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Photoperiod and genetics of growth and maturity in cod *(Gadus morhua)*

Guðmundur Óli Hreggviðsson
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Helgi Thorarensen

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Guðmundur Óli Hreggviðsson[1,2)]

Ólafur Friðjónsson[1)]

Þorleifur Ágústsson[1)]

Sigríður Hjörleifsdóttir[1)]

Kjell Hellman[1)]

Filipe Figueiredo[1)]

Helgi Thorarensen[3)]

[1)] Matís ohf, Vínlandsleið 12, 113 Reykjavík

[2)] Háskóli Íslands, Líffræðiskor, Askja, Sturlugata, 101 Reykajvík

[3)] Háskólinn á Hólum, 551 Sauðárkrókur

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Report summary

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Titill / Title	**Photoperiod and genetics of growth and maturity in cod (*Gadus morhua*)**		
Höfundar / Authors	Guðmundur Óli Hreggviðsson[1,2)], Ólafur H. Friðjónsson[1)], Þorleifur Ágústsson[1)], Sigríður Hjörleifsdóttir[1)], Kjell Hellman[1)], Filipe Figueiredo[1)], Helgi Thorarensen[3)]. *[1)]Matís, [2)]Háskóli Íslands, [3)]Hólaskóli*		
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Ágrip á íslensku:

Eitt af megin vandamálum þorskeldis er ótímabær kynþroski sem leiðir af sér hægari vöxt og mun lengri eldistíma. Forrannsóknir sem framkvæmdar voru í kerjum á landi bentu til þess að ný tegund ljósa, svonefndra CC ljósa, sem varpa ljósi af einni bylgjulengd (grænt ljós) hefði mun meiri áhrif á vöxt (allt að 60% vaxtaraukning) og tímasetningu kynþroska en hefðbundin halógen ljós. Í þessari rannsókn var kannaður hver væri þáttur arfgerðar og tjáningu ákveðinna lykilgena í svörun við ljóslotu, ákvörðun ótímabærs kynþroska og tengsl við aukinn vöxt þorsks í eldi. Kannað var hvort, líkamsstærð, þyngd og ótímabær kynþroski væru fjölskyldulægir eiginleikar.

Að loknu sex mánaða innanhúss foreldi frá klaki við tvenns konar lýsingu, annars vegar við hvítt halógen ljós og hins vegar við samfellt CC-ljós var þorskurinn stríðalinn í sjókvíum. Í sjókvíunum var hann einnig undir mismunandi ljóslotumeðferð annars vegar við samfellt CC-ljós og hins vegar við náttúrlegt ljós. Að loknu tveggja ára sjóeldi var fisknum slátrað, svipgerðir vaxtar og kynþroska ákvarðaðar og hann greindur í systkinahópa, alls u.þ.b. 2000 fiskar úr hvorri meðferð fyrir sig. Í ljós kom að CCL ljós hafði greinleg áhrif til seinkunar kynþroska og var það mun meira áberandi hjá hængum. Kynkyrtlar þroskuðust verulega hægar og alls ekki til fulls miðað við þorsk sem var alinn við náttúrulega ljóslotu á sama tíma. Þá var nokkur breytileiki milli systkinahópa hvað varðaði áhrif ljóss á þroskun kynkyrtla. Á hinn bóginn gætti þessarar seinkunar kynþroska ekki í auknum vaxtarhraða svo nokkru næmi. Fjölskyldulægni vaxtarsvipgerða var einnig könnuð og kom fram greinlegur munur á meðalþyngd milli systkinahópa. Svörun við CCL meðferðar var hins vegar mjög misvísandi og virtist breytileg milli systkinahópa. Virtist CCL meðferð auka vaxtarhraða í sumum hópum en hægja á vaxtarhraða í öðrum. Þetta gat svo verið breytilegt eftir kynjum. Þá var kannað hvort formeðhöndlun seyða með CCL ljósi á strandeldiskeiði gerði þorskinn næmari fyrir áhrifum CCL ljóss á sjókvíastigi. Svo reyndist ekki vera.

Einangruð voru ákveðin lykilgen í vaxtar- og kynþroskastýringu ásamt innröðum þeirra að nokkru eða öllu leyti. Þetta voru gen fyrir vaxtarhormón (GH), viðtaka vaxtarhormóns, viðtaka fyrir vakningarþátt vaxtarhormóns (Growth hormone releasing factor (GHRF)) og viðataka insúlínlíks vaxtarþáttar2 (IGF2). Í innröðum þriggja þessara gena, GH, GHR og IGF2, fundust breytilegar stuttraðir og var þróuð aðferð til erfðamarkagreiningar sem byggði á þessum röðum. Áhrif ljóslotu á vaxtarsvipgerð voru einnig metin með mælingum á tjáningu tveggja þessara gena, GH og GHR. Hlutfallsleg tjáning GHR reyndist ekki vera meiri í stærri fiski og ekki fannst neitt samband milli seinkunnar á kynþroska af völdum CCL og tjáningar á GHR. Tjáning GH var einnig könnuð í fiski á sjókvíastiginu. Marktækur munur í tjáningu GH mældist einungis í einum sýnatökupunkti, snemma á fyrsta ári í sjókvíunum í hópnum sem hlaut CCL meðferð. Þessi aukning átti sér ekki stað í tilsvarandi breytingum í þeim sviðgerðareinginleikum vaxtar og kynþroska sem mældir voru á sama tíma.

Lykilorð á íslensku:	*Ljóslota, kynþroski ,þorskur, vaxtarhormón, erfðir CC-ljós*
Summary in English:	Light and photoperiod is a powerful environmental regulator of growth and sexual maturation in fish. Initial studies had indicated that a new type of lights, Cold Cathode-lights (CCL), had much greater effect on growth and sexual development than white halogen light traditionally used in fish farming. In this study we investigated selected candidate gene expression in cod in response to CC-light photoperiod treatment and possible genetic contribution to this response. This effect was evaluated by quantitatively comparing phenotypic traits under the different treatments. On December 2008 ("*"), significant differences were foundd fish farmed under natural light, in combination with gene expression studies and genotypic family assignments. After hatching the fish was reared from 6 months in indoor tanks under two different light regimes, white halogen light and CC-light. The fish was then transported to sea cages and divided into two groups, one that received continuous CC-light and another that received only natural light treatment. The fish was reared in these sea cages for additional two years until harvesting. Approximately 2000 individuals from each treatment were genotyped and assigned to different full sib groups. Total body weight, length and gonadal weight were also measured for all individuals. Physiologically, the fish responded clearly to CCL treatment regarding maturity related traits, with less gonadal development in the CCL treated fish. The difference was substantial and the same trend could be observed in all families. The degree of response however differed somewhat between families to some which may signify underlying genetic differences. The effect of CCL treatment on growth related traits was less clear. Apparent growth responses to CCL treatment varied greatly between families and they appeared to be either negative or positive, depending on family and sex. Opposite effect were even observed within families on different sexes. In this project genes associated with growth and maturity were retrieved partly or completely from cod (*Gadus morhua*). These genes were: Growth hormone (GH), growth hormone receptor (GHR), growth hormone releasing factor (GHR F) and insulin like growth factor 2 receptor (IGF2R). A number of their introns were also obtained and variable microsatellite regions could be identified in intron regions of three of these genes, GH, GHR F, and IGF2R. A method was developed based on the GH and GHR gene sequences to amplify and evaluate expression of these genes in different tissues of cod. GHR expression levels were measured at different sampling points both during the indoor stage where different size groups and treatments were compared and at the outdoor stage where different light treatments were mainly compared. Differences in expression levels between different size groups and between different light treatment groups were insignificant. The light influence is on the GH gene expression, was only observed in the beginning of March early at the sea cage stage and could not be associated with increased growth or delayed reproductive development. The CCL (Cold-Cathode Light) has a single green wavelength that diffuses more effectively throughout the water column than white light. It may therefore mask natural light more efficiently. Still it may be necessary to train fish for the CCL lights and at the indoor stage one half of the juveniles received CCL treatment before transportation to the sea cages. When imprinted and not imprinted were compared negligible difference in gonadal development were, however, observed strongly indicating that prior imprinting to sea cage rearing had no effect.
English keywords:	*photoperiod, secular maturity, growth hormone, CC-light*

Contents

1. Introduction

1.1. General background

Atlantic cod is predicted to become economically important for the European aquaculture industry. Global production figures (in excess of 4000 tons in 2004, and predictions close to 7000 tons in 2005: Pricket, 2004) are relatively low, however, industry analysts predicted annual harvests of 175,000 tons (just under 20% of present consumption rates) by 2010 (Rosenlund and Skretting, 2004). This would represent (at first point of sale) an industry with gross value of around 880 million Euro (at present market prices). The industry is presently focusing on identifying and addressing production bottlenecks that limit the later stages of the production cycle i.e. those experienced during the on-growing phase (rearing from juvenile to harvestable adult).

The single most important bottleneck identified in all countries where farming is already performed is that of sexual maturation during the grow-out cycle. Cod raised in culture conditions will mature at 2 years of age, prior to the attainment of a profitable harvest weight. Once a stock has matured it will do so each year thereafter until harvested. Sexual maturation in cod as with most species, suppresses growth, has significant impacts on flesh composition/quality and causes the release of fertilized gametes into the surrounding environment which leads to genetic pollution of local native populations. Over and above the numerous environmental concerns that this brings, the main economic impact is a minimum extension of 10 months to the targeted 29 month production cycle. To date there has been only very limited success in the regulation of maturation in commercial scale farming conditions.

1.2. From the environment to the biological cascade

The physiological cascade leading to reproductive maturation has been characterized in many species. However, differentiation between genetic components and plasticity responses to environmental conditions and the mechanisms involved in triggering puberty is mostly not known in the cod and needs further investigation.

The growth process of animals is controlled by a number of physiological pathways that regulate amongst other, energy metabolism, muscle growth and reproductive development. The most important pathways are the somatotropic axis, brain-pituitary-gonad axis and pathways mediating structural development. Somatogenesis is consequently a polygenic trait and involves a multitude of interacting genes and gene products.

The main hormones of the somatotropic axes are growth hormone (GH) secreted from the pituitary gland, and insulin like growth factor I (IGF-I), secreted from the liver in response to GH binding to its hepatic growth hormone receptors (GHRs) , but also expressed locally in many other tissues. Growth hormone expression/secretion is under complex regulatory control, both stimulatory by e.g. dopamine and ghrelin, and inhibitory, in particular by somatomedin releasing inhibitory factor (somatostatin, SRIF) (for review, see Björnsson *et al* 2002). With a recent discovery (Leo *et al* 2007), the possibility that GH in fish is also under a stimulatory control by a GH-releasing hormone (GHRH) has re-emerged. Both GH and IGF-I mediate their actions by binding to their membrane receptors, GHRs and IGFIRs, respectively. The GHR is highly expressed in the liver, but is to some extent expressed in all tissues. The GHR may be found in various splice variants, which may be differentially expressed in different

tissues. Truncated isoforms may act as dominant negative forms by binding GH without eliciting intracellular signalling; other splice variants may give rise to a soluble form of the receptor which acts in plasma as a GH binding protein (GHBP). For IGF-I, there are at least 4 different binding proteins (IGFIBPs) which are at least partly differentially regulated (for review, see Reinecke *et al* 2005).

Not only does GH have growth-promoting effects in fish, but the hormone also has extensive metabolic actions, affecting metabolic rate and pathways (for review, see Rousseau and Defour 2007)

A number of growth-regulating and/or metabolic hormones such as insulin, leptin, glucocorticoids and thyroid hormones interact on various levels with the GH-IGF-I system.

Puberty occurs following gonadal sex differentiation and is characterized by the onset of spermatogenesis in males and oogenesis in females. The hypothalamo-pituitary- gonadal (HPG) axis has a direct influence on this process. Gonadotropin-releasing hormone (GnRH) expressed in the hypothalamus stimulates the synthesis and release of the gonadotropins, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and aromatase from the anterior pituitary which act on the gonads to induce oogenesis and spermatogenesis by stimulating production of sex steroids. The axis also consists of other gene/gene-products that modulate their expression and availability. The release of GnRH is itself governed by the interplay of excitatory and inhibitory signals (neurohormones and neurotransmitters) acting at the level of the hypothalamus (De-Santis, Dean R. Jerry. 2007, Filby *et al*, 2008)).

The onset of puberty can be modulated by regulation of environmental conditions such as the period, intensity and wavelength of light and it is likely that food availability and therefore growth rate plays a part. However, the physical or molecular triggers and genetic mechanisms involved in the activation of puberty are not known for any fish species. A few candidate genes have been identified that may respond to or mediate environmental stimuli such as kisspeptin and its receptor in mammals. A possible involvement of this protein in triggering maturation has recently been demonstrated in *Pimephales promelas* (Filby *et al*, 2008). Environmental signals triggering puberty may vary in importance for different species of fish and responsiveness may be genetically determined, and vary between and within species.

Research into relationship between specific candidate gene variability and phenotypic traits of teleosts associated with growth and maturity is sporadic and only few major genes have been investigated. Relationship has been found for GHRH –in arctic char that explains 9, 4% of early growth (Tao and Boulding, 2003), for GH and growth of salmon (Gross and Nilsson, 1999) the flounder *Paralichthys olivaceus* (Kang et al.. 2002) *Sparus aurata*, (Almuly et al., 2005)) and in "Seabass" (*Lates clacarifer*) (Yue et al. 2001). It is also noteworthy that variability was found in the parvalbumin gene that explains 82% of differential growth in Seabass (Xu et.al. 2006).

Fish have a luminescent and chromatic response in the retina and pineal gland, the two principle light sensitive organs. This sensitivity is due to the molecular structures called opsins. Opsins are proteins which, when coupled to a chromophore (11-*cis* retinal), form functioning photopigments. These photopigments are located in a number of regions in fish (including retina, pineal complex and brain) and provide the physical interaction between light and the subsequent endocrinological and physiological cascades that regulate e.g. maturation or growth. These vital photic structures remain largely uncharacterized in cod despite their obvious value. Both the pineal gland and retina have been demonstrated to regulate many of the crucial physiological life stages from hatching to the sexual maturation. It has been suggested in other teleosts that principally the pineal gland would play this regulatory role. This gland using the above described opsin proteins converts this environmental signal into a number of neuro-endocrinological responses. It is believed at present that the principle one of interest is the hormone melatonin (Bromage NR, Porter MJR & Randall CF (2001). Aquaculture

197, 63-98), which is released into the blood and cerebral spinal fluid by the pineal gland in direct response to the light-dark cycle the fish is exposed to. Melatonin expression varies in direct response to the dial light cycle with raised levels during the dark phase and basal levels during the day. By introducing artificial light during the natural dark phase the night-time melatonin levels are reduced in proportion to the spectral and intensity quotient of the illumination (Bayarri *et al*. 2002, Porter *et al.* 2000).

1.3. The challenge of photoperiod regulation.

When cod are held in intensive culture conditions they mature within 2 years from hatching (Hansen *et al* 2001, Davie 2005, Ágústsson et al 2008 unpublished data), much earlier than their wild counterparts (Godo and Moksness, 1987; Johansen *et al*., 2000; Rey and Junquera, 1998). This is a reflection of the favorable growing conditions in culture allowing the individuals to reach a suitable size several years in advance of wild counterparts. Such "early" maturation significantly impacts on potential production profitability as maturation reduces growth potential thus extending the production cycle (by a minimum of 6 months) and negatively impacts on product quality and farmed animal welfare. Furthermore allowing farmed stocks to mature in captivity also has significant environmental implications both in terms of the potential excessive environmental pollution through feed wastage and the unnecessary extension of the production cycle along with the genetic pollution through the mass release of fertilized gametes into the local environment. It is therefore clear that research into techniques that will allow the delay or cessation of maturation during on growing is of paramount importance to the industry.

The problem of unwanted maturation during the production cycle is not new to the aquaculture industry. It is common in a range of popular farmed species including Atlantic salmon, Rainbow trout, European Sea Bass and Oysters (Bromage *et al* 2001). As such a number of remediation techniques have already been successfully trailed in these species and are now routinely used in commercial operation worldwide. These are:

Single sex production: In cases where one sex matures later than the other, genetic manipulation of brood fish (e.g. the production of "neo males") allows the production of single sex populations for on growing, which can be harvested later with no maturation. (Such a system is used in the production of portion size Rainbow trout and Tilapia spp.)

Ploidy manipulation: In cases where neither sex show a preferential performance, temperature or pressure shock treatments of fertilized gametes produces "triploid" individuals which are characterized as animals which retain a 3rd set of chromosomes. Such animals are physiologically incapable of sexually maturing allowing extension of the grow-out cycle. (Such a system is used in the production of Oysters and large sized Rainbow trout)

Photoperiod manipulation: In cases where neither sex show a preferential performance, the application of artificial illumination overlaid on an ambient photoperiod signal can mask the ambient environmental cues which recruit individuals into sexual maturation so causing the population to "skip" maturation that year. (Such a system is used in the production of Atlantic salmon).

With regards to which strategy to adopt for farming of Atlantic cod, both the industry and scientific community have moved in favor of developing photoperiod manipulation techniques for a number of fundamental reasons. Under farmed conditions, while it is apparent that male cod may mature at a smaller size than females both are capable of maturing below 1kg in weight so the adoption of a single sex population is not appropriate. Then with regards to ploidy manipulation the technique itself is very time sensitive and modern cod juvenile production methods do not lend themselves to the application of the technique, furthermore

the consumer acceptance of such “manipulated” individuals was felt to be lower than non manipulated stocks. Hence studies have been ongoing into the cessation of maturation during grow-out using artificial photoperiod manipulation.

With regards to the entrainment of reproduction in Atlantic cod specifically, there have been a number of recent studies (Dahle *et al*., 2000; Karlsen *et al*., 2000; Hansen *et al.,* 2001; Davie *et al*., 2003, 2004; Kristoffersen, *et al*., 2004; Taranger, *et al*., 2004; Davie 2005). Together these works have been able to define the natural photoperiod cycle which entrains reproduction in cod (Davie 2005) and furthermore have also consistently demonstrated in laboratory based studies how reproduction can therefore be prevented for up to two additional years (i.e. up to 4 years of age).

An understanding of light transmission and perception by fish has led to the recent developments in the use of new lighting technologies. A number of novel technologies have been investigated for their potential use in the aquaculture industry. These include Light emitting diodes (LED) and Cold cathode ray tubes (CCRT). Benefits of such systems will include:-

- The ability to produce unfiltered narrow bandwidth light as required in comparison to standard metal halogen bulb currently used within the aquaculture industry.
- Lower power requirements which will entail lower electrical running costs
- Increased durability
- Improved lamp life (up to 20 times the life of incandescent bulbs).
- The CCL narrow green bandwidth light penetrates water more effectively and is better perceived by the fish compared to the best practice metal halide lights (Northmore and Muntz 1970; Ziv *et al.,* 2007).

1.4. Objectives

Previous studies have shown that fish species respond differently to photoperiod manipulation and that results cannot be directly transferred from ones species to another without evaluating key phenotypic properties. In this study the effect of CCL on cod growth and maturation of cod was investigated. The main objectives were to

- Evaluate the effect of CCL on sexual maturation of cod in sea cage farming.
- Evaluate the effect of CCL on growth rate in sea cage farming
- Investigate if significant variation could be observed between families regarding growth traits indicating genetically based differences
- Investigate if significant variation could be observed between families regarding onset of sexual maturity indicating genetically based differences
- Investigate if differences could be observed between families in response to light treatment (e.g. delay of sexual maturity or direct effect on growth traits independent of the onset of sexual maturity or “inherent” family growth-phenotype).

1.5 Project overview, funding, participants, management and time scale.

Light and photoperiod is a powerful environmental regulator of growth and sexual maturation in fish. The project goal is to delay sexual maturation and obtain improved growth rate by using novel light source and to evaluate the genetic contribution to the determination of these phenotypes. The use of novel cold cathode light (CCL), which emits a single wavelength with improved distribution pattern in water compared with normal light, will allow for manipulation

of key developmental processes during sea cage culture of cod. Light induced stimulation of growth will improve feed utilization and shorten the production cycle, as well as decreasing the environmental impact of sae cage culture. The delay or inhibition of sexual maturation will improve product quality and taken together, the project will contribute to increased efficiency and profitability of industrial cod farming. The study reported herein was a part of a larger investigation set up for investigating the potential benefits of CCL-technology in cod farming and consisted of three interrelated parts that were funded by different funding bodies.

Project overview and funding

Part 1, '*Cod-Light-Tech*' was a 6th framework EU, Craft-project. Development of efficient light regulation in fish farming. (Year: 1, 2, 3): To ensure optimal dispersion of the light in sea cages and to solve pertinent technical and practical problems related to structural features (Size and shape) and placement of equipment in sea-cages. Results published in the final report June 2009.

Part 2, "ÞRÓUN IÐNAÐARVÆDDS ÞORSKELDIS: Stjórn vaxtar og kynþroska með háþróuðum ljósabúnaði", was funded by the Icelandic funding body: AVS rannsóknasjóður í sjávarútvegi. Investigation of the biological effects of photoperiod manipulations on cod. The aim was to evaluate the effect of light manipulation on sexual maturity (Year 2, 3)Measurements of Steroid levels in blood: Evaluation of gonad development (Year 2, 3)the effect of light manipulation on growth traits (Year 1, 2 ,3) Measurements of Growth Factor concentrations in blood (Ár 1,2, 3). Results published in the final report June 2009.

Part 3, "*Inheritance of photoperiod and sexual maturation in cod"* which is reported herein was funded by The Technical Development Fund of the Icelandic Research Council. This part is built upon the other two (**Objectives 1.4**)

Participants

The Project was carried out in cooperation between Matís ohf (Þorleifur Ágústsson, Rannveig Björnsdóttir, Guðmundur Óli Hreggviðsson, Ólafur H. Friðjónsson), Hraðfrystihússins Gunnvarar hf. (Kristján Jóakimsson), Hafrannsóknastofnunar (Agnar Steinarsson), The University of Göteborg (Björn Þrándur Björnsson) and University of Hólar, (Helgi Thorarensen).

The Matis division of Genetics and Breeding of (formerly: Rannsóknastofnun fiskiðnaðarins) had the overall management of the project, development of light regulation technology, sampling and phenotypic measurements (length, weight, and gonad development) and carried out the investigation of biological/physiological effects of light treatments. Hafrannsóknastofnun provided broodstock and carried out the initial hatching work and subsequent initial rearing of larvae and juvenile cod. The biotechnology division of Matis (formerly Prokaria) had the overall management of genetic work (Part III), the gene isolations, the genetic analysis and family assignments, and supervised the analysis of selected growth factor gene expression. Professor Björn Þrándur Björnsson from the University of Goteborg served as a consultant to the project at the early stages and Dr. Helgi Thorarensen from the University of Hólar oversaw the statistical data analysis. Hraðfrystihúsið Gunnvör, under the management of Kristján Jóakimsson, supervised work related to the sea-cage and CCL-equipment operation and maintenance.

Two M.Sc. students at the University of Hólar worked in the project: Kjell Hellmann (Provisional title of thesis: *Effect of continuous light on Expression of Growth hormone and Growth hormone receptor in cod*, MSc degree estimated end of spring 2010) and Filipe Figueiredo (Provisional title of thesis: *Influence of continuous light on the sexual maturation and growth of Atlantic cod*, MSc finish estimated end of spring 2010).

Time scale

The project took approximately one year more than planned in the as setting up the breeding/farming experiments.

1. The project started after having received funding in April 2006, with the genetic work of isolating candidate genes from the cod.
2. Farming experiments started in August 2006
3. Harvesting was in the end of the year 2008 and beginning of the year 2010
4. Expression studies in the years 2007 and 2008
5. Genotyping work and analysis was done in the year 2009

1.6. Organization of the report

Besides introduction and chapter on materials and methods the study is divided into three interrelated parts and the report is organized accordingly. There are three separate results chapters including discussion of obtained results. Following the results chapters are conclusions.

The three results chapters are following, each with discussion at the end of results:

Isolation of previously unknown selected candidate genes associated with growth and maturity in cod and identification of variable genetic markers associated with those candidate genes.

Analysis of candidate gene expression of selected candidate genes under different light regimes (with and without CCL).

Genotypic and phenotypic analysis of cod families reared under different light regimes in sea cages (with and without CCL).

2. Material and methods

2.1. Experimental set up

2.1.1. CCL-equipment

Recent years innovations and developments in narrow bandwidth light sources have led to a series of new and highly intense light sources. The main area of innovation is within the area of high intensity LEDs (Light emitting diodes) – where new generations of bright LEDs has found new applications within products ranging from the auto industry, to biotechnology and medicine. Intravision has for example experienced recent success using the brightest LEDs developed by the industries innovation leader Lumileds Lighting Ltd; Netherlands (EUREKA 3232) in developing advanced light systems for production of natural plant carotenoids (Unpublished). However using high intensity LEDs in a system for creating an even light field in a large commercial sea cage for production of cod – will result in an extremely expensive light system. Intravision developed in 2002 a series of narrow bandwidth lights for use in salmon farming – using a light source named Cold Cathode Light Tubes. The CCL technology is related both to the better known Neon light and the standard Hot Cathode light tubes currently dominating indoor office lighting. The salmon test using the low energy CCL system did compete evenly with high power Metal Halides in the depression of Melatonin (Migaud et al – unpublished). Intravision therefore established a cooperation with the leading supplier of materials to the CCL industry (Masonlite Ltd, England), while experiencing with different light tube constructions, materials and a slight increase in power consume the first generation of new CCL tubes (2005) resulted in a new system with and improved light intensity of 240% compared to the old salmon light setup, and a production price of estimated only 10 – 15% of a LED setup of similar intensity. The problem of making an even light field in a sea cage however is far more complex than choosing between light sources. The CCL light tubes are easily arranged in columns making it easier to establish an even light field sea-cages.

Results from the first year of the Codlight Tech project that was carried out in parallel to this project revealed that little or moderate light intensity had little effect on growth and maturation of the fish. It was therefore decided to use only the greatest intensity in the CCL regime and compare its effect to natural photoperiod.

Two 180cm long CCL light-tubes were connected and placed in three locations within each sea cage (Figure 1). The higher light tube was 50 cm below the sea surface and the lower light tube was 3 m below the surface.

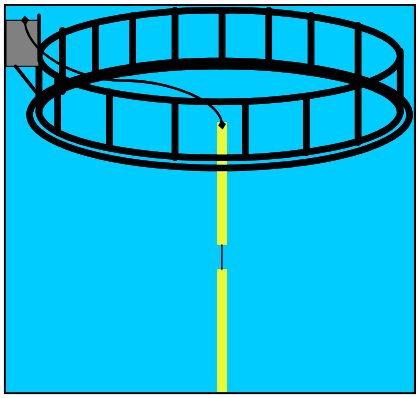

Figure 1: Placement of light tubes in the sea cages

2.1. 2. Photoperiod manipulation

The project started 1. August 2006 and the first 7th months of the work was carried out at the Hatchery of Hafrannsóknastofnun Íslands at Staður in Grindavik under the supervision of Agnar Steinarsson, where the larvae and juvenile cod was reared under different photoperiod regimes in indoors in tanks. In May 2007 the fish were transported to sea cages in Súðavík. Figure 2 gives a schematic overall presentation of the experimental set up.

Coastal tank stage – indoor rearing of juveniles from hatching to approx. 160 g.

Sea cage stage –outdoor rearing of the fish after transfer to sea from approx. 160 g to harvesting size

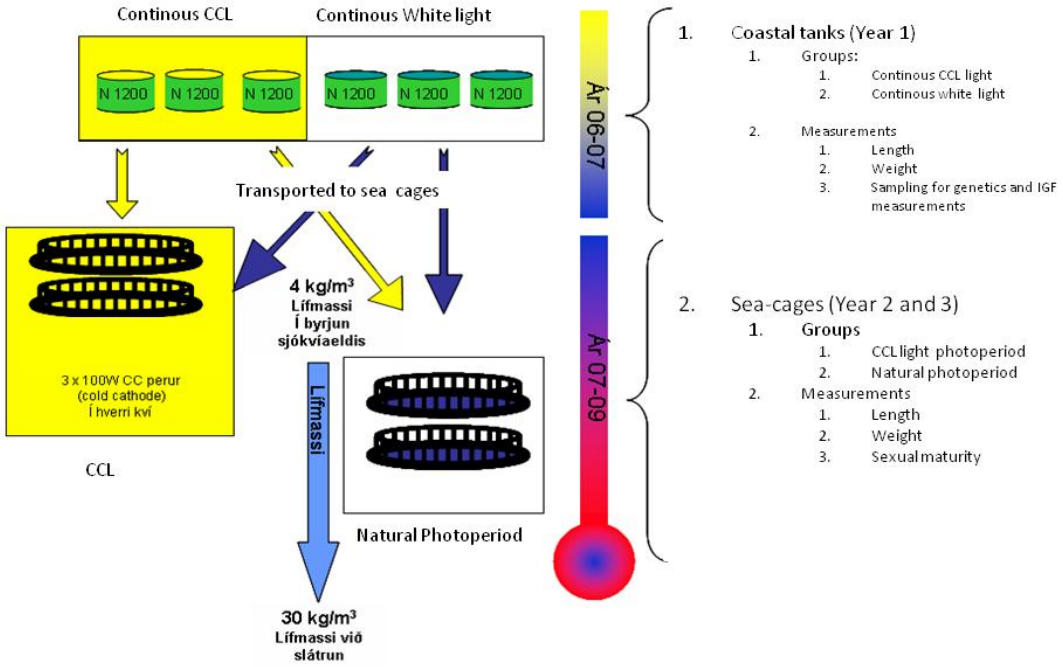

Figure 2: Schematic representation of the experimental set up for the photoperiod manipulation study

2.1.3. Coastal Tanks - indoor stage

The brood fish were wild cod caught in the Ísafjarðardjúp and reared from juvenile size to slaughter size in sea cages at Súðavík. Sexually mature individuals that were selected on basis of large size were transported to the Hatchery in Staður in Grindavík at the south coast of Iceland, where they were used for producing fish for the experiment. 33 sibling groups were produced. (One sibling group: offsprings of one male and two females). The eggs hatched on 1 May 2006 and feeding started 4th May. The larvae were reared under continuous light.

At the average weight of 25 g the fish (n= 72 000) were distributed randomly into 6 rearing tanks (see Figure 1.) of 3 m^3 (~12 000 fish per tank) and reared under continuous light. Three of the tanks had traditional lights (700 lux at the surface, 200 lux at 20 cm depth, 150 at 40 cm depth) and the other three CC-lights from Intravison (4000 lux 5 cm from the tube, 1000 lux 20 cm from the tube, 500 lux 40 cm from the tube). After 14 weeks, prior to transportation to sea cages, the fish in each of the group were transferred to a 30m^3 tank (í mars 2007) and kept under the same light regime until the date of transportation.

Samples were taken each month from November 2006 to April 2007. Each time 100 individuals were weighted from each tank and thereof 30 measured for length. 10 individuals were put to

sleep and blood and tissue samples, (liver, brain, pituitary gland, muscle and gills) taken for IGF measurements and RNA and DNA for sibling group assignment, and growth factor gene expression studies. Three times in the period from January to April 2007 30 fish were tagged then captured again 8 weeks later for specific growth rate measurements; (SGR). More detailed information on the sampling procedures can be found in MSc thesis of Guðbjörg Stella Árnadóttir (*The effects of cold cathode lights on growth of juvenile Atlantic cod, (Gadus morhua L).: use of IGF-I as an indicator of growth*", MSc from University of Akureyri November 2008)

2.1.4. Sea cage stage

The fish was transported to sea cages on 6 May 2007. The average weight was 166 g (150±30g). A total of 19.880 fish were transported to Álftafjörður in Westfjords and placed in four circular sea cages, (each 155 m^3, 6 meters in diameter and 5-6 meters deep). In two of the cages was natural light but the other two were under CCL illumination, each with 2 x 3 CCL tubes (a total of 6 lights) from Intravision (263W $cage^{-1}$). The fish was randomly distributed into the sea cages (approximately 5 000 juveniles per cage. The fish that had been reared under CCL from hatching was fin-clipped to distinguish it from fish that had been reared under white light. Density was rather low or 5 kg m^{-3}.

The sea cages in Álftafjööður were located 200 m offshore in two separated places (N 66°01.522' - W22° 58.906' og 66°01.519' - W 22°58.776') at the depth below the cages were 40-50 meters. Samples were taken every second or third month until final harvesting in January 2009.

2.2. Methods used in isolation and analysis of candidate genes associated with growth and maturity of *Gadus morhua*

2.2.1. DNA isolations

Chromosomal DNA was isolated from cod fins using isolation kits and protocols according to manufacturers' instructions from Macherey-Nagel (Nucleospin 96 Tissue) from Agowa GmbH (Magnetic bead kit).

2.2.2. RNA isolations

The RNA extraction was carried out using Trizol reagent as follows; 500 µl were added to the samples which were vortexed to carry out tissue homogenization. After vortexing, 500 µl of Trizol were added, making a total volume of 1 ml. Samples were then incubated for 5 min at room temperature after which 200 µl of chloroform were added and mixed in the tube by shaking. Samples were again incubated for 5 min, at room temperature, followed by a centrifugation for 15 min. The aqueous phase was transferred into a new tube, and 500 µl of isopropyl alcohol were added. Samples were incubated for 15 min, at room temperature, followed by a centrifugation for 10 min. The RNA formed a gel-like pellet at this stage. The aqueous phase was discarded. 1 ml of 75% ethanol was added, followed by a short vortexing. Samples were centrifuged for 5 min. Supernatant was discarded, and the pellet was allowed to air-dry for 5-10 min. Pellet was dissolved in 100 µl RNase free water, and incubated for 10 min at 57ºC. All centrifugations at this stage were performed at a speed of 12000 g, and at a 4ºC temperature. Sample final volumes were of 100 µl.

2.2.3. cDNA synthesis

cDNA was synthesized from the extracted RNA, using iScrip™t cDNA synthesis Kit (BioRad). 4 µl of 5x iScript™, 1 µl of iScript™ reverse-transcriptase, RNA template contenting approximately 500 ng of template RNA, and RNAse free water were added, performing a total volume of 20 µl per well. Thermo cycler protocol was as follows: 5

The cDNA purification was performed using the illustra™ GFX PCR DNA and Gel Band purification kit (GE Healthcare). Manufacturer protocol was used, and is as follows:

1. 500 µl of buffer 1 were added to the sample
2. The capture buffer-sample mix was then transferred to a GFX column, followed by centrifugation (13000rpm, 30s)
3. flow-trough was discarded and 500µl of buffer 2 were added (using same collecting tube), followed by centrifugation (13000rpm, 30s)
4. Collecting tube was discarded, and the GFX column was transferred to a 1,5ml centrifuge tube (not supplied)
5. 50 µl of elution buffer 3A were added directly on top of the glass fibre matrix
6. Sample was incubated at room temperature, for 1 min, and then centrifuged (13000rpm, 1min).
7. The RNA clean-up from reaction mixtures was carried out using NucleoSpin® RNA II. Manufacturer protocol was followed, and is as described: cDNA was isolated

2.2.4. Conserved-region PCR amplification with CODEHOP primers

For the primer construction, amino acid sequences of various growth factor genes were retrieved from protein sequence databases and aligned using CLUSTAL_X version 1.8. A number of different forward primers reverse primers, aimed to target sequences encoding conserved amino acid sequence regions were constructed. The primers were designed according to the CODEHOP strategy: They were degenerate at a 3' core region of 11 or 12 nucleotides, across four codons encoding highly conserved amino acids in regions A and B. In contrast, they were non-degenerate at a 5' region (consensus clamp region) of 18 to 25 nucleotides, with the most probable nucleotide predicted for each position. The degeneracy of the primer pools ranged from 8-fold to 16-fold, and the primers were 29 to 32 bp in length. The primers were used in a matrix of PCRs where every forward primer was used with every reverse primer.

2.2.5. PCR amplifications and cloning

The PCR was carried out with DyNAzyme DNA polymerase (Finnzymes, Espoo, Finland) with a PTC-0225 MJ Research thermal cycler. The reaction mixture was first denatured at 95°C for 5 min, followed by 30 cycles of denaturing at 95°C for 50 s, annealing at 52°C for 50 s, and extension at 72°C for 3 min and finally an extension for 7 min at 72°C to enhance formation of A overhangs. PCR products were separated on gels and purified using GFX spin columns (Amersham Biosciences, Piscataway, NJ). The gene fragments with sizes of 300 to 600 bp were selected and were cloned into conventional pUC-based sequencing vectors by the TA-cloning method (1). Eight to 12 clones from each band were sequenced with M13 forward and reverse primers on an ABI 3700 DNA sequencer, using a BigDye Terminator cycle sequencing ready reaction kit (PE Applied Biosystems, Foster City, CA). To ensure clarity a special nomenclature was adopted to keep track of gene fragments obtained and facilitate the subsequent work. Each gene fragment obtained was designated as Primary fragment with an appropriate abbreviation denoting the gene targeted (e.g. GH for Growth Hormone GHR for growth Hormone Receptor) and a running number if more than one fragment was obtained for the same gene.

2.2.6. Gene mining amplification

Known genes homologous to the target growth factor genes were analyzed in order to define the number of exons, possible size of introns and to locate exon/intron boundaries. To obtain full coding regions, cDNA was used. Following sequencing of the obtained target primary gene fragments, upstream and downstream flanking regions the selected fragments were amplified from the corresponding genomic cDNA in a series of nested PCRs, using one gene-specific, 5'-biotin-labeled primer and one arbitrary primer (Arb1 or Arb2 [see Table 1]), targeting the unknown flanking sequence. The PCR product was purified with streptavidin-coded Dynabeads (Invitrogen) and further with QIAquick PCR purification spin columns (Qiagen, Hilden,

Germany) prior to a second PCR with a nested gene-specific primer upstream of the previous one and a primer targeting the 5' consensus sequence of the previously used arbitrary primer. The PCR product of the latter amplification was cloned and sequenced as described above, and the sequence information was used to make new gene-specific primers for the next nested PCR amplification until the complete genes were obtained. Similarity searches by BLAST were performed on the NCBI server (http://www.ncbi.nlm.nih.gov). The ClustalW tool on the EBI server (http://www.ebi.ac.uk/clustalw) was used to create multiple sequence alignments which were displayed using Gene doc 2.6.02. Pair wise alignments were made using LALIGN (http://www.ch.embnet.org/software/LALIGN_form.html). To obtain intron sequences long PCR was used with primers targeted to adjacent up- and downstream exons. Gene walking as described above was also used in introns could that could not be obtained by long PCR.

2.3. Methods used in expression studies of candidate genes

2.3.1. Sampling in hatchery:

Samples were taken each month from November 2006 to April 2007. Each time 100 individuals were randomly netted from each tank and measured for length and weight. Of the 100 sampled, a total of 30 individuals from each treatment group were slaughtered and blood and tissue samples, (liver, brain, pituitary gland, muscle and gills) taken for hormonal measurements, beside sibling group assignment, and gene expression studies (At the 4 first sampling points from November 2007 to February 2008 10 individuals were taken from each tank). Three times in the period from January to April 2007 30 fish were tagged then captured again 8 weeks later for specific growth rate measurements (SGR). After transportation to sea cages samples were taken every second or third month until harvesting in January 2009. For the gene expression profiles analysis, tissue samples (liver and pituitary) were placed directly on dry ice after being collected, then stored at -80°C. Gill samples were extracted for genotyping purposes. Equipment was rinsed and sterilized between samples in order to avoid cross contamination. Gill samples were stored in 76-80% ethanol. Figure 3. Shows the experimethal design.

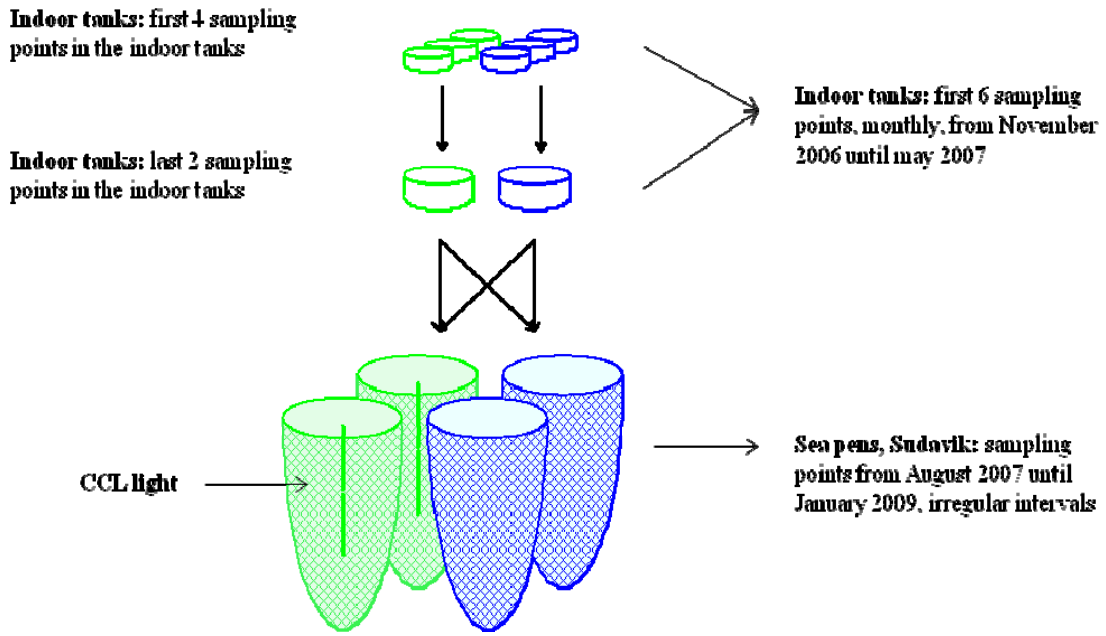

Figure 3: A basic schematic of the experimental design, representing the different rearing conditions and replicates. Green tanks and sea pens represent the light treatment, while blue represent the control.

2.3.2. Phenotypic measurements and selection of samples for gene expression analysis

For growth assessment, fish were weighed (to the nearest gram) and measured for length (to the nearest centimetre). Liver and gonads, when present, were weighed in order to calculate the gonadosomatic index (GSI), and the somatic weight (SW), which will be considered as the whole bodyweight (BW) minus the gonad and liver weight, GW and LW respectively.

The individuals at each sampling point were grouped into one of three groups with 10 individuals in each group as follows: large individuals, intermediate size individuals and small individuals. Gene expression profiles of Growth Hormone (GH) and hepatic Growth Hormone-Receptor (GHR) were investigated and compared between the groups of the largest 10 individuals and smallest 10 individuals at each sampling point. The 10 intermediate size fishes were not analyzed for gene expression.

2.3.3. RNA extraction

Extraction, purification and handling of RNA, was carried out in an RNAse free chamber, with exception of the centrifugations.

Trizol reagent (500 µl) was added to the tissue samples and the mixture homogenised by vortexing. Further 500 µl of Trizol were then added and the samples incubated for 5 min at room temperature. 200 µl of chloroform were added, and the liquid in the tubes mixed by hand. Samples were again incubated for 5 min, at room temperature, followed by a centrifugation for 15 min.

The aqueous phase was transferred to a new tube, and 500 µl of isopropyl alcohol added. Samples were incubated for 15 min, at room temperature, followed by a centrifugation for 10 min. The RNA formed a gel-like pellet at this stage. The aqueous phase was now discarded and 1 ml of 75% ethanol added, followed by a short vortexing. Samples were centrifuged for 5 min, supernatant discarded, and the pellet dried in air for 5-10 min. After air drying, the pellet was dissolved in 100 µl RNase free water, and incubated for 10 min at 57ºC. All centrifugations at this stage were performed at 12000 g and 4ºC temperature. Final sample volumes were 100 µl.

The RNA clean-up from reaction mixtures was carried out using NucleoSpin® RNA II. Manufacturer protocol was followed, and is as described:

2.3.4 Pituitary tissue

1. 1 Buffer RA1 was added (3,5x sample volume)
2. 96% ethanol was added and mixed by vortexing (same volume as RA1 buffer)
3. The content was loaded into a NucleoSpin® RNA II column, followed by centrifugation (30 s, 11000g, 4ºC)
4. 350 µl of MDB were added, followed by centrifugation (30 s, 11000g, 4ºC)
5. 95 µl of DNAse reaction mixture were added, and samples were allowed to incubate at room temperature for 15 min (DNAse reaction mixture was prepared with 10 µl DNAse plus 90 µl reaction buffer for DNAse, provided with the kit)
6. 200 µl of RA2 buffer (DNAse inactivation) were added, and centrifuged (30s, 4ºC, 11000g)
7. 600 µl of RA3 buffer were added, followed by centrifugation (30s, 4ºC, 11000g)
8. 250 µl of RA3 buffer were added, followed by centrifugation (2min, 4ºC, 11000g)
9. RNA was eluted in 60 µl RNAse free water, and centrifuged (1min, 4ºC, 11000g)
10. Final sample volume of cleaned RNA was of 60 µl.

2.3.5 Liver tissue

1. Buffer RA1 was added (3,5x sample volume)
2. Sample was filtered through a filter column (violet) in a collecting tube (centrifuged for 1min, 4ºC, 11000g)

3. 75% ethanol was added and mixed by vortexing (same volume as RA1 buffer)
4. The content was loaded into a NucleoSpin® RNA II column, followed by centrifugation (30 s, 11000g, 4ºC)
5. 350 µl of MDB were added, followed by centrifugation (30 s, 11000g, 4ºC)
6. 95 µl of DNAse reaction mixture were added, and samples were allowed to incubate at room temperature for 15 min (DNAse reaction mixture was prepared with 10 µl DNAse plus 90 µl reaction buffer for DNAse, provided with the kit)
7. 200 µl of RA2 buffer (DNAse inactivation) were added, and centrifuged (30s, 4ºC, 11000g)
8. 600 µl of RA3 buffer were added, followed by centrifugation (30s, 4ºC, 11000g)
9. 250 µl of RA3 buffer were added, followed by centrifugation (2min, 4ºC, 11000g)
10. RNA was eluted in 60 µl RNAse free water, and centrifuged (1min, 4ºC, 11000g)

2.3.6 cDNA synthesis and purification

cDNA was synthesized from the extracted RNA, using iScrip™t cDNA synthesis Kit (BioRad).

4 µl of 5x iScript™, 1 µl of iScript™ reverse-transcriptase, RNA template contenting approximately 500 ng of template RNA, and RNAse free water were added, performing a total volume of 20 µl per well.

Thermo cycler protocol was as follows: 5 min at 25ºC, 30 min at 42ºC and 5 min at 85ºC (held at 4ºC after finished).

The cDNA purification was performed using the illustra™ GFX PCR DNA and Gel Band purification kit (GE Healthcare). Manufacturer protocol was used, and is as follows:

1. 500 µl of buffer 1 were added to the sample
2. The capture buffer-sample mix was then transferred to a GFX column, followed by centrifugation (13000rpm, 30s)
3. flow-trough was discarded and 500µl of buffer 2 were added (using same collecting tube), followed by centrifugation (13000rpm, 30s)
4. Collecting tube was discarded, and the GFX column was transferred to a 1,5ml centrifuge tube (not supplied)
5. 50 µl of elution buffer 3A were added directly on top of the glass fibre matrix
6. Sample was incubated at room temperature, for 1 min, and then centrifuged (13000rpm, 1min)
7. cDNA was stored at -20ºC.

2.3.7 Quantitative PCR

The method used to profile both the hepatic GHR and the pituitary GH expression, was to calculate the relative quantity of a fragment of each gene mRNA, relative to a normaliser gene, also known as housekeeping gene. The housekeeping gene selected was β-actin (reference).

Gene expression is given in “Fold Change” units. This fold change determined in relation to a calibrator sample, which was the same in ever qPCR run. This is necessary in order to be able to compare samples which run in different sets (maximum of 20 samples per qPCR run).

2.3.8 Numerical and statistical analysis

Statistical tests were chosen according to the data distribution. When the data met the requirements for a parametric test (normal distribution and equal variance), nested ANOVA were performed. When this test could not be performed, Krushkal-Wallis test was performed. Statistical significant difference was not found between replicates for the same groups of treatments, and therefore all the replicates were treated together (different tanks and cages were run as a random factor).

2.4. Materials and methods used in genotypic and phenotypic analysis of cod families reared in sea cages under different light regimes

2.4.1 Sampling

Approximately 2000 individuals, 1000 from each light treatment, were sampled for phenotypic and genotypic analysis in December 2008 and another 2000 individuals in January 2009. Weight and length measurements were taken for growth assessment. Liver and gonads were weighed in order to calculate the gonadosomatic index (GSI), and the somatic weight (SW), which defined as the whole bodyweight (BW) minus the gonad and liver weight (GW and LW respectively). Gill samples were taken for DNA isoaltina and subsequent sibling group assignment.

2.4.2 DNA isolation and PCR

Genomic DNA was isolated from gill tissue preserved in 90% ethanol using Puregene DNA isolation kit (Gentra). A total of 266 individuals were collected at two different spawning grounds located in Iceland (sampling sites 511, 911 and 931, see Pampoulie *et al*. 2006). DNA was isolated using Chelex 100 Resin (Walsh *et al*. 1991). Polymerase chain reactions (PCR) were performed in a 10-µL volume containing 3 µL of 1/10 diluted DNA, 200 µM of each dNTP, 1× Teg buffer (100 mM Tris-HCl, pH 8.8; 500 mM KCl; 15 mM $MgCl_2$; 1% Triton X-100), 0.2 U Teg polymerase (Matis-Prokaria, *Taq* comparable), 0.04–0.10 µL of the labelled forward (100 µM) and 0.04–0.1 µL of the reverse primer (100 µM). PCR were performed on GeneAmp2700 thermal blocks as follows: initial denaturation step of 4 min at 94 °C followed by 30 cycles of 50 s at 94 °C, 50 s at 55 °C (multiplex-1) or 58 °C (multiplex-2) and 2 min at 72 °C, final elongation step of 7 min at 72 °C .

2.4.3 Genotyping

Samples were analyzed on an ABI PRISM 3730 sequencer using the GeneScan-500 LIZ size standard and genotyping performed with GeneMapper 4.0 (Applied Biosystems). Tissue samples from cod were genotyped at ten microsatellite loci (PGmo38, PGmo61, PGmo87, PGmo49, PGmo94, PGmo124, PGmo100, PGmo134, PGmo71, PGmo74). Samples were analyzed on an ABI PRISM 3730 sequencer using the GeneScan-500 LIZ size standard and genotyping performed with GeneMapper 4.0 (AppliedBiosystems).

2.4.4 Family analysis

Levels of genetic relatedness were calculated for pairs of individuals within the group using the program Kinship 1.3.1 (Queller & Goodnight, 1989) method. The program was used to investigate possible sibling relationships between individuals in the population. This software carries out maximum likelihood tests of pedigree relationships between pairs of individuals in a population. It uses genotype information for single-locus, codominant genetic markers (such as DNA microsatellite loci). Two hypothetical pedigree relationships are used, a primary hypothesis and a null hypothesis, and the program calculates likelihood ratios comparing the two hypotheses for all possible pairs in the data set.

3. Results

3.1. Isolation and analysis of candidate genes associated with growth and maturity of *Gadus morhua*

3.1.1. Retrieval of genes

Following genes were targeted for isolation, Insulin like Growth factor 1 (IGF1), Growth Hormone (GH), Growth Hormone Releasing Hormone (GHRH), Growth Hormone Receptor GHR and Insulin like Growth factor- Receptor 1 (IGF2R). The first four proteins are the main hormones of the somatropic axis. The growth hormone (GH) is secreted from the pituitary gland into the blood stream. One of the main target tissues of GH is the liver where GH binds to its receptor and induces the production of IGF-I that in turn acts as a growth factor. In fish it is known that GH is regulated by both stimulatory factors, such as growth hormone releasing hormone (GHRH) as well as inhibitor factors such as somatomedin releasing inhibitory factor (SRIF) both of which are expressed in the brain (Agustsson *et al.* 2000) . Both GH and IGF-I mediate their actions by binding to their membrane receptors, GHRs and IGFIRs, respectively. The role of IGF2- and its receptor is more unclear, but studies indicate thatIGF2 has a role in the autocrine/ paracrine regulation of growth and metabolism and responds to levels of GH (Shamblot *et al* 1995).

Varying amount of sequence information was available. No information was available for IGF1 and GHR. Sequence fragment was available for IGF2, and cDNA sequence for GHRH. Only the amino sequence existed for GH. Various gene mining techniques were used to retrieve missing sequences. Homologous sequences were aligned and PCR- primers designed in highly conserved regions and where amino acids of low redundancies, when DNA sequences fragments were known the primers were targeted outwards into flanking regions from the sequence ends. These gene specific primers were used singly for gene walking into adjacent flanking regions against arbitrary reverse primers (in both introns and exon regions (Figures 4 and 5). They were also used against each other as forward and reverse primers in a typical PCR of in-between regions both in coding regions from cDNA and across introns from chromosomal DNA. Up to fifteen primer pairs were used for retrieval of a particular gene in a matrix of PCRs where every forward primer was used with every reverse primer (Figure 4. for GHR). When introns sequence was predicted to be very long on the basis of information on genes from other fish a long-PCR was carried out.

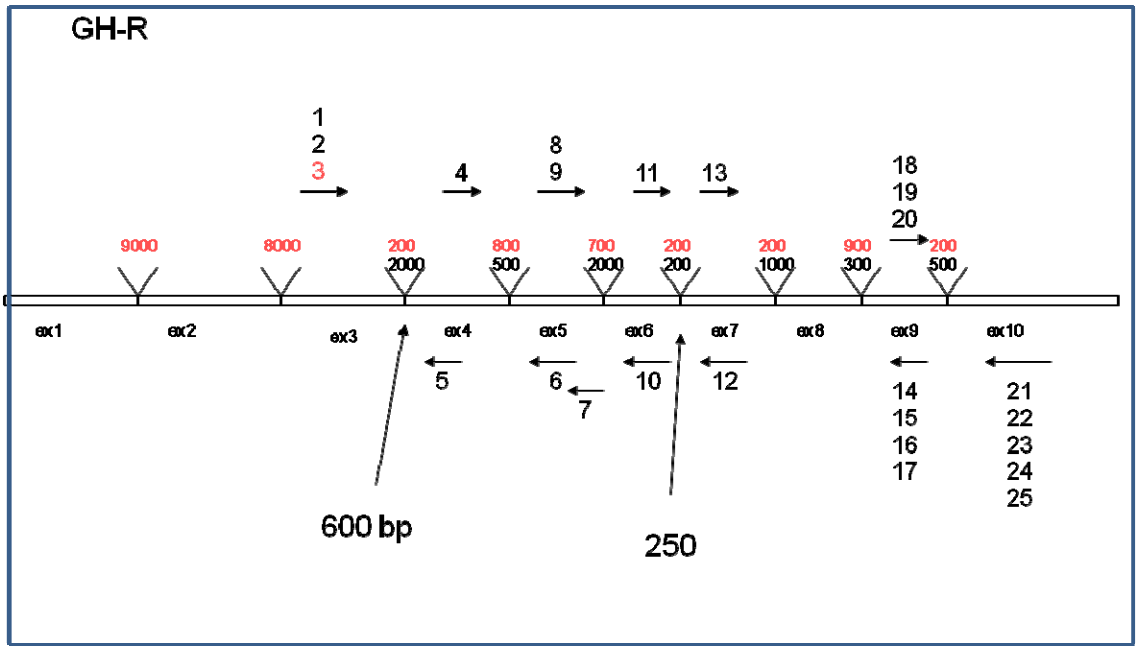

Figure 4: Schematic overview of the gene amplification strategy for GHR gene.

The figure shows the exons and introns of the gene and predicted length of the introns. The arrows show the sites of forward and reverse primers that were designed on the basis of protein alignments from related fish. The PCR amplifications were carried out on cDNA and also on chromosomal DNA in order to amplify across introns.

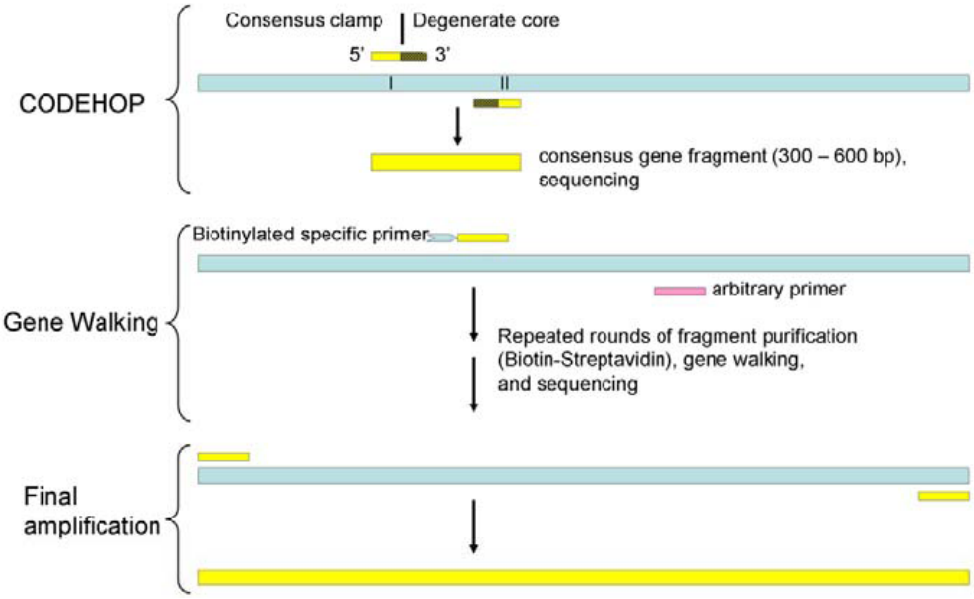

Figure 5: Schematic overview of the gene amplification strategy.

Primers to conserved regions were constructed and used in PCR amplifications with chromosomal DNA from od as a template. Resulting fragments in the size range 300–600 bp were selected and sequenced. Specific biotinylated primers were then constructed and used in PCR reactions together with an arbitrary primer and the same template as above. Resulting fragments were again sequenced, allowing construction of new specific primers.

IGF1

The IGF1 could not be retrieved despite number of attempts using a great number of primer pairs from the various sites in the genes, inter-exon amplifications from either chromosomal DNA or exon independent amplifications using cDNA. The reason for this is unclear.

GHRH

The Growth hormone releasing hormone (GHRH) has only four exons and three introns. The cDNA sequence was known and we managed to obtain the sequence of the three missing introns.

GH

An amino acid sequence existed for Growth Hormone (GH) and a partial cDNA sequence. We managed to get the whole GH cDNA sequence and four of the five introns. Only intron 4 is missing.

GHR

The Growth hormone receptor (GHR) is a very large gene with a great number of introns. No sequence information was available. A few primary gene fragments were obtained by using primers designed on the basis of conserved regions in various exons. After subsequent gene mining, all the exons were obtained and 8 of the 9 introns by gene mining and by long-PCR across intron regions.

IGF2R

A small partial cDNA sequence was available for Insulin like growth factor 2 receptor (IGF2R) This sequence was used to obtain two introns 42 and 44.

3.1.2. Tandem repeats

The introns and flanking regions of obtained genes were analysed with the TANDEM Repeat software for repeats and microsatellite type sequences. A great number of tandem repeats were found in these regions. They were of all kinds, both simple and compound and from 2 to 194 bp long, Copy numbers also varied greatly.

For potential utilization for genotyping analysis of cod, six short promising microsatellite regions were selected for further analysis in the different genes (Table 1).

Gene	Intron	Type	Copy	Position
GHRH	2	GT	50,5	1527 1278
GH	2	GT	10,5	1569 1702
GH	3	Compound	2,7-3,8	17 294
GHR	8	ATA	7,7	1541 1696
GHR	1	GT	52	72 171
IGF2R	42	GT	8,5	20 230

Table 1. Repeats selected for variability analysis.

The table gives the name of the gene, intron location, type of repeat, the copy number and the repeat position.

These included a very long dinucleotide repeat, (GT) in50 copies in intron 2 of the GHRH, but none were found in introns 1 and 3. In GH two microsatellites were found in introns 2 and 3 One was a dinucleotide repeat, (GT) in 10 copies in intron 2 the other was a compound repeat ((ACCT)2(ACC))3 in intron 3. Despite a great number of introns in GHR only two microsatellites were found, a long dinucleotide repeat in intron 1, 52 copies long and a tri nucleotide (ATA) in 7 copies in intron 8. In intron 42 in IGF2R one long dinucleotide region was found, a GT repeat in 52 copies located in intron 42.

3.1.3 Analysis of genetic variability of markers

The sequence information obtained was used to design primer pairs complementary to the flanking regions of the repeats for PCR amplification of the microsatellites (See table 3). DNA was isolated from 24 cod individuals collected from a brood stock in Iceland and wild cod from far apart geographic locations in the North Atlantic Ocean.

Three of the repeat sequences showed variability and good readability on the ABI sequencer. The GH-4 marker showed 4 alleles and The IGF2F showed six good alleles The GHRH-1 marker gave high variability but some stutter was also observed. Table 2 gives detailed information on the primers used, including the sequence and the labeling of the primers

Marker	Label	Primer	Length	Tm C°	Posistion	Marker size	Sequence
GHRH-1	Pet	GHRH-Min2-f	32	64	1527	249	CATATCTAGTAGCCTAAAATGCTGATGTAAATTC
	pigtail	GHRH-Min2-r	27	64	1278		**GTTTCTT**GAATGATCAGAAGTAGCCCAAAATGTG
GH-4	6fam	GH-Min2-f1	29	61	1569	133	CCTTGCCTAGATAGTTAATAGATAAAACG
		GH-Min2-r1	23	62	1702		**GTTTCTT**TCAGCTGTCTCTGTTCTTCAATC
IGF2-2	Ned	GF2r-M2in42-	20	62	460	125	CCTTACTCAACGCAGTCAGG
	pigtail	GF2r-M2in42-	28	60	585		**GTTTCTT**AAATATATAGATAGGTTGTGCGTAGATG

Table 2. Primers and conditions for amplification of candidate gene markers

3.1.4 Discussion

The aim of the study was to isolate and obtain markers that could be associated with two particular genes GHR and IGF1 of which noting was known at the start of project. We obtained the whole GHR gene and most of its introns, but IGF1 could not be isolated despite an intensive effort. We therefore targeted three additional genes belonging to the somatropic axes to the original two. These were: the growth hormone gene (GH), the GH-releasing hormone gene (GHRH) and the insulin like growth factor 2 receptor gene (IGF2R) . The growth hormone gene (GH) is the central growth factor in the somatotropic axis and is secreted from the pituitary gland. Only the amino acid sequence was known. The complete cDNA sequence for the gene was obtained and 4 of 5 introns. The IGF2R, responds to level of IGF2 and plays a role in the autocrine/ paracrine regulation of growth and metabolism. A fragment of the gene was known, but this is a very large gene with more than 40 introns. Two introns were isolated with clear microsatellite sequences. The third gene was the growth hormone releasing hormone (GHRH), expressed in the brain. The GHRH stimulates expression of GH. The complete cDNA was known for GHRH, but none of the introns. We isolated all 3 introns and identified one microsatellite region.

Six microsatellite type repeats were analysed in these 5 genes and variability was observed in three of them: IGF2R, GH and GHRH.

The sequence information of GHR and GH was used in making primers for real time PCR to measure levels of expression during growth under different light regimes from juvenile stage to slaughter size two years later.

3.2 Analysis of candidate gene expression of selected candidate genes under different light regimes (with and without CCL).

The goal of this part of the study was to assess if there was any significant differences in the expression of growth hormone (GH) and hepatic growth hormone receptor (GHR) in juvenile development that could be related to size, maturity, light regime, light imprinting or to more specific family differences in response to light treatment.

3.2.1. Indoor tanks - juvenile fish

The effect of CCL light regime on juvenile Atlantic cod was investigated in indoor tanks.

The fish was reared for 6 months at this stage under two different light regimes. One group was held under continuous CCL and therefore receiving imprinting for CCL regime at the following sea cage stage. The other group, the control, was held under regular hatchery conditions for the same period of time receiving standard hatchery ambient light (white). Both treatment groups were held under 24 hours light photoperiod during this stage. 30 fish were sampled randomly each month during the first 6 months in the indoor tanks. The fish at every sampling point from each treatment were classified into three groups. Group I, with the 10 heaviest fishes, Group II, with the 10 intermediate sized fishes and Group III, with the 10 lightest fish. Gene expression profiles of Growth Hormone (GH) and hepatic Growth Hormone - Receptor (GHR) were investigated and compared between the groups of the largest 10 individuals (Group I) and smallest 10 individuals (Group III) at each sampling point. The intermediate 10 fishes (Group II) were not analyzed for gene expression.

3.2.2. Growth

The length and weight of individuals from each treatment were measured at every samplings point. As can be seen in Figure 6 A &B no significant difference was observed between the treatment groups. Figure 7shows the weight distribution of the fish in Group I and Group II the largest and smallest fish respectively that were used for gene the expression profiling. It may be noteworthy that greater variation in both weight and length was observed in the control than in the group that received only ambient light.

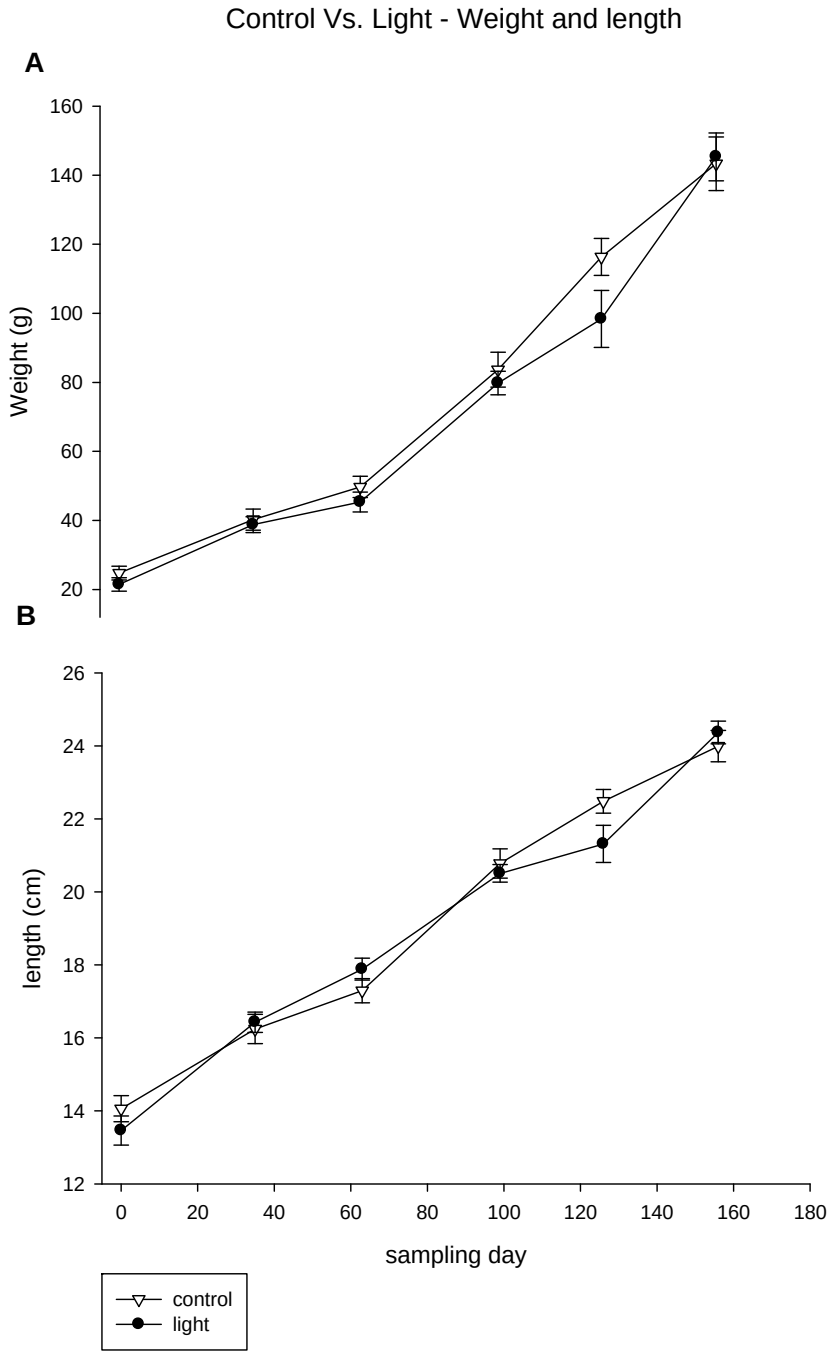

Figure 6: Weight (A) and length (B) for the juvenile fish held in the indoor tanks. First sampling point is set as day 0 in the time scale. Black circles represent the average values (+/- SEM) for the light treatment while the empty triangles represent the average values (+/- SEM) for the control treatment.

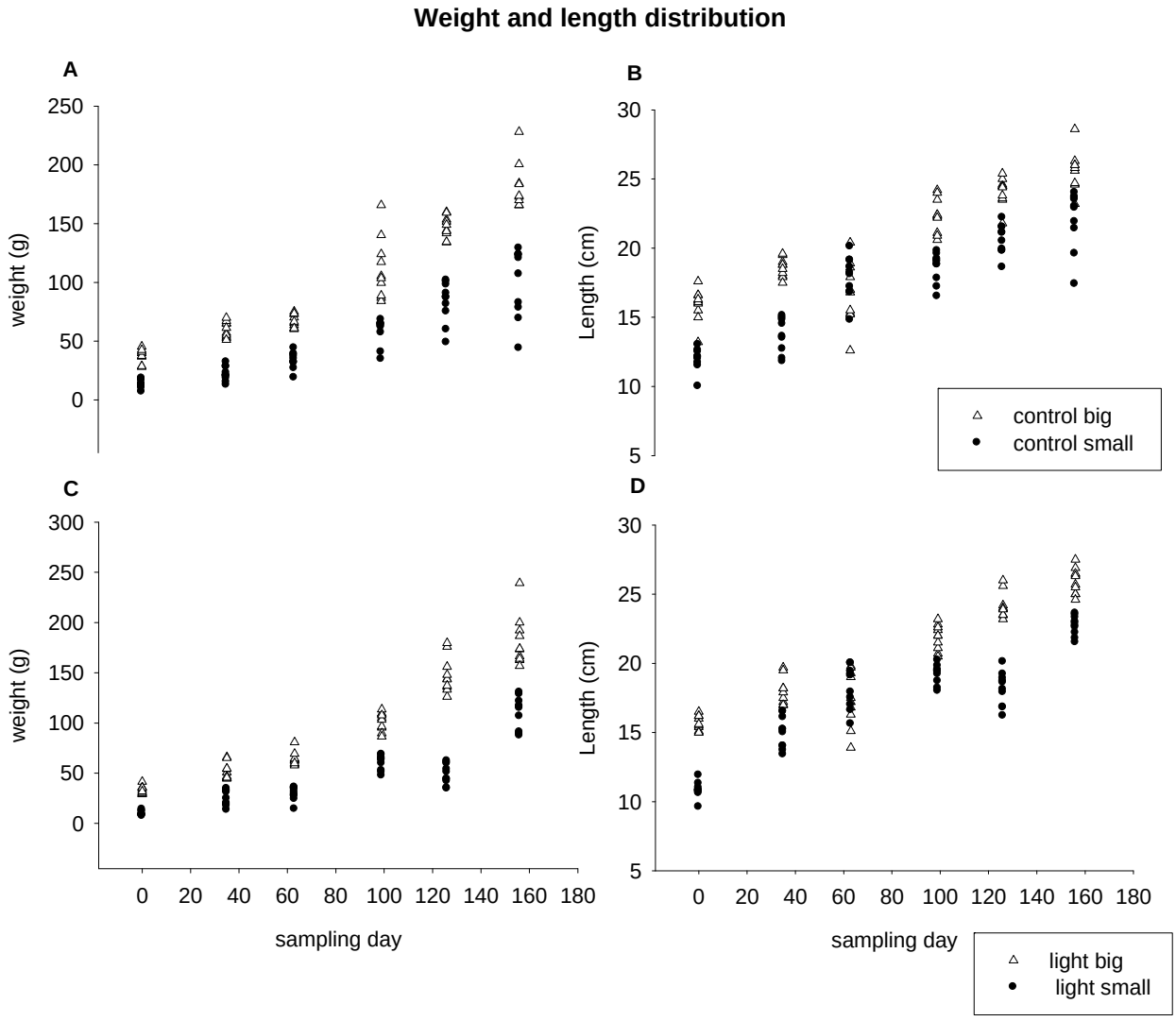

Figure 7: Weight (A,C) and length (B,D) distribution for the small (Group III) and large subgroups at different sampling points (Group I). The error bars represent the standard error. Control fish are represented in graphics A (weight) and B (length), while the fish reared under the CCL light treatment are represented in graphics C (weight) and D (length). Each symbol represents one sample.

3.2.3. GHR expression between light treatment and control

The differences in the hepatic growth hormone receptor (GHR) gene expression in different fish groups were assessed. The comparisons were made between the different treatment groups and different size classes within the same treatment. Figure 8 shows the mean GHR expression at the different sample points for the different light treatments and Figure 9 shows the mean GHR expression in the two different sizes groups at the different sample points. Difference in GHR expression levels between fish receiving different light treatments was only observed at the very early sampling points. This difference evened out towards the end of the indoor stage. When different size groups were compared (Figure 10 clear difference in level of GHR was seen towards the end of the indoor period, with apparent higher levels in the small size group receiving CCL treatment. The relative expression was however similar in the control group.

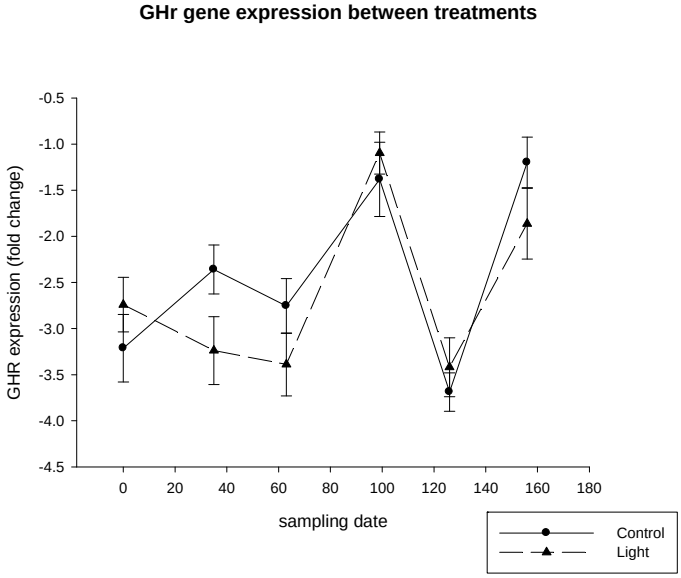

Figure 8: GHR expression in fold change (in relation to the calibrator sample). for the control treatment (full line) and for the light treatment (broken line). 2.1.3 GHR expression between different size grades

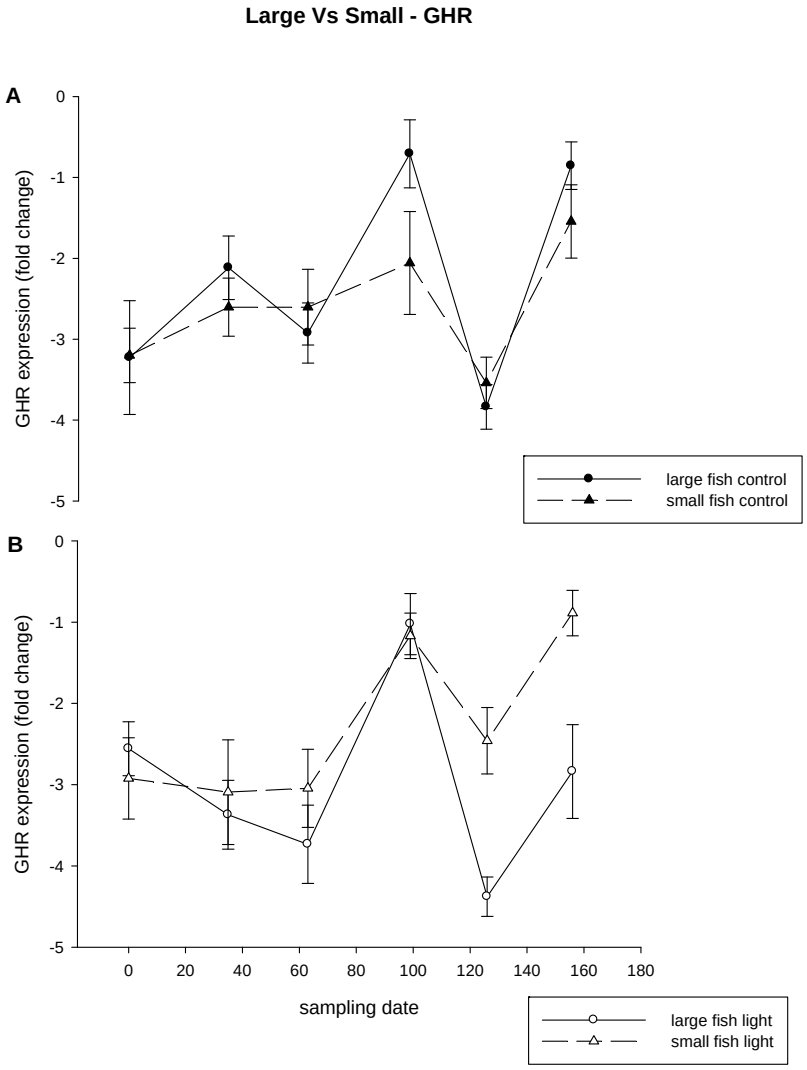

Figure 9: GHR expression, in fold change. Broken line, small fish, Full line large fish (in each treatment - A) control treatment (black symbols) and B) light treatment (empty symbols).

3.2.4 Outdoor sea cages

After being held in the indoor tanks, fish were transferred to four outdoor sea cages. Two tanks were subjected to natural light and the other two for CCL light treatment under 24 hour photoperiod. To be able to assess the effect of an early light imprinting, the fish from each of the two indoor light regimes were distributed evenly into all four cages.

Fish held under CCL light treatment at the indoor stage were marked by a fin cut on the rear dorsal fin – (referred as cut fish from now on), while fish held under ambient light regime at the indoor stage (without the CCL lights) were not marked (referred to as uncut fish). From this set up four treatment groups were formed.

1. Group that received natural light only.
2. Group that received CCL light during the indoor stage and natural light during the sea cage stage
3. Group that received natural light during the indoor tank stage and CCL light during the sea cage stage
4. Group that received continuous CCL treatment during both the indoor tank stage and the outdoor sea cage stage.

Phenotypic traits and gene expression profiles of Growth Hormone (GH) and hepatic Growth Hormone -Receptor (GHR) were investigated and compared between the two light regimes and whether they had received CCL imprinting or not.

The fish was transported to sea cages in May 2007 and samples were taken every second or third month until harvesting in January 2009. Figure 10 shows the distribution of weight at the different sampling points during this period.

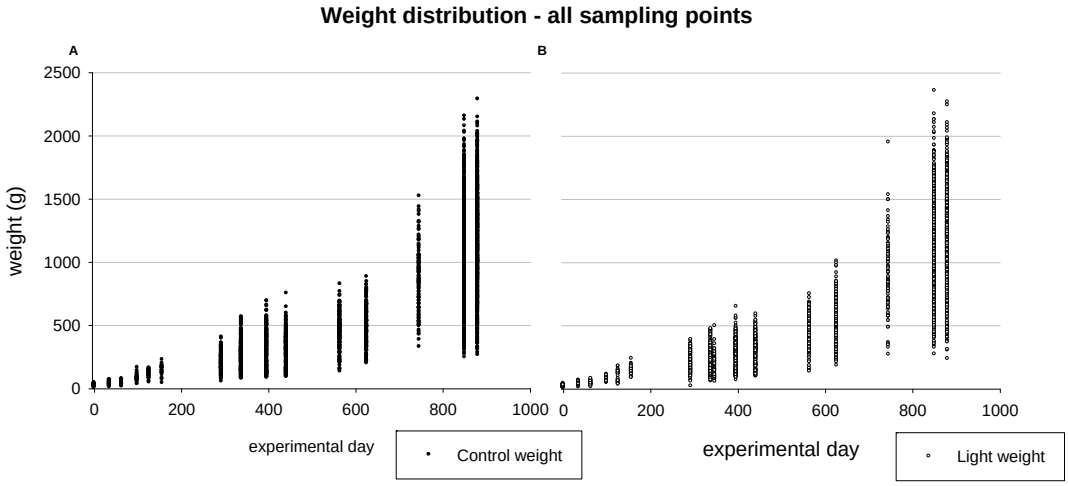

Figure 10: Weight (g+/- SEM) distribution throughout the experimental time. A) control B) light

Body and gonadal weight of individuals from each treatment were measured at every samplings point and compared between treatments. Figure 12a and 12b compare the effect of hatchery CCL imprinting on response to subsequent CCL light and natural light treatments in sea cages. No significant difference could be seen in both body and gonadal weight between imprinted and non-imprinted fish. They were therefore treated as one and same group in following analyses.

When the effect of natural light and CCL is compared (Figure 11a) no significant difference was found between the treatment groups in body weight. However, significant gonad weight differences were found in March 2008 ($p=0.005$), December 2008 ($p<0.001$) and January 2009 ($p<0.001$), with the average gonad weight being significantly higher in fish receiving natural light treatment. It was also evident that the responses of the sexes were different; with the effect of CCL treatment having much more pronounced effect in males (Figure 13). Significant differences between males and females held under the control treatment were found in March 2008 ($p=0.004$), September 2008 ($p=0.001$) December 2008 ($p<0.001$) and January 2009 ($p<0.001$).

In contrast, the only significant differences found between males and females, held under the natural light treatment, were found in December 2008.

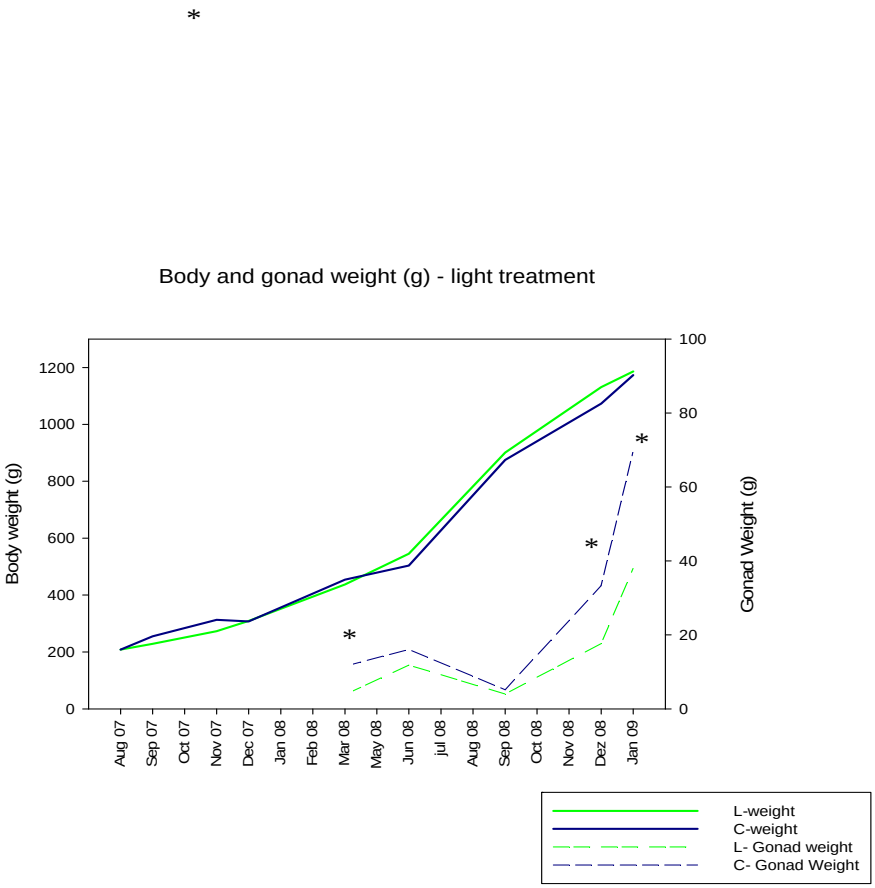

Figure 11a: Comparing whole body weight and gonad weight for the different treatments. Green lines represent the light treatment while blue lines represent the control treatment. Full lines represent the whole body weight while broken lines represent the whole gonad weight

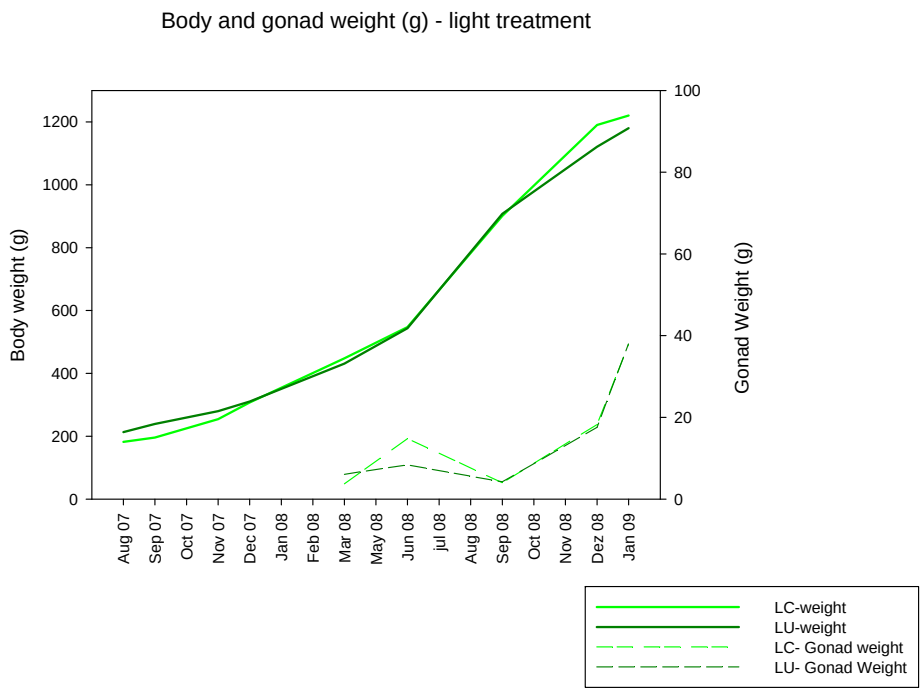

Figure 12a: Comparing whole body and gonad weight, between trained fish (cut fish) and non-trained fish (uncut) reared under the light treatment.

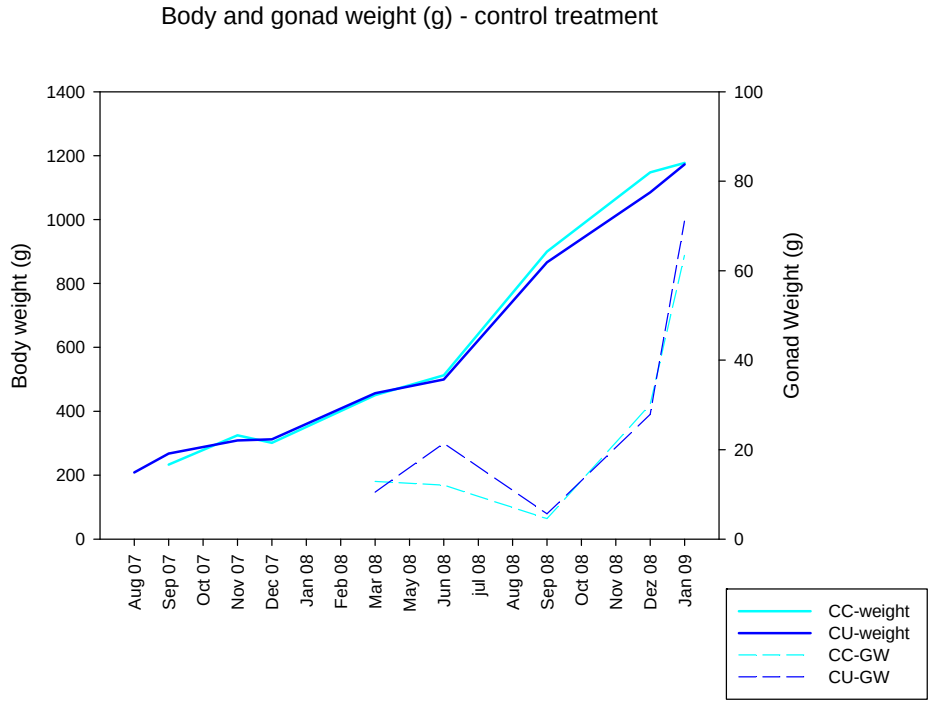

Figure 12b: Comparing whole body and gonad weight, between trained fish (cut fish) and non-trained fish (uncut) reared under the control treatment (natural light).

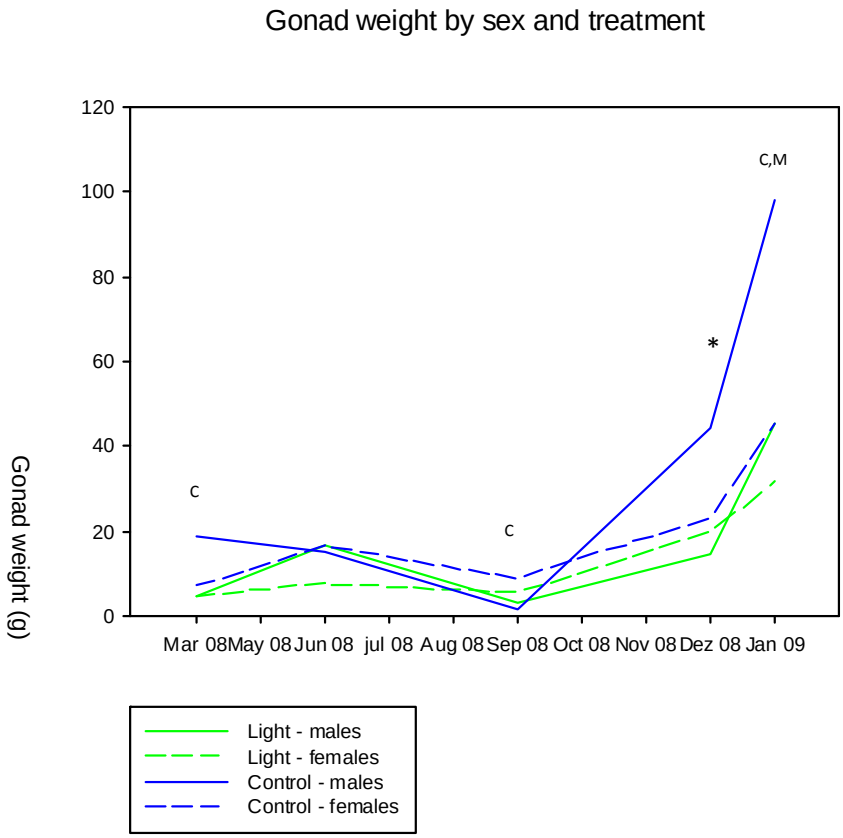

Figure 13: Comparing gonad weight between sexes. "C" denotes significant differences between sexes under the control treatment while "M" denotes significant differences between males held under the different treatments. On December 2008 ("*"), significant differences were found at every levels with $p<0.001$ - same sexes between treatments, and between sexes in each treatment.

3.2.5 GH and GHR expression levels

The differences in the hepatic growth hormone receptor (GHR) and growth hormone GH gene expression were also assessed during the sea cage stage. The comparisons were made between the two different treatment groups: The CCL treatment group and the control group that was held under natural light. As no phenotypic differences either in weight or gonadal development could be observed between imprinted or non-imprinted fish they were treated as one and the same group.. Figure 14 shows the mean and GH and GHR expression levels at the different sample points for the different light treatments. Significant difference was only found on the first time point, between the light and control treatment GH expression (p=0.007), denoted by the "*".

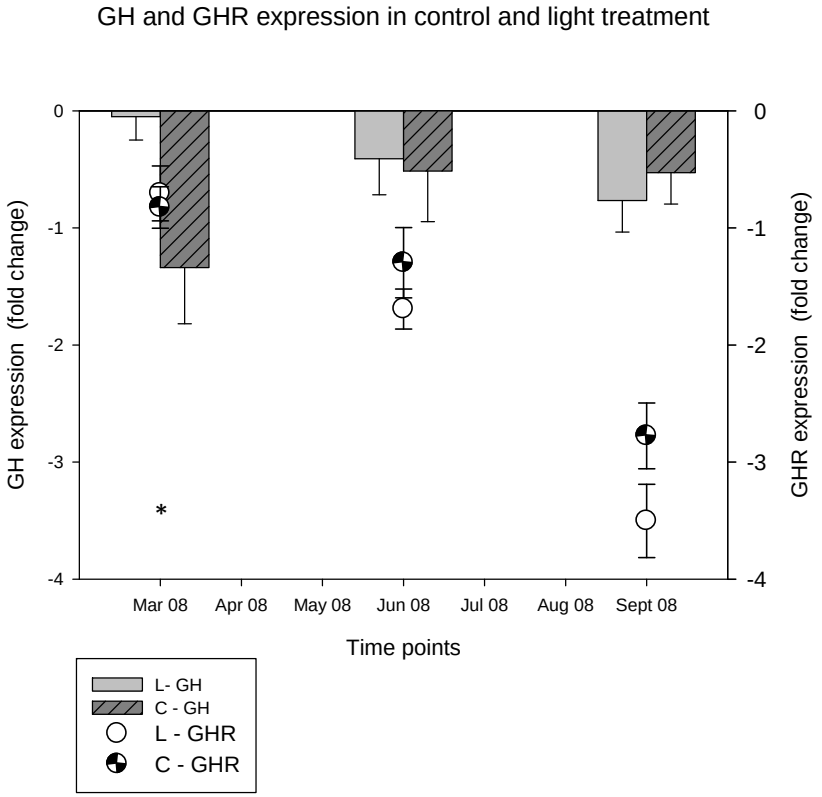

Figure 14: GH and GHR expression levels on control and light treatment. Error bars represent the SEM. Significant difference was only found on the first time point, between the light and control treatment GH expression (p=0.007), denoted by the "*".

3.2.6 Discussion

The CCL light treatment has an effect on the fish development. This is however, only visible in the gonadal development and GH gene expression. However, instead of completely arresting the gonadal development, CCL treatment appears to delay it and is most clearly visible in the last two sampling points during the outdoor rearing stage.

The hypothesis is that differences in development especially in growth related traits might be the result of differences in GHR expression levels in the liver. This, however, could not be verified. GHR expression levels were measured at different sampling points both during the indoor stage where different size groups and treatments were compared and at the outdoor stage where different light treatments were mainly compared. No significant differences were observed at all sampling points both during the indoor and outdoor levels, except, at the point just before the transportation to sea cages. This defies interpretation and is likely an experimental error as the high level of GHR expression is observed in fish group with the smallest fish. Furthermore, this appears to be not an effect of CCL light treatment as the control group receiving natural light has similar levels of expression as well as control group for the Group of the largest individuals.

A major concern regarding the use of photoperiod manipulation in outdoor cages is that fish development may still be regulated by the natural photoperiod. The CCL (Cold-Cathode Light) has a single green wavelength that diffuses more effectively throughout the water column than white light. It may therefore mask natural light more efficiently. Still it may be necessary to train fish for the CCL lights at the indoor stage before transportation to the sea cages. In this part of the study the effect of juvenile imprinting was examined. Fish imprinted for CCL light were expected to be less affected by natural light photoperiod when receiving CCL treatment in the outdoor sea cages. The effect of CCL light was observed in gonadal development but none were observed in the growth related traits, body weight and length. When imprinted and not imprinted were compared negligible difference in gonadal development were, however, observed strongly indicating that prior imprinting to sea cage rearing had no effect.

The growth effects of GH are indirect, through the insulin like growth-factor produced in the liver, the most important one being Insulin like growth-factor 1 (IGF-1). Greater quantity of IGF-1 is produced when higher levels of GH are released to the blood by the pituitary. This response is mediated by the Growth hormone receptor in the liver. The greater the concentration of receptors the more sensitive is the systems to increases in GH concentration. The light influence is on the GH gene expression, is only observed in the beginning of March. and is apparently not associated with growth increase. The effects are probably being applied in other physiological processes than growth. GH is known to influence varied physiological processes, the fat metabolism (Norbeck et al, 2007) protein metabolism (Mauras et al, 2005) and the fish immune system as an example (Yada et al, 2007).

3.3. Genotypic and phenotypic analysis of cod families reared under different light regimes in sea cages

The aim was to i) Evaluate the effect of CCL on sexual maturation of cod in sea cage cod farming ii) Evaluate the effect of CCL on growth in sea cage cod farming. iii) Investigate if significant variation could be observed between families regarding growth traits indicating genetically based differences iv) Investigate if significant variation could be observed between families regarding onset of sexual maturity indicating genetically based differences v) Investigate if differences could be observed between families in response to light treatment (e.g. delay of sexual maturity or direct effect on growth traits independent of the onset of sexual maturity or "inherent" family growth-phenotype).

In this last part of the study - family trait association analysis - all the fish in the experiment was harvested and approximately 4000 individuals geno- and phenotyped from all 4 groups. Growth performance and maturity were compared between the groups receiving different light treatments as well as between within and between families receiving different light regimes.

The fish was transported from indoor rearing to sea cages on 6 May 2007. The average weight was 166 g (150±30g). A total of 19.880 fish were transported to Álftafjörður in Westfjords and placed in four circular sea cages, (each 155 m^3, 6 meters in diameter and 5-6 meters deep). In two of the cages was natural light but the other two were under CCL illumination, each with 2 x 3 CCL tubes (a total of 6 lights) from Intravision (263W $cage^{-1}$). The fish was randomly distributed into the sea cages (approximately 5 000 juveniles per cage. The fish that had been reared under CCL from hatching was fin-clipped to distinguish it from fish that had been reared under white light. Density was rather low or 5 kg m^{-3}.

The sea cages in Álftafjööður were located 200 m offshore in two separated places (N 66°01.522' - W22° 58.906' and 66°01.519' - W 22°58.776') at the depth below the cages were 40-50 meters.

3.3.1. Sampling

Samples were taken every second and third month during the sea cage stage. Growth and maturity related traits were measured and gene expression profiles of two candidate genes, GH and GHR, evaluated at each sampling point. The results of these studies are recounted in part 2. In short the results showed that a CCL regime affected first and foremost development of gonads by delaying it. This delay was not reflected in overall growth. Furthermore training of juveniles for CCL in the indoor tanks did not appear to affect subsequent response to CCL in sea cages.

3.3.2 Pheno- and Genotyping

At total of 4000 individuals were sampled in December 2008 and January 2009. Growth and maturity related traits were evaluated. Length and weight were measured and liver and gonads were weighed in order to calculate the gonadosomatic index (GSI), and the somatic weight (SW), which was defined as the whole bodyweight (BW) minus the gonad and liver weight (GW and LW respectively). Gill samples were taken for DNA isolation and subsequent microsatellite genotyping and family group assignments.

10 microsatellite markers were used and approximately 3517 individual were scored in all loci. Of the 33 families that were initially used members of only 13 could be detected. Family is defined as offsprings of one male and two females, or two full sib groups sharing a father. The number of individuals in the different families ranged from 50 to approximately 1000 and some families were only represented by one full sib group. The distribution of individual between treatments within families was also sometimes unequal.

3.3.3 Family comparisons

The genotyping revealed 13 families in the population. The families consisted often of only one full sib group with the other completely missing. The number of individuals in the groups also varied greatly, from below 100 to approx. 1000. One family, F15 that consisted of one full -sib group (H01) dominated the population (approx.30% of all fish, Figures 15 and 16). Proportional representation of families at the two sampling points, December 2009 and January 2010, were similar (see Figures 15 and 16).

Those groups that had fewer members than 100 were excluded from the analysis giving a total 9 full-sib groups used. These were the following: Group F01H16 with 282 members (Family01 and Full sib group H16), group F02H19 with 156 members, group F03H02 with 164 members, group F06H05 with 194 members, group F08H09 with 105 members, group F10H04 with 306 members, group F11H07 with 171 members, group F15H01 with 1065 members and group F20H03 with 164 members. This gave a total of 2067 sampled fish for analysis. The data from December and the January samplings were treated separately (see Table 3 for details).

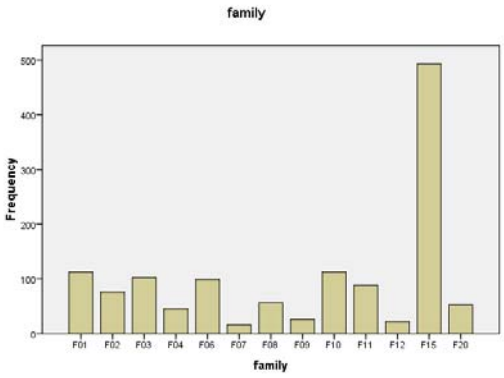

Figure 15: Number of individuals sampled in each family in December.

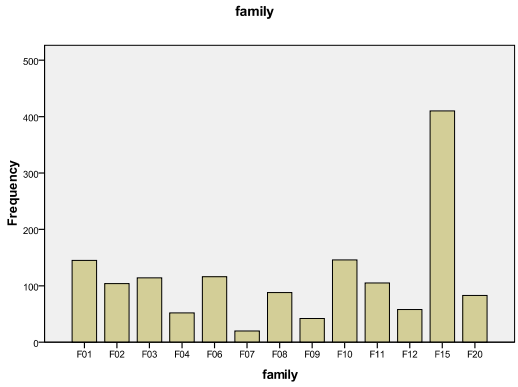

Figure 16: Number of individuals sampled in each family in January

Family	Sib-group + treatment			sm	gr	hold	sex	G.weight	GSI
F01	H16 Nat	male	55	46,55	1054,3	1,05	1	50	4,74
F01	H16 Nat	female	33	46,48	1046,0	1,04	9	26	2,49
F01	H16 CCL	male	35	44,51	946,9	1,07	1	13,4	1,42
F01	H16 CCL	female	30	45,43	965,5	1,03	9	24	2,49
F01	H16 Nat	male	39	46,90	1117,6	1,08	1	97,4	8,72
F01	H16 Nat	female	36	47,17	1063,4	1,01	9	39,8	3,74
F01	H16 CCL	male	29	46,55	1071,6	1,06	1	50,9	4,75
F01	H16 CCL	female	25	47,28	1105,5	1,05	9	35,2	3,18
Family			282	sm	gr	hold	sex	G.weight	GSI
F02	H19 Nat	male	18	43,4	936,7	1,15	1	32	3,42
F02	H19 Nat	female	22	43,5	991,6	1,20	9	22	2,22
F02	H19 CCL	male	17	45,4	1127,7	1,21	1	13,1	1,16
F02	H19 CCL	female	27	42,7	955	1,23	9	22,7	2,38
F02	H19 Nat	male	13	44,1	990,9	1,16	1	76,2	7,69
F02	H19 Nat	female	24	44,5	1013,4	1,15	9	36,9	3,64
F02	H19 CCL	male	15	45,6	1090,7	1,15	1	36,86	3,38
F02	H19 CCL	female	20	46,5	1169,7	1,16	9	31,2	2,67
Family			156	sm	gr	hold	sex	G.weight	GSI
F03	H02 Nat	male	21	46,7	1190,9	1,17	1	54	4,53
F03	H02 Nat	female	17	47,3	1240,9	1,17	9	20	1,61
F03	H02 CCL	male	26	47,2	1181,7	1,12	1	19,4	1,64
F03	H02 CCL	female	27	45,9	1170,9	1,21	9	19,2	1,64
F03	H02 Nat	male	24	48,1	1268,1	1,14	1	80,2	6,32
F03	H02 Nat	female	21	48,8	1305,6	1,12	9	50,6	3,88
F03	H02 CCL	male	11	47,9	1328,9	1,21	1	51,5	3,88
F03	H02 CCL	female	17	48,4	1344,1	1,19	9	38,2	2,84
Family			164	sm	gr	hold	sex	G.weight	GSI

F06	H05 Nat	male	18	45,6	1146,3	1,21	1	57	4,97
F06	H05 Nat	female	34	46,4	1062,8	1,06	9	16	1,51
F06	H05 CCL	male	24	46,2	1092,3	1,11	1	8,4	0,77
F06	H05 CCL	female	25	45,2	1149,6	1,24	9	13,4	1,17
F06	H05 Nat	male	24	45,7	1106,1	1,16	1	74,3	6,72
F06	H05 Nat	female	17	46,2	1065,4	1,08	9	23,4	2,20
F06	H05 CCL	male	23	48,7	1271,8	1,10	1	42,1	3,31
F06	H05 CCL	female	29	47,9	1217,1	1,11	9	22,5	1,85
Family			194	sm	gr	hold	sex	G.weight	GSI
F08	H09 Nat	male	14	46,8	1349,9	1,32	1	78	5,78
F08	H09 Nat	female	18	47,6	1363,6	1,26	9	37	2,71
F08	H09 CCL	male	15	46,5	1255,5	1,25	1	18,5	1,47
F08	H09 CCL	female	9	47,6	1429,4	1,33	9	23	1,61
F08	H09 Nat	male	9	44,8	1207,4	1,34	1	149,1	12,35
F08	H09 Nat	female	17	48,5	1389,2	1,22	9	64,1	4,61
F08	H09 CCL	male	5	47,6	1242,6	1,15	1	43	3,46
F08	**H09 CCL**	**female**	**18**	**48,3**	**1298,5**	**1,15**	**9**	**38,4**	**2,96**
Family			105	sm	gr	hold	sex	G.weight	GSI
F10	H04 Nat	male	31	47	1198,5	1,15	1	55	4,59
F10	H04 Nat	female	41	48,2	1307,1	1,17	9	32	2,45
F10	H04 CCL	male	42	48	1292	1,17	1	20,3	1,57
F10	H04 CCL	female	46	48,7	1410,3	1,22	9	29,4	2,08
F10	H04 Nat	male	38	48,8	1337,4	1,15	1	125	9,35
F10	H04 Nat	female	46	49	1368,5	1,16	9	60,1	4,39
F10	H04 CCL	male	17	48,4	1269,1	1,12	1	66,3	5,22
F10	H04 CCL	female	45	49,7	1444,7	1,18	9	43,8	3,03
Family			306	sm	gr	hold	sex	G.weight	GSI
F11	H07 Nat	male	24	44,3	828,2	0,95	1	35	4,23
F11	H07 Nat	female	21	46,1	1011,6	1,03	9	21	2,08
F11	H07 CCL	male	22	45,1	915	1,00	1	15,4	1,68

F11	H07 CCL	female	30	45,5	1009,9	1,07	9	19,2	1,90
F11	H07 Nat	male	20	46,2	999,1	1,01	1	95,1	9,52
F11	H07 Nat	female	23	46,7	1076,9	1,06	9	41,1	3,82
F11	H07 CCL	male	17	47,4	1112,3	1,04	1	53,5	4,81
F11	H07 CCL	female	14	48,4	1162,1	1,02	9	28,6	2,46
Family			171	sm	gr	hold	sex	G.weight	GSI
F15	H01 Nat	male	155	46,8	1107	1,08	1	40	3,61
F15	H01 Nat	female	161	47,1	1133,1	1,08	9	21	1,85
F15	H01 CCL	male	170	47	1146,1	1,10	1	13,9	1,21
F15	H01 CCL	female	169	47,9	1240,9	1,13	9	18,8	1,52
F15	H01 Nat	male	124	48,5	1204,3	1,06	1	124	10,30
F15	H01 Nat	female	131	49,8	1321,5	1,07	9	44,5	3,37
F15	H01 CCL	male	76	48,6	1238	1,08	1	45,8	3,70
F15	H01 CCL	female	79	49,1	1251,3	1,06	9	29,9	2,39
Family			1065	sm	gr	hold	sex	G.weight	GSI
F20	H03 Nat	male	23	48,1	1210,5	1,09	1	43	3,55
F20	H03 Nat	female	19	48,3	1178,1	1,05	9	27	2,29
F20	H03 CCL	male	13	45,9	1021,9	1,06	1	16,9	1,65
F20	H03 CCL	female	26	48,2	1199,9	1,07	9	21,6	1,80
F20	H03 Nat	male	14	47,9	1144,4	1,04	1	90,8	7,93
F20	H03 Nat	female	26	49,8	1278,7	1,04	9	51,8	4,05
F20	H03 CCL	male	17	46,7	1054,8	1,04	1	46,1	4,37
F20	H03 CCL	female	26	49,7	1335,1	1,09	9	39,6	2,97
Family			164	sm	gr	hold	sex	G.weight	GSI

Table 3. Full sib groups with more than 100 members classified in groups of sex and treatment. Average, length, body and gonadal weight, GSI are given for the different groups.

3.3.4 Growth related traits

The two sampling points December 2009 and January 2010 were treated separately and average growth increase of approximately 6 % was observed in the period (Figure 17). Significant differences in mean weights (up to 40% between families F08 and F11 in December) could be observed between the different families and the same pattern was observed at the

two harvesting time points in January and in December (Figure 17). The effect of CCL on the weight and length size is contradictory. In some families the light seemed to have negative effect and sometimes positive. Sometimes the effect was only observed at one of the sampling points and sometimes only in one sex. The apparent responses of the families are so different that it cannot be state unequivocally if CCL is beneficial or not to growth. However, the differences between treatments are relatively large in some families, up to +20 % and – 14%, which indicates different responses to CCL light in different families (Table 3). These responses may have an underlying genetic basis.

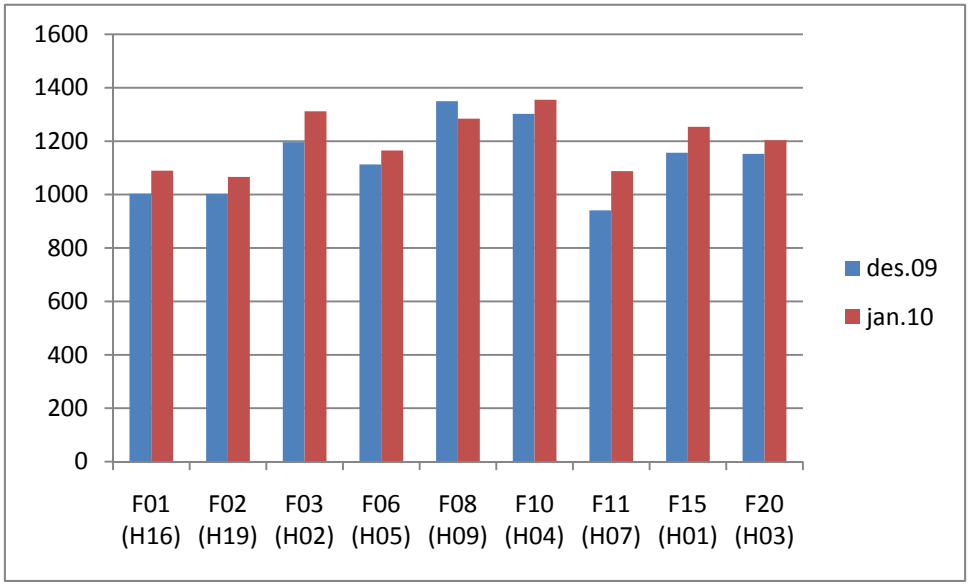

Figure 17: Average weights in full sib groups sampled in December 2009 and January 2010.

3.3.5 Maturity related traits

Increase in gonadal development between the different sampling points, December and January, was substantial. The gain in gonadal weight in fish under natural light treatment amounted to 105% for males and 85% for females. Furthermore, in contrast to the effect of growth related traits, CCL treatment had a clear and consistent effect on the maturity related traits, gonad development and GSI. The effect was much more pronounced in males than in females. Thus, the average gonadal weight of males in December under natural light was 49,3g whereas the average gonadal weight of the male fish receiving CCL treatment was only 14,1 g or 71 % less. At the later sampling point, in January, the average gonad weight of males receiving CCL treatment was 48% less than that of males under natural light conditions (Table 3). The effect on female gonad weight was less, at the December sampling point the average weight of gonads of females receiving CCL treatment was in comparisons only 14% less compared to that of males that were 71 % less. The decrease in weight of female gonads compared with natural light treated females was, however, greater at the later sampling point in January. In Figure 18 and 20, comparisons are made between treatments at the December and January sampling points respectively. It can clearly be seen that the mean gonad weight is much lower in the group that received CCL treatment than in the group reared under natural light. This difference is clearly significant. Similar results were obtained with GSI (Figure 19 and 21). There are clear differences between families in gonadal weight under CCL treatment. This could be genetically based, but needs further studies.

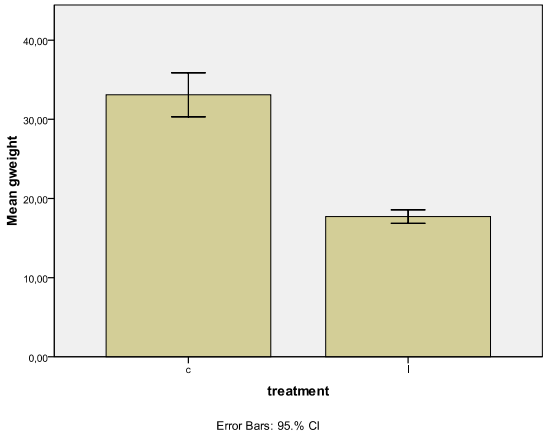

Figure 18: Mean gonad weight in the different treatments. (c – Control; l – Light) in December sampling

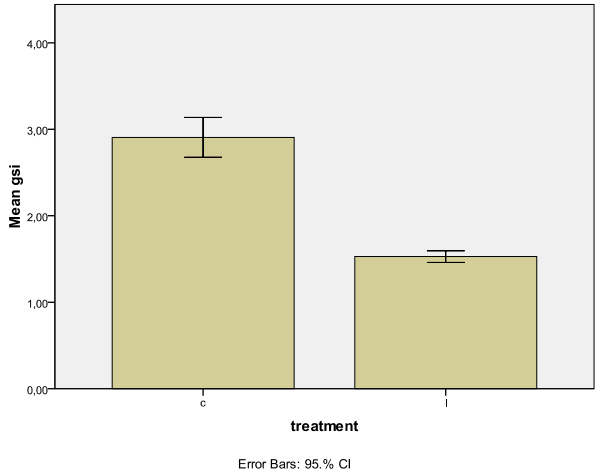

Figure 19: Mean GSI in the different treatments. (c – Control; l – Light) in December sampling

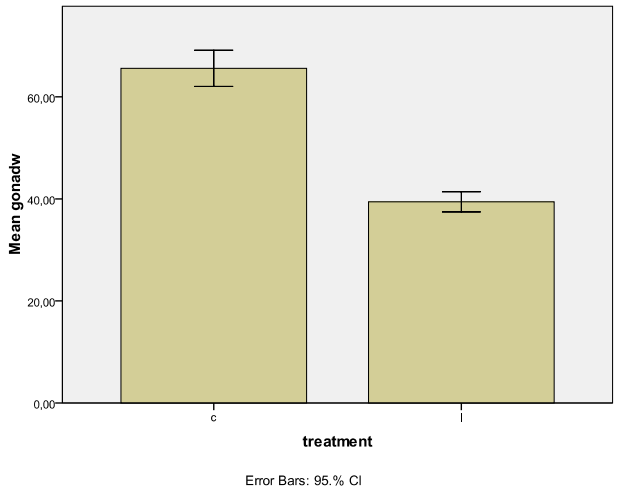

Figure 20: Mean gonad weight in the different treatments. (c – Control; l – Light) in January sampling

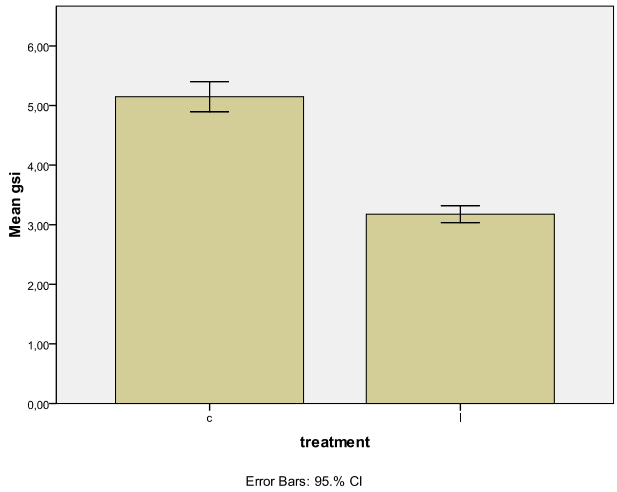

Figure 21: Mean GSI in the different treatments. (c – Control; l – Light) in January sampling.

3.3.6 Discussion

The same families are consistently larger, both in length and weight at the different sampling points indicating clear genetic differences between them.

Physiologically, the fish responds clearly to CCL treatment regarding maturity related traits, with less gonadal development in the CCL treated fish. It was substantial and the same trend

could be observed in all families. The degree of response however differed between families to some extent which may signify underlying genetic differences.

The effect of CCL treatment on growth related traits was less clear. Apparent growth responses to CCL treatment varied greatly between families and they appeared to be either negative or positive, depending on family and sex. Opposite effect were even observed within families on different sexes. Furthermore, the same trend was not observed in all families at both sampling points. This makes interpretation difficult and while these responses may have an underlying genetic basis the genetic background is quite complex.

The less gonadal growth in CCL treated fish is not clearly reflected in increased weight (or length) as might have been expected. The overall weight is more or less the same between the treatments.

The mapping of the three candidate related markers obtained in the project onto the complex phenotypic family pattern observed is not expected give any additional information.

More detailed statistical analysis will be given in the masters theses of Kjell.

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