation cycle. Various photoperiod manipulations have been investigated in relation to cod (Davie et al., 2003; Hansen et al., 2001; Hemre et al., 2004), and these results show that the manipulations delayed fish maturation beyond the second summer of the production. However, all of these experiments were conducted in indoor tanks. Experiments which were conducted in sea pens showed a much lower

level of inhibition of maturation as a result of continuous additional light, with a delay of maturation by only 3 to 5 months (Davie et al., 2007a; Taranger et al., 2006).

It has been suggested that it is more challenging to influence the maturation cycle using light manipulation in cod than it is in salmon (Taranger et al., 2006; Oppedal et al., 1997).

Experiments, such as the study conducted by Hansen et al. (2001), demonstrate that cod can have an earlier maturation date, if they are subjected to additional artificial light. Hansen et al. (2001) added light on December 1st to cod which were kept in indoor tanks, and this resulted in an earlier maturation when compared to cod kept under ambient light. In addition, Hansen et al. (2001) propose that there is less impact on the maturation for cod grown in these conditions, compared to fish undergoing a normal spawning cycle. A similar trial was performed by a group of researchers at the Institute of Marine Research at Austevoll Research Station. In that trial cod were kept in 5x5x5 net pens with LED lights, in the blue, green and yellow spectra, added to the pens from mid-October. The results of this trial showed that these fish experienced maturation 6 weeks earlier than the fish in the control group (Pers. comm. Karlsen, 2007).

The photoperiodic influence on spawning behaviour in fish is thought to be dependent on dark phase melatonin levels. The pineal gland plays an important role in spawning behaviour as it receives photoperiod signals and transfers a signal via photoreceptor cells to the brain/gonad axis which determines the onset of maturation (Porter et al., 2000). In salmonids, it has been suggested that the dark phase plasma melatonin levels have to reach a threshold level in order to have an effect on the timing of maturation (Porter et al., 2000). This might explain the fact that it is difficult to get the same effect of light manipulation of cod in sea cages compared to cod kept in covered tanks. The day and night light intensities fluctuates greatly in uncovered sea cages with additional lighting, thereby allowing the signal of the normal diurnal rhythms to be the more pronounced (Porter et al., 2000) .

Suggestions made by Kristoffersen et al. (2006) that only some of the wavelengths in the visible spectre of light are required to effect cod, stimulated the choice of wavelengths picked out for this trial.

Light Emitting Diode technology

Light Emitting Diodes (LED) are 2.5 times more effective compared to metal halogen lights for the given wave length, and can therefore be maintained with a much lower voltage than halogen lights.

LED's emit light in a given spectra without the use of filters, and when dimming is applied, the quality of light does not change during the process. LED's have an extremely long lifespan, up to 100,000 hours, and can easily be mounted on mini circuit boards. In addition they do not burn out but slowly fade away. However, LED lights are more expensive than conventional light technologies. This is the main disadvantage of this light technology for aquaculture today (Pers. comm. Lysså, 2007).

Geographical differences

Most experiments conducted in sea cages in Norway are conducted by the Institute of Marine Research in the south-western part of Norway (60° N). At that latitude daylight cycles vary less (Fig. 1) and water temperatures are higher than in the northern part of Norway (67°N) where this research was conducted (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 16° C. This means that growth conditions are different between these two locations. Cod (*Gadus morhua*) which has a optimal growth temperature around 13.5° C (Jobling, 1988), depending on fish size (Björnssson et al., 2001) and blood type (Johnston et al., 2006), has therefore very little growth potential in south western Norway during the summer months. However the summer period from June to November is the optimal growing time for cod in northern Norway (Solberg et al., 2006).

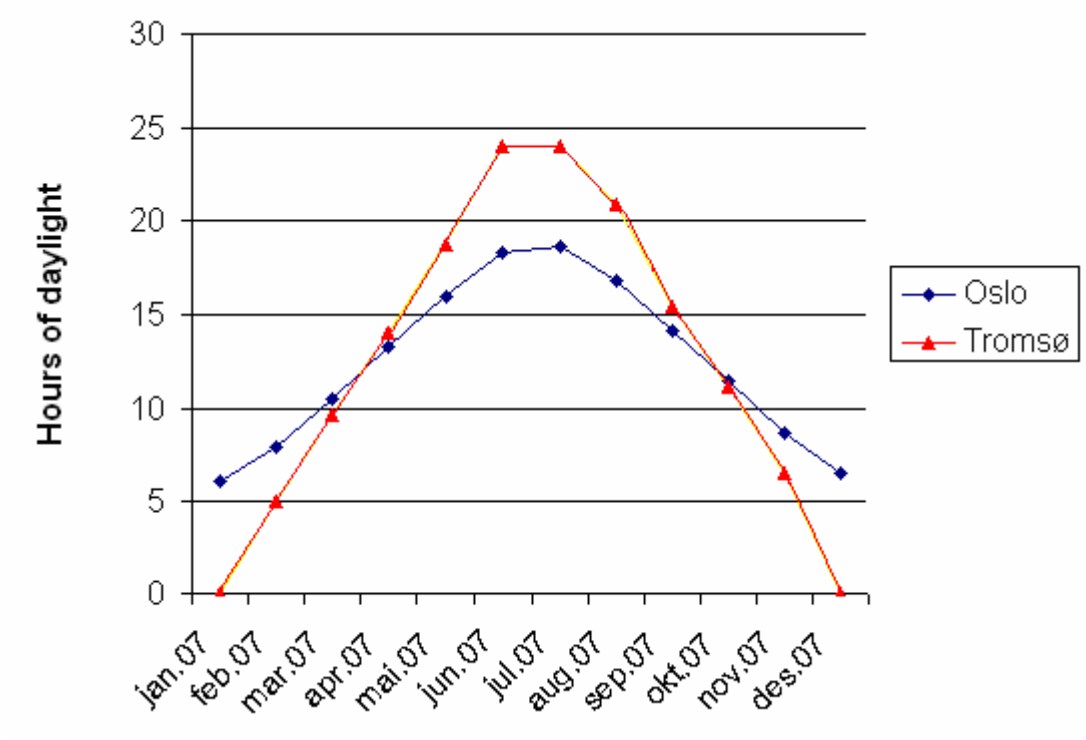

Figure 1: Graph showing hours of daylight in two different cities in Norway, Oslo at 60° north and Tromsø at 69° north. Tromsø is the only city which experiences the midnight sun. Tromsø also has two months of very limited light in the winter. Bodø lies at 67°north and is therefore most comparable to Tromsø. Source: The Norwegian Almanac.

It should be noted that sea temperatures are very dependent on location, and it is therefore difficult to generalize regarding this. The sea temperature depends on salinity, vicinity to freshwater and glacier outflows, depth, local and global current systems as a result of the tidal and wind systems.

Muscle fibres and connective tissue

The swimming apparatus of the teleost fish consists mainly of muscle fibres which are built up by sarcomeres stacked into muscle fibrils. Each fibre is in turn encapsulated by a thin basal lamina and reticular fibres, called the endomysium. Bundles of fibres are held together by the perimysium, a sheet of connective tissue matrix. The myotomes are separated from each other by a layer of connective tissue, mainly consisting of the protein collagen. In fish, this collagen is mainly type I and IV. The connective tissue is referred to as the myosepta or myocommata. The connective tissue is the connection which tendon use to transfer the myotomal energy of contraction to the axial skeleton and caudal fin, enabling locomotion. (Bjørnevik, 2003a; Johnston, 1981; Sänger and Stoiber, 2001)

Fibre diameter varies between red and white fibres. Red fibres can be between 25 to 45 µm, whereas white fibres can be between 150 and 300 µm. Fibre diameter seems to be regulated by a

trade-off between diffusional constraints and the energy cost of maintaining fewer larger diameter

muscle fibres according to Johnston et al.'s (2006) hypothesis of optimal fibre number. Muscle fibres grow by two distinct mechanisms. The 1st mechanism works by recruiting additional fibres from a possible separate muscle stem cell population. This process is known as hyperplasia. Hyperplasia is separated into two different stages. The first stage of hyperplasia occurs early in the life cycle producing the first muscle fibres of the fish. The second stage of hyperplasia creates additional fibres in later stages of the life cycle. The latter stage is most pertinent in fish which grow to a large final size and is known as mosaic hyperplasia (Bjørnevik, 2003a; Rowlerson and Vegetti, 2001). The second mechanism of muscle fibre growth is hypertrophy. In hypertrophy, fibres grow in size by adding additional nucleus to existing muscle fibres, and thereby create the foundation for additional muscle fibrils and increased fibre size (Bjørnevik, 2003a).

Davie et al. (2007b) suggests that some families of the Gadiforms exhibit sexual differences in muscle cell recruitment. Females were found to recruit muscle fibres over a longer period of time and therefore, had a steeper growth curve.

Influential factors in fibre recruitment (hyperplasia) and growth (hypertrophy) include the length of the photoperiod, exercise, diet composition, and feeding regimes (Johnston, 2001).

Fish muscle quality

There are many variables which contribute to muscle quality in cod and there are many ways to measure the quality. Flesh quality is the result of a range of factors including production methods, diets, slaughter techniques, processing and packaging. The measurable qualities include freshness, storage properties, taste, nutritional value and texture of the fillet (Johnston, 2001).

There is variation in how consumers perceive fish quality. Currently, the market wants farmed cod that is white, high in protein and has a thick loin. The producers need to produce a fish of this quality within a reasonable time and with minimal costs.

Texture

Hyldig and Nielsen (2001) describe texture as the attribute of a substance resulting from a combination of physical properties as perceived by the senses of touch (including kinaesthesis and mouth-feel), sight, and hearing.

Textural characteristics are often divided into mechanical, geometrical and other categories. Instrumental testing can only investigate mechanical and geometrical characteristics (Bjørnevik 2003a). The main techniques used for measuring the quality in fish are: puncture, compression, shear and tensile techniques (Bjørnevik, 2003a). In cod, shear resistance is found to be negatively correlated to water content in cooked flesh (Love et al., 1974).

Texture in cod flesh is reported to vary with the season, as the amounts of collagen, lipid content and post-mortem pH vary seasonally (Love, 1979). More recent research suggests that both the connective tissue matrix (consisting of mainly collagen I and IV) and muscle fibres have a role in the texture of raw fish (Johnston, 2001). Cooking of raw fish results in a structural change in the connective tissue matrix and gives it a lesser role in texture; as the fish is cooked the myotomes slide apart (Johnston, 2001).

The amount of connective tissue found, consisting mainly of the protein collagen, is found to be related to the firmness of meat (Bjørnevik, 2003a). Changes in the amount of pyridinolin crosslinking and water content in halibut are found to effect the softness of texture (Hagen et al., 2007). Studies comparing various species of fish concluded that fish with a high fibre density had a firmer flesh compared to fish with a low fibre density (Johnston, 2001).

Material and methods

Fish husbandry and sampling

The fish used in this research came to Gildeskål Research Station from Lofilab in January 2004. The average weight of the fish was sixty grams, and they were fed every day using an automatic feeding station. Some of the fish (both sexes) matured in the spring of 2005 at approximately 1 kg round weight. This was confirmed by samples taken in November 04 and April 05 by personnel at the research station (data not shown). From the spring 2005, the fish were fed to satiation three times a week (Monday, Wednesday and Friday) with a commercial cod feed from Skretting.

On 1st November, 2005, the fish were distributed into two different sea net pens, each consisting of 5x5x5 mesh in aluminium frames. The net pens were sixty meters apart. All the fish in the trial were taken from the same holding pen, and sixty fish designated for each net pen were individually weighed and measured in length. The rest of the total of 240 fish per pen was bulk weighed using a plastic container placed on a Marel animal weight (Marel m2000) using a 10 second weighing interval. 50 fish from each holding pen were killed by a blow to the head, gutted, bled in plastic containers for an 1/2 hour, put in styrofoam fish boxes, iced and transported to the laboratory at Bodø University-College for further analysis. The transport time was about 1.5 hours. 50 fish were taken from the holding pen at the start of the trial; thereafter about 50 samples were collected from each net pen in February, March, April, May and July. Some deviation from the original sample size occurred in the March and May samplings due to an otter attack. The smaller sample size was chosen in order to secure a good sample size in late June. Table 1 shows the number of fish of each sex in each of the treatment groups at the different sampling dates.

Table 1: Dates, sample sizes, temperature and sex ratios for each sampling from the control group (C) and the light treated group (L) throughout the trial period.

Date	Number of experimental weeks	Mean water temperature, °C	Number of fish from each net pen		Sex C		Sex L	
			C	L	M	F	M	F
Biological analysis of slaughtered fish								
1st November 2005	0	7.9	50 (start)		22	27	22	27
2nd – 3rd February 2005	12	4.3	50	50	25	25	27	23
2nd – 3rd March 2005	16	3.8	47	45	23	24	21	24
30th – 31st March 2005	20	4.2	45	45	17	28	26	19
4th – 5th May 2005	24	8.2	45	45	27	18	28	17
29th – 30th June 2005	28	10.2	45	53	33	12	38	15

Experimental design

From the 1st of November until May 2006, the two experimental groups were subjected to two different environments: Natural light (control group, C) and LED light treatment during the night time (Light manipulated group, L). Both groups were fed to satiation using feed for cod supplied by Skretting during the trial period.

Light source

The light source in this experiment was handmade by personnel at Gildeskål Research Station (GIFAS). The main light component was low energy diodes (LED's) placed in a 2.5 meter plexiglass tube. A total of 156 diodes were used, 12 diodes at each of the 13 circuits distributed evenly over the length of the tube. 78 of the diodes were HLMP-CB15-ROOxx (Agilent Technologies) and 78 were HLMP-CM15-WOOxx (Agilent Technologies).

The HLMP-CB15-ROOxx has a dominant wavelength of 472 nm, which is in the blue light spectrum. The HLMP-CM15-WOOxx has a dominant wavelength of 526 nm which is in the green light spectrum. The light source used very little electricity; in fact two 12 volt batteries were enough to keep it lit with full intensity for 7 nights. The battery pack was changed two times a week to ensure the full function of the light.

A daylight sensor was connected to a programmable logic system (Saia®PCD1 PLC based controller) that dimmed the light source off and on according to the signal from the daylight sensor.

The light dimmed up and down over a 20 minute time interval. The light increase and decrease was exponential. The daylight sensor was calibrated to give an off and on signal at early dawn and dusk, in order to ensure that the ambient light, in the light manipulated net pen, never dropped below the intensity given by the light source. The light source was placed in the centre of the 5x5x5m trial pen at a depth 0.5 meter.

Light measurements

Light measurements were performed in both of the light manipulated and the control pens in the beginning of March 2005 using a Li-Cor model Li-1400 data logger equipped with a Li-193 underwater Spherical Sensor. The sensor was mounted in a 009S Lowering Frame. Light measurements were performed at three points in the light manipulated pen: one at a depth of five and a half meters in the centre of the pen; one at a depth of four meters in the centre of the pen; and one in the corner of the pen at a depth of five meters. The locations were selected after visual inspection of the behaviour of the cod at night, resulting in an optimal placement of the sensor in relation to the area of the net pen the fish occupied. The light sensor measured the amount of light for 24 hours at each measuring point. The control pen was measured only at a depth of five meters.

Light sensors measure light in the photo synthetically active range (PAR) from 400 to 700 nm wave length (http://www.licor.com/env/Products/Sensors/rad.jsp), which covers the band of the emitted light from the LED in the trial. Data was collected in μmol / second per m^2. Light intensity measurements are shown in Fig. 2. The light measured during day-time (in February) varied from 20 up to 100 mikromol/second m^2 depending on the environmental conditions.

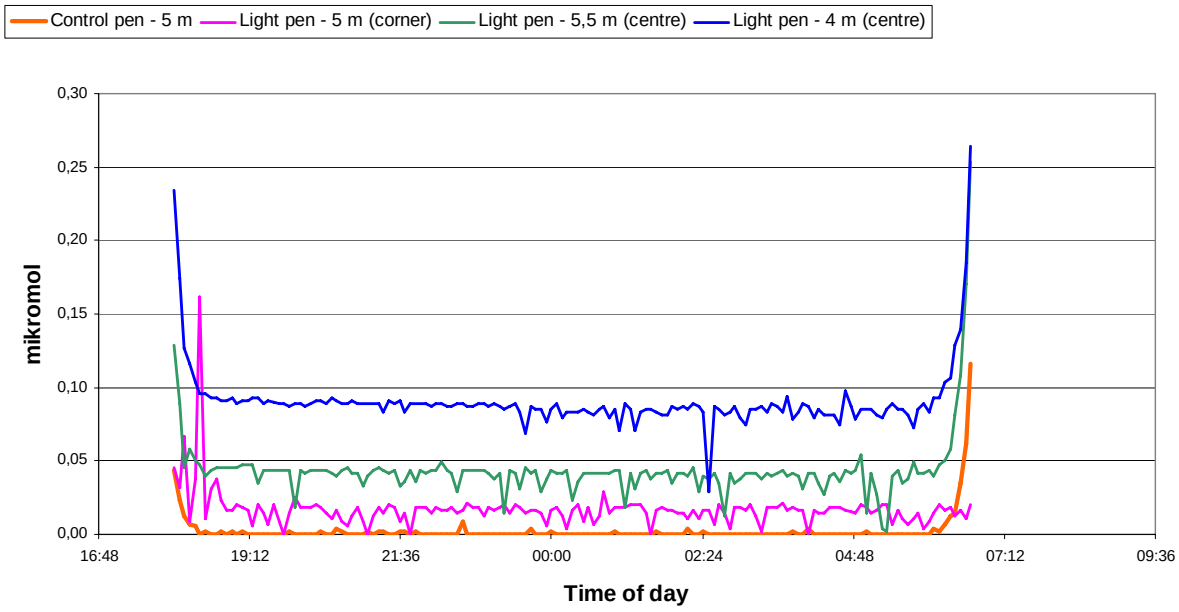

Figure 2: Light measurements at different depths in control and light pen, from dusk till dawn. They represent the light conditions the cod experienced during the night.

Biological measurements

Each fish was tagged, length measured and weighed before and after removal of the gut content. The liver and the gonads were weighed. The gonads were kept for later development assessments by a trained eye.

Calculations

Slaughter loss (%) = (Gutted Weight / Round Weight) * 100 (1)

Gonado Somatic Index (GSI) (%) = (Gonad Weight / Gutted Weight) * 100 (2)

Hepato Somatic Index (HSI) (%) = (Liver Weight / Gutted Weight) * 100 (3)

ConditionFactor = (Gutted Weight / Length^3)* 100 (4)

Muscle cell collection

Following the biological analysis performed on all of the fish in the sample pool from both of the treatment groups (50+50), 5 male and 10 female fish were randomly selected from each group. Each of these fish was cut at a place which was measured to be approximately 70 % of the length (just in front of the third dorsal fin) as shown in Fig. 3. Following that, a 5 mm thick cutlet was removed from the anterior part of the cut. This cutlet was placed so that the right side seen from above represented the right side of the fish in its direction of swimming. A picture was then taken and saved digitally for later investigations. Six pieces of fish muscle at 5 x 5 x 5 mm were removed from pre-designated areas on each of the cutlet's right side (as shown in Fig. 3), placed on pre-cut

and marked cork pieces, covered with Shandon Cryomatrix (Thermo Electron Corporation, Anatomical Pathology International, Chadwick, England) and frozen in isopentane (C_6H_{12}) cooled down in liquid nitrogen (N_2). Each fish piece (from now on: cryo sample) was held in isopentane for 1 minute, removed from the isopentane, wrapped in aluminium foil and stored in liquid nitrogen (N_2) for further analysis.

Muscle cell measurements

Cryosamples were removed from the liquid nitrogen (N_2) storage tank and placed in the Cryostat (Microm HM 550, Microm, Waldorf, Germany) at -20°C, where they were left to acclimatize for 1 hour before the cutting commenced. Cutting was performed at -20°C, with a setting resulting in 8 mm thick sections. The sections were placed in pairs on object glasses, dried and then coloured for 8 minutes in Harris Hematoxilin Solution (Accustain, Sigma Aldrich, Steinheim, Germany). Sections were then washed in tap water for 10 minutes and mounted using Glycerol Gelatin (Sigma Aldrich, Steinheim, Germany). 800 to 1200 muscle cells from each fish were counted after recommendation of from Johnston at Gatty Marine Laboratory, St. Andrews University in Scotland. Mounted sections were transferred to the microscope (Carl Zeiss, Axioskop 2 Mot Plus with Axiocam HRC, Göttingen, Germany) and cells were counted using Axiovision 4 V4.2.0.0 software (Carl Zeiss Vision, Göttingen, Germany). Cutlet area was estimated using the picture editor software (Sigma Scan).

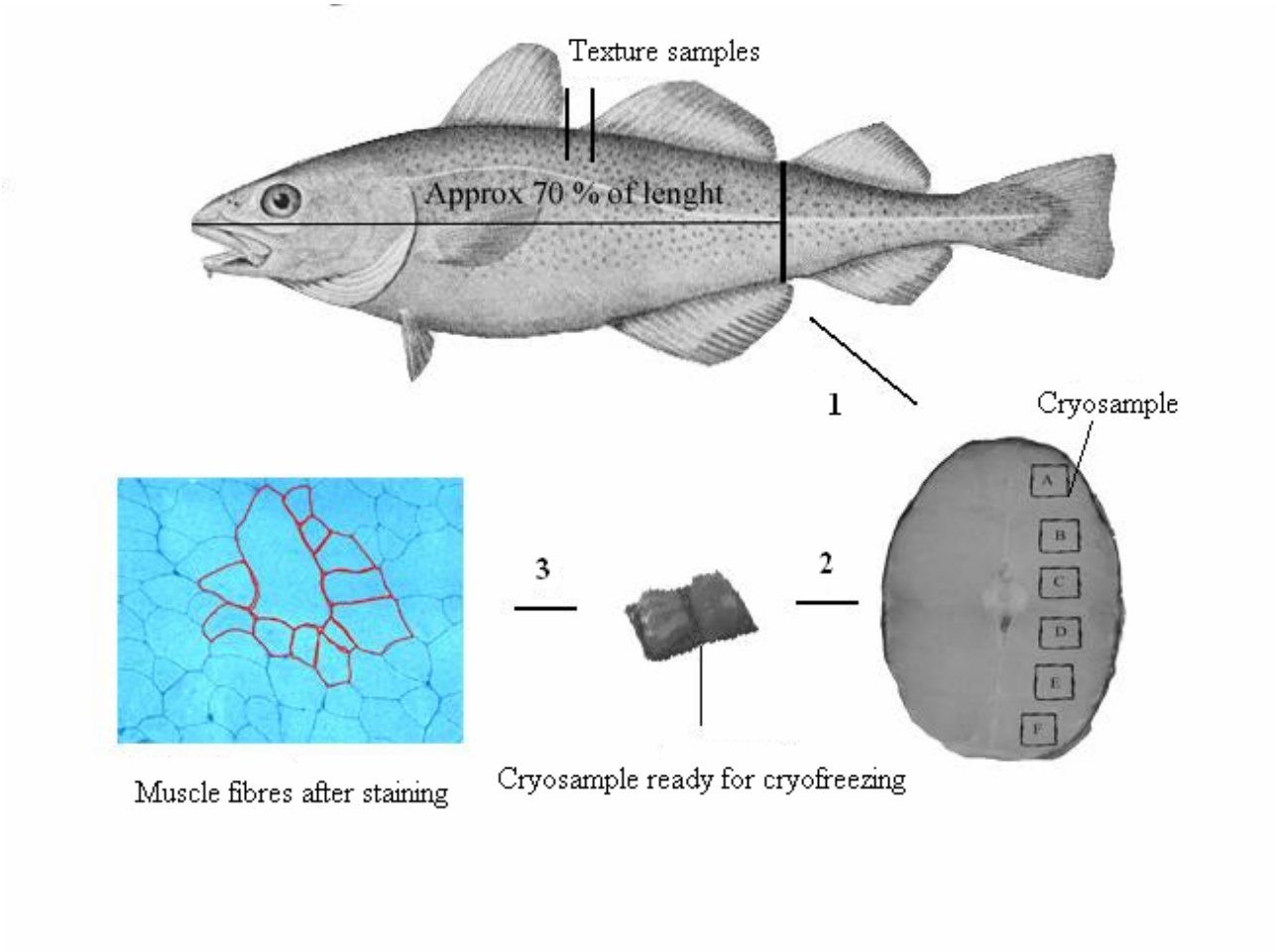

Figure 3: Overview of the different stages in muscle cell sampling, from cutting a cutlet at 70 % of the length (1) to dissecting the cryosample (2) and looking at the muscle sample under the microscope (3).

Muscle cell calculations

In estimating the muscle fibre number, the following function was used:

10^6 x (Total Cross sectional Area x Fibres counted / Total Counted Area) (6)

In estimating the muscle fibre density the following function was used:

10^6 x (Number of Fibres Counted / Total Counted Area) (7)

Average muscle diameter was calculated by taking the total number of different diameters measured divided by the total number of muscle cells counted.

Muscle cell distribution was estimated by dividing the total number of cells counted into size groups, with a 10 µm interval. Each distribution interval was then tested between groups to reveal differences.

Gonadal development assessment

The gonads sampled in February, March, April and May, were blind tested to give each gonad a designated stage in maturation. A scale developed by the Institute of Marine Research, Norway, was used (Fotland et al., 2000). This scale is comparable to the maturation scale for cod which is found in Katsiadaki et al. (1999). The blind test was performed by placing a qualified assessment personnel (J.T. Nordeide, Bodø University College) behind a curtain. Without knowing the origin of the gonad (control or light treated group) J.T. Nordeide designated a maturation stage to each gonad shown to him. The maturation scale is found in Appendix 4: The maturation scale for cod (Katsiadaki et al., 1999; Fotland et al., 2000). The scale values range from stage 1 (immature) to stage 6 (spent).

Stage in maturity was only assessed for female gonads, due to the difficulties in assessing male gross gonads and the high level of uncertainty related to this. No blind test was performed in July since all fish were spent at this point.

Texture analysis

After biological measurements were recorded and cryosamples taken, the fish were stored on ice for three days until rigor mortis had passed. Following the cold storage, the right side of the fish body, was filleted by a sharp knife. Texture analysis was performed on all of the fillets (with skin) on all of the sample dates using a blade with a TA-XT2texture analyser, Stabile Micro Systems (Haslemere, England). The fillets were kept on ice until minutes before the analysis was performed.

Each texture measurement was performed twice, one directly behind the second dorsal fin in the middle of the fillet, and the second, 2 cm posterior of the first slice (Fig. 3). The blade cut 90 % of the fillet and used 5 grams to trigger the measurement. The blade had a speed of 1mm per second when triggered. The average of the total force used in both measurements was recorded and used in the results.

Chemical analysis

After textural measurements were performed, each fillet was de-skinned and minced into a homogeneous mass using a conventional food processor with cutting blades. Near infrared transmission measurements were performed on every sample using a Infratec 1255, Food & Feed Analyzer (Foss Analytical AB, Höganäs, Sweden). 5 minced fillets from a male origin and 5

minced fillets from a female origin were randomly selected out of the sample pool. Protein and water content analysis were performed on each of these minced fillets. The chemical analysis was duplicated for each analysed sample.

Protein analysis was performed by accurately weighing 0.8 – 1.2 grams of the homogeneous mince from each sample and placing it into a nitrogen free weighing vessel. Then it was transferred into a Kjeldahl reagent tube, and 7 grams of Cu-Kjeltabs (Foss Analytical AB, Höganäs, Sweden) were added. In the next step, 15 ml 96 % sulphuric acid was added to each of the reagent tubes and the tubes were then placed in a rack and transferred to a boiler block. The boiler block was set at 420° C and the samples stayed in the block for 1 hour. After boiling, the samples were cooled and 75 ml of water was added to each reagent tube. After water addition, each sample tube was analysed using a Kjeltec 2300, (Foss Analytical AB, Höganäs, Sweden). The average of the double analysis was used in the results.

Water analysis was performed by first weighing 5 grams of the minced sample twice (double analysis) into separate aluminium foil cups. Then the samples were dried at 104°C for 18 hours. The water content was then estimated by subtracting the dried weight from the initial weight. The average of the double samples was used in the results.

Near infrared analysis

In order to determine the protein and water content of the trial's samples, a NIT analysis was administered to all of the samples. This was done by analysing the minced sampled with the Infratec 1255 Food & Feed Analyzer (Foss Analytical AB, Höganäs, Sweden). This analyzer used 23 mm thick samples (red rings) in the sample cup holder. The sample cup holder has 5 rings, and each of them was filled with the same minced sample. Measures were taken to assure that no pockets of air got caught within the mince placed in the sample ring in order to prevent diffraction of the light and an inaccurate measurement. Near infrared light passed through each of the samples, and the amount of near infrared light passing through each sample was recorded. The data was then transferred to the analytical software used (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.

pH measurements

After NIT analysis was performed, a set quantity of mince from each sample was placed in small plastic beakers. A pH electrode (Radiometer pHC2401) was used to estimate the pH in the mince by insertion. The electrode was washed in distilled water and put back in a buffer after each measurement. The electrode was calibrated before the sample run and during analysis, depending on the quality of the buffer values between each sample run.

Statistical analysis

Statistical analysis was performed using the JMP IN 5.1.2 (Statistical Discovery^{TM}, SAS Institute Inc., 2006). The student t-test was used to show the differences between treatments and sexes. A t-test was applied were assumptions of homogeneity of variance were not fulfilled. All of the material was tested for normality using the Goodness of Fit test (0.05) (Sall, 2005). Were normality was not found, a non-parametric Mann-Whitney *U test* (Zar, 1999) was used for two levels. In cases were there were more than two levels, the Kruskal–Wallis (Zar, 1999) test was applied. Homogeneity in variance was tested using the Levenes and Bartlett's test (Zar, 1999).

GSI (%) values were ArcSinH-transformed in order to achieve a better normality distribution. This was done by converting the precentage to a proportion. The resulting value was then square rooted and then the inverse of sin was applied (Dytham, 2003). This process was performed using JMP IN 5.1.2.

Stage of maturity was not statistically tested between the groups because this was not found appropriate considering the nature of the data.

Partial least square regression

Partial least square regression, performed with Uncrambler (Ver. 9, Camo A/S), was used to predict values for the protein and water content in all of the samples. The values were based on chemical measurements of ten samples per treatment group on each test date. A model was made using the chemical measurements as a reference and the NIT data as the prediction data.

Principal component analysis

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

Results

Gutted weight

Fig. 4 shows that the female gutted weight in the C-group is significantly higher than in the L-group ($p<0.0003$) in February and March. Female fish in the L-group lost weight and female fish in the C-group gained weight between November and February. Female fish in the L-group reached the lowest gutted weight in March (1.67 ± 0.27), and the females in the C-group reached their lowest weight in May (1.62 ± 0.25). In July there were no significant differences in the gutted weight between groups.

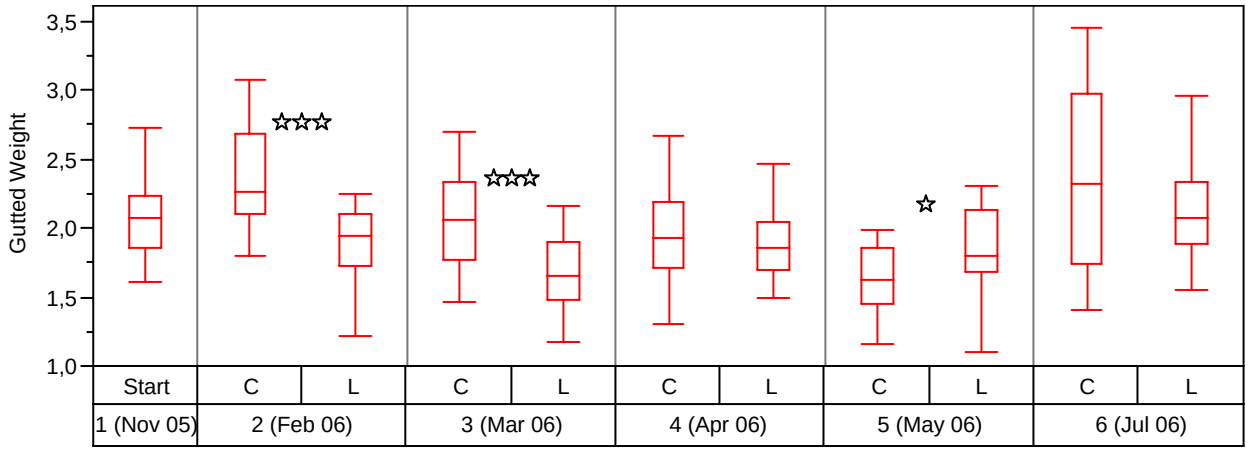

Figure 4 : Female gutted weight(kg) during the trial period, for the control (C) and the light treated group (L). Box plots show upper75 and lower 25 percentile, median and standard deviation. The treatments were significantly different when indicated with stars and not significantly different when left without stars. A description of significant levels is found in Appendix 1.

The results in Fig. 5 showes that there was a tendency ($p<0.086$) for a higher gutted weight in male fish in the C-group in February (2.08 ± 0.28). However on the other sample dates, no further tendencies of difference were seen.

The female fish varied more in gutted weight when compared to male fish within both groups. This indicates that female fish experience more muscle loss during spawning compared to male fish. Average weight for female fish in July in both groups (C and L) was lower than their the male counterparts; however this was not significant.

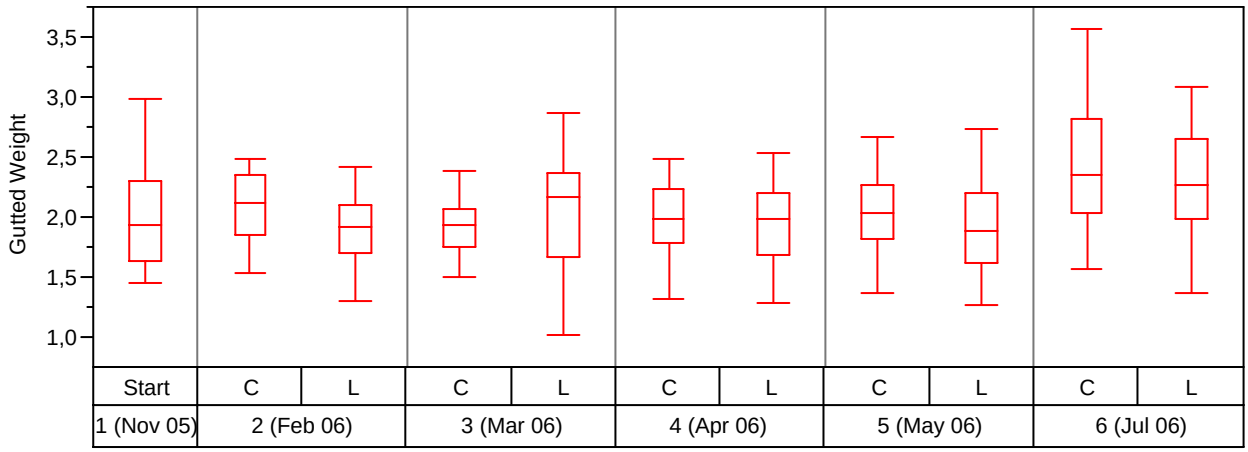

Figure 5 : Male gutted weight (Kg) during the trial period, for the control (C) and the light treated group (L). Box plots show upper (75) and lower (25) percentile, median and standard deviation. The treatments were significantly different when indicated with stars and not significantly different when left blank. A total description of significance levels is found in Appendix 1.

Condition factor

The female Condition-factor (C-factor) was 1.08 at the start of the experiment (Fig.6), but dropped more in the L group compared to the C group until February (Fig.6). A significantly ($p<0.0001$) lower C-factor in the L-group (0.86 ± 0.09) was found in comparison to the C-group (0.97 ± 0.07). This tendency continued until April, but shifted in May, when the significantly ($p<0.0003$) lower C-factor was found in the C-group (0.68 ± 0.05). The C-factor is still higher in the L group in July, but this is only close to significant ($p<0.088$).

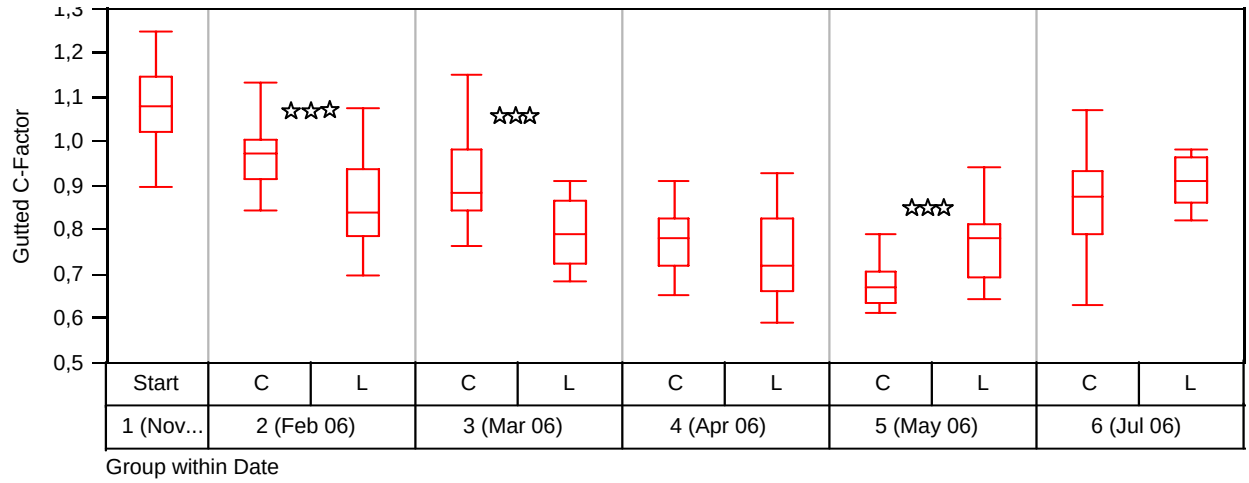

Figure 6 : Female gutted C-factor during the trial period, for the control (C) and the light treated group (L). Box plots show upper (75) and lower (25) percentile, median and standard deviation. Treatments are significantly different when indicated with stars and not significantly different when left blank. A description of significance levels is found in appendix 1.

There were no significant differences in the male fish between treatments in C-Factor (Fig. 7). However, a drop in C-factor was registered from November (1.08 ± 0.099) through April (0.86 ± 0.09), The C-factor started to increase towards July (0.97 ± 0.11).

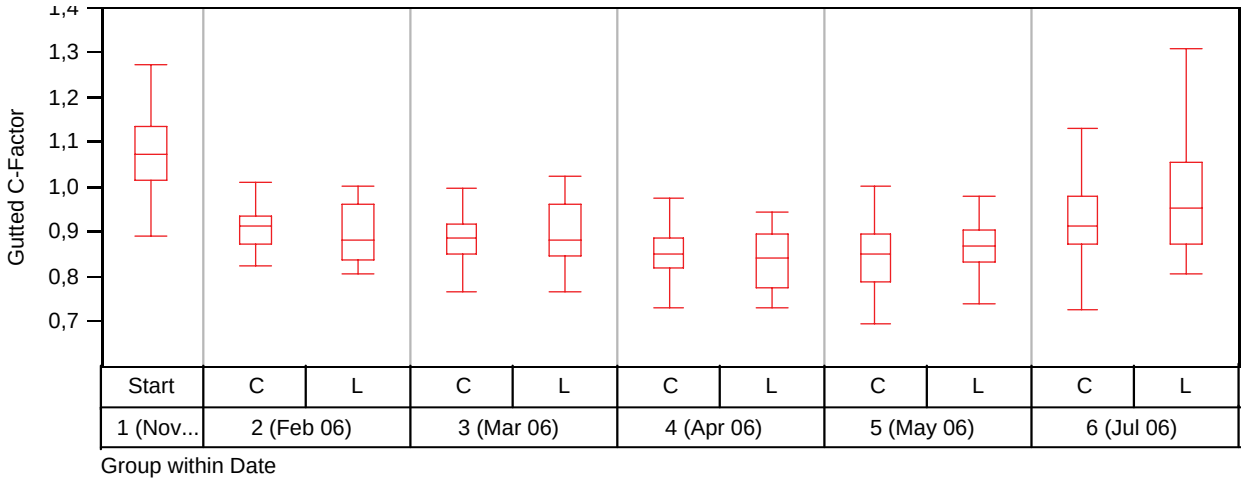

Figure 7: Male gutted C-Factor during the trial period, for the control (C) and the light treated group (L). Box plots show upper (75) and lower (25) percentile, median and standard deviation. Treatments are significantly different when indicated with stars and not significantly different when left blank. A description of significance levels is found in appendix 1.

Fibre distribution

No significance was found between the fibre distribution between the C- and the L-group in July 2006. However the results indicate that male fish in the L-group have more fibres in a lower micrometer category compared to the male C-group (Fig. 8). For female fish, indications show the opposite tendency (Fig. 9).

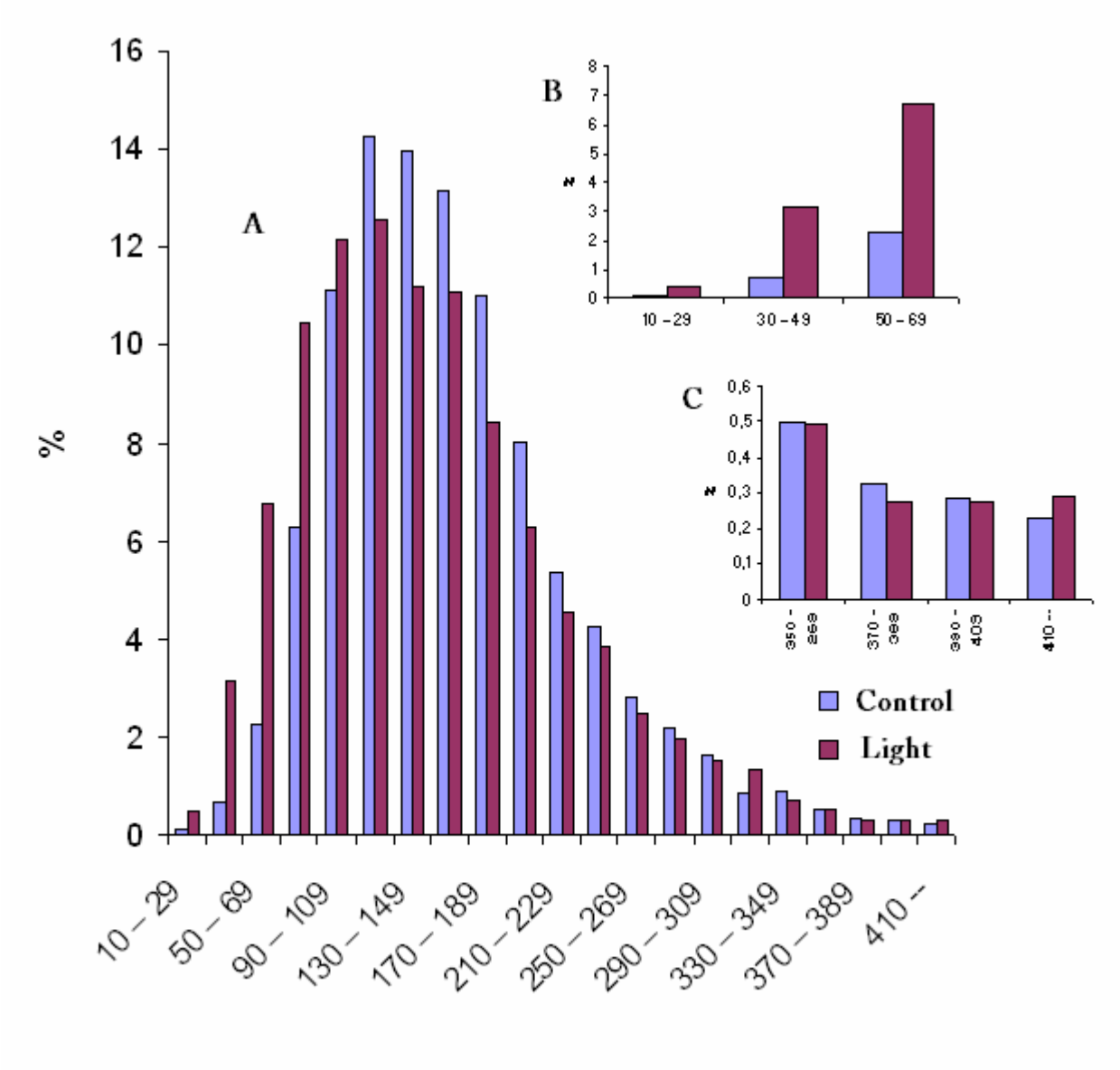

Figure 8: (A) Fibre distribution in % fibres within a 20µm interval for ***Male*** *fish in July between the C- and L-groups. (B) Show the distribution between fibres below 70µm and (C) shows the distributions between fibres above 350µm between groups. There are no significant differences between groups within intervals.*

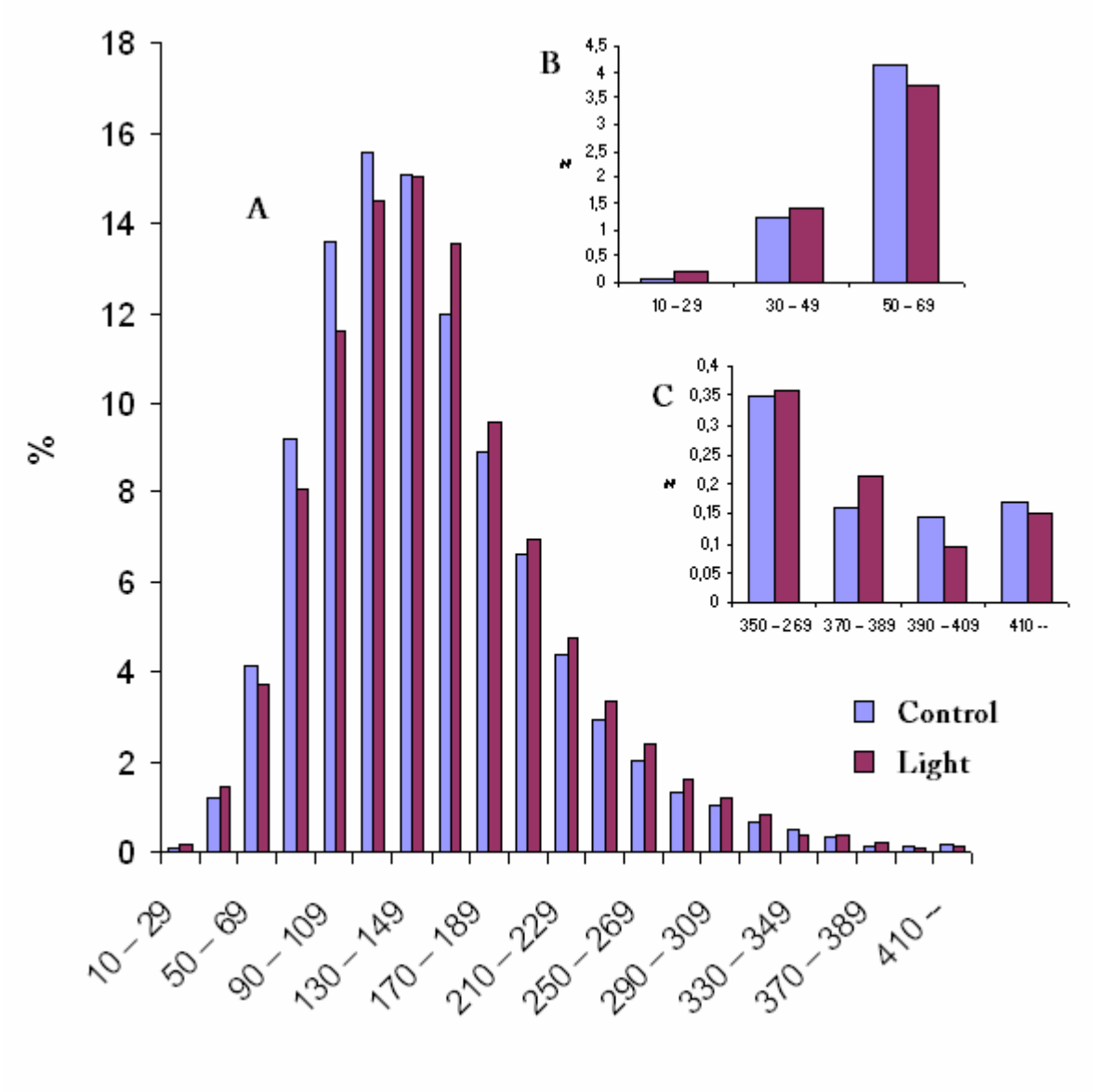

Figure 9: (A) Fibre distribution in % fibres within a 20µm interval for ***Female*** *fish in July between the C- and L-group, (B) shows the distributions between fibres below 70µm and (C) shows the distributions between fibres above 350µm between groups. There are no significant differences between groups within intervals.*

When looking at both female and male fish as one group, the results do not show any tendencies of differences in fibre distributions between L-group fish and C-group fish.

Fibre diameter, number and density

A significantly higher number of white muscle fibres were found in the male fish in July compared to the fish at the start of the trial (Table 2). There where only tendencies for an increase in fibre number from November to July in the female fish. These results indicate that hyperplasia occurred between November and July in male fish.

Table 2: Number of cells, diameter and density for Female and Male fish in July and November

Month	Group	Cell #	Std.+/-	Cell dia µm	Std.+/-	Cell density #/mm^2	Std.+/-
July	***C Female***	122 700	22 500	148	21	55	17
	L Female	117 900	19 200	152	16	50	10
July	***C Male***	122 700	15 100	160	16	44	7
	L Male	140 400	53 400	150	26	51	17
November (Start)	***Start Female***	95 600	25 600	161	21	42	11
	Start Male	78 200	21 500	173	23	38	13

Neither tendencies nor significant differences in cell density or fibre diameter were found between groups and dates (data not shown). The cell number was not found to be significantly different between sexes within the groups (data not shown).

Gonado somatic index

Fig. 10 shows that the female fish had a significantly higher ($p<0.0001$) GSI value in February in the L-group (32 ± 12), compared to the C-group (16 ± 4.). Similar GSI values were still found in March in both groups, but in April the situation changed when a significantly ($p<0.0001$) higher recorded GSI value in the C-group (45 ±12) occurred. The highest GSI value in the trial was found in female fish in the C-group in April, which was significantly ($p<0.002$) higher than the highest value found in the L-group in March.

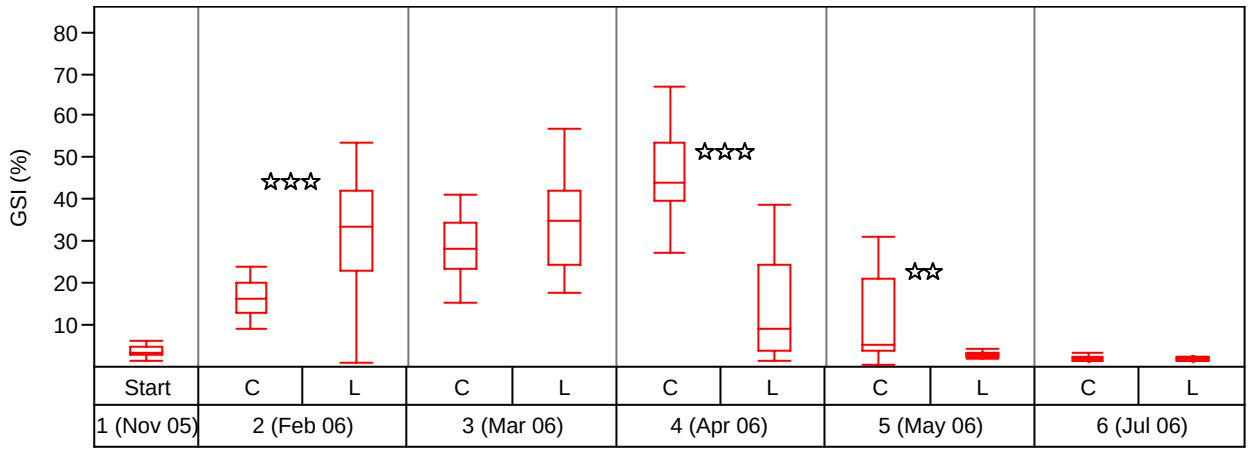

Figure 10: Female GSI (%) calculated as a precentage of the gutted weight during the trial period, for the control (C) and the light treated group (L). Box plots show upper (75) and lower (25) percentile, median and standard deviation. Treatments are significantly different when indicated with stars and not significantly different when left without stars. A description of significant levels is found in appendix 1.

Male fish in the C-group (19 ± 6) had significantly higher GSI value in February ($p<0.0001$) compared to the L-group (14 ± 4) (Fig. 11). This continued until May when all male fish in both groups had finished spawning.

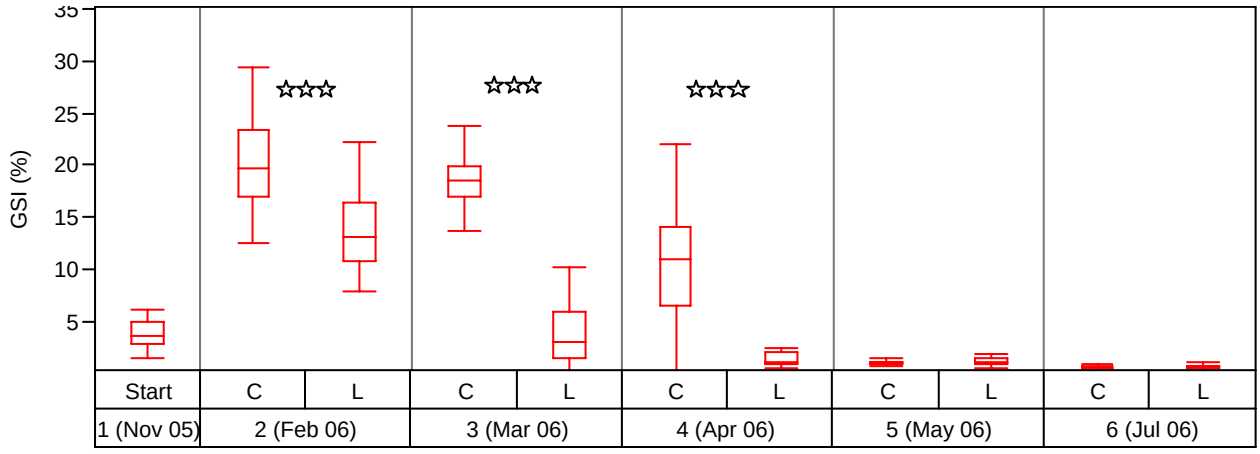

Figure 11: Male GSI (%) during the trial period, for the control (C) and the light treated group (L). Box plots show upper (75) and lower (25) percentile, median and standard deviation. Treatments are significantly different when indicated with stars and not significantly different when left blank. A description of significance levels is found in Appendix 1.

Male fish developed gonads earlier than female fish; however female fish had a much higher GSI than male fish during spawning. The GSI (%) developed differently between sexes during the trial period for fish in the C-group. The male group peaked in February and the female peaked in April. The same pattern was found in the L-group, where male fish had the highest GSI (%) values in January/February compared to the female peak values in February/March. There were significantly lower peak GSI (%) values in the L-group compared to the C-group (Appendix 2).

Gonad index assessment

Table 3 shows that the majority of female fish were assessed as stage two maturity in November (96.5 %). In February all of the fish in the C-group were assessed to be in stage 2, while 74 % of the fish in the L-group were between stage 3 and 5, with 30.4 % of the fish being in stage 4.

Table 3: Maturity stage for female fish throughout the trial, from November to July. All values are given in percentage within each stage and group.

Stage	**1**		**2**		**3**		**4**		**5**		**6**	
Group	**C**	**L**	**C**	**L**	**C**	**L**	**C**	**L**	**C**	**L**	**C**	**L**
November	4	4	96	96	0	0	0	0	0	0	0	0
February	0	4	100	22	0	22	0	30	0	22	0	0
March	0	0	74	4	13	21	4	46	9	29	0	0
April	0	6	0	0	14	0	68	39	18	0	0	56
May	6	0	0	0	6	0	24	0	0	0	65	100
July	0	0	0	0	0	0	0	0	0	0	100	100

In March, 30 % of the female fish in the L-group were in stage 5, compared to 9 % of the female fish in the C-group. This is supported by the fact that as much as 56 % of the female fish in the L group were in stage 6 (spent) in April, compared to no spent fish in the C-group. In May, 100 % of the female fish were spent in the L-group, but only 65 % of the fish in the C-group were assessed to the same stage. All female fish were spent in July.

Table 4: Maturity stage for male fish throughout the trial from November to July, All values are given in percentage within each stage and group

Stage	**1**		**2**		**3**		**4**		**5**		**6**	
Group	**C**	**L**	**C**	**L**	**C**	**L**	**C**	**L**	**C**	**L**	**C**	**L**
November	4	4	87	87	9	9	0	0	0	0	0	0
February	4	0	96	0	0	0	0	7	0	93	0	0
March	0	0	0	0	13	0	0	0	87	74	0	26
April	0	0	0	0	0	0	0	0	100	12	0	88
May	0	0	0	0	0	0	0	0	4	0	96	100
July	0	0	0	0	0	2.5	0	0	0	2.5	100	95

Male fish showed the same trends (Table 4), but since no blind test was performed on these gonads, the result is of less value.

Hepato somatic index

Fig. 12 shows that the female HSI increased slightly more in the L-group (17 ± 3) compared to the C-group (16 ± 3) in February ($p<0.026$). A significantly ($p<0.004$) higher HSI value was found in the C-group in April (18 ± 3) than in the L-group (15 ± 4). On the next two sample dates a difference was not found between the two groups. The highest HSI value was found in the C-group in April (18 ± 3).

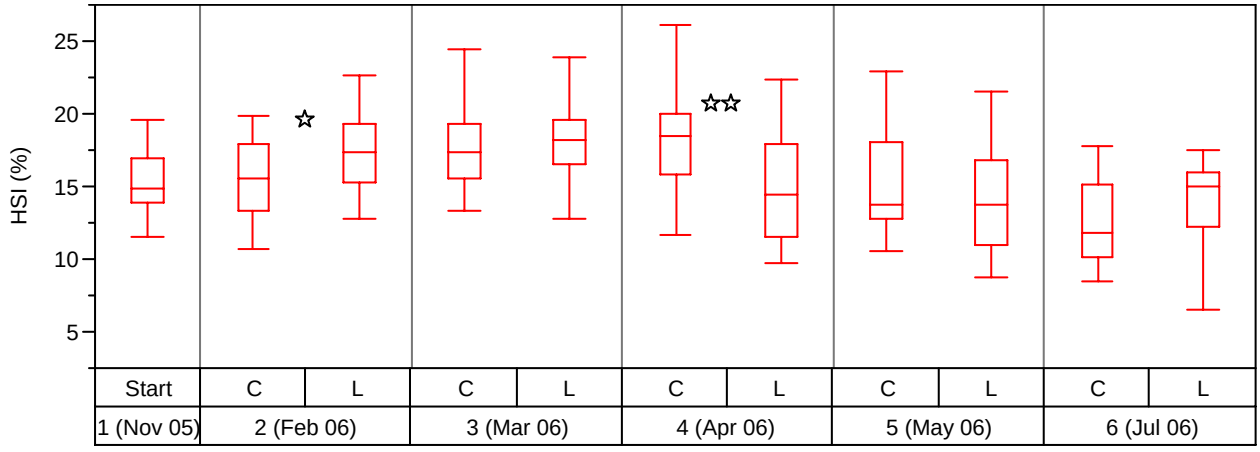

Figure 12: Female HSI (%) during the trial period, for the control (C) and the light treated group (L). Box plots show upper (75) and lower (25) percentile, median and standard deviation. Treatments are significantly different when indicated with stars and not significantly different when left blank. A description of significance levels is found in appendix 2.

For the male cod (Fig.13), the HSI drops to a significantly ($p<0.005$) lower level in the male L-group (13 ± 2) from November to February when compared to the male C-group (14 ±2). From February on, there was a tendency for a lower HSI in the L-group (only significant in April) until May, when the tendency disappeared and the means were equal.

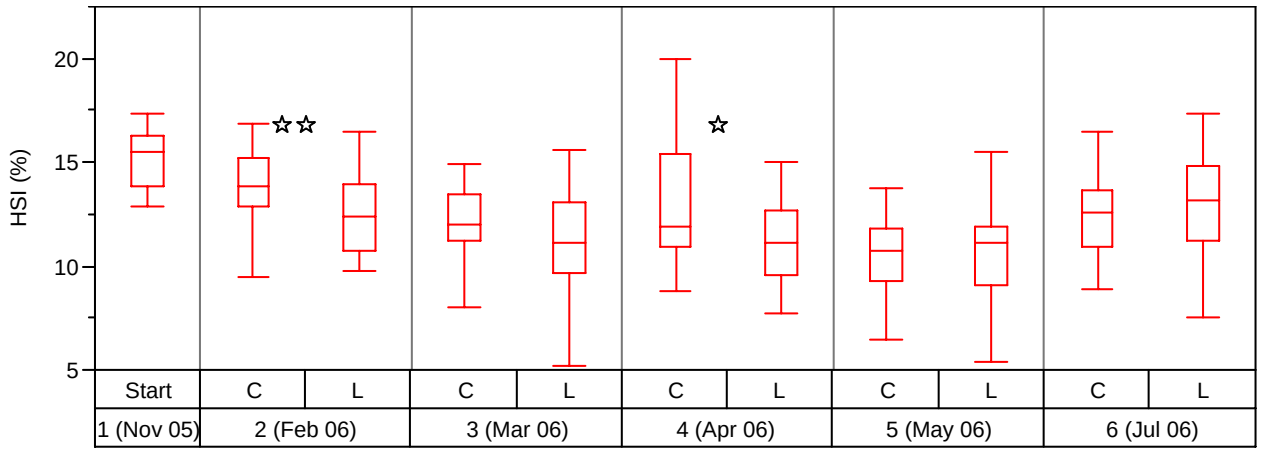

Figure 13: Male HSI (%) during the trial period, for the control (C) and the light treated group (L). Box plots show upper (75) and lower (25) percentile, median and standard deviation. Treatments are significantly different when indicated with stars and not significantly different when indicated when left blank, a description of significance is found in Appendix 1.

Female HSI was significantly higher than male HSI on all sample dates within both groups, except in November and July. However, the variation in HSI seems higher for the male fish in both groups.

Feed usage and temperature

Fig. 14 shows that the percentage of feed given per day in relation to the calculated ingoing biomass in each month, markedly dropped earlier in the L-group compared to the C-group. The L-group had increased feeding activity earlier in the spring than the C-group.

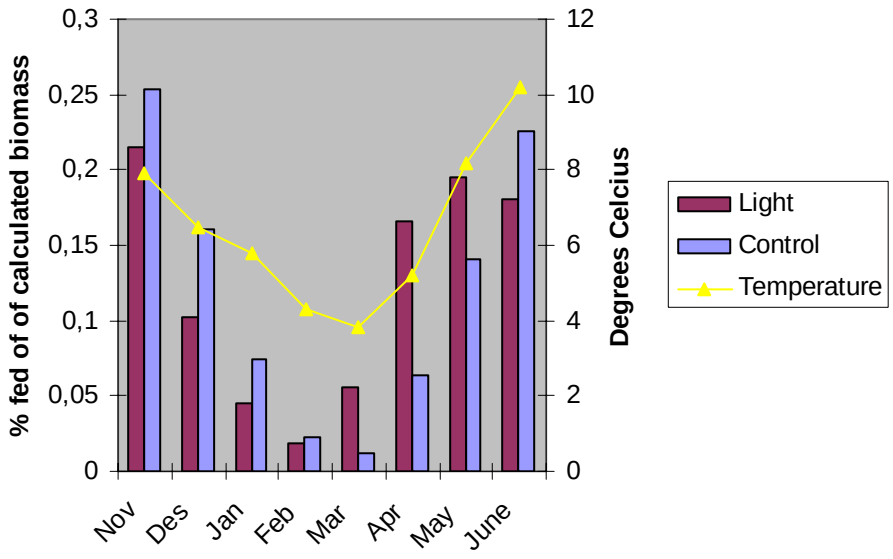

Figure 14: Amount of feed used in % per day in relation to the ingoing calculated biomass per month throughout the trial (bars), and average monthly temperature (yellow target line).

Slaughter loss varied with GSI and HSI values, and the size of the other internal organs in the fish (Table 5). A strong correlation between GSI and slaughter loss was found (0.93). Female and male fish in the L-group had a higher slaughter loss earlier than fish in the C-group. The differences in slaughter loss between the sexes were also pronounced with an earlier peak in male slaughter loss compared to female.

Slaughter loss

Table 5: Slaughter loss, in precentage for all groups and sexes, on all the dates throughout the trial.

Group	Sex	Nov	Feb	Mars	Apr	May	Jul
Light	*Female*	21	**35**	**37**	28	24	24
Control	*Female*	21	27	**34**	**41**	26	26
Light	*Male*	**20**	**23**	18	18	19	19
Control	*Male*	20	**27**	**25**	22	18	18

Muscle water content

In February, the female fish in the light manipulated group had a significantly ($p<0.0001$) higher water % (81.5 ± 1.4) than fish in the C-group (79.7 ± 0.4) (Fig. 15). This continued until April when the L-group reached its highest value (83.8 ± 1.8), compared to the C-group (82.3 ± 1.1). The C-group reached a significantly ($p<0.011$) higher value (84.2 ± 1.2) compared to the L-group (83.0 ±1.4) in May. This value is not significantly different than the peak in the L group (83.8 ± 1.8) a month earlier.

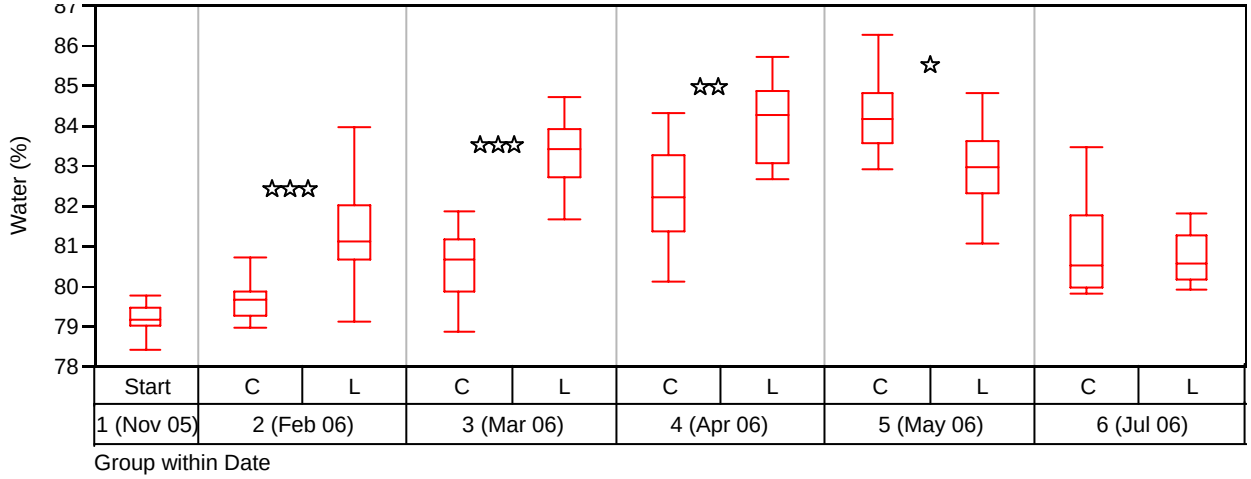

Figure 15: ***Female*** *Water content in % between groups during the whole trial period, for the control (C) and the light treated group (L). Box plots show upper (75) and lower (25) percentile, median and standard deviation. Treatments are significantly different when indicated with stars and not significantly different when blank. A description of significance levels is given in Appendix 1.*

In February, the male fish in the L-group (80.0 ± 0.6) had a significantly ($p<0.0001$) higher water content than the fish in the C-group (78.9 ± 0.4) (Fig. 16). This continued until April reaching values as high as 80.9 ± 0.7 in the L-group. In May and July the means were equal and steadily descended towards the November value of 78.9 ± 0.3.

Male fish did not exceed 81 % in water value, compared to the significantly higher peak of 84 % in the female fish in the L-group in April (Appendix 1: Table 8).

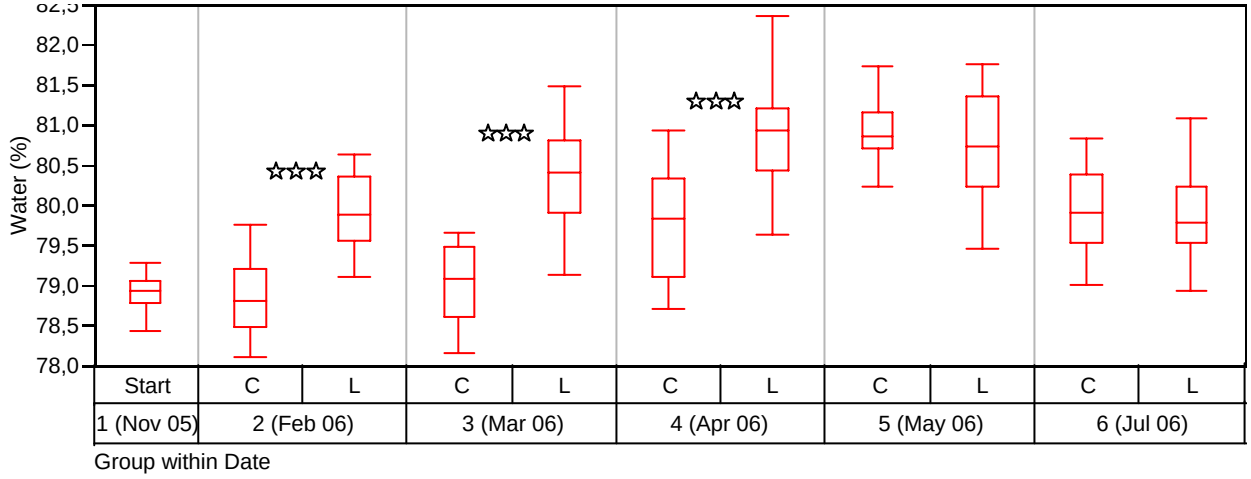

*Figure 16: **Male** water content in % between groups during the whole trial period, for the control (C) and the light treated group (L). Box plots show upper (75) and lower (25) percentile, median and max/min. Treatments are significantly different when indicated with stars and not significantly different when blank. A description of significance levels is given in Appendix 1.*

A strong correlation (r=0.998) was found between the water and protein content (Fig. 17). The amount of protein found is calculated into a percentage using the calculation:

Protein (%) = 101.63 – 1.04 Water (%) (9)

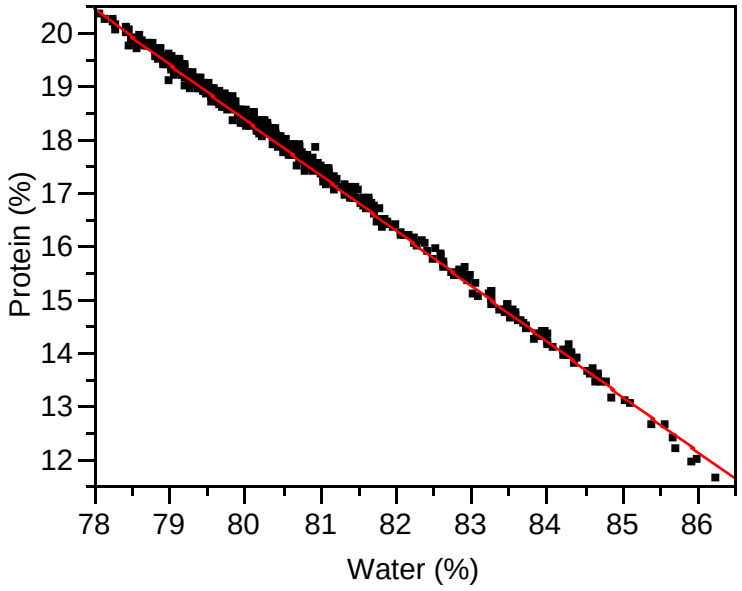

Figure 17: Showing almost perfect linear fit between protein and water(0.998).

A strong linear relationship between the water and protein shows that almost all protein lost is replaced with water.

pH

Significant variations in the post-rigor pH between the groups were not found throughout the trial, neither in relation to treatment nor sex. However a lower pH value was found in November than in February, March, April, May and July.

Shear resistance

Shear resistance (kg) increased considerably earlier for the females in the L-group compared to those in the C-group (Fig. 18). In March the shear resistance was significantly ($p<0.0001$) higher in the L-group. This continued until April when the L-group reached its peak (18 ± 4). The shear resistance evened out for both groups in July.

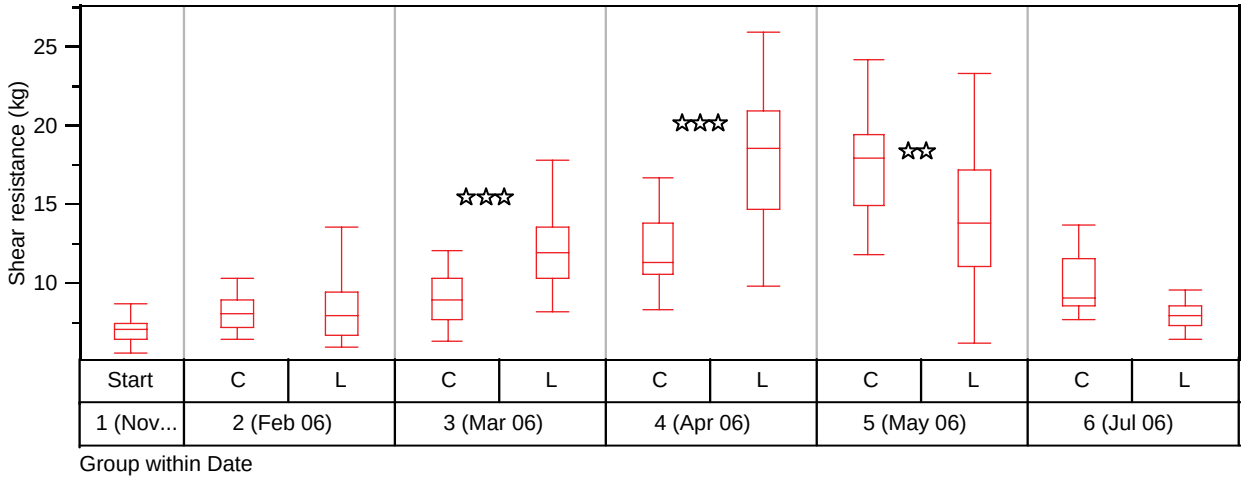

Figure 18: Shear resistance (kg) development between the two groups of female fish during the trial period, for the control (C) and the light treated group (L). Stars indicate level of significance and when blank no significant difference exists. Box plots show the 25 and 75 percentile, mean and max and min values. A table describing significance levels is found in Appendix 1.

There was less variation between the two groups of male fish (Fig.19). It was only in May that a significantly ($p<0.02$) larger shear resistance was seen in the C-group (11 ± 2) when compared to the L-group (10 ± 2). A month earlier, there was a tendency to a higher shear resistance in the L-group (11 ± 3) compared to the C-group (9 ± 2). This however is not significant based on a non-parametric test, due to lack of normality.

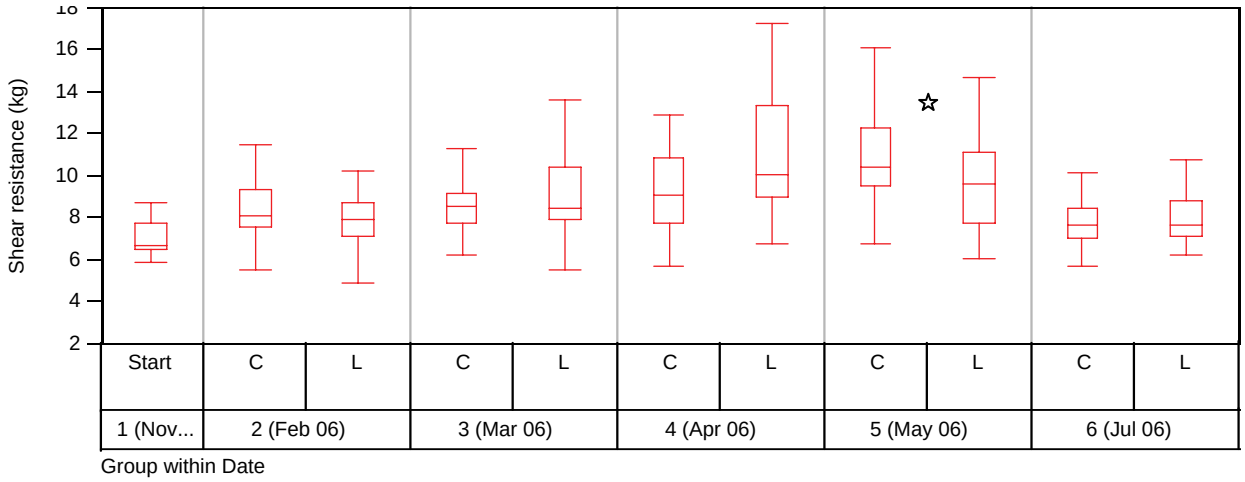

Figure 19: Shear resistance (kg) development between the two groups of male fish during the trial period, for the control (C) and the light treated group (L). Stars indicate level of significance and when blank no significance difference exists. Box plots show the 25 and 75 percentile, mean and max and min values. A table describing significance levels is found in Appendix 1.

Shear resistance varied considerably between sexes in the C-group and was found to vary the most in the female fish. However there were no significant differences between sexes in February and March, where the values were approximately 7 to 8 kg.

Female fish had approximately 3 months of higher shear resistance than male fish during the trial for both groups, reaching a peak of 17.6 kg in May and 18.1 kg in April for the C-group and the L-group respectively.

In April, the shear resistance increased considerably for female fish in both groups and was significantly higher than that of the male fish. The shear resistance reached a maximum value in both sexes in the C-group in May, a month later than the fish from the L-group.

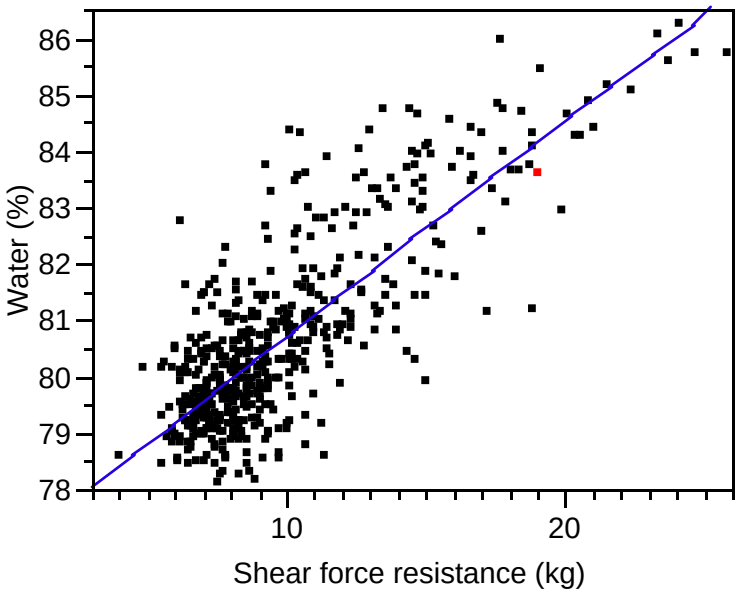

Figure 20: Showing 0.76 linear fit between Water % and texture measured in shear force resistance (kg).

Lower water content results in a lower shear resistance (Fig.20). This means that starved fish or fish undergoing maturation have a higher shear resistance compared to fish in a normal condition.

Principal component analysis (PCA)

As shown in Fig. 21, the female fish had more seasonal variation than male fish. The female fish in the L-group (LFeb1--> LJul) experienced variation over a slightly lower mean than the C-group (CFeb1--> CJul1).

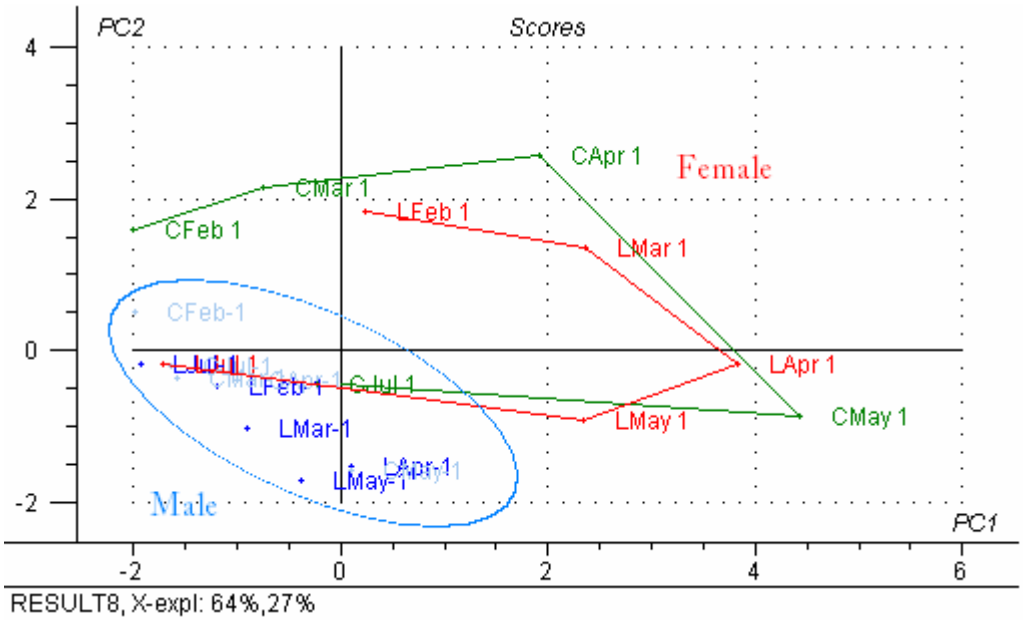

Figure 21: Score plot showing variation in PC1 and PC2 .Female fish have the most variation and are coloured red and green. L indicates fish in the light manipulated group and C indicates fish in the control group. Male fish have little variation and is represented with blue and light blue. The November sampling is not included in the PCA analysis.

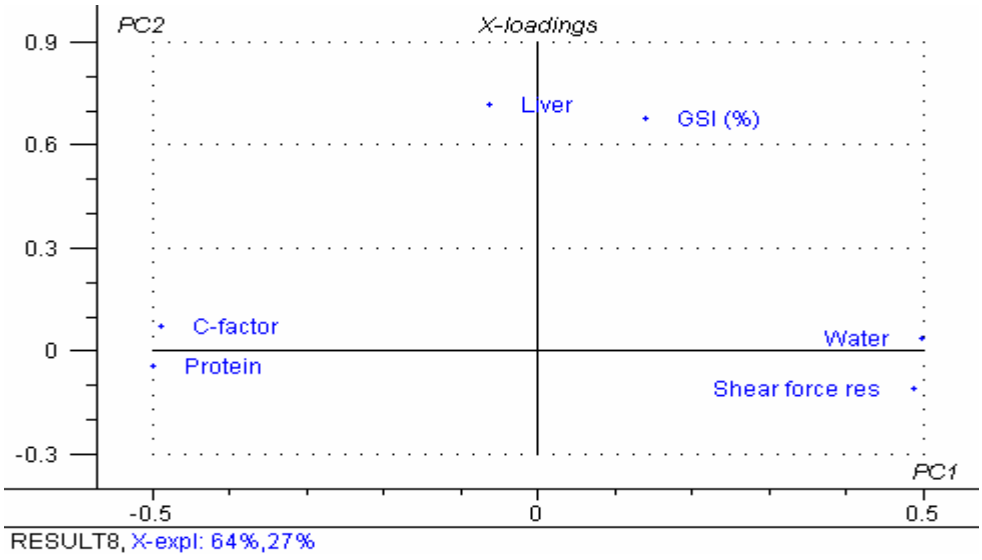

Figure 22: Loadings plot showing 6 parameters which explain 91 % of the variation between sampling dates, groups and sexes.

Multivariate analysis uncovered that 91 % of the variation between groups and sexes was explained by the differences in: GSI, liver size, C-Factor, shear resistance, water and protein content (Fig. 22).

Discussion

The trial was performed using non-replicated cages in a small scale, which added a degree of uncertainty to the results and therefore, the results serve as indications rather than well documented effects. However, two years prior to this trial, a feeding trial with cod was conducted with six net pens at the same site (Solberg et al., 2006). Results from that feeding trial showed that there were no significant differences in muscle growth between the fish in the six netpens. In this trial the cod in the C-group matured at the same rate as those fish in their trial. This supports the assumption that fish in the C-group in this trial matured at the expected time for cod at this latitude.

The design of this experiment was created based on economical considerations, work load and analytical quality.

Since the fish within each cage were not totally independent of each other, and most of the results were reached using traditional parametric tests, the results are considered as an act of pseudo-replication (Oksanen, 2001). However, non-parametric tests were used where material was not normally distributed or where homogeneity of variance was not found. The non-parametric tests performed on the same data generally showed the same results as those reached by using parametric tests.

Growth differences

Fish in the trial were not individually tagged and all of the fish were not weighed on each of the sample dates. Therefore statistical tests could not be applied to the specific growth rates (SGR) between the L-group and C-group, and thus these results could not be included in the results.

Gutted weight differed markedly between the groups of female fish. The female cod from the L-group had a lower gutted weight earlier in the spring compared to female fish in the C-group, which indicates that spawning influences female muscle mass. No statistical differences in gutted weight were found between the groups of male fish during the trial. Female fish in the C-group had the lowest gutted weight in the trial, which was measured to be 1.62 kg in May. This low gutted weight supports previous observations that female fish suffer a more pronounced loss of fish muscle during spawning than male fish (Solberg and Willumsen, 2007).

Differences in the C-condition factor of the slaughtered fish between the treatment groups were more pronounced in the female fish. This is probably in large part due to the fact that female fish

loose more muscle mass during spawning. Since male fish loose less muscle mass during spawning, there were no significant differences in the C-factor between the male treatment groups due to the suggested accelerated maturation. The female fish on the other hand, had a significantly lower C-factor in the L-group compared to the C-group, in both February and March, indicating accelerated maturation in the L-group. Gutted weight was used instead of round weight when calculating the C-factor because this method removes the variation related to the gonad and liver size, and therefore gives a more accurate picture of the condition of the fish (Solberg et al., 2006).

Factors effecting fibre diameter distribution and growth

It is difficult to see any clear patterns in fibre distribution between the groups within the sexes. However, there seems to be a pattern of fibre distribution in the sexes in relation to the treatment groups, with a tendency towards a higher proportion of larger fibres in the L-group for female fish (Fig. 9), and a smaller proportion of larger fibres for male fish in the L-group (Fig. 8) in comparison to the respective sexes in the C-group. The fish fibres had a unimodal distribution, like the distribution pattern found by Bjørnevik et al. (2003b). There was a significantly higher number of white muscle fibres in male fish in July compared to the numbers seen earlier in November. This suggests that fibre recruitment had occurred in that period of time. Davie et al. (2007b) have suggested that fibre recruitment is more dominant in female fish. However this was not apparent in this trial since the male fish seemed to recruit more fibres than the females during or after maturation between November and July (Table 2). The significant increase in fiber number seen in male cod undergoing maturation is clearly opposite to a similar investigation done on Atlantic halibut in the same geographical region, where the maturation in male halibut resulted in an arrest in the growth of the fish and no further fiber recruitment (Hagen et al. 2006).

Factors effecting biological differences

The sex ratio of the sampled fish in the trial was stable around 1:1 on the first 3 sample dates, but then deviated considerably through the last two sample dates as there were fewer females sampled than males. There is no simple explanation for this, but it must be considered when assessing the significance of the analysis done between the sexes in May and July. This is especially true when looking at the results for differences in GSI, where the variation in numbers of sampled fish within sexes is considerable in May.

The GSI values were significantly influenced by the added light in both sexes, indicating that the light added to the L-group in November 2005 accelerated the peak of maturation in both sexes by approximately one month. Hansen et al. (2001) found similar results when 1500 W quartz halogen lamps were mounted above indoor tanks in December. The result showed an accelerated maturation in the fish (Hansen et al., 2001).

Differences in GSI values were marked between the sexes in both groups, where the highest GSI values in males were found earlier in the spawning cycle, compared to female fish, and were far less pronounced. These results are in accordance with Kjesbu's (1989) results.

Male fish in the L-group did not have a higher GSI value than the male fish in the C-group in February. The GSI value for the male fish in the L-group possibly peaked early in January, and was therefore not seen at the sampling in February. This could be possibly explained by the fact that the male fish in the L-group had already started spawning at the sample date in February, which was seen as the high percentage of spawning fish (maturity stage 5, table 4) in this group. This possibility could only be disclosed with a narrower sampling interval, and this is an important consideration when planning further trials with accelerated maturation in cod.

Female cod shed eggs in regular intervals of up to 2-3 days, and can in total spawn over a period of 50 to 60 days (Kjesbu, 1989). This means that the sample dates used in this trial could have also missed the peak GSI values within females in each of the L- and C-groups. Missing the peak GSI values for either one or both of the sexes could result in variation in GSI.

The HSI values of male fish in both groups were found to be lower than those values seen in the female fish during the trial. The HSI in male fish dropped to <11 % during spawning, in contrast to the HSI in female fish which was >12 %. In addition, findings by both Solberg and Willumsen (2007) and Dahle et al. (2003), suggest that male cod have a lower HSI value than female fish during spawning. These results suggest that male cod use more energy from the liver than from muscle during spawning when compared to female cod, or that they possibly use more energy during the "lek" and spawning activities (Nordeide and Folstad, 2000). Since the feed intake of individual fish was not recorded in this trial, it is impossible to know whether the reduced HSI value in males was caused because the male fish ate less than the females during spawning. If this had been the case, the male fish would have used more energy from the liver due to starvation, as it is well known that starvation results in a reduced liver index (Black and Love, 1986).

However, in contrast to this possibility, studies done by Fordham and Trippel (1999) indicate that male cod eat more during spawning than female cod, and it is therefore not likely that the large reduction in HSI in males is due to sex related differences in eating patterns. Karlsen et al. (1995) found that liver size in male cod was reduced earlier than in its female counterparts. The authors suggested that this could be explained by either enlarged hepatocytes as a result of vittellogenesis in females, or that the males prepare for spawning earlier than females. The observations done in this trial show an increase in HSI values towards female ovulation, thereby supporting the enlargement of hepatocytes in females as suggested by Karlsen et al. (1995).

The percentage of feed in relation to the monthly ingoing biomass is presented (Fig. 14) to give an impression of the feeding response in relation to the sea temperature and their maturation status, as the feeding patterns of cod are very dependent on both temperature (Björnsson et al., 2001) and spawning status (Fordham and Trippel, 1999).

Fig. 14 indicates that the percentage of feed input in relation to the biomass was different between the groups, were the L-group experienced an earlier drop in the specific feeding rate when compared to the C-group. A drop in the specific feeding rate is an indication of fish spawning (Fordham and Trippel, 1999). The drop in the specific feeding rate in the L-group suggests that the fish in this group matured earlier than those in the C-group. The L- group's feeding rate also increased earlier than the C-group after the initial drop, suggesting that the compensatory growth following maturation initiated earlier in this group. Compensatory growth is observed in post-spawning cod in studies by Pedersen and Jobling (1989).

Slaughter loss is positively correlated (0.93) with GSI (%) in fish which reflects the size of the gonads; large gonads represent a large slaughter loss. Slaughter loss varied with GSI and therefore, also with the stage of maturity. There is a low slaughter loss in a fish that has not begun maturing (in stage 1), and a high slaughter loss in a maturing fish. In fact the highest slaughter loss was found in both female and male fish in stage 4. However male fish at stage 2 had a higher slaughter loss compared to fish in stage 3. This could be explained by the difficulties encountered when assessing male gonad stages in maturity. Due to human error, some gonads assessed to be in stage 2 could have been in stage 3 and vice versa (Pers. Comm. Nordeide, 2007a).

Chemical measurements

Chemical measurements were only performed on a number of the samples. The rest of the samples were analysed using a near infrared transmission (NIT) spectroscopy. Results from the traditional chemical measurements were used as reference values for the NIT values when creating a regression model with the Unscrambler software. The regression model created was used to predict values for all of the samples in the trial. This method is less toxic to the environment, takes less time and is more precise (Solberg, 1992) than traditional analytical methods. However, the regression model needs accurate reference data in order to obtain an accurate result (scrap in, scrap out) (Esbensen, 2006).

To ensure the best possible measurements of protein and water percentage, the tissue samples were taken at the same time interval after the death of the fish. All of the samples used for biochemical analysis were taken directly after the fish fillet was homogenized in a food processor and kept on ice until weighing.

Chemical differences

Fig. 17 shows that water and protein content were positively correlated ($r = 0.998$). The differences between the sexes and the treatment groups can therefore be sufficiently studied by focusing on one of these variables. When looking at water content, this study revealed that female fish had a much higher increase in water content throughout maturation when compared to male fish. This was also found in the study by Solberg and Willumsen (2007). The female muscle water content in the L-group peaked a month earlier than fish in the C-group, supporting the idea that fish in the L-group had an accelerated maturation. Female fish need more protein for regulated proteolysis to assure access to essential amino acids needed in developing the oocyts (Martin et al.,1993). There is no doubt that egg production is a strenuous process for female cod, and observations done by Kjesbu (1991) indicate that during spawning female fish mobilize protein and fat from muscle and the liver to the ovaries.

Other results in this trial show that male fish in the L-group had significantly higher water content than male fish in the C-group in February, March and April. This suggests that the male fish from the L-group lost some of its muscle protein during maturation. The muscle water content of the male fish did not reach the levels of the female fish. This could be explained by the differences in sexes in relation to their needs for developing gonads during maturation and spawning.

Texture measurements

There is no recommended or universally accepted way of measuring the texture of a fish (Hyldig and Nielsen, 2001). The method used for measuring texture in this trial was performed at the same location in the fish fillet, at the same temperature, and after the same amount of hours post-slaughter. This was done in order to minimize the noise. There is a risk that the thickness of the fillet might be of importance to the textural properties of each fillet, and this may have introduced some uncertainty to the result. However, in this trial there was little variation in the thickness of the fillets. Temperature is shown to also effect the shear resistance in fish (Veland and Torissen, 1999) and the temperature of the fish sample was therefore kept constant.

Factors effecting texture

Texture, which was measured by the total resistance against a blade when cutting 90 % of a fillet's height, was positively correlated to the water content ($r = 0.74$) (Fig. 20). It is therefore not surprising that female fish had a larger variation in shear resistance (kg) compared to male fish. The higher shear resistance in maturing female fish is also a strong indication that female fish suffer a higher protein loss during spawning than male fish (Bjørnevik, 2003a). In addition, as seen in meat, it is possible that a specific amount myofibril protein loss results in a relatively higher amount of connective tissue, which leads to a firmer texture (Bjørnevik, 2003a). The loss of protein could be explained by the fact that female fish need more protein for regulated proteolysis which assures access to essential amino acids needed in developing the oocyts. This suggestion was presented in a study on salmon *(Salmo salar)* by Martin et al. (1993).

Both sexes of cod have a higher shear resistance when maturing. The higher shear resistance seen in maturing fish could be explained by the occurrence of a larger amount of cross-linking in collagen, as well as a higher water content, which was observed in a study on halibut (Hagen et al., 2007). The seasonal stability of shear resistance seem to be greater in male fish compared to female fish, as the standard deviation in the shear resistance measurement on male fish didn't exceed 2.8 kg when the corresponding value for females cod was 4.6 kg.

Loosing myofibrillar protein within each muscle fibre could also result in cell shrinkage, which in turn, gives a higher fibre density (Johnston et al., 2001). Evidence suggests that a higher muscle cell density is associated with a firmer texture in salmon (Johnston et al., 2001), which makes it also plausible for cod. However, nothing was done in this study to confirm the relationship between muscle cell density and firmness of the flesh, which is a sensory trait. In this study, a weak

correlation between shear resistance and muscle cell density (Appendix 3) suggests a potential relationship between the two.

Principal component analysis

Principle component analysis (PCA) is a valuable method which uses a multivariate approach to assess the differences between the treatment groups and the sexes. This analysis was done to clarify which samples varied most between the groups. The score plot (Fig.21) clarifies that female fish varied considerably more than the male fish. This score plot also supports the notion that female fish in the L-group were less negatively impacted than the female fish in the C-group during spawning. In turn, this information suggests that accelerated maturation could result in a reduced production loss compared to fish undergoing a normal maturation cycle. Hansen et al. (2001) also suggest this and therefore, light manipulation could be a possible production strategy to increase the production yield, as seen in this trial. However, more research is needed to quantify the possible economic gain.

General observations

This trial suffered several otter attacks during January and February. These attacks killed several fish in both groups. The C-group was hit the hardest, and this could have influenced the results. However, previous cod-trials done at Gildeskål Research Station have shown that fish growth is not effected by otter attacks in any measurable way.

Sea temperatures in northern Norway lie around 12° C in the summer, and therefore the growth potential for cod is high in this area during the summer. The situation is unlike that in the sea pens longer south, where the sea temperatures are often high enough in the summer to inhibit cod growth. This means that a delayed maturation occurrence, which would delay the maturation of cod until summer, would be potentially more damaging for the growth of cod in aquaculture in northern Norway in comparison to such a strategy in southern Norway. In fact, it could be beneficial to speed up the maturation of cod in northern Norway (instead of delay it) because attempts to delay or inhibit maturation have often resulted in maturing fish during the summer (Davie et al., 2007; Taranger et al., 2006), which wastes the optimal growth period in the North.

Light manipulation to speed up maturation could be an alternative strategy to cost-effectively reduce the problem followed by maturation of cod in aquaculture. This strategy could be potentially be used for harvest weight fish, but more likely to reduce juvenile spawning in the early stages of

production (300 to 700 gram).

These findings only suggest the potential of this strategy and more thorough investigations are needed to produce a more comprehensive result. These further investigations need to research light manipulation in regard to the light intensities, light quality, light regime timing, effects on growth and flesh quality in the summer and autumn after spawning.

Conclusion

Additional artificial light added in a sea net pen on the 1st November 2005, resulted in approximately a one month earlier maturation in cod, in comparison to cod under ambient conditions.

The earlier maturation resulted in a lower muscle water content and higher C-factor of the fish earlier in the spring which are indications of a better fish quality.

Results also revealed tendencies of a reduced impact of maturation for the L-group compared to the C-group, however these are not clear evidence only indications.

Results presented also revealed differences in the impact of maturation between the sexes. These differences were most pronounced in water content, shear resistance and GSI values. However, all the parameters reflected that female fish matured slightly later than male fish, and that the energy cost of maturation is more dramatic in female in comparison to male fish.

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Appendix 1: Mean values and test results

Table 6: Mean values, standard deviations and test results for female fish, between the C-group and the L-group for all dates throughout the trial.

Type	Month	Control			Light			P<	Test	Eq Var.
		Mean	SD	#	Mean	SD	#			
GW (Kg)	Nov	2.14	0.39	28					No test	
GW (Kg)	Feb	2.38	0.37	25	1.92	0.39	23	0.0003	Student t	Y
GW (Kg)	March	2.04	0.34	24	1.67	0.27	24	0.0001	Student t	Y
GW (Kg)	April	1.94	0.34	28	1.89	0.24	19	0.3694	Student t	Y
GW (Kg)	May	1.62	0.25	18	1.87	0.39	17	0.0129	t-test	N
GW (Kg)	July	2.33	0.68	12	2.14	0.35	15	0.1462	t-test	N
C - Factor	Nov	1.07	0.09	28					No test	
C - Factor	Feb	0.97	0.07	25	0.86	0.09	23	0.0001	Student t	Y
C - Factor	March	0.91	0.09	24	0.8	0.07	24	0.0001	pooles t-test	Y
C - Factor	April	0.77	0.09	27	0.75	0.1	19	0.2928	Student t	Y
C - Factor	May	0.68	0.05	18	0.77	0.08	17	0.0003	Student t	Y
C - Factor	July	0.86	0.12	12	0.93	0.09	15	0.0878	t-test	N
HSI (%)	Nov	15.1	2.9	28					No test	
HSI (%)	Feb	15.5	2.8	25	17.4	2.8	23	0.0258	Student t	Y
HSI (%)	March	17.7	2.9	24	17.9	3	24	0.7587	Student t	Y
HSI (%)	April	18.0	3.1	27	14.8	3.9	19	0.0041	Student t	Y
HSI (%)	May	15.2	3.4	18	14.4	4	17	0.5426	Student t	Y
HSI (%)	July	12.4	2.9	12	14.1	2.9	15	0.1448	Student t	Y
GSI (%)	Nov	4	1	27					No test	
GSI (%)	Feb	16	4	25	31	12	23	0.0001	t-test	N
GSI (%)	March	30	9	24	35	11	24	0.0651	Student t	Y
GSI (%)	April	45	12	27	15	16	19	0.0001	Student t	Y
GSI (%)	May	12	11	18	3	1	17	0.0027	t-test	N
GSI (%)	July	2	0.7	12	2	0.6	15	0.6914	Student t	Y
Water (%)	Nov	79.2	0.3	28					No test	
Water (%)	Feb	79.7	0.4	25	81.5	1.4	23	0.0001	t-test	N
Water (%)	March	80.6	0.2	24	83.3	0.2	24	0.0001	Student t	Y
Water (%)	April	82.3	1.1	27	83.8	1.8	19	0.0026	t-test	N
Water (%)	May	84.2	1.2	18	83	1.4	17	0.0105	Student t	Y
Water (%)	July	80.9	1.1	12	79.9	3.3	15	0.2852	t-test	N
Protein (%)	Nov	19.3	0.4	28					No test	
Protein (%)	Feb	18.8	0.4	25	16.9	1.5	23	0.0001	t-test	N
Protein (%)	March	17.8	0.9	24	15	0.9	24	0.0001	Student t	Y
Protein (%)	April	16.1	1.2	27	14.5	1.9	19	0.0027	t-test	N
Protein (%)	May	14.0	1.3	18	15.4	1.5	17	0.0094	Student t	Y
Protein (%)	July	17.5	1.2	12	18.6	3.8	15	0.3116	t-test	N
Shear res. (kg)	Nov	7.1	1	28					No test	
Shear res. (kg)	Feb	8.1	1	25	8.91	3.2	23	0.25	t-test	N
Shear res. (kg)	March	9.1	1.7	24	12	2.3	24	0.0001	Student t	Y
Shear res. (kg)	April	12.0	2.5	27	18.1	4.5	19	0.0001	t-test	N
Shear res. (kg)	May	17.6	3.3	18	14.1	4.2	17	0.0093	Student t	Y
Shear res. (kg)	July	10.2	2.7	12	8.1	1	15	0.0211	t-test	N

Table 7: Mean values, standard deviations and test results for male fish, between the C-group and the L-group for all dates throughout the trial.

Type	Month	Control			Light			P<	Test	Eq Var.
		Mean	SD	#	Mean	SD	#			
GW (Kg)	Nov	2.03	0.43	23					No test	
GW (Kg)	Feb	2.08	0.28	25	1.92	0.36	27	0.0856	Student t	Y
GW (Kg)	March	1.95	0.31	23	2.09	0.56	21	0.3013	t-test	N
GW (Kg)	April	2.03	0.42	17	1.96	0.34	26	0.5252	Student t	Y
GW (Kg)	May	2.05	0.33	27	1.91	0.36	28	0.1506	Student t	Y
GW (Kg)	July	2.43	0.49	33	2.29	0.43	38	0.1798	Student t	Y
C - Factor	Nov	1.08	0.10	23						
C - Factor	Feb	0.91	0.05	25	0.89	0.06	27	0.2358	Student t	Y
C - Factor	March	0.89	0.07	23	0.90	0.08	21	0.5375	Student t	Y
C - Factor	April	0.86	0.07	17	0.85	0.09	26	0.5021	Student t	Y
C - Factor	May	0.85	0.07	27	0.87	0.07	28	0.2131	Student t	Y
C - Factor	July	0.93	0.10	33	0.97	0.11	38	0.1127	Student t	Y
HSI (%)	Nov	15.1	1.4	23					No test	
HSI (%)	Feb	14.1	1.8	25	12.6	1.8	27	0.0045	Student t	Y
HSI (%)	March	12.2	1.7	23	11.1	2.6	21	0.1226	t-test	N
HSI (%)	April	13.0	2.9	17	11.2	1.9	26	0.0315	t-test	N
HSI (%)	May	10.6	2.2	27	10.8	2.3	28	0.7417	Student t	Y
HSI (%)	July	12.4	2.2	33	13	2.4	38	0.251	Student t	Y
GSI (%)	Nov	4	2	22					No test	
GSI (%)	Feb	19	6	25	14	4	27	0.0001	Mann Whitney	Y
GSI (%)	March	18	4	23	4	4	21	0.0001	Student t	Y
GSI (%)	April	11	6	17	2	2	26	0.0001	Mann Whitney	N
GSI (%)	May	1	1	27	1	0.4	28	0.1548	Mann Whitney	N
GSI (%)	July	0.5	0.2	33	0.9	1	38	0.2261	Mann Whitney	N
Water (%)	Nov	78.9	0.3	23					No test	
Water (%)	Feb	78.9	0.4	25	80.0	0.6	27	0.0001	Student t	Y
Water (%)	March	79.0	0.5	23	80.4	0.7	21	0.0001	Student t	Y
Water (%)	April	79.8	0.7	17	80.9	0.7	26	0.0001	Student t	Y
Water (%)	May	80.9	0.5	27	80.7	0.6	28	0.25	t-test	N
Water (%)	July	79.9	0.5	33	79.9	0.5	37	0.596	Student t	Y
Protein (%)	Nov	19.5	0.3	23					No test	
Protein (%)	Feb	19.6	0.4	25	18.5	0.6	27	0.0001	Mann Whitney	N
Protein (%)	March	19.3	0.5	23	18.1	0.7	21	0.0001	Student t	Y
Protein (%)	April	18.7	0.7	17	17.5	0.7	26	0.0001	Student t	Y
Protein (%)	May	17.5	0.5	27	17.7	0.7	28	0.2283	t-test	N
Protein (%)	July	18.5	0.5	33	18.5	0.5	38	0.9181	Student t	Y
Shear res. (kg)	Nov	7.0	0.8	23					No test	
Shear res. (kg)	Feb	8.3	1.4	25	8.0	1.4	27	0.4528	Student t	Y
Shear res. (kg)	March	8.4	1.5	23	9.1	2.0	21	0.1967	Student t	Y
Shear res. (kg)	April	9.3	1.9	17	11.0	2.8	26	0.0778	Mann Whitney	Y
Shear res. (kg)	May	11.0	2.2	27	9.6	2.3	28	0.0178	Student t	Y
Shear res. (kg)	July	8.1	1.8	33	8.1	1.5	38	0.8087	Mann Whitney	Y

Table 8: Mean values, standard deviation and test results between female and male fish in the light manipulated group throughout the trial, GSI and HSI is in procentage of the gutted weight.

Type	Month	Light			Light			P<	Test	Eq Var.
		F Mean	SD	#	M Mean	SD	#			
GW (Kg)	Nov	2.12	0.39	27	1.998	0.42	22	0.29	Student- t	Y
GW (Kg)	Feb	1.92	0.39	23	1.92	0.36	27	0.99	Student t	Y
GW (Kg)	March	1.67	0.27	24	2.09	0.56	21	0.0036	t – test	N
GW (Kg)	April	1.89	0.24	19	1.96	0.34	26	0.4423	Student t	Y
GW (Kg)	May	1.87	0.39	17	1.91	0.36	28	0.72	Student t	Y
GW (Kg)	July	2.14	0.35	15	2.29	0.43	38	0.23	Student t	Y
C - Factor	Nov	1.07	0.10	27	1.08	0.1	22	0.79	Student t	Y
C - Factor	Feb	0.86	0.09	23	0.89	0.06	27	0.13	t - test	N
C - Factor	March	0.8	0.07	24	0.9	0.08	21	0.0001	Student t	Y
C - Factor	April	0.75	0.10	19	0.85	0.09	26	0.001	Student t	Y
C - Factor	May	0.77	0.08	17	0.86	0.066	28	0.0001	Student t	Y
C - Factor	July	0.93	0.09	15	0.97	0.11	38	0.3056	Student t	Y
HSI (%)	Nov	15.1	2.91	27	15.13	1.43	22	0.94	Student t	Y
HSI (%)	Feb	17.4	2.76	23	12.61	1.82	27	0.0001	t - test	N
HSI (%)	March	17.9	2.95	24	11.12	2.61	21	0.0001	Student t	Y
HSI (%)	April	14.8	3.93	19	11.2	1.89	26	0.001	t – test	N
HSI (%)	May	14.4	3.97	17	10.78	2.33	28	0.0024	t – test	N
HSI (%)	July	14.1	2.85	15	13.02	2.38	38	0.1817	Student t	Y
GSI (%)	Nov	4	1	27	4	2	22	0.9822	Student t	Y
GSI (%)	Feb	32	12	23	1	4	27	0.0001	Mann Whitney	N
GSI (%)	March	35	11	24	4	4	21	0.0001	t - test	N
GSI (%)	April	16	16	19	2	2	26	0.0001	Mann Whitney	N
GSI (%)	May	3	1	17	1	0.4	28	0.0001	Mann Whitney	N
GSI (%)	July	2	0.6	15	0.9	1	38	0.0001	Mann Whitney	N
Water (%)	Nov	79.2	0.3	27	78.9	0.3	22	0.0008	Student t	Y
Water (%)	Feb	81.5	1.4	23	80.0	0.6	27	0.0001	Mann Whitney	N
Water (%)	March	83.3	0.8	24	80.4	0.7	21	0.0001	Mann Whitney	Y
Water (%)	April	83.8	1.8	19	80.9	0.7	26	0.0001	Mann Whitney	N
Water (%)	May	83.0	1.4	17	80.7	0.6	28	0.0001	T – test	Y
Water (%)	July	80.7	0.6	14	79.9	0.5	37	0.9989	Student t	Y
Protein (%)	Nov	19.2	0.4	27	19.5	0.3	22	0.68	Student t	Y
Protein (%)	Feb	16.9	1.5	23	18.5	0.6	27	0.0001	T – test	N
Protein (%)	March	15.0	0.9	24	18.1	0.7	21	0.0001	Student t	Y
Protein (%)	April	14.5	1.9	19	17.5	0.7	26	0.0001	Student t	Y
Protein (%)	May	15.4	1.5	17	17.7	0.7	28	0.0001	Student t	N
Protein (%)	July	18.6	3.8	15	18.5	0.5	38	0.948	T – test	N
Shear res. (kg)	Nov	7.1	1.0	27	7.0	0.9	22	0.8741	Student t	Y
Shear res. (kg)	Feb	8.9	3.2	23	8.0	1.4	27	0.9845	Mann Whitney	N
Shear res. (kg)	March	12.0	2.3	24	9.1	2.0	21	0.0001	Student t	Y
Shear res. (kg)	April	18.1	4.5	19	11.0	2.8	26	0.0001	T – test	N
Shear res. (kg)	May	14.1	4.2	17	9.6	2.3	28	0.0004	T - test	N
Shear res. (kg)	July	8.1	1.0	15	8.1	1.5	38	0.4833	Mann Whitney	N

Table 9: Mean values. standard deviation and test results between female and male fish in the control group throughout the trial. GSI and HSI is in procentage of the gutted weight.

Type	Month	Control			Control			P<	Test	Eq Var
		F Mean	SD	#	M Mean	SD	#			
GW (Kg)	Nov	2.12	0.39	27	1.998	0.42	22	0.2947	Student t	Y
GW (Kg)	Feb	2.38	0.37	25	2.08	0.28	25	0.0019	Student t	Y
GW (Kg)	March	2.044	0.34	24	1.95	0.31	23	0.351	Student t	Y
GW (Kg)	April	1.94	0.34	28	2.03	0.42	17	0.4267	Student t	Y
GW (Kg)	May	1.62	0.25	18	2.05	0.33	27	0.0001	Student t	Y
GW (Kg)	July	2.33	0.68	12	2.43	0.49	33	0.5543	Student t	Y
C - Factor	Nov	1.07	0.096	27	1.08	0.1011	22	0.7881	Student t	Y
C - Factor	Feb	0.97	0.07	25	0.91	0.05	25	0.0024	Student t	Y
C - Factor	March	0.91	0.09	24	0.89	0.07	23	0.2429	Student t	Y
C - Factor	April	0.77	0.09	28	0.86	0.07	17	0.0009	Student t	Y
C - Factor	May	0.68	0.05	18	0.85	0.07	27	0.0001	Student t	Y
C - Factor	July	0.86	0.12	12	0.93	0.099	33	0.0752	Student t	Y
HSI (%)	Nov	15.1	2.91	27	15.13	1.43	22	0.9409	Student t	Y
HSI (%)	Feb	15.5	2.79	25	14.12	1.83	25	0.045	T – test	N
HSI (%)	March	17.7	2.88	24	12.18	1.7	23	0.0001	T – test	N
HSI (%)	April	18.0	3.07	28	13.02	2.93	17	0.0001	Student t	Y
HSI (%)	May	15.2	3.4	18	10.58	2.16	27	0.0001	T – test	N
HSI (%)	July	12.4	2.88	12	12.38	2.22	33	0.9919	Student t	Y
GSI (%)	Nov	4	1	27	4	2	22	0.9822	Student t	Y
GSI (%)	Feb	16	4	25	19	6	25	0.6376	T – test	N
GSI (%)	March	30	9	24	18	4	23	0.0001	Student t	Y
GSI (%)	April	45	12	28	11	6	17	0.0001	T - test and M.W.	N
GSI (%)	May	12	11	18	1	1	27	0.0001	T - test and M.W.	N
GSI (%)	July	2	1	12	0.5	0.2	33	0.0001	T – test and M.W.	N
Water (%)	Nov	79.2	0.3	27	78.9	0.3	22	0.0008	Student t	Y
Water (%)	Feb	79.7	0.4	25	78.9	0.4	25	0.0001	Student t	Y
Water (%)	March	80.6	0.8	24	79.0	0.5	23	0.0001	t- test	N
Water (%)	April	82.3	1.1	28	79.8	0.7	17	0.0001	T – test	N
Water (%)	May	84.2	1.2	18	80.9	0.5	27	0.0001	T – test	N
Water (%)	July	80.9	1.2	12	79.9	0.5	33	0.0172	T – test	N
Protein (%)	Nov	19.2	0.4	27	19.5	0.3	22	0.0041	Student t	Y
Protein (%)	Feb	18.8	0.4	25	19.6	0.4	25	0.0001	Student t	Y
Protein (%)	March	17.8	0.9	24	19.3	0.5	23	0.0001	T – test	N
Protein (%)	April	16.1	1.2	28	18.7	0.7	17	0.0001	T – test	N
Protein (%)	May	14.0	1.3	18	17.5	0.5	27	0.0001	T – test	N
Protein (%)	July	17.5	1.2	12	18.5	0.5	33	0.016	T – test	N
Shear res. (kg)	Nov	7.1	1.0	27	7.0	0.9	22	0.8741	Student t	Y
Shear res. (kg)	Feb	8.1	1.0	25	8.3	1.4	25	0.4748	Student t	Y
Shear res. (kg)	March	9.1	1.7	24	8.4	1.5	23	0.1958	Student t	Y
Shear res. (kg)	April	12.2	2.6	28	9.3	1.9	17	0.0003	Student t	Y
Shear res. (kg)	May	17.6	3.3	18	11.0	2.2	27	0.0001	T – test	N
Shear res. (kg)	July	10.2	2.7	12	8.1	1.8	33	0.0008	Mann Whitney	Y

Appendix 2: Maximum and minimum value tests

Table10: Tests between minumum and maximum values in the trial, between groups and sexes.

Type	Month	Maximum L			Maximum C			P<	Test	Sex
		Max L	SD	#	Max C	SD	#			
GSI (%)	Mar/Apr	35	11	24	45	12	28	0.002	Student t	F
GSI (%)	Feb	14	4	27	19	6	25	0.0001	Mann Whitney	M
Shear res. (kg)	Apr/May	18.1	4.5	19	17.6	3.3	18	0.7041	Mann Whitney	F
Shear res. (kg)	Apr/May	11.0	2.8	26	11.0	2.2	27	0.4933	Mann Whitney	M
Type	**Month**	**Minimum L**			**Minimum C**			**P<**	**Test**	**Sex**
		Min L	**SD**	**#**	**Min C**	**SD**	**#**			
Protein (%)	Apr/May	14.5	1.9	19	14.0	1.3	18	0.454	Student t	F
Protein (%)	Apr/May	17.5	0.7	26	17.5	0.5	27	0.9331	Student t	M
HSI (%)	Jul	12.4	2.9	12	14.1	2.9	15	0.1448	Student t	F
HSI (%)	May	10.8	2.3	28	10.6	2.2	27	0.7417	Student t	M

Appendix 3: Correlations

Table 11: Correlations between parameters in the trial. correlations is found based on all the samples at all sample dates. Density. Cell # and Cell Dia. is only calculated in November and July and correlations for these parameters is therefore only based on samples taken in November and July.

	Lenght	***RW***	***GW***	***Liver***	***Gonad***	***Water***	***Protein***	***Shear Resistance***	***Density***	***Cell #***	***Cell Dia***
Lenght	**1.000**	0.726	0.760	0.464	0.096	0.079	-0.076	0.200	-0.434	-0.013	0.522
RW	0.726	**1.000**	0.844	0.815	0.446	-0.164	0.163	-0.116	-0.506	0.185	0.521
GW	0.760	0.844	**1.000**	0.620	-0.072	-0.383	0.381	-0.254	-0.588	0.082	0.622
Liver	0.464	0.815	0.620	**1.000**	0.397	-0.123	0.115	-0.120	-0.387	0.243	0.410
Gonad	0.096	0.446	-0.072	0.397	**1.000**	0.265	-0.264	0.148	0.103	-0.055	-0.044
Water	0.079	-0.164	-0.383	-0.123	0.265	**1.000**	-0.998	0.764	0.546	-0.009	-0.452
Protein	-0.076	0.163	0.381	0.115	-0.264	-0.998	**1.000**	-0.756	-0.549	0.006	0.453
Shear Resistance	0.200	-0.116	-0.254	-0.120	0.148	0.764	-0.756	**1.000**	0.559	0.140	-0.431
Density	-0.434	-0.506	-0.588	-0.387	0.103	0.546	-0.549	0.559	**1.000**	0.654	-0.961
Cell #	-0.013	0.185	0.082	0.243	-0.055	-0.009	0.006	0.140	0.654	**1.000**	-0.657
Cell Dia	0.522	0.521	0.622	0.410	-0.044	-0.452	0.453	-0.431	-0.961	-0.657	**1.000**

Appendix 4: Maturation scale for cod

Table12: 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.

Table13: Maturation scale for male cod (Fotland et al., 2000)

Stage	Description
1	Umoden. Tynn streng.
2	Modnende. Klemmes i stykker i små biter. Tykk melke. Hvitaktig.
3	Klemmes i stykker. Flytende seig melke, hvit.
4	Klemmes i stykker, men mer lettflytende melke, hvit.
5	Rennende melke, gytende, hvit.
6	Utgytt, blå/rød knudrete streng.

Appendix 5: Predictions

Water

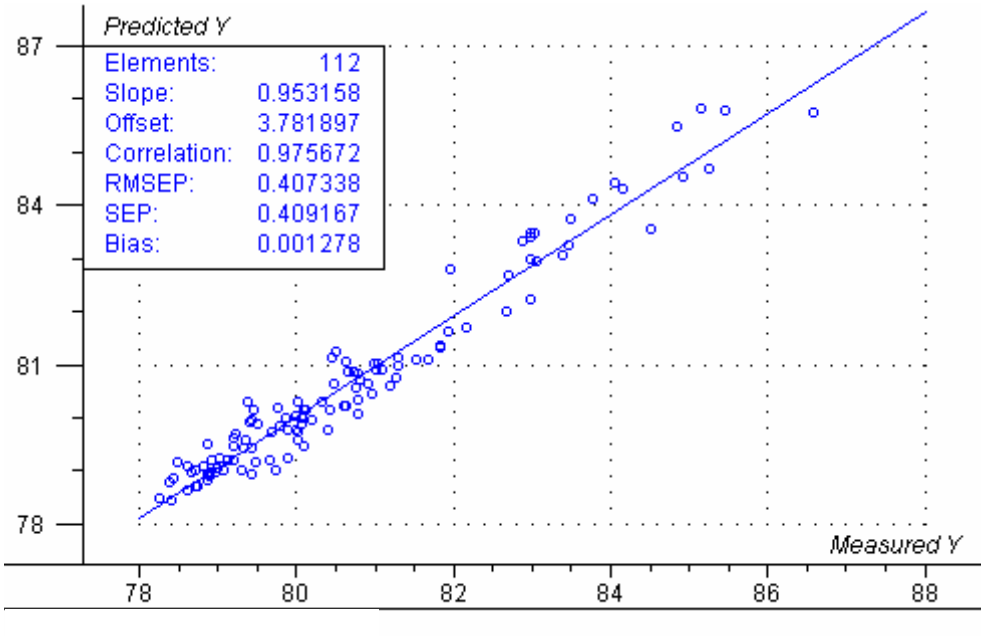

Figure 23: Regression showing the result of PLS regression, for measured NIR values against Water values after drying for 16 hours in 106°C of 112 samples. Standard error of prediction is 0.41 and the correlation is very strong (0.98). The PLS is done using 5 Principal Comoponents,

Protein

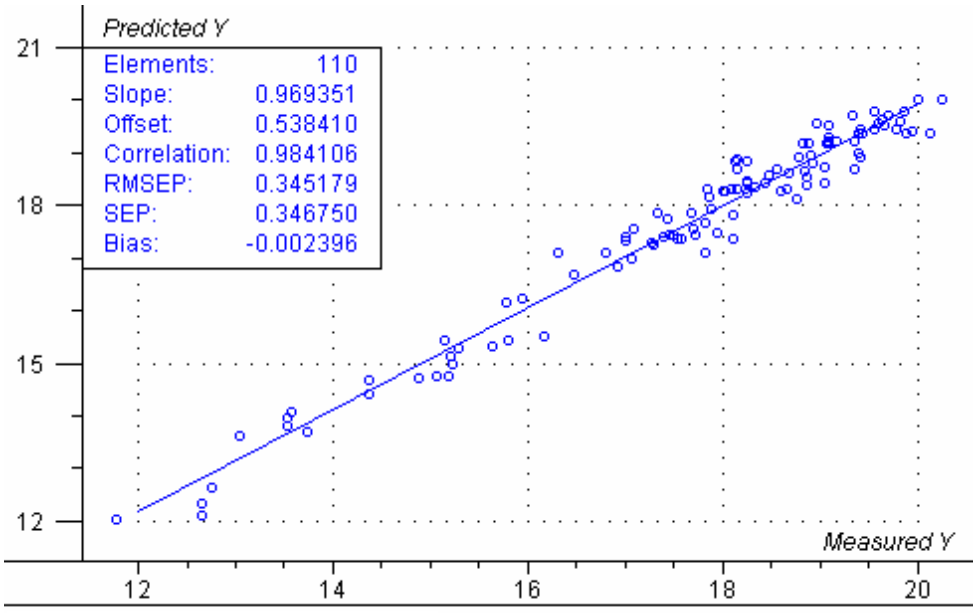

Figure 24: Regression showing the result of PLS regression, for measured NIR values against Kjeldahl Protein values of 110 samples. Standard error of prediction is 0.35 and the correlation is very strong (0.98). The PLS is done using 5 Principal Components.