University of Akureyri
Department of Business and Science

The effects of cold cathode lights on growth of juvenile Atlantic cod (*Gadus morhua* L.): use of IGF-I as an indicator of growth

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Statement

„I hereby confirm that I am the author of this work

and it is the product of my own research".

Guðbjörg Stella Árnadóttir

„We, the undersigned, confirm that this work satisfies examination requirements for

M.Sc. degree in Natural Resource Sciences "

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Ágrip

Ljóslota er einn af þeim umhverfisþáttum sem hefur hvað mest áhrif á vöxt þorsks líkt og hjá öðrum fiskitegundum. Nýlegar rannsóknir benda til að unnt sé að auka vaxtarhraða þorsks með því að nota ljósastýringu til þess að lengja dag yfir haust og vetrarmánuðina.

Í þessari rannsókn, sem er hluti af stærra verkefni, eru þorskseiði ljósameðhöndluð með nýrri ljósatækni (Cold-Cathod Lights; CCLs) frá því að þau eru um 10 grömm að þyngd. Með ljósameðhöndluninni er seiðunum skapaðar sérstakar umhverfisaðstæður mjög snemma á lífsferlinum og er markmiðið að ná fram aukinni næmni fyrir þessari bylgjulengd ljósa, sem vonir eru um að skili sér þegar seiðin eru síðar á lífsferlinum flutt í ljósastýrðar sjókvíar. Áhrif ljósameðhöndlunar á magn IGF-I vaxtarþáttar (insúlín-líkur vaxtarþáttur-I) í blóði eru einnig metin. IGF-I er mælt með radioimmunoassay (RIA) aðferð. Í verkefninu er jafnframt rannsakað hvort unnt sé að mæla IGF-I í blóði þorsks með einfaldari mæliaðferð, enzyme-linked immunosorbent assay (ELISA). Sömu sýni eru mæld með báðum aðferðum og skoðað hvort niðurstöður eru sambærilegar.

Helstu niðurstöður rannsóknarinnar benda til þess að meðhöndlun með þessari gerð ljósa hafi ekki áhrif á vöxt eða lifun þorsksseiða fyrsta árið í eldi. Niðurstöður sýna ennfremur að mögulegt er að mæla magn IGF-I vaxtarþáttar í blóði með RIA aðferð en ekki reyndist vera samband á milli vaxtarhraða og styrks IGF-I í blóði þorsks.

Lykilorð: Þorskur, ljóslota, vöxtur, IGF-I, CCL ljós

Aknowledgements

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Guðbjörg Stella Árnadóttir

Abstract

Photoperiod is an environmental regulator of growth and sexual maturation in Atlantic cod as well as other species of fish. In this study, cod juveniles are exposed to a novel cold cathode light (CCL) system in order study the effects on growth as well as a possible delay of sexual maturation later in the production process. CCL emit a single wavelength green light with improved distribution pattern in water compared to standard white light systems used in aquaculture. By starting continuous light treatment (24L:D0) at an early stage, while juveniles are still in hatchery tanks, "light adaptation" may occur, making photoperiod regulation more effective after transfer of the fish to sea-cages.

The aim of the study was to determine the effects of light treatment (24L:D0) using a new light technology (CCLs) on Atlantic cod juveniles at an early stage and study the relationship between growth and the concentration of insulin-like growth factor-I (IGF-I) in Atlantic cod plasma. The levels of IGF-I in plasma are measured using a radioimmunoassay (RIA) method. The light treatment did not significantly affect growth or survival of the Atlantic cod juveniles, but importantly, the CCL regime resulted in reduced incidence of skeletal deformities. In contrast to numerous studies on fish, no correlation was found between growth rate and plasma IGF-I levels.

Key words: Atlantic cod, photoperiod, growth, IGF-I, cold-cathode light

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1 Introduction

Photoperiod is an environmental regulator of growth and sexual maturation in Atlantic cod as well as other species of fish (Norberg *et al.,* 2001; Esteban *et al.,* 2006). Regulatory mechanisms of growth, early maturation and the effects of photoperiod have been studied during the development of Atlantic halibut (Roth *et al.,* 2007), Atlantic salmon (Björnsson *et al.,* 2000; Nordgarden *et al.,* 2005; Nordgarden *et al.,* 2006; Pooedal *et al.,* 2007) and Eurasian perch (Migaud *et al.,* 2006a). The results indicate that extended photoperiod leads to faster somatic growth, reduced gonad development and thus, a shorter production cycle (Davie *et al.,* 2003). In farming of Atlantic halibut, the main purposes of photoperiod manipulation is to control and alter time of spawning periods of broodstock fish, and to facilitate delay or inhibit sexual maturation of production fish (Norberg, 2001; Unwin, 2005). In Atlantic salmon, the main use of photoperiod manipulation is to get rapid juvenile growth and smoltification at the 0+ stage (Björnsson *et al.* 2000).

Early maturation is one of the major problems in intensive farming of Atlantic cod (*Gadus morhua* L.), and much work has focused on delaying or completely arresting sexual maturation, which can result in up to 60% increase in growth in addition to improved quality of the final product (Davie *et al.,* 2007a; 2007b).

In this study, juvenile Atlantic cod were exposed to continuous light (24L) throughout the first year in the hatchery, using a novel light system, cold cathode lights (CCL), emitting a single wavelength of green light. The objective was to mask the seasonal light cycle and to prepare the juveniles for transfer to photoperiod-regulated sea cages. This new light technology may be a step towards regulation of photoperiod in sea cages (Oppedal *et al.,* 2005). The length and weight of the fish was measured on a regular basis throughout the project and the specific growth rate (SGR) of individually tagged fish determined. Furthermore, blood samples were collected for the analyses of insulin-like growth factor I (IGF-I) and for studying the correlation between IGF-I levels and growth rate.

Atlantic cod belongs to the teleost group which is the largest group of vertebrates, approximately 20,000 species. The main features of the teleosts are bonelike support tissues and gill slits with opercula on both sides. During the development, a swim bladder arose within this group, but some species have subsequently lost it.

Fish comprise in to two main groups, Actinopterygians and Sarcopterygians. Actinopterygians include Chondrosteans, holosteans and teleosts. Sacropterygians include Crossopterygians, and lungfish (Figure 1).

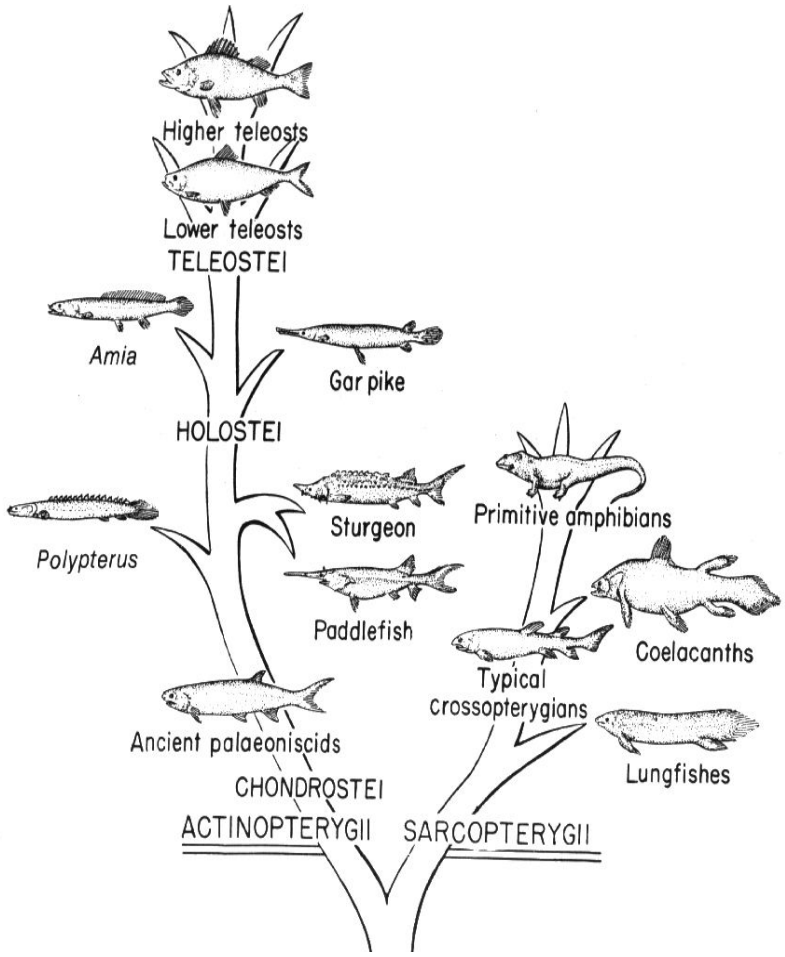


Figure 1. An evolutionary phylogenetic tree.
The tree shows how fish are divided into two main groups, Actinopterygii, and Sarcopterygii. The Atlantic cod belongs to the Actinopterygii group and is classified as a higher teleost species.
(http://bill.srnr.arizona.edu/classes/182/Lecture-10.htm.)

Table 1 Systematic classification of Atlantic cod

Kingdom:	**Animalia**
Phylum:	**Chordata**
Class:	**Actinopterygii**
Subclass:	**Neopterygii**
Order:	**Anacanthini (*Gadiformes*)**
Family:	**Gadidae (*Gadidae*)**
Genus:	**Atlantic cod (*Gadus*)**
Species:	**Atlantic cod (*Gadus morhua* L.)**

Atlantic cod are found on both sides of the North-Atlantic Sea, by Japan, Alaska, Korea, Canada and the west cost of USA. It is a demersal fish that can live from depth of approximately 600 meters up to a few meters, but is most commonly found at 100 - 400 meters depth. The optimal temperature for wild Atlantic cod is 2 - 7°C (Gunnarsson, 1998), but the Atlantic cod moves around in the sea in search of food (Comeau *et al.,* 2002). It mostly prefers clay, lava, coral and sand bottom surfaces (Gunnarsson, 1998). The harvesting size of Atlantic cod is 55 - 90 centimeters at the weight of 1.5 - 7 kilos, but the fish can grow up to 200 centimeters in length. The Atlantic cod changes color according to the environment and the age of the fish. However, the Atlantic cod is often yellow or gray on the back and down to the sides, with small dark spots, and white belly (Figure 2). In the area south of Iceland, the Atlantic cod is often near to black, but it is gray or blue in the North and East of Iceland (Gunnarsson, 1998).

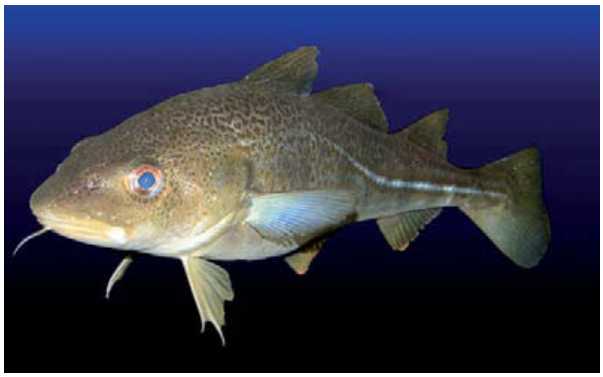


Figure 2. Atlantic cod (Gadus morhua L.*)*

The Atlantic cod spawns during a 6 – 8 weeks period in late winter. During spawning, females release eggs in many small doses, from 500 thousand up to 15 million eggs, depending of the size and age of the fish. After approximately 2 - 3 weeks, pelagic larvae hatch at approximately 5 millimeters in length. When the larvae reach 5 - 8 cm, they move down to the bottom of the sea (Gunnarsson 1998).

Capelin is the main feed of adult Atlantic cod in its natural environment. Data collected between 1979 and 1995 show that capelin represented 49% of the stomach content of Atlantic cod captured around Iceland (Gunnarsson 1998). The Atlantic cod also eats other fish species such as redfish, blue whiting, catfish and herring. The feed of choice in the natural environment is relatively well known, but the appropriate combination of nutrients for farmed Atlantic cod is not fully understood (Árnason, 2004).

1.1 Cod fisheries and farming

The earliest records of Atlantic cod fishing in Iceland date back to the 12th century and the first written source of export is from 1624 (Karlsson, 2007). Ever since, the Atlantic cod has been one of the most important coldwater marine fish species for commercial fisheries. The Atlantic cod became a part of the Icelandic heritage and has been extremely important for the Icelandic economy. Export of cod has been gradually decreasing during the last decades as the fishing quota for Atlantic cod have been decreased from 530,000 tons in 1955 to 215,000 tons in 2005 (Figure 3) (Sigurðsson, 2006).

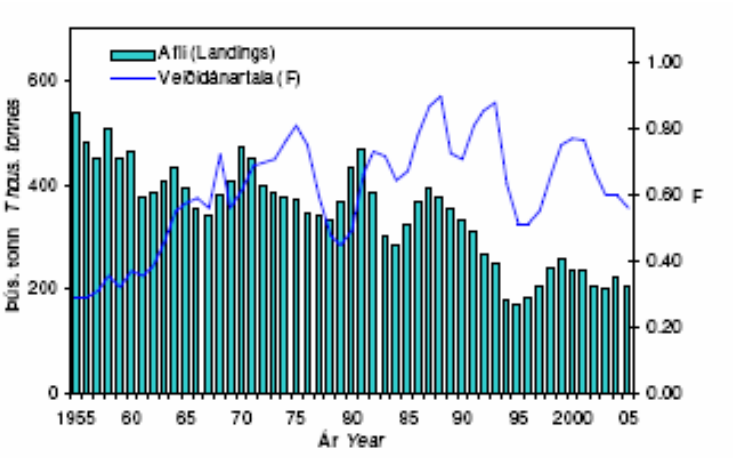


Figure 3. Atlantic cod. Total landings (thousand tons) 1955-2005 (Sigurðsson, 2006).

The Icelandic fishing quota for Atlantic cod was further scaled down by 30,000 tons in 2007, representing enormous difficulties for many companies and the fish industry as a whole.

Due to declining stocks and the decrease in Atlantic cod fishing, the fish industry has to seek other ways to maintain their market share of cod that have been established over the last decades. One possibility is farming of Atlantic cod which is a growing industry in countries of the Northern hemisphere including Iceland.

The first reports on farming of Atlantic cod date back to 1886 in Norway (Rosenlund and Halldórsson, 2007), but farming of Atlantic cod was not established in Iceland until 1992 (Gunnarsson, 2007). Being a North Atlantic species and adapted to cold water, the Atlantic cod is an ideal species for farming in Icelandic waters. Research indicates that the optimal temperature for farmed Atlantic cod is a few degrees higher than for wild cod or between 8 - 12°C for fish over 1000 g (Björnsson *et al.,* 2007). Also, optimal temperature for growth of Atlantic cod has been found to decrease with increasing weight of the fish (Björnsson *et al.,* 2007). These findings imply that it should be attractive to farm Atlantic cod in the sea around Iceland compared with farming of species with higher temperature optimum for growth.

The conditions of farmed Atlantic cod, including continuous supply of feed and the absence of predators, allows faster growth under farming conditions. Results from studies indicate that the feeding regimes, nutrient combination and the amount of feed is critical in fish farming. Improved feed formulas, adjusted to the species needs, are therefore likely to lead to improved growth and health of the fish (Árnason, 2004; Solberg *et al.,* 2006).

However, the fast growth of farmed cod may result in a number of biological and production-related problems. One is the increased incidence of abnormal skeletal development during the earliest stages, and premature maturation during which growth is arrested for 2 - 4 months. This leads to a prolongation of the production time with increased cost for the farmers. Light manipulation is commonly applied with the aim of delaying or arresting sexual maturation. Lights, however, impose increased production costs, but energy requirements differ between light technologies. The present study is a part of a larger project where the overall goal is to improve the production of farmed cod by the use of novel, energy-efficient CCL technology.

1.2 Photoperiod manipulation

The seasonal changes in photoperiod are reflected in fish behavior (Karlsen *et al.,* 2006). In the northern hemisphere, fish growth is fast during the short, bright summer. After the long, dark winter, fish such as cod are hungry because of low availability of nutrients and adverse feeding conditions (darkness). As soon as the day gets longer, the temperature of the sea increases and more nutrients become available.

Photoperiod manipulation is commonly used in fish farming, using light intensity and/or photoperiod duration controlled by the fish farmer. The effects of such manipulation have been intensively studied in various fish species and photoperiod manipulation is becoming an important factor in the farming of a number of fish species. Photoperiod manipulation of broodstock fish is commonly applied for the year around production of gametes in commercial farming of various fish species including Atlantic halibut (*Hippoglossus hippoglossus*), Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), turbot (*Scophthalmus maximus*), sea bass (*Dicentrarchus labrax*) and sea bream (*Sporus aurata*) (Norberg *et al.,* 2004). A number of studies have been carried out in order to investigate the effects of light during the juvenile and larval stages of various fish species (Han *et al.,* 2005), such as juvenile haddock (Trippel and Neil, 2003), Atlantic cod larvae (Huse, 1994) and flatfish larvae (Blaxter, 1986).

Hormones have been found to be affected by photoperiod and altered levels of hormones, especially growth hormone (GH) and thyroid hormones, as well as survival and growth rates, have been reported in studies of photoperiod manipulation (Boeuf and Le Bail, 1989; Björnsson *et al.,* 2000; Han *et al.,* 2005). Specific activity of hormones such as trypsin and chymotropsin did not significantly affect survival, cannibalism or growth in post-larval sea bass during the weaning period when 24L photoperiod was used (Cuvier-Péres *et al.,* 2001), but there was a tendency towards increased cannibalism among sea bass and perch larvae held at higher candle power (Cuvier-Péres *et al.,* 2001; Kestemont *et al.,* 2003). The results indicate that it is the candle power rather than the number of light hours that affects cannibalism of these species. Aggression in catfish was also found to be affected by photoperiod and light intensity where 24L resulted in about twice as many scars and wounds compared to normal

photoperiod (Almazán-Rueda *et al.*, 2004). On the other hand, survival and growth were improved in larval haddock subjected to higher light intensities (Downing and Litvak, 1999).

Photoperiod manipulation also affects feeding behavior. It has been documented that most marine fish larvae, including Atlantic cod, are visual feeders (Puvanendran and Brown, 2002). The suitable light levels and wavelengths are therefore important for successful larval growth and Atlantic cod seems to grow better in continuous light compared with natural daylight (Imsland *et al.,* 2005; 2007). The results from an extensive study on salmon imply a preference by salmonids for feeding at low light intensities if pellets are easily available and detectable. Feed discharge rates were, however, found to decrease with increasing light intensity (Petrell and Ang, 2001). Light manipulation of young catfish caused a change in behavior, with increased movements and eating at higher light intensity compared to lower (Almazán-Rueda *et al.,* 2004).

Photoperiod manipulation and swimming activity have also been studied as well as effects on skin color of the fish. Experiment in juvenile catfish showed that the fish spent more time swimming under continuous high light intensities compared to 12D:12L (12 hours daylight and 12 hours darkness) low light intensities (Almazán-Rueda *et al.,* 2004). The skin color of fish depends on light exposure as well as the color of the environment. Increased light intensities often cause the skin color to lighten, but in some species the skin color will darken in response to stress often caused by exposure to light (Rotllant *et al.,* 2003).

The above mentioned as well as numerous other studies show that different fish species react in various ways to light manipulation and consequently appropriate photoperiod conditions can not be transferred from one species to another without a comprehensive validation of the effects on key issues such as growth and development.

1.2.1 Effect of photoperiod on sexual maturation

Sexual maturation in teleosts is controlled by endogenous hormonal rhythms. Spawning takes place at the same time every year and is mainly controlled by the natural photoperiod (Skjæraasen *et al.,* 2004). In nature, Atlantic cod normally mature at the weight of 1.5 - 3 kg (4 - 6 years old). Under farming conditions, cod may, however, mature at 2 years of age (Karlsen *et al.* 2006) and sexual maturation of farmed cod has been reported for fish as small as 500 g in weight, with significant negative impact on the production economics (Skjæraasen *et al.,* 2004). This is because sexual maturation of Atlantic cod is associated with loss of weight as well as appetite during spawning. As a consequence, prolonged production time is needed in order to reach the optimal harvesting size of 3 - 5 kg. Sexual maturation also negatively affects food conversion efficiency and leads to increased mortality. Gonad growth and spawning are energy-demanding processes (Karlsen *et al.,* 2006), and during one spawning season, large and well-fed female Atlantic cod can loose up to 50% of their weight (Kjesbu *et al.,* 1991). Methods applied in order to delay or arrest maturation is therefore urgently needed (Karlsen *et al.,* 2006). Photoperiod affects growth and puberty in many fish species (Skjæraasen *et al.,* 2004) and a control of the spawning process has been shown to be manageable by photoperiod manipulation (Dave *et al.*, 2003). Studies have shown that Atlantic cod maintained in continuous light during an experimental period did not mature, while fish in the control groups were spawning (Hemre *et al.,* 2002). Similar effects have been observed in Eurasian perch (Migaud *et al.,* 2006a). Studies furthermore show that continuous light during the juvenile stage of Atlantic cod has a growth-stimulating effect and may delay sexual maturation (Imsland *et a.,*. 2005; 2007). Photoperiod blocking of maturation has furthermore been found to persist up to the age of three years (Taranger *et al.,* 2006).

1.2.2 The effects of light wavelength

The lights mainly used in fish farming are white metal halide lamps (Karlsen *et al.,* 2005; Taranger *et al.,* 2006; Nordgarden *et al.,* 2006). This type of lights is energy consuming and therefore costly for the farming industry. White light consists of many wavelengths. Blue and green wavelengths pass well though water (Figure 4), while red and yellow light do not and are therefore not well suited for lighting up large water masses. Results have shown that fish are highly sensitive to the wavelength between 500 and 600 nm, i.e. the green to yellow spectrum of light (Northmore and Muntz 1970; Ziv *et al.,* 2006). Atlantic cod larvae have shown improved growth in water containing high amounts of algae compared with clear water. The results shown the green light impact from the algae has a beneficial effect on the larvae, providing the appropriate distraction of light in their environment (Meeren *et al.,* 2007).

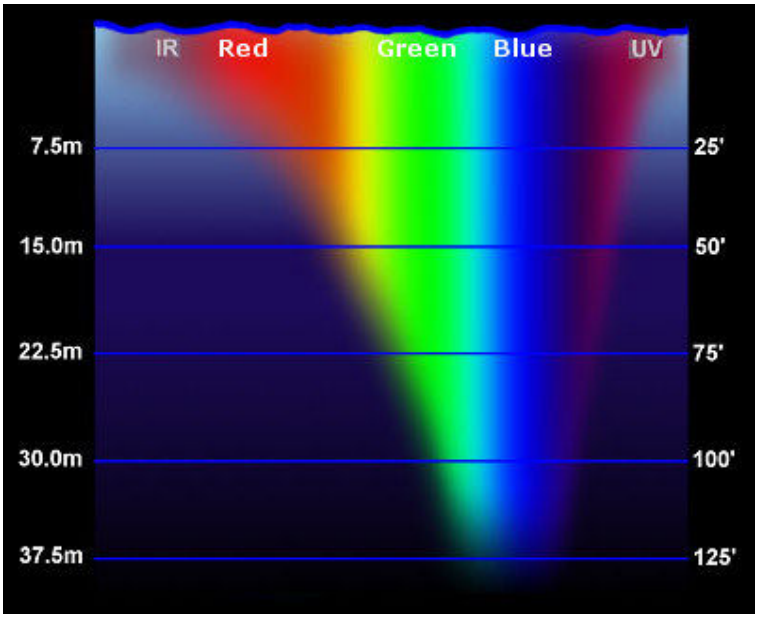


Figure 4. The light spectrum in the sea
Visible light is from 400 - 700 nm and is divided into eight different colors. Blue lights have the shortest visible wavelength 400 - 450 nm, green from 450 - 500nm, yellow is from 550 - 650nm and red is from 650 - 700 nm. Above 700 nm infra red light takes over and ultraviolet under 400 nm

1.2.3 Cold-cathode light

A cold cathode lighting (CCL) system is a new technology of light that works similarly to neon light i.e. a gas discharge lamp, containing primarily neon gas under low pressure. The CCL waves are emitted when an electrical current passes through gas or vapor chamber and the lights can be made in various colors and sizes. In addition to easy transmittance of the waves through seawater, the green light has been found to consist of wavelengths optimal for the Atlantic cod with respect to light perception (Northmore and Muntz 1970; Ziv *et al.,* 2006). The CCLs are up to five times brighter than neon lights and have an extended lifespan of approximately 50.000 hours. Furthermore, the CCLs use approximately 7.5 watts of electricity per 0.3 meters compared with 150 watts used by the metal halogen lights traditionally applied in fish farming. The CCLs are also flicker-free, which is preferable, as unstable light can cause a stress response in the fish (Taylor *et al.,* 2008).

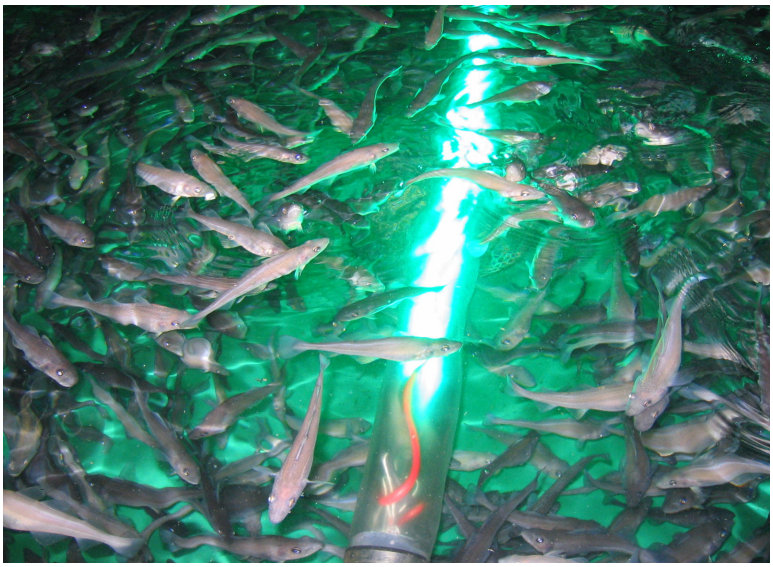


Figure 5. A cold-cathode light unit in cod juvenile tank

1.3 The endocrine growth axis in fish

Several hormones have been found to affect growth of fish with each hormone affecting the physiological responses in a different way and through different pathways.

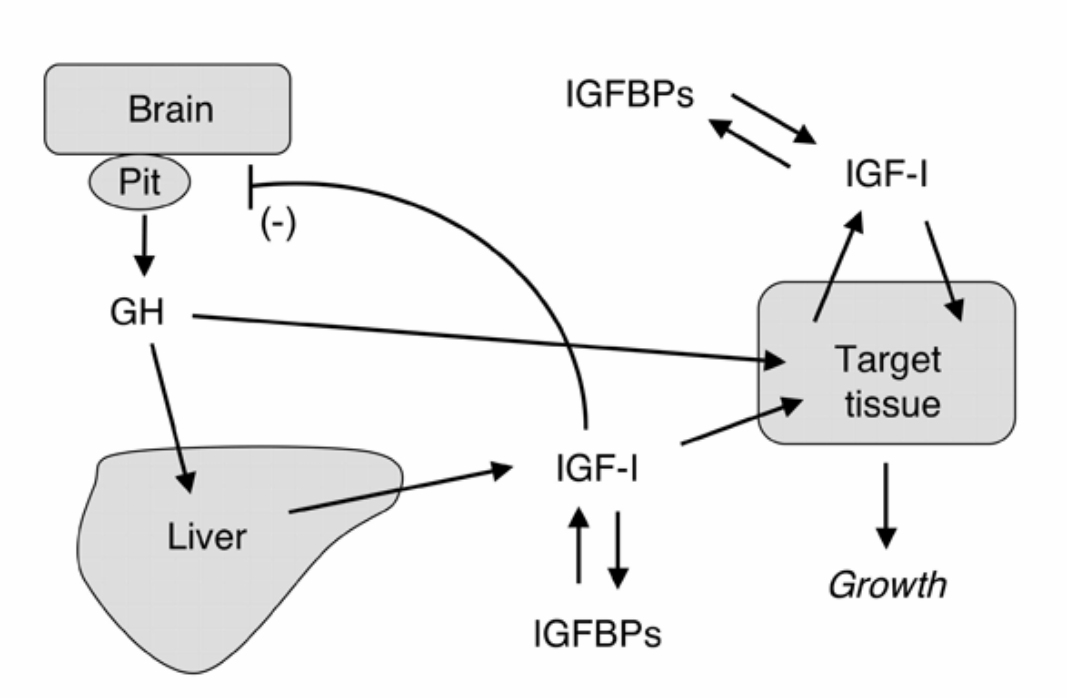


Figure 6. The GH-IGF-I system.
GH produced and secreted from the pituitary stimulates the production of IGF-I in the liver. From the liver, IGF-I is carried in the blood, largely bound to IGF binding proteins (IGFBP's) to its target tissues where it stimulates growth. IGF-I also affects the production and secretion of GH through negative feedback.

1.3.1 Insulin-like growth factor I

IGF-I plays a key role in the complex system that regulates growth (Figure 6), differentiation and reproduction of teleost fish (Björnsson *et al.,* 2003; Castillo *et al.,* 2004; Dyer *et al.,* 2004; Berishcili *et al.,* 2006), and much research has therefore been focused on assay methods and measurements of IGF-I in plasma and tissues. These studies have shown that the structure, function and regulation of IGF-I are similar in fish and mammals (Reinecke *et al.,* 2005).

The secretion of IGF-I is stimulated by plasma levels of insulin and GH. Insulin is secreted from the pancreas and GH is released from the pituitary gland (Pierce *et al.,* 2004; Pierce *et al.,* 2005; Nordgarden *et al.,* 2005; Eppler *et al.,* 2006; Very and Sheridan, 2007). The secretion of IGF-I is also stimulated by low levels of plasma glucocorticoids which potentiate the expression of GH-stimulated IGF-I mRNA as well as thyroid hormones (Pierce *et al.,* 2005).

IGF-I is a 7.5-kDa polypeptide of 70 amino acids, which is an evolutionarily ancient and highly conserved polypeptide, closely related structurally to pro-insulin (Moriyama *et al.,* 1994; Shimizu *et al.,* 1999; Otteson *et al.,* 2002). In plasma, IGF-I can bind to a number of different IGF binding proteins (IGFBPs) which slow the clearance of IGF-I, exert IGF-I independent effects and modify the effects of IGF-I on target tissues (Shimizu *et al.,* 1999; Pierce *et al.,* 2003; Shimizu *et al.,* 2003). In mammals, six IGFBPs have been identified and characterized and at least five different IGFBPs have been found in fish (zebrafish). Each of the identified IGFBP types found in fish has an important role in regulating IGF-I action in vertebrates (Shimizu *et al.,* 2003; Shimizu *et al.,* 2006).

IGF-I is mainly produced and secreted from the liver (Figure 6). GH treatment leads to increased production of hepatic IGF-I mRNA and increased levels of IGF-I in plasma of many fish species (Pierce *et al.,* 2005). IGF-I producing cells also occur in other organs, including the gonads where it may exert paracrine or autocrine effects (Moriyama *et al.,* 1994; Pierce *et al.,* 2000; Berishvili *et al.,* 2006; Eppler *et al.,* 2006), possibly independently of GH-stimulation, When IGF-I is produced locally in the gills of salmonids, it is thought to have osmoregulatory effects, an example of its paracrine actions. Studies have also suggested that in marine fish, IGF-I mediates the

osmoregulatory actions of GH. However, increased salinity tolerance only lasts for a short time period when mediated by IGF-I (Very and Sheridan, 2007).

IGF-I has been proposed as a growth index factor in fish (Dyer *et al.,* 2004). In coho salmon, plasma levels of IGF-I where found to be significantly related to growth rate. Endocrine IGF-I plays an important role in mediating body growth, and stimulates growth in fish (Beckman *et al.,* 2004).

The levels of IGF-I in plasma are affected by a number of environmental/seasonal variables (Nordgarden *et al.,* 2005; Reinecke *et al.,* 2005). Temperature is one of those factors (Gabillard *et al.,* 2003; Beckman *et al.,* 2004; Eppler *et al.,* 2006). At lower environmental temperatures (7°C), the plasma levels of IGF-I will decrease but increase with higher water temperatures (11°C). This may be an indication of slower growth at reduced temperatures (Beckman *et al.,* 2004; Kling *et al.,* 2006). Feeding activity is also linked to IGF-I levels in fish plasma. Restricted food access lead to decreased plasma levels of IGF-like, IGF-I and IGF-II as well as decreased IGF-I mRNA levels in the liver (Gabillard *et al.,* 2003; Pierce *et al.,* 2004; Small and Peterson, 2005). Fish that were fed at higher rates grew faster and had higher plasma levels of IGF-I (Beckman *et al.,* 2003; Gabillard *et al.,* 2003). The plasma IGF-I levels have been found to be significantly increased with increasing ration size in barramundi (Dyer *et al.,* 2004) and IGF-I levels were positively correlated to growth rates in barramundi and Atlantic salmon when fed different diet formulations. It has been documented that in salmon, IGF-I plasma levels peak in October when the fish prepare for spawning (Nordgarden *et al.,* 2005), and similar hormone profile could be expected in Atlantic cod (Dyer *et al.,* 2004).

It has therefore been suggested that measuring IGF-I levels in plasma may be a useful tool for monitoring growth rate in fish and as a method to rapidly evaluate the effects of various diets used in aquaculture (Dyer *et al.,* 2004).

1.3.2 Growth hormone and melatonin

Numerous studies have been carried out over the last two decades in order to elucidate the multiple functions of growth hormone (GH) in fish (Björnsson *et al.,* 1997; Björnsson *et al.,* 2002, Reinecke *et al.,* 2005). GH is a versatile hormone that is produced in the pituitary gland. The hormone has been found to play an important role in all main body functions in fish, such as regulation of salinity, metabolism of fat, protein and carbohydrates as well as the growth of bone and soft tissue, reproduction, immunity and behavioral factors (Fleming *et al.,* 2001; Ágústsson *et al.,* 2002; Björnsson *et al.,* 2003; Reinecke *et al.,* 2005; Canosa *et al.,* 2006; Nordgarden *et al.,* 2006). Along with IGF-I, GH appears to be a key factor in embryonic development, nutritional condition and growth regulation of teleosts. Consequently, GH production, stimulation and regulation are highly important with respect to aquaculture of various fish species (Pedroso *et al.,* 2006).

Melatonin is a hormone associated with the daily dark-light cycle. This hormone is synthesized by the retinal cells and the pineal organ that has changed considerably during the evolution of vertebrates. Early in vertebrate evolution, this was a peripheral photo sensory organ, but has subsequently changed into a profound intra-cranial endocrine gland with the substitution of indirect for direct light sensitivity (Boeuf and Le Bail, 1998). In teleosts, only the pineal gland promotes the release of melatonin to blood. In salmonids, the pineal endocrine melatonin message mostly depend on the irradiance of the incident light, but in other teleosts the pineal photoreceptors commonly comprehend an endogenous circadian oscillator, or clock, which is synchronized to 24 hour daily cycles and regulates melatonin secretion (Boeuf and Le Bail, 1998).

It has proven difficult to link the growth of fish to the plasma levels of melatonin (Boeuf and Le Bail, 1998). However, as melatonin secretion and thus plasma levels reflect the day/night cycle, this hormone remains a chief candidate for relaying photoperiod information to other endocrine systems, such as the GH-IGF-I system, which is clearly affected by photoperiod through the classically described “light-pituitary axis” (Komourdjian *et al.,* 1976).

1.4 Measuring hormone levels in fish

The invention of an immunological method, the radioimmunoassay (RIA), for measuring circulating levels of hormones, was a major break-through in endocrinological research (Yalow and Berson 1960), and its inventor, Prof Rosalyn Yalow was awarded the Nobel Prize in Physiology or Medicine in 1977 "for the development of radioimmunoassays of peptide hormones". The RIA methodology makes use of radioisotope labels and β- or γ-counters as analytical instruments. Subsequently, a number of methods have been developed, based on the same immunological principles, but using non-radioactive labeling and detection. The main such method is the enzyme-linked immunosorbent assay (ELISA), but others such as dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) and enzyme immunoassay (EIA) exist.

Unknown samples to be measured in RIAs may be biological fluids such as urine, saliva, serum, cerebrospinal fluid or, as used in this study, plasma. RIAs can also be used to measure hormone content in tissues by establishing extraction protocols which allow the hormone to come into solution (Wegner, 2000). In the RIA method, a fixed concentration of antigen marked with a tracer is incubated with antiserum. The antigen binding sites of the antibody are limited and only a certain percent of the total tracer molecules can be bound to the antibodies. Unlabeled antigens are added to the mixture and the labeled tracer will then compete with the unlabeled antigen for the binding sites (Figure 7). With increased concentration of the unlabeled antigen, the amount of tracer bound to the antibodies will decrease. The bound antibodies are then separated from the free tracer and the free fractions and the bound fractions can be counted. To determine the amount of the antigen, a standard curve is obtained by analyzing the specific binding of a dilution series of standards of known concentration. The results are plotted on a graph with the specific binding (%) on the Y-axis and the concentration on the X-axis. Such graphs are often logit-log transformed to obtain a straight line over the measurable range. The amount of antigen in the unknown sample can then be calculated from this curve, based on its measured specific binding (Wegner, 2000).

In the IGF-I RIA method, the γ-radiating isotope iodine-125 (I^{125}) is bound to the IGF-I molecule antigen. Sodium iodide (radioactive labeled antigen) is added to the samples,

emitting a flash of yellow light in the gamma counter when it absorbs gamma radiation. The amount of emitted light can be measured using a photomultiplier which records "counts per minute" (CPM). When a RIA is set up to measure hormone levels, exact volumes of the antigen (known or unknown hormone levels), the radiolabel (radiolabeled antigen), and a hormone-specific antibody are added to all tubes, together with an appropriate buffer. Apart from tubes containing known concentrations (the standard curve) and tubes containing unknown amounts (samples to be measured), a number of extra tubes are included, which do not contain all the ingredients. This includes tubes with radiolabel only (T) to determine exactly how much labeled antigen has been added to each RIA tube. Tubes missing the antigen are included to estimate maximum binding (B_0) and tubes missing the antibody are included to estimate non-specific binding activity (NSB). Specific binding of receptor and I^{125} labeled hormone is calculated by subtracting the amount of bound radioactivity hormone (non-competed) to the unlabeled hormone (competed) (Brooks *et al.,* 1982).

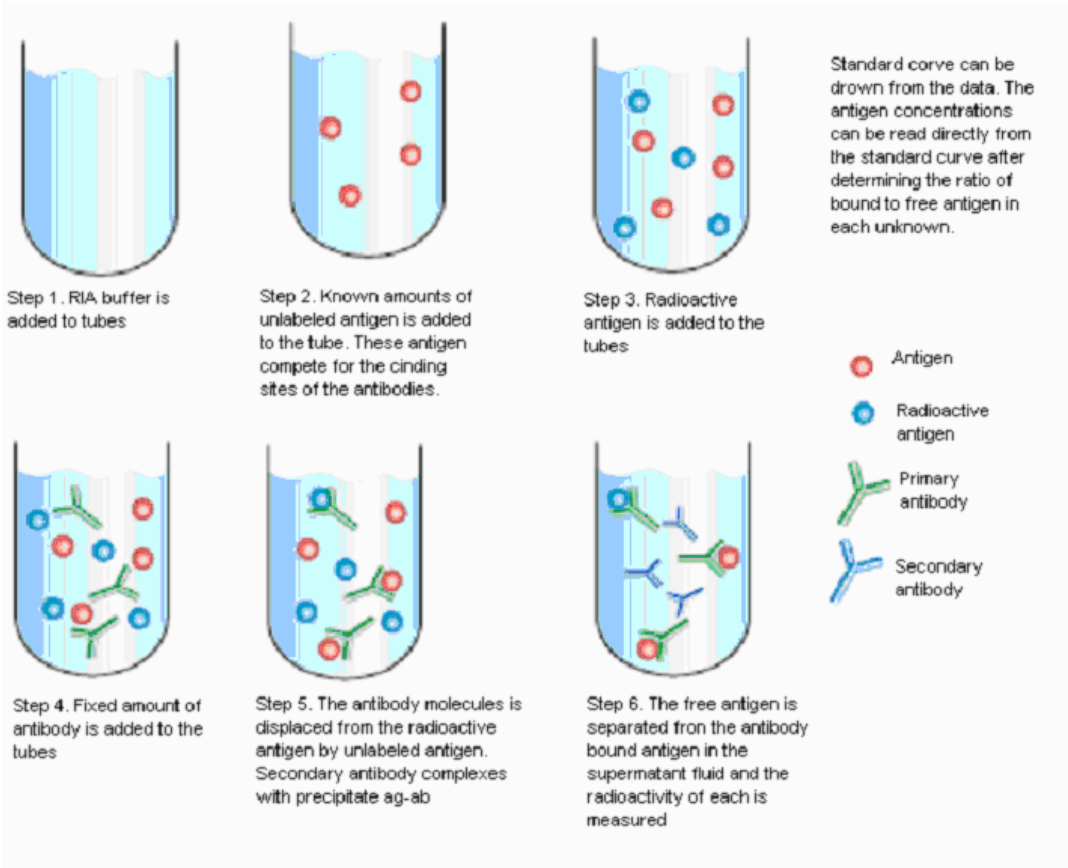


Figure 7. RIA diagram

For measuring IGF-I levels in fish plasma, a RIA method has been developed and optimized by Professor Björn Þrándur Björnsson at the Fish Endocrinology Laboratory at Gothenburg University in Sweden. Björnsson and his research team have measured IGF-I in salmon, trout, halibut and turbot (McCormick *et al,* 1998; Björnsson *et al*., 2002; Nordgarden *et al.,* 2005; Nordgarden *et al.,* 2006; Hildahl *et al.,* 2007, Imsland *et al*., 2007), but not previously in the Atlantic cod

The ELISA method is a highly sensitive and suitable method for measuring antigen or antibody levels in plasma and was the first screening test commonly for testing human immunodeficiency virus (HIV) positive patients as well as various nutritional factors (Maskarinec *et al.,* 2004). The ELISA method has also been commonly used for detection of food allergens. Only a few micro liters of plasma are needed for measuring antigen or antibody levels. The ELISA method has the advantage of being safer and less costly compared to the RIA method (Kindt *et al.,* 2007). However, measuring some antigen, especially peptide hormones, the ELISA method notes to be less sensitive (Pridal *et al.,* 1995)

The ELISA methodology has been widely used for the analysis of steroid and thyroid hormones, but only to less extent for analysis of peptide hormones. In this study, a sandwich ELISA technique was tested for measuring the concentration of IGF-I in Atlantic cod plasma, using standard ELISA approach and the same antibodies as were used in the RIA method. The ELISA method has previously been used to measure GH in rainbow trout (*Oncorhynchus mykiss*) (Mercure *et al.,* 2001) and rabbitfish (*Siganus guttatus*) (Funkenstein *et al.,* 2005) but not in Atlantic cod.

1.5 Statistics

When analysing data as collected in the present study, regression is an important tool for achieving correct results. In the current study, regression time-series analysis, according to Greene (1997) was used

Regression represents and estimates the connection between a dependent variable, often symbolized as Y, and one or more independent variables, symbolized as X_1, X_2, X_3 etc. (Greene, 1997). The purpose of using regression is threefold. Firstly, regression is used for analysing whether the X variable significantly affects the Y variable. As an example, the position of the cages in the present experiment (control versus light treated group) may affect the weight of the fish recorded in the experiment. Secondly, regression may be used to analyse if the presence and size changes in the independent variable may impact the dependent variable (Greene, 1997). As an example, the weight of the fish might be affected by altering the photoperiod. Thirdly, regression is used to predict changes in the Y value in response to X value changes. As an example, the weight of the fish may be predicted in relation to various treatments of the fish.

If a linear relationship exists between the variables, such as i.e. the length and the weight of the experimental fish, a linear regression is used to estimate if the variables affect each other. The formula for the regression line will then be:

$$\boldsymbol{Y = \alpha + \beta X}$$

Where Y is the independent variable, β is the coefficient of the line, X is the dependent variable and α is the intersection of the lines on the Y-axis. In this formula, the correlation of X and Y is linear (Björnsdóttir, 2004).

If the connection between the variables is defective, an imponderable disturbance term will be added to the formula which will be:

$$\boldsymbol{Y = \alpha + \beta X + \varepsilon}$$

Using this formula, values can only be predicted using determinintic models. The values therefore can't be calculated precisely as if there was a linear relationship between the variables. Many Y values can be related to each X value, and the best value has to be estimated (Björnsdóttir, 2004).

Time-series analysis is a method used to analyse any relation in data that have been arranged in a series over a period of time (Greene 1997). To make it easier to analyse the data and construct the model, it is often assumed that the measurements have been carried out on a time scale which is usually not the case. The procedure represents a collection of statistical methods applied for analyses of the nature of stochastic processes. Time-series analysis are used in many fields of science, for instance for measuring changes in the climate, fish stocks, demographics and for measuring various environmental effects that can be related to a time scale.

Panel data, a combination of time series and cross section data, was used for statistical analysis of the results in the present study. The model can be used to measure a number of independent variables and their connection to the independent variable (Greene 1997).

1.6 Objective

The aim of this project was to study the effects of supplementary 24h CCL treatment compared to standard metal halogen light systems commonly used in hatcheries on the growth of Atlantic cod at an early life stage. The aim was further to investigate if the levels of IGF-I in plasma may be a good indicator of growth rate of cod juveniles. An additional objective was to examine if IGF-I levels in Atlantic cod plasma could be measured by the RIA method and the ELISA method.

2 Materials and methods

The experimental fish were kept in indoor tanks at Staður, an experimental hatchery station run by the Icelandic Marine Research Institute in Grindavík on the south coast of Iceland. The hatchery was built in 1988 and is 1350 m^2. Access to warm seawater pumped from an underground well makes it possible to adjust the seawater temperatures accordingly. The main experimental fish species kept at the research station are Atlantic halibut (*Hippoglossus hippoglossus*), Atlantic cod (*Gadus morhua*), turbot (*Scopthalmus maximus*) and red abalone (*Haliotis rufescens*).

Figure 8. Experimental facilities at Staður, Grindavík.

2.1 Experimental conditions

The broodstock spawned on May 1st 2006. The parent fish were wild Atlantic cod, captured south of Iceland. The onset of feeding was May 4th 2006. The first 30 days the larvae were fed enriched live feed (rotifers) using Protein Selco Plus (Inve, Belgium) and *Artemia* gradually introduced between 15 - 45 days in feeding. Dry feed pellets were introduced from day 15. The fish were hand fed three times per day in addition to 12 hours feed rationing through automatic feeders.

Figure 9. Smaller tanks (3m^3)

The experiment was started on October 8th 2006 when the fish weighed 25 ± 15 g. During the first weeks, approximately 1200 fish were kept in each of the 6 tanks of 3 m^3 (Figure 9). After 14 weeks (February 14th 2007) the fish were transferred to larger tanks of 30 m^3 (Figure 10). The CCL treated group was kept in one tank and the control fish in another.

The density in the tanks in the beginning of the experiment was 14 kg / $m^3$and the average water temperature was 8.0°C (± 0.5 °C), with 20 L min^{-1} run through of water without any recycling. Pure seawater of 30 ‰ salinity was pumped from a 40 meters deep well.

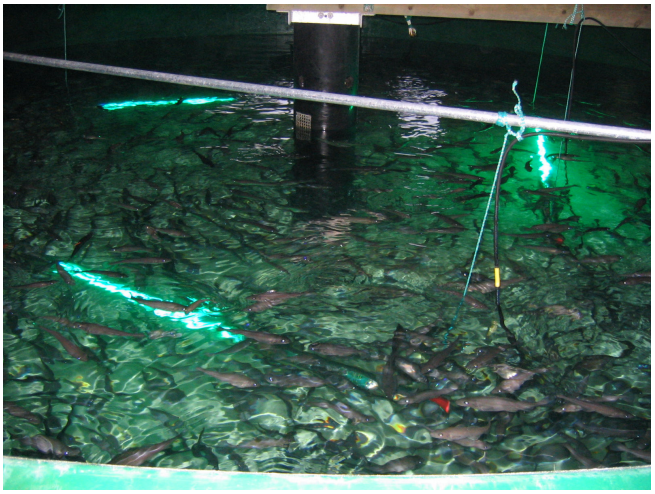
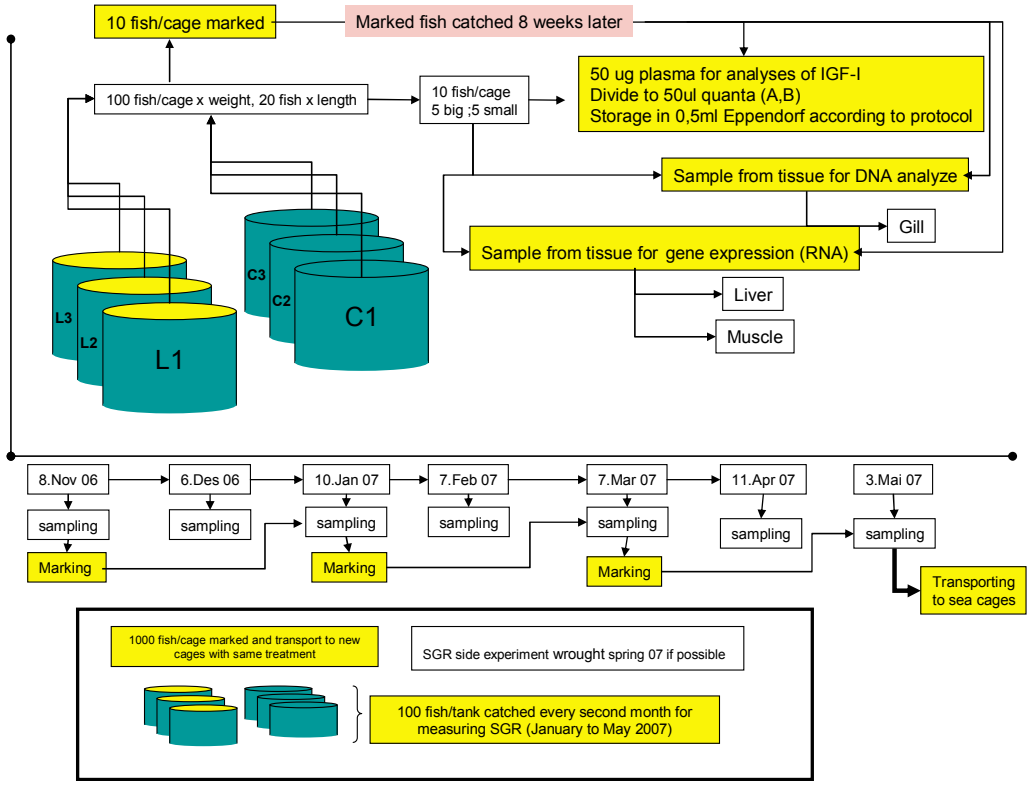


Figure 10. Larger tank (30 m^3)

Standard metal halogen light units were placed above the surface of the control group. The light was keep on for 24 hours at day photoperiod (24L:0D). The light intensity was 700 lux at the surface, 200 lux at 20 cm depths and 150 lux at 40 cm depth. In smaller tanks, one sub-surface CCL unit was placed in each treatment tank (six tanks), with the light intensity reaching 4000 lux at 5 cm distance from the light, 1000 lux at 20 cm distance and 500 lux at 40 cm distance from the light unit. In larger tanks, three CCL units were placed in the treatment tank.

Samples of fish were collected at approximately monthly intervals during November 2006 through May 2007 (Figure 11).



(Ágústsson, 2006).

Figure 11. Experimental design.
At monthly intervals, 100 fish were measured for weight and length and blood collected for analysis of IGF-I. During January to May 2007, individual fish were sampled for analysis of specific growth rate (SGR) and samples of liver, gills and muscle collected for genetic analysis and parental assignment in a parallel project.

2.2 Sampling and sample analysis

Needles and syringes were prepared the day before sampling. One drop of heparin (5000 IE/ml; Kabi Pharmacia) was drawn into each syringe, the syringe shaken and the drop then expelled. This is an important step to prevent coagulation for the blood to be collected.

Figure 12. Small storage tanks (1m^3)

The Eppendorf tubes were labeled using small printed labels, listing the sample number (1 to 354), sampling number (1 to 6), project ID and A or B replicate. The labels were taped onto the tubes using narrow semi-transparent tape (Scotch Magic Tape 810).

Figure 13. Sleepy fishes

Prior to each sampling, the experimental fish were netted and transferred to smaller tank units (Figure 12), ten fish at each sampling. Five large and five small fish were then chosen from each tank, for genetic analysis and parental assignment in a parallel project.

The netted fish were then transferred to a small bin (Figure 13) containing lethal

dose of anesthetic medium (1 g/L Benzocain in seawater). When fish were successfully anaesthetized seized, blood was drawn and the weight and length of individual fish measured and recorded.

During sampling of blood and tissues, the experimental fish were placed on their back on a towel and held tightly with one hand. The needle was inserted vertically into the mid section of the caudal peduncle. Approximately 1ml of blood was drawn from the caudal vessel in to a heparinized syringe (Figure 15), the needle then removed from the syringe and the blood sample transferred to a 1.5 ml Eppendorf tube. Tubes containing blood were centrifuged at 3000 rounds per minute (rpm) for 5 minutes. Plasma was carefully pipetted off and two aliquots transferred to 0.5 ml Eppendorf tubes, snap frozen on dry ice at -80°C. During each sampling, blood was collected from 30 fish from each tank, a total of 180 fish at each sampling. The tubes were stored at -80°C until subsequent analysis.

Figure 14. The sampling table

Figure 15. Blood sampling

A small part was furthermore cut from the gills of each fish and placed in a tube containing 98% ethanol. Muscle and liver samples were also collected from all fish (Figure 16), placed in 0.5 ml Eppendorf tubes and snap frozen on dry ice

before storage at -80°C for subsequent analysis of genetic markers as a part of another project.

Individual fish were tagged for analysis of specific growth rate (SGR). The weight and length of a total of 100 fish from each tank was measured and the fish then individually marked (Figure 17). Two months later these fish were recaptured and their length and weight measured for the SGR analysis.

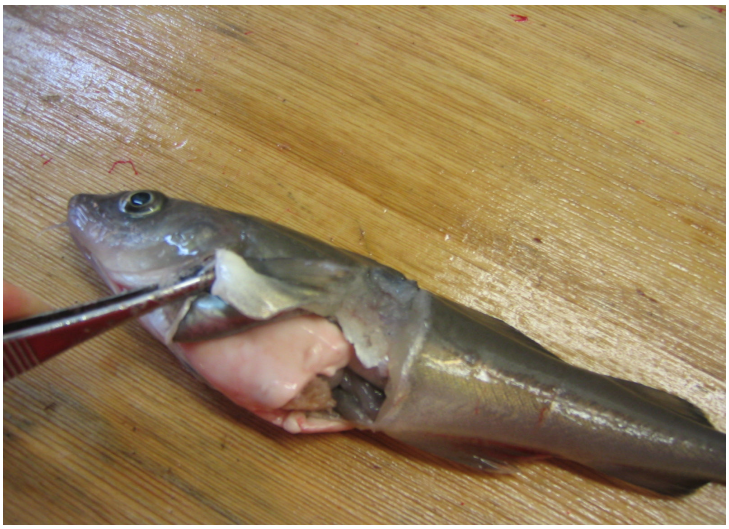


Figure 16. Liver and muscle sampling

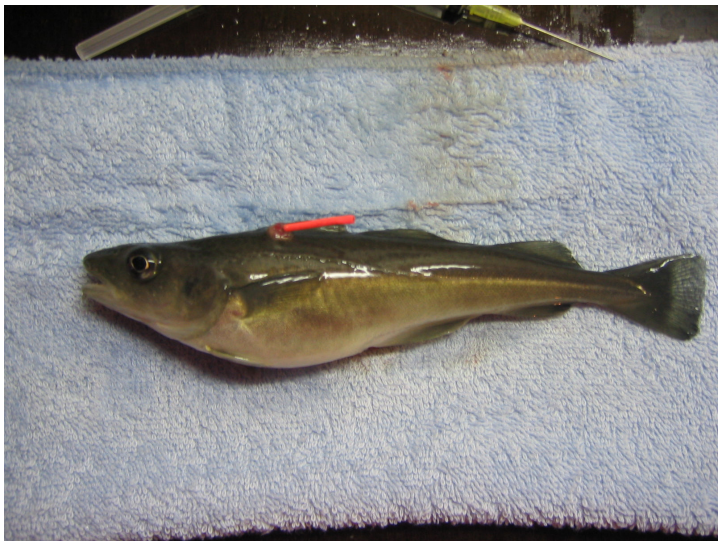


Figure 17. Individually tagged fish.
The tags were attached to the dorsal fin.

2.2.1 Measuring IGF-I by the RIA method

The plasma levels of IGF-I in all samples were measured during the fall of 2007 using RIA method. The possible effects of IGFBPs in plasma of Atlantic cod were unknown and extracted as well as untreated plasma therefore analysed for comparison of results.

Figure 18. From the RIA lab at the Zoology institute in Gothenburg.

Plasma extraction

Figure 19. An Eppendorf centrifuge, (Allegra X-12R).

Plasma samples were extracted according to the method of Breier *et al.* (1991). A solution of acid-ethanol was prepared (Shimizu *et al.*, 2000) containing 13.125 ml of 87.5% ethanol mixed with 2.5 ml of 2 M HCl (12.5%), sufficient for the extraction of a total of 60 samples to be run in one assay. 50 µl of plasma samples were pipetted into 1.5 ml Eppendorf tubes and thoroughly mixed with 200 µl of the acid-ethanol solution before incubation at room temperature (RT) for 30 minutes. It was noted, an Eppendorf tubes have to be used in stead of polystyrene tubes,

as recommended in the original protocol as the polystyrene tubes will break in the centrifuge. 100 µl of Tris 0.855M (kept refrigerated) were added to each tube and vortexes thoroughly for a few seconds. The tubes were centrifuged (Figure 19) at 3.200 rpm, for 30 minutes at 5°C and the supernatant then transferred to polystyrene assay tubes in duplicates, a 100 µl/tube.

Iodination

Sephadex (G-75) was incubated in an elution buffer (0.1M TRIS HCL mixed with 0.1% triton X-100, pH 8.5) over night at 4°C. The mixture was acclimated to room temperature and allowed to evaporate for 30 minutes and then poured gently into an Econo-column (Kemila 737-1030) filled with the sephadex buffer. The column was allowed to acclimate over night. 3 ml of elutionbuffer containing 1% triton x-100 and 10 ml elution buffer were then applied and the run-through of the column adjusted (should be approximately 400 µl per. 30 seconds). The sampling tubes were prepared with 50 µl of 2% bovine serum albumin mixed with the elution buffer. 4.6 µg of recombinant IGF-I (rIGF-I) were mixed with 40 µl of the elution buffer, giving the total volume of 50 ml. 2 µl of I-125 and 20 µl of Chloramine T (30 mg / 10 ml elution buffer) was added to the solution that was mixed thoroughly. After incubation for 55 seconds the process was stopped by adding 25 µl of SMB (Sodiummetabisulfide; 30 mg / 5 ml elution buffer). The solution was then applied to the column. The first 5 ml were sampled into the first tube and then sampled for 30 seconds per tube. After approximately 7 ml, free Iodine started to elute. The four fractions emitting the highest amounts of gamma radaiation were analyzed for total binding (B0) and non specific binding (NSB). Fractions were then pooled, each pool aliquoted and then frozen at -20°C until used.

Radioimmunoassay for measuring IGF-I

The concentration of IGF-I in extracted samples was measured using radioimmunoassay (RIA) and according to the method of Moriyama *et al.* (1994). A total of 314 plasma samples were measured in 6 separate runs. Plasma was stored at -80°C and used for repeated analyses of selected samples that proved to be less successful compared to the analyses of unfrozen samples. The conclusion was therefore that the plasma cannot be analysed following repeated thawing from -80°C.

Figure 20. A Wizard 1470 Automatic, Wallac Gamma counter

Samples were analysed in a Gamma counter (Wizard 1470 Automatic, Wallac) (Figure 21) which measures gamma radiation emitted by a radionuclide, analysing duplicate samples every second for one minute and calculating average values for these.

Barramundi recombinant IGF-I (receptor grade) produced in *Escherichia coli* (E. *coli)* at 95% purity (GroPep, Australia) were used as a standard. A polyclonal rabbit antiserum against barramundi IGF-I (GroPep, Australia) was used as primary (1^{st}) antibody, diluted according to the manufacturer's instructions. The antiserum displays < 0.5% cross reactivity with recombinant human IGF-I and < 1% cross reactivity with recombinant human IGF-II (GroPep, 1998). Pansorbin cells (Calbiochem cat#507858, Lot#B60306-1, Calbiochem) were used as secondary (2^{nd}) antibody.

A total of 151 polystyrene tubes were labeled for each assay and placed in a rack with 3 tubes labeled T (total counts), 3 tubes labeled NSB (non-specific binding), 3 tubes labeled B_0 (binding without plasma), 2 tubes labeled PB (plasma blank), 20 tubs labeled S (standard duplicate from 1 to 10) and 120 tubes labeled with sample numbers.

The RIA-buffer was prepared from Phosphate Buffered Saline (PBS; 20 mM phosphate, 0.15M NaCl, 0.05% NaN_3, pH 7.5) with the addition of 0.69 g NaH_2PO_4,H_2O, 2.13 g Na_2HPO_4, 8.76 g NaCl and 0.50 g NaN_3 diluted in 900 ml dH_2O. The pH was adjusted

to 7.5 with 1 M NaOH and the volume finally made up to 1 liter. Bovine Serum Albumin (BSA; 0.5% w/v) and triton X-100 (0.05% v/v) were finally added to each liter of the PBS.

The standard (100 ng ml^{-1}), was prepared by transferring 925 µl of the RIA-buffer to 50 µl of the RIA-standard and labeled (# 10). Nine Eppendorf tubes were labeled from 1 to 9 and 500 µl of RIA-buffer pipetted to each tube. Dilution series were prepared by transferring 500 µl from the first tube, vortexing and then transferring 500 µl to the next tube. This was repeated for all 9 Eppendorf tubes. Each tube then contained 500 µl of various dilutions of the standard solution (except tube number 1 that contained 1 ml). From each tube, 100 µl aliquots were pipetted to new tubes for the assay analyses.

The non-specific binding tubes (NSB) contained labeled antigen, assay buffer and 2nd antibody, but never 1st antibody. These tubes will have the lowest counts per minute (CPM) because they contain no 1st antibody to bind to antigens of the samples. These tubes are used to determine if there are any errors or background counts (Wegner, 2000). 200 µl aliquots of RIA-buffer were pipetted to 3 NSB tubes for each assay.

Maximum binding tubes (B_0) contained labeled antigen, 1st antibody, assay buffer and 2nd antibody. These tubes therefore did not contain any antigen and are used to determine how much of the antigen can possibly bind. These solutions will give a little lower CPM counts compared to the total count tubes (Wegner, 2000). 100 µl aliquots of RIA-buffer were pipetted to the B0 and PB tubes

The standard contained concentrations of IGF-I comparable to the sample IGF-I concentrations. The standard is used to make a standard curve for the assay and will have decreasing CPM counts with decreasing concentrations of the standard (Wegner, 2000).

The control consists of pooled plasma samples and 100 µl pipetted to 2 tubes every time the assay was run. The same control samples were measured in every assay for comparison of assays (Wegner, 2000).

Unknown samples are the samples to be analysed. 100 µl aliquots of Atlantic cod plasma were transferred to each sample tube in duplicates. 100 µl of 1st antibody, diluted 1:1800 in RIA-buffer were transferred to each B_0, standard and sample tubes. The rack was vortexed gently and then stored at 4°C overnight. 100 µl of a solution

containing labeled hormone were added to each tube which were then vortexed gently and stored at 4°C overnight. 100 µl aliquots of a 0.25% solution of Pansorbin cells were added to each tube except the T- tubes, the tubes vortexed gently and then stored at 4°C overnight.

A 200 µl aliquots of the RIA-buffer were added to each tube except the T- tubes. The tubes were vortexed and then centrifuged at 3.200 rpm for 60 minutes at 5°C (Allegra X-12 R, Beckman Coulter). The supernatant was removed from all tubes, except the T-tubes.

All tubes were placed in a gamma counter and the radioactivity of the pellet measured. The results were uploaded to the ASSAY32 format and the IGF-I concentration of the samples calculated.

2.2.2 ELISA method for measuring IGF-I

The levels of IGF-I in Atlantic cod plasma were also analysed by the ELISA method. ELISA is a biochemical technique that is not only used in studies within the field of endocrinology, but also in other fields where exact biochemical analyses are needed. As in the RIA, the prerequisite is that specific antibodies can be raised against the substances (antigens) to be measured. The antibodies are then coupled to an enzyme and when a substrate solution is added, the enzyme substrate reaction produces a signal that is detectable. The antibodies can also be tagged with a fluorescent dye and the immune complexes containing these fluorescently labeled antibodies then detected by colored light emission when excited by light of the appropriate wavelength (Hennies *et al.,* 2002; Yun *et al.,* 2002).

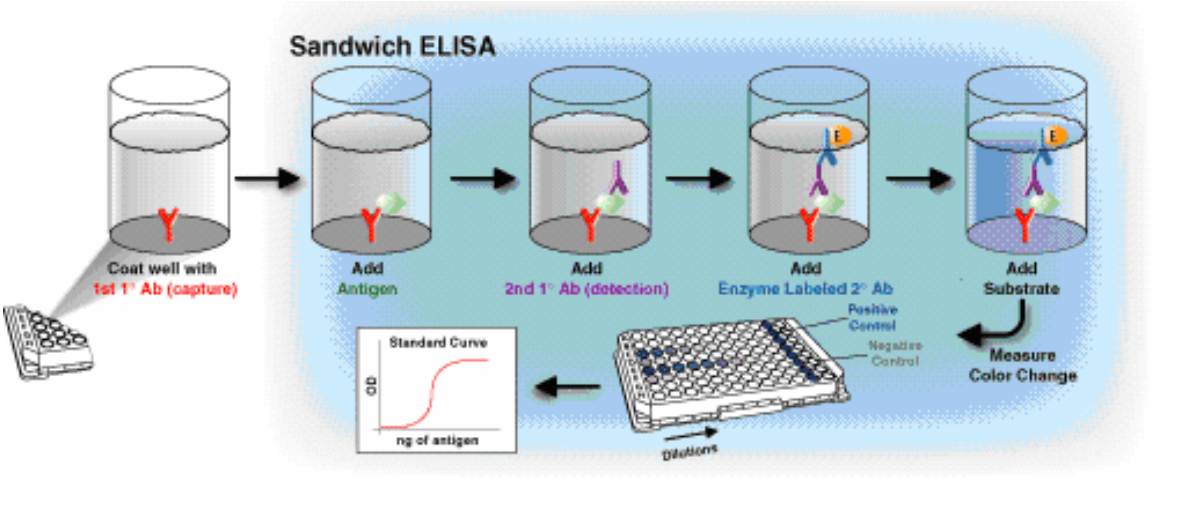


Figure 21. A sandwich ELISA diagram and ELISA standard curve

The method was adjusted according to the RIA method using technical information of the Long R^3IGF-I ELISA (Novozymes GroPep Limited in Australia) as well as growth factor products and protocols 1998-99 (GroPep) and ELISA protocols used at the laboratory at the University of Akureyri in Iceland. The reagents used have been developed as a support product for companies using LONG R^3IGF-I in serum-free media for cell culture (GroPep, 1998).

LONG R^3IGF-I has a low affinity for IGFBPs but the binding to the IGFBPs in cod plasma is not known and consequently extracted as well as untreated plasma samples were analysed.

The monoclonal antibodies against IGF-I were prepared in mice immunized with rat IGF-I (GroPep, Australia). The antibody solution was diluted in coating buffer according to the manufacturer's instructions (1:5000) and used for coating the wells of the ELISA plates. A polyclonal antiserum against Barramundi IGF-I made in rabbit (GroPep Australia) was used as capture antibodies in a sandwich ELISA. The antiserum is designed to recognize IGF-I in frozen and paraffin embedded human, mouse, rat and barramundi (fish) tissue. The antibody-enzyme conjugate (Alcalic Phosphatase conjugated goat anti-rabbig Ig; Sigma) was diluted 1:2000 in assay diluents.

The standard used in these experiments was Barramundi IGF-I produced in recombinant *E. coli* to 95% purity (GroPep Australia).

Assay procedure

Coating Antibody: The vial of lyophilized coating antibody was reconstituted in 200 µl of sterile water and placed at RT for approximately 10 minutes. 100 µl of the solution were transferred to each well of a 96 well flat-bottomed ELISA plate (NUNC) (Figure 22). Wet paper towels were put on the bottom of a plastic box and the plates were covered with a lid before placing on top of the towels. The box was then closed carefully and the plates incubated over night at RT.

The blocking solution was prepared by dissolving 2% (w/v) Bovine serum albumin (BSA; Sigma) in PBS. The assay diluents were prepared by dissolving in PBS containing 0.5% (w/v) BSA.

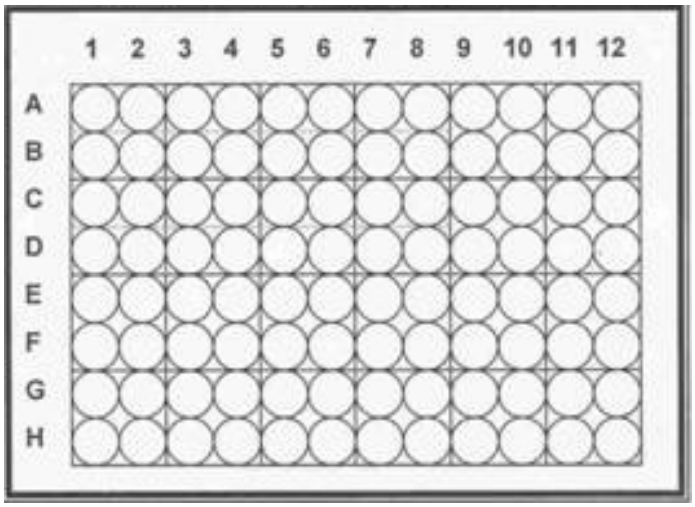


Figure 22. A schematic drawing of an ELISA plate, 96 wells

Efficient removal of all liquid from the wells at each step is essential for good performance. The wells were washed 3 times with phosphate buffered saline containing 0.05% v/v Tween 20 and the plates then blotted thoroughly against paper toweling between each step in the assay.

Solution A (1 µg μl^{-1}) was prepared by reconstituting 1 vial of Barramundi IGF-I (receptor grade) standard with 100 µl of a 10 mM solution of HCl. 50 µl of the solution were transferred to 4.95 ml assay diluents to make solution B (10 ng ml^{-1}) and mixed thoroughly. 50 µl of solution B were pipetted to 4.95 ml assay diluents to make solution C (1 mg ml^{-1}) and mixed thoroughly. Assay standards were prepared as shown in Table 2 and used immediately.

Table 2 Dilution of assay standard

Concentration	Volume of stock solution	Volume of Assay Diluent
100 ng/ml	2000µl Solution C	0 µl
50 ng/ml	1000µl (100 ng/ml standard)	1000 µl
25 ng/ml	1000µl (50 ng/ml standard)	1000 µl
12.5 ng/ml	1000µl (25 ng/ml standard)	1000 µl
6.25 ng/ml	1000 µl (12.5 ng/ml standard)	1000 µl
3.125 ng/ml	1000 µl (6.25 ng/ml standard)	1000 µl
1.56 ng/ml	1000 µl (3.125 ng/ml standard)	1000 µl
0.78 ng/ml	1000 µl (1.56 ng/ml standard)	1000 µl

100 µl of blocking buffer were transferred to each well of the plate, the plate then covered and incubated for 90 minutes at RT. After washing, 100 µl of standards and samples in duplicates were pipetted into each well, the plates then covered and incubated at RT for 90 minutes.

The vial containing capture antibody was reconstructed in assay diluents by adding 500 µl of assay buffer to the glass and gently mixing at RT for approximately 10 minuets to allow to completely dissolving. The solution was then diluted to 1:10000 using assay diluents and 100 µl pipetted into each well. The plates were then covered and incubated at RT for 2 hours.

The antibody-enzyme conjugate was diluted 1:2000 in assay diluents and 100 µl of the solution pipetted into each well of the assay plate, the plate then covered and incubated for 90 minutes at RT.

Figure 23. The microplate reader.

For preparation of the substrate, 10 mg of p-nitrophenyl phosphate were added to each 10 ml of substrate buffer within 30 minutes before use and carefully protected from light. 100 µl of the substrate solution were transferred to each well, the plate covered and incubated under aluminum foil in a humidified chamber in the dark for 30 minutes at RT. The absorbance was measured at 405 nm in a microplate reader (Multiscan EX and software, Thermo Electron Corporation, Finland) (Figure 23).

2.3 Statistical calculations

The formula used for time-series analysis was:

$$\ln W_{it} = \alpha + \beta \ln L_{it} + \varepsilon_{it} \quad \text{(Greene, 1997)}$$

The formula works for imperfect linear relationships, where the natural logarithm (ln) W_{it} is the fish weight in grams, α is a factor obtained by regression (b0), β is the time that is obtained by regression (b1) and ln L_{it} is the length of the fish in centimeters while ε_{it} is a random variable that gratifies the Gauss-Markow condition, witch is a complex mobility model. The variable randomizes the direction and speed of each node based on the standard deviation and current values (Kullberg, 2005).

To estimate the variability between both cages within one treatment as well as between treatments, dummy variables are put in to the formula and the estimated values then calculated.

The data was analysed by regression using the mean values of fish in individual tanks. The numerical values were formularized to logarithm because live organism does not grow just for length or weight, but both. The formula $W = A * L^n$ is therefore uncrowned for organism growth, where W is the weight of the fish, A is a constant and L is the length of the fish. The exponent can be expected to be close three (cubic measure), representing the growth in more than one way. With the natural logarithm of the weight of the fish, this relationship is linear:

$$\ln W = \ln A + n * \ln L$$

Three different regressions analyses were carried out:

ln length against ln weight of the experimental fish and by using either two dummy variables, (0 is the control fish and 1 is the light treated fish) or three dummy variables, where each tank is numbered from 110 to 000 (using only 1 and 0 digits).

Table 3 Dummy variables
Each tank was labeled by a certain dummy variable in order to look for variations between the tanks.

Dummy variable			
Tank	**Treatment**	**1 dummy**	**3 dummy**
E-4	Control	0	000
E-5	Control	0	100
E-6	Control	0	010
E-7	Light	1	001
E-8	Light	1	110
E-9	Light	1	011
U-3	Control	0	111
U-1	Light	1	101

The coefficient of variance (CV) for regression was calculated by the formula:

$$CV_R = \text{standard error} / \text{coefficient}$$

The coefficient of variance (CV) for specific growth rate (SGR) was calculated using the formula:

$$CV_{SGR} = (\text{standard deviation} / \text{mean}) * 100$$

% SGR was calculated as the mean weight of fish in each treatment group according to the formula:

$$\frac{\ln(\text{final weight}) - \ln(\text{start weight})}{\text{Number of days}} * 100$$

Condition factor (CF) is used to expresses the condition of a fish, such as relative robustness, fatness and well being. CF is calculated according to the formula:

$$K = \text{weight (g)} / \text{length (cm)}^3$$

Data was also analysed using Correlation, Kruskal-Wallis one-way ANOVA on ranks and pair wise multiple comparison procedures (Dunn's Method), using linear regression and two-way ANOVA from SigmaStat.

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3 Results

3.1 Growth

The mean weight and survival of fish was not significantly different between the two groups (P = 0.985) (Figure 24).

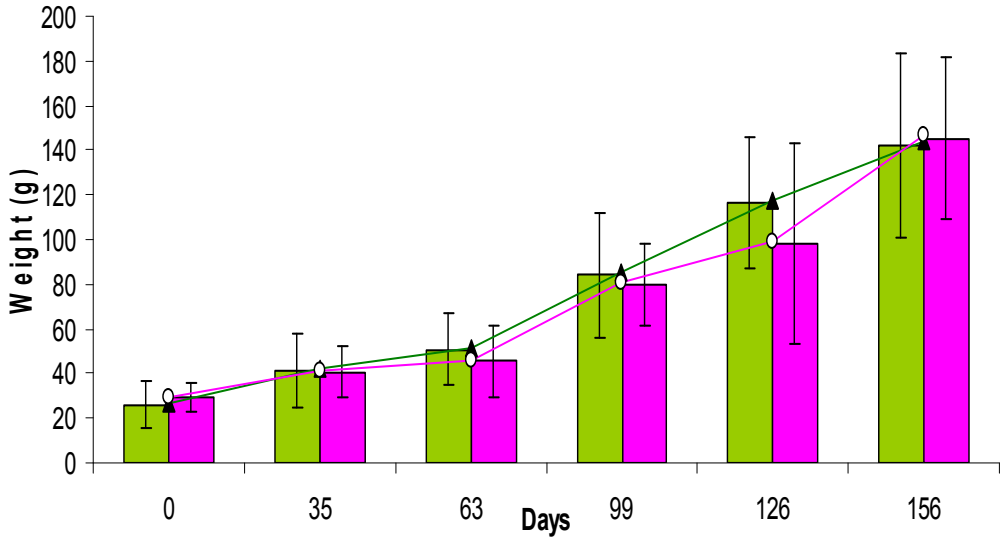

Figure 24. The mean weight of fish.
The figure shows mean values (± S.D.) at various sampling points throughout the period. Green bars represent control fish and pink bars the light treated fish. Green and pink line represents a growth model for Atlantic cod proposed by Björnsson et al., (2007).

At the first sampling point (November 2006), the fish were 25 ± 15g and grew to 145 ± 25g during the 156 day period (Figure 24). The weight- and length growth measured by fork length were found to correlate using regression (R^2 = 0.997) analysis (Figure 26) and CV_R (0.710). The mean length of the fish was 15 ± 2 cm at the onset of the experiment and 24 ± 2 cm at the last sampling point (Figure 25).

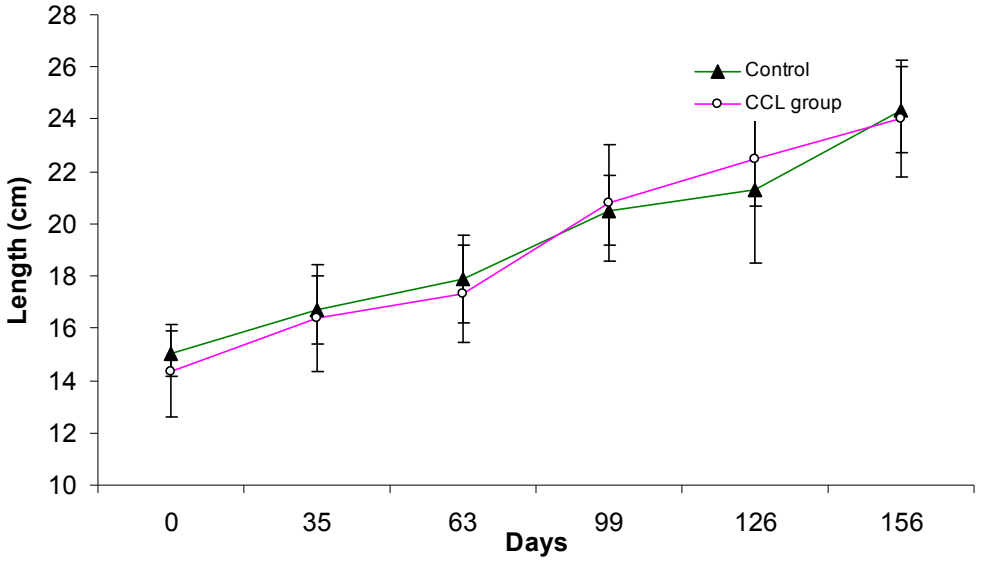

Figure 25. The mean length of 20 fish (± S.D) from each treatment at various samplings points.

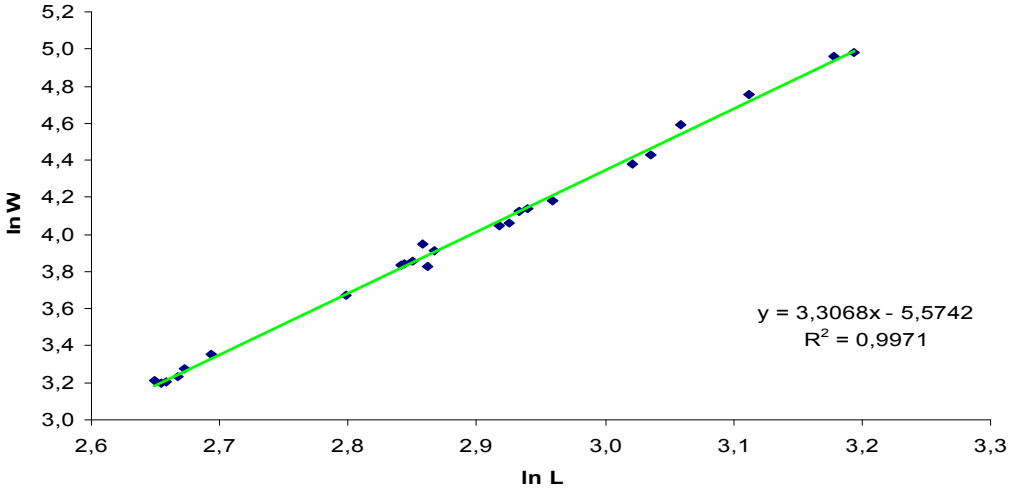

Figure 26. The mean weight (ln weight) plotted against the mean length (ln length) of the experimental fish at all samplings through the period.

The specific growth rate (SGR%) varied considerably between different growth intervals (mean 1.03% d^{-1}) (Figure 27) but the difference between the two groups was not significant.

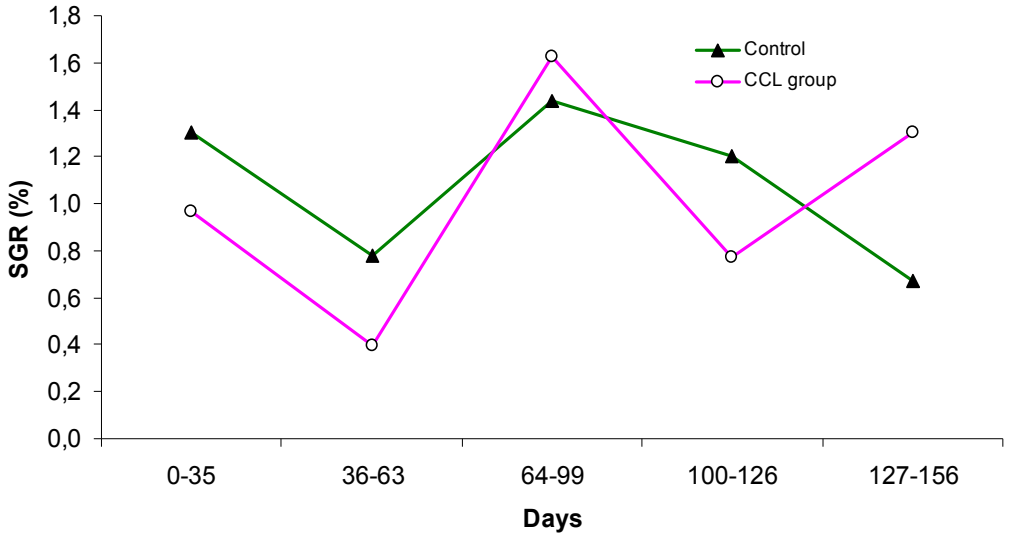

Figure 27. Specific growth rate (% SGR)
The figure shows the SGR of the experimental fish during the experimental period.

The condition factor (CF) was close to 1 during the whole experiment (Figure 28). No significant differences were observed between the treatment groups using t-test (0.55).

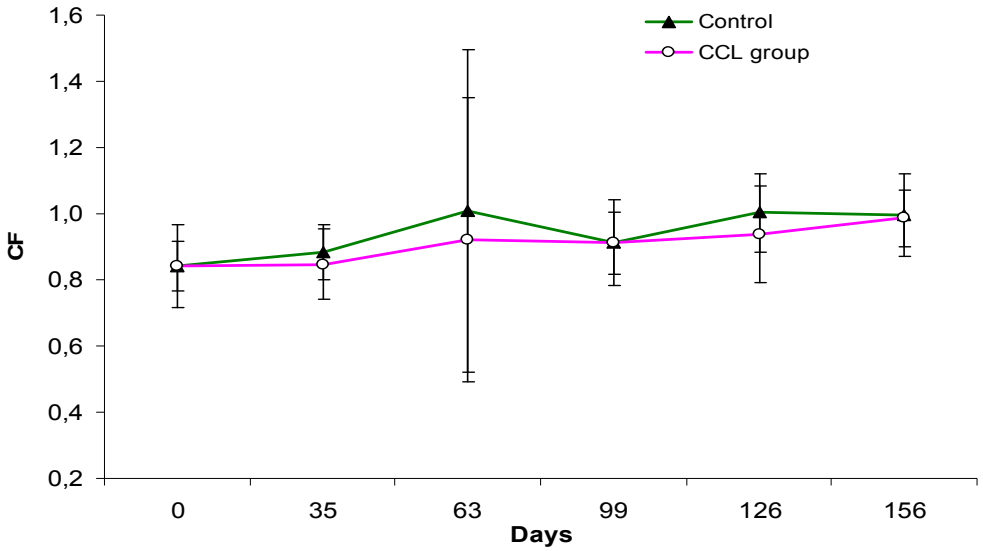

Figure 28. Condition factor (CF) of the experimental fish
The figure shows the CF during the experimental period and shows mean values ± S.D. at various sampling points.

A formula for CF was calculated from the relationship between weight and length of the fish; y = 3.307x - 5.574, where y is ln weight and x is ln length. The calculated CF will then be weight × 263.5 / length$^{3.308}$. No significant difference was observed between the treatment groups using the new formula.

The results furthermore, indicate reduced spinal deformities of fish in the CCL group compared to the control group, estimated at the end of experimental period. A total of 200 fish from each group were visually examined and the incidence of spinal deformation was found to be considerably higher in the control group (61 of 200 fishes) compared to the CCL group (23 of 200 fishes) (results not shown).

3.2 Plasma extraction

The IGF-I measurements of plain (un-extracted) plasma gave more variable results than IGF-I analysis of extracted plasma (Figure 29). The standard deviation for extracted plasma was lower (± 1.3) than for un-extracted plasma (± 9.8) and t-test showed high variation between un-extracted plasma and extracted plasma (0.69)

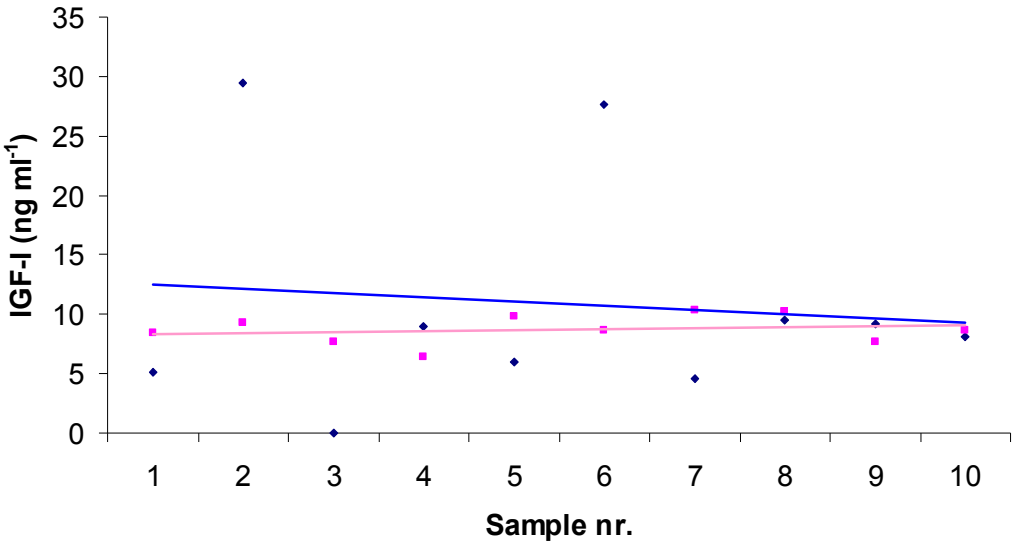

Figure 29. A comparison of IGF-I levels in plasma and extracted plasma. Ten plasma samples were collected from each group and measured both as extracted and un-extracted. Blue dots represent samples from un-extracted plasma and pink dots samples from extracted plasma.

3.3 IGF-I measured by the RIA method

Plasma levels of IGF-I measured by the RIA method were higher at the beginning of the experiment (24 ng ml^{-1}) when the mean weight of fish was less than 30 grams. Two months after the onset of the experiment, the mean levels of IGF-I were reduced to 8.7 ng ml^{-1} and stayed close to that level throughout the experiment (Figure 30).

No significant difference was observed in mean values of IGF-I in plasma of CCL treated fish compared with control fish using two-way ANOVA (P = 0.518).

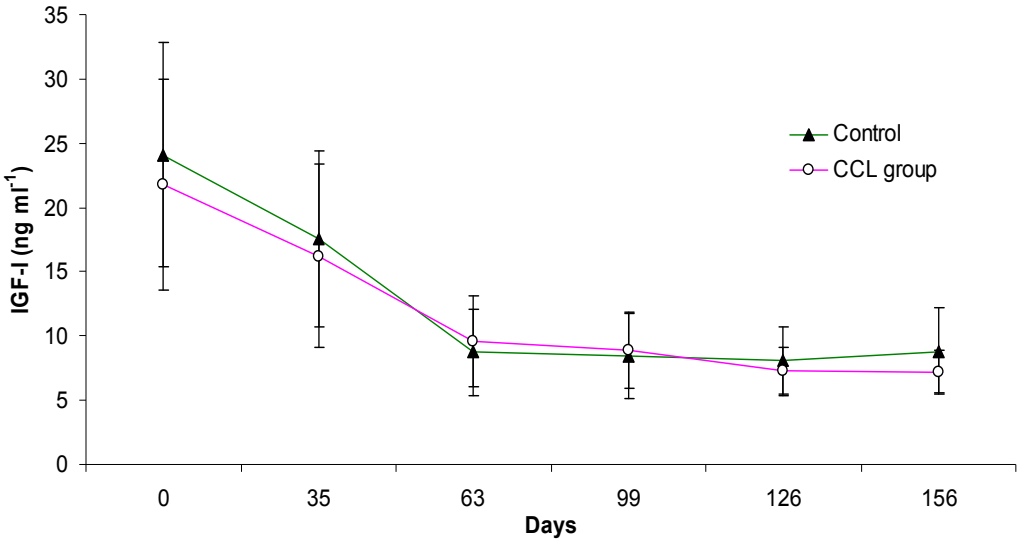

Figure 30. Plasma IGF-I levels in experimental fish.
Dots and triangles represent the mean values (ng ml^{-1}) of 30 fish from the CCL and the control group, respectively. The figure shows mean values ± S.D. at various sampling points.

A negative correlation was observed between weight of the fish and plasma IGF-I levels for both groups ($R^2 = 0.46$) in the beginning of the experiment. When the fish weighed 25 ± 15g, the IGF-I levels were approximately 23 ng ml^{-1}. At sampling day 63 the fish weighed 47g ± 16g and the plasma IGF-I levels were 9 ng ml^{-1} (Figure 31). At the end of the experiment, the mean weight of the fish was 145 ± 25g but the mean levels of IGF-I was still as low as 9 ng ml^{-1}. No significant difference was found when the two experimental groups were compared (regression and t-test).

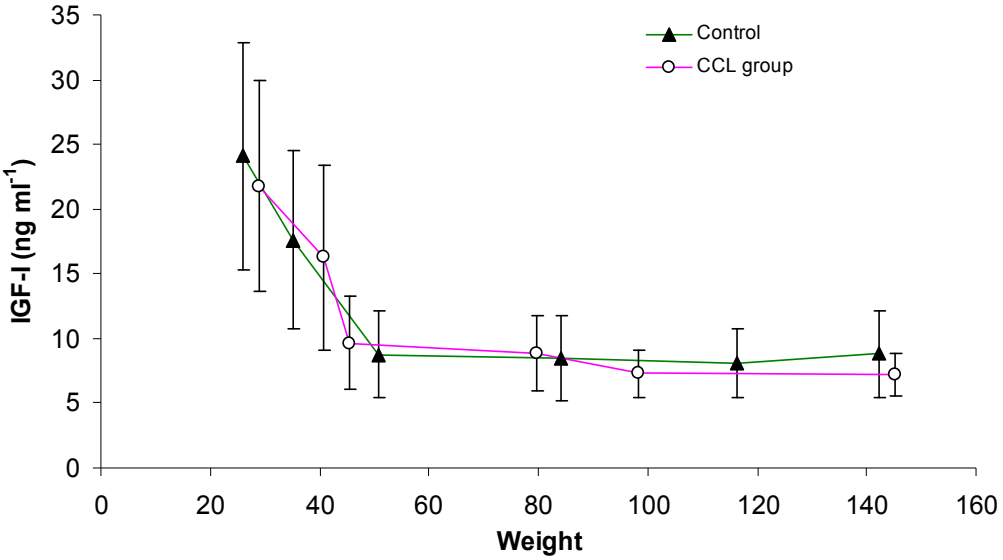

Figure 31. Plasma IGF-I levels in relation to the weight of experimental fish
Each dot represents the mean values (ng ml^{-1}) of 30 fish from each treatment (± S.D.).

Mean values of plasma IGF-I levels were plotted against mean values of SGR at various sampling points throughout the period (Figure 32). There was no significant correlation between plasma IGF-I levels and SGR, neither in the CCL group nor the control group.

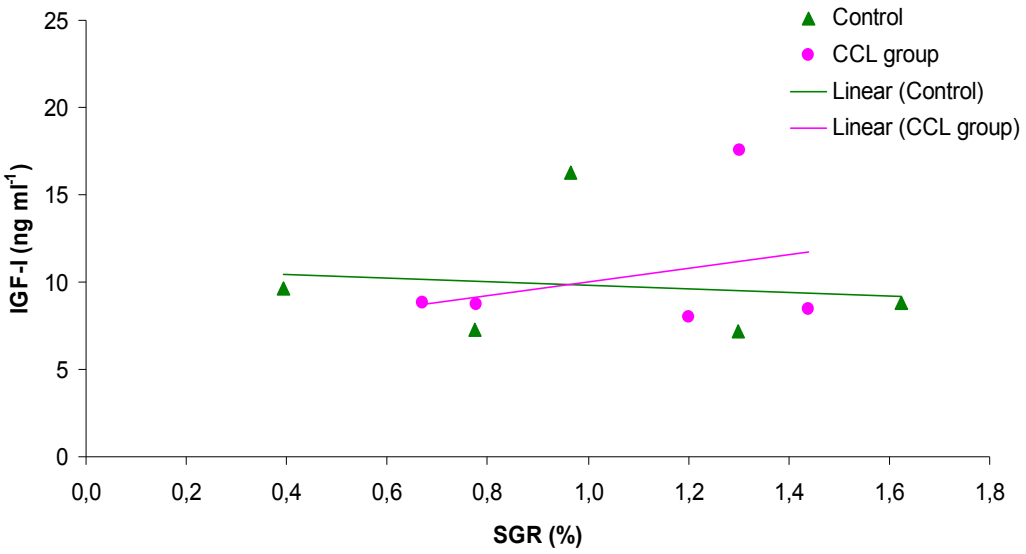

Figure 32 Plasma IGF-I levels (ng ml^{-1}) and specific growth rate (SGR).
Each dot represents the mean value of 30 fish from each treatment.

Specific growth rate of individually tagged fish was also plotted against the plasma IGF-I levels of fish sampled at two distinct sampling points (Figure 33). No significant differences were observed when the two groups were compared using t-test.

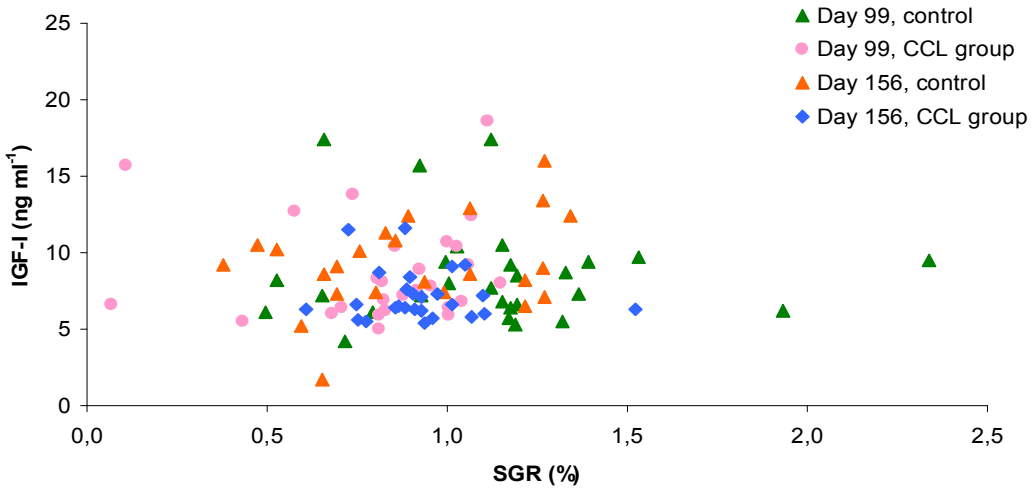

Figure 33. Specific growth rate (SGR).
The figure shows SGR plotted against the plasma IGF-I levels (ng ml^{-1}) of individually tagged fish, sampled at two distinct sampling points, day 99 and day 156

Considerable individual differences were observed within both groups, with a tendency to higher levels of IGF-I following high SGR% (0.148) in the CCL group, but lower levels of IGF-I following lower SGR% (-0.047) in the control group. Coefficient of variance (CV) for IGF-I values was measured for individual fish at each sampling day, resulting in 32% for day 99 and 29% for day 156.

3.4 ELISA for measuring IGF-I

Numerous approaches were made in using the ELISA method for analysis of plasma IGF-I levels. The values measured for extracted and un-extracted plasma were similar as for the RIA method. Compared to the RIA results, the measured values for extracted plasma were furthermore more consistent when individual fish were compared. However, several approaches in creating a successful standard curve using various dilutions of the purified IGF-I (barramundi recombinant IGF-I) proved ineffective. As a consequence, the IGF-I levels in plasma samples measured by ELISA could not be verified and the results therefore not presented.

4 Discussion

The growth of the fish in this study was comparable to what is predicted by the model of (Björnsson *et al*. 2007) reaching approximately 150 g during the first 11 months. Fish following this growth curve would be expected to be about 200 g after the first year and 1100 g after two years in farming conditions (Björnsson *et al.,* 2007).

Light is an important modulator of physiological functions in fish (Boeuf and Le Bail, 1999; Björnsson *et al*., 2000; Han *et al.,* 2005; Karlsen *et al.,* 2005). Light sensitivity varies among different fish species observed in their natural environment. The long dark winters and short bright summers in the North Atlantic area affect behavior of various fish species (Norberg *et al.,* 2001; Nordgarden *et al.,* 2005), including the Atlantic cod (Norberg *et al.,* 2004; Karlsen *et al.,* 2005; Taranger *et al.,* 2006). Photoperiod manipulation during late fall and winter is used in aquaculture to increase growth rate and reduce maturation of fish. Metal halogen lights are commonly used, positioned over the sea cages or tanks on land and emitting white light. Photoperiod manipulation has been used for Atlantic cod, both during the larval and the juvenile stages, as well as for adult fish. The main reason for using photoperiod manipulation during the early stages is to stimulate appetite, increase survival and enhance growth (Boeuf and Bail, 1999; Meeren *et al.,* 2007). In the current study, the experimental fish were subjected to light treatments at an early stage, long before the onset of maturation. The data reveal that there was no significant difference in survival between the CCL group and the control group. While this shows that the CCL treatment does not confer any obvious positive effects, it also demonstrates that the CCL treatment does not harm the fish at juvenile stage. This is an important observation for further studies on photoperiod manipulation of Atlantic cod.

In general, negative effects of light treatment of Atlantic cod have not been reported and studies on cod larvae show that the larvae capture prey more efficiently in brighter light (2400 lux) and a 24L:0D treatment compared with exposure to lower light intensity and shorter photoperiod (Puvanendran and Brown, 2002). Furthermore, mortality rates have been found to be lower under a 24L photoperiod than under other photoperiod regimes (Puvanendran and Brown, 2002). Similar results have also been obtained for juvenile

Atlantic cod as growth rate was found to be highest under a 24L:0D photoperiod (Imsland *et al.,* 2005).

Teleost species are more sensitive to wavelengths in the range of 500 to 600 nm (green and yellow light) compared with wavelengths between 400 and 700 nm that characterize white light (Northmore and Muntz 1970; Ziv *et al.,* 2006). The CCLs emit green light of wavelengths between 450 and 500 nm were therefore selected in the present study. The wavelengths of the CCLs used are similar to the wavelengths of light found in the depths of the sea which is the natural environment of the Atlantic cod.

In this study, there were no significant differences in SGR between the two groups. SGR decreased by increasing weight of the experimental fish, with values of 1.3% detected ~ 40 g cod juveniles. Björnsson (1995) showed in his study on 2 kg Atlantic cod that the SGR was only 1/10 of the values calculated for a 12 g Atlantic cod.
The present study is a part of a larger study, where Atlantic cod will be kept on CCLs until harvesting size is reached. The results show that cod juveniles are not harmed by the lights and that a 24L:0D photoperiod may be used during the early growth phase is of importance. Also, it should be noted that the CCL lights are highly cost effective compared with standard metal halogen lights, and their use is thus of economical importance in the future establishment of cod farming procedures. The results therefore indicate that CCL can be used at juvenile stage to prepare further 24L:0D photoperiod manipulation on Atlantic cod in pubescent stage in order to delay or arrest maturation in coastal farming conditions. It is important to delay maturation of farmed fish as long as possible, as early maturation does not only cause growth arrest, but may in fact cause up to 25% decrease in the weight of the fish at harvesting (Davie *et al.,* 2003). Obviously, any such decrease or reversal of somatic growth represents a significant economic loss to the farming industry. Atlantic cod normally show acceptable levels of feed intake during maturation, but may still lose up to 30% of muscle weight during maturation as proteins and lipids are mobilized to support gonadal development (Lambert and Dutil, 2000). Furthermore, feed conversion efficiency (FCE) of CCL manipulated fish is lower compared to the control fish which will further improve the cost effectiveness of the light manipulation (Ágústsson, unpublished data). In farming conditions, up to 100% of 2 years old Atlantic cod mature under normal farming conditions (Karlsen *et al.,* 1995; Taranger *et al.,* 2006). Preliminary results indicate that the use of CCLs and 24L photoperiod regime in sea cages minimizes premature developing of gonads in on-

growing cod kept in sea cages (Ágústsson, unpublished data). Under farming conditions, the Atlantic cod matures at a much earlier age (2 years), but at a similar size to wild Atlantic cod of 1.5 to 2 kg (Taranger *et al.,* 2006). Farmed fish reach the natural spawning weight earlier, mainly due to continuous feeding throughout the year and good growth conditions (Hemre *et al.,* 2002; Norberg *et al*., 2004; Skjæraasen *et al.,* 2004; Karlsen *et al.,* 2006). Karlsen *et al.* (1995) have shown that lack of nutrition for Atlantic cod in the weeks before maturation delays the maturation time. It has also been reported that the growth during the autumn prior to spawning affects the time of spawning. Less amounts of feed will result in later spawning compared with well-fed fish (Kjedbu and Holm, 1994).

As in other animals, growth and the maturation processes in fish are regulated by hormones (Björnsson *et al*., 2002). In general, it is known that photoperiod affects the hormone systems of Atlantic cod and increasing photoperiod after a period of darkness, causes the fish to prepare for spawning process (Skjæraasen *et al.,* 2004). However, in natural environment, this usually happens when the fish reaches a certain age (4 - 9 years depending of temperature of the sea) or a size of 1.5 – 3 kg (http://www.hafro.is/undir.php?ID=1&ID=120).

An increased understanding of how and which hormones affect the maturation process, will hopefully open the possibility to solve the problem of early and unwanted maturation in farmed Atlantic cod. Studies have been carried out on a number of fish species, emphasizing the numerous endocrine effects of light (Boeuf and Le Bail, 1999; Cuvier-Péres *et al.,* 2001; Davie *et al.,* 2003). Beckman and coworkers (2004) observed that the levels of IGF-I in coho salmon increased by increasing length of the photoperiod during the spring. The authors measured approximately 14.5 ng ml^{-1} of IGF-I in the blood fish that had not matured. These results are in agreement with our results where IGF-I concentrations of 7 – 13 ng ml^{-1} were measured in cod juveniles of 45 g.

Appetite, growth rate and levels of GH as well as IGF-I have been found to increase by increasing day length during the spring (Suttie *et al.,* 1991; Webster *et al.,* 1996).

In order to further explore the functional link between light conditions and plasma IGF-I levels in juvenile Atlantic cod, plasma IGF-I levels were analysed in the present study. Plasma levels of IGF-I were analysed using a radioimmunoassay (RIA), a method

successfully applied for analysis of plasma GH and IGF-I levels in a number of fish species including coho salmon (Beckman *et al.,* 2004; Dyer *et al.,* 2004), rainbow trout (Moriyama et al 1994; Biga *et al.,* 2005), Atlantic salmon (Björnsson *et al.,* 1994), barramundi, bluefish tuna (Dyer *et al.,* 2004), turbot (Imsland *et al.,* 2007b) and Atlantic halibut (Einarsdóttir *et al,*. 2007).

The RIA method was successfully developed for analyses of plasma IGF-I levels in Atlantic cod, using antibodies commonly used for analysis of IGF-I levels in Atlantic salmon (Björnsson *et al.,* 1994). Moriyama *et al.* (1994) have shown that IGFBPs do not affect results in the RIA IGF-I method used for Atlantic salmon (Björnsson *et al.,* 1994). Shimizu *et al.* (2003), on the other hand, found that IGFBPs may affect the RIA results. The different results obtained are likely to be due to the different antibodies used in the IGF-I RIAs by Moriyama *et al*. (1994) and Shimizu *et al*. (2003). Different polyclonal antibodies bind to different epitopes on the IGF-I molecule, and these can be the same or different from the binding sites of the IGFBPs, and thus, the IGFBPs can either interfere with antibody binding or not. In order to examine possible interference by IGFBPs, plasma extraction was tested in this study. The current results indicate greater measurement variability when unextracted plasma is used, compared with the use of extracted plasma. This indicates the possible interference of IGFBPs and therefore, all samples were extracted in this study. Several methods of extracting IGF from plasma have been established. In the present study, acid-ethanol extraction was used, mainly due to its simplicity. Extraction using acid-ethanol will cause a slight modification of the molecular weight, but the quantity of immunoreactive IGF-I will not be affected

The concentration of IGF-I in the blood of parr salmon has been estimated to be 100 - 140 ng ml^{-1} using the RIA method (McCormick *et al*., 1998). The highest values for IGF-I in this study were observed at the beginning of the experiment when the experimental fish were smallest (25 g ± 15 g). The mean levels of IGF-I detected in plasma of the smallest fish were approximately 23 ng ml^{-1} but levels gradually decreased to approximately 10 ng ml^{-1} as the fish grew larger (45 g and above). These levels of IGF-I are comparable to the levels observed in coho salmon by Beckman *et al.* (2004). The results therefore indicate that the concentration of IGF-I in plasma may decrease with increasing weight of the fish. The results of Moriyama *et al.* (1994) on the other hand, showed the highest plasma (extracted) IGF-I values for coho salmon smolt

(117.4 ± 19.1 ng ml^{-1}) but lower both for parr (45.3 ± 2.5 ng ml^{-1}) and adult fish (45.2 ± 5.4 ng ml^{-1}). The measured values are considerably higher than observed in the present study. Furthermore, Dyer *et al.* (2004) reported increased plasma levels of IGF-I of juvenile barramundi and salmon with increasing growth rate and Imsland *et al.* (2007b) showed similar results for turbot with values up to 500 ng ml^{-1} with SGR 1.5 to 3%. This may be explained by rising IGF-I levels when growth becomes GH dependent (Moriyama *et al.,* 1994).

In the present study, no significant differences were found between the two experimental groups when plasma levels of IGF-I were compared to growth rate. Coefficient of variation (CV) compares the relative level of variation in groups having different means and is commonly used to compare variability in growth and hormone levels in other biological samples (Sokal and Rohlf, 1995). The CV represents the difference between two populations that can be compared. A comparison of the calculated CV values for the SGR showed no significant differences between the two experimental groups.

The concentration of IGF-I in plasma of the experimental fish was also measured by ELISA. This was done as an attempt to develop a method for measuring hormones which does not require facilities and equipment needed for radioisotope work. Besides requiring simpler and less costly equipment and facilities, the ELISA method is more easily implemented and results are available within 24 to 48 hours (Hennies *et al.,* 2002; Yun *et al.,* 2002) compared with 72 - 96 hour analysis using RIA methods (Björnsson *et al.,* 1994; Moriyama *et al.,* 1994; Shimizu *et al.,* 2003).

The ELISA method has to our knowledge not previously been used to measure IGF-I in Atlantic cod, but the method has been used to measure various hormones in many fish species such as GH in common carp (Wu *et al.,* 2007) and cortisol in rainbow trout (Velasco-Santamaria and Cruz-Casallas 2007), channel catfish, largemouth bass, red pacu and golden shiner (Sink *et al.,* 2007).

The establishment of new assay methods is a time-consuming process and every aspect of the protocol needs to be optimized and validated, both for the hormone to be assayed and also for the species to be analysed. This is especially true for peptide hormones such as IGF-I, which are species-specific in their amino acid composition. Thus, parameters such as buffers and antibody concentrations, as well as incubation times and

temperatures need to be optimized. The standard curve represented a major problem with highly variable values commonly registered in various concentrations of the standard solution. Without a stable and reproducible standard-curve values, any assessment of plasma values is questionable. However, most plasma values obtained were comparable to the values measured by RIA method, which is promising. The results indicate that the ELISA method may be successfully used to measure the levels of IGF-I in plasma of cod. However, to do so, the variability of values measured for the standard used needs to be resolved, and the method further optimized.

4.1 Conclusions

No significant difference was observed between the two experimental groups. The results therefore indicate that Atlantic cod can be treated with CCL from early stages, without negatively affecting the growth or survival of the fish. The project will continue until spring 2009 and it will be interesting to see if the light manipulation during the first stages affects the growth of the fish after transferring to sea-cages, and whether the CCL treatment will result in a delay or arrest of sexual maturation at later stages.

IGF-I can be measured in Atlantic cod plasma, using the RIA method and it is concluded that the ELISA method has the potential of becoming an important research tool, if current assay problems can be resolved.

No significant correlation was observed between the levels of IGF-I and growth of Atlantic cod during the juvenile stage. The IGF-I levels furthermore decreased with increasing weight of the fish. Therefore, it can't be concluded if IGF-I levels in plasma may be an indicator of growth in Atlantic cod during the early growth phase. Further studies are needed to determine if the relationship between the levels of IGF-I and growth of Atlantic cod may change during later growth phases and if the levels of IGF-I may be used as an indicator of growth rate or maturation at later stages. Further studies are furthermore needed to investigate the indication of reduced spinal deformities following the use of the cold cathode lights.

4.2 Closing words

Fish endocrinology has been studied for several years by numerous scientists all over the world (Björnsson *et al.,* 2002; Reinecke *et al.,* 2005). Endocrinology is a complicated concept and various aspects have to be considered in order to successfully analyse the role of individual hormones. The present study may have contributed to further progress in aquaculture as successful farming techniques have to be based on knowledge of the fish and its physiological responses.

Aquaculture has been growing fast during the last decade and is expected to keep on growing due to the wild fish stocks won't grow very fast for the next years.

Any knowledge we gain on fish behavior will result in improvements in farming technology and farming of fish. However, knowledge of one species of fish can not automatically be transferred to another species, as each species has its own characteristics and even related species can react differently to the same stimuli.

A number of Icelandic companies have supported studies related to aquaculture. The support may be through financing of M.Sc and Ph.D students, research and experimental facilities, travel costs and conferences where results are presented. This is a preferable way to transfer knowledge from the research sector to the farmer.

It has been my honor to be supported by the cod aquaculture industry to carry out my work. The support gave me an opportunity to finish my M.Sc degree and present the results of my work at an Icelandic conference as well as at an international conference. The work as a whole has been an invaluable experience and I have met people working in the same field. I want to thank companies and organizations that supported my studies and, in general, the research work of students.

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5.2 Figures

Figure 1. Fiskfysiologi, Grundkurs. University of Gothenburg. (http://vivaldi.zool.gu.se/Fiskfysiologi_2001/Course_material/)

Figure 2. Jørstad, K.E., Fjalestad, K.T.,. Ágústsson, T., Marteinsdottir, G., 2008. Biology, ecology and genetics. Genimpact final scientific report.

Figure 3. Sigurðsson, 2006. State of marine stocks in Icelandic waters 2005/2006. Prospects for the quota year 2006/2007. Hafrannsóknarstofnun Íslands, Reykjavík

Figure 4. The website of environmental biology. Chapter 4. Aquatic environment. (http://staffwww.fullcoll.edu/tmorris/elements_of_ecology/images/light_spectral_absorption_water.jpg)

Figure 6. The website of geocites (http://www.geocites.co.jp/CollegeLife/3854/GH-IGF-I.gif)

Figure 7. Based on picture in the website of Millipore.
(http://www.millipore.com/immunodetection/id3/radioimmunoassay)

Figure 8. The website of the Icelandic marine research institute.
(http://www.hafro.is/undir.php?REFID=5&ID=48&REF=1)

Figure 11. Þorleifur Ágústsson.

Figure 21.The website of the World health organization
(http://www.who.int/vaccines/en/poliolab/webhelp/Chapter_08/8_1_3_Test_procedure.htm)

Figure 22. The website of Herbal analysis services.
(http://www.herbalanalysis.co.uk/ELISA.gif

Other figures: G. Stella Árnadóttir

6 Appendixes

6.1 ELISA buffers and materials

Bovine Serum Albumin (RIA grade; Sigma Chemical Company)

Coating Buffer
(0.05 M Carbonate buffer pH 9.6)
Na_2CO_3 0.795 g
$NaHCO_3$ 1.465 g
Add ultra filtered (0.22 μm) distilled water to 500 ml store at 4°C for a max 14 days

PBS (phosphate buffered saline)
NaCl 146.1 g
KH_2PO_4 1.0 g
Na_2HPO_4 4.6 g
Add ultra filtered (0.22 μm) distilled water to 5.000 ml store at 4°C for a max 14 days

Wash Buffer (phosphate buffered saline containing 0.05%(v/v) Tween 20, pH 7.6)
PBS (5.000 ml)
Tween 20 2.5 ml
Store at 4°C for a max 14 days

Substrate Buffer (citric acid in phosphate buffer, pH 9.8)
97 ml (1M) dietanolamin diluted to 900 ml with ultra filtered (0.22 μm) distilled water
pH set to 9.8 with 6M HCl
100 mg $MgCl_2$ * 6 H_2O mg (0.005M) put in
Store in dark bottle at 4°C for a max 14 days

p-nitrophenyl phosphate (1 mg / ml)

Blocking Buffer

PBS containing 2% BSA (10 g in 500 ml)

Assay diluent

PBS containing 0.5% BSA (2.25 g in 500 ml)

96 well ELISA plates (NUNC).

Note: Assay plates should be coated the day before the assay is performed.

6.2 Dilution of antibodies

Standard:

Each mix = 50 µl of solution B sustain on 3 plates.

Dilution: 100 µl 0.01 M HCl is transfered to 100 µg Receptor Grade = solution A 1 µg / µl

50 µl of solution A is transfered to 4.95 ml of assay dilute = solution B 10 ng / µl

50 µl of solution B is transfered to 4.95 ml of assay dilute = solution C (stock solution) 0.1 ng / µl

See table 2.

Standard solutions are prepared immediately before use.

Transfer 50 µl of solution A to ependorf and in -80°C freezer, one glass. Mark 99 ependorf IGF-I standard 10 ng / µl and add 50 µl of solution B. Store at -80°C.

Antibody 1 (coating antibody):

200 µl of sterile water is transfered to the vial (1:2 dilutions) and the powder dissolved for at least 10 min at room temperature.

Diluted 1/5.000

100 µl for each well = 10 ml for each plate

1/5000 x 10 ml = 1/2 x X X = 0.004 ml = 4µl to 9.996 ml of Coating Buffer

Pipett 12 µl aliquots to eppendorf tubes and store at -80°C

Antibody 2 (capture antibody)

10 µg per glass. Dissolved in 500 µl of Assay Buffer (1/50)

Dilution 1/10.000

10 ml for each plate.

1/10000 x 10 ml = 1/50 x X X = 0.05 ml = 50µl to 9.95 ml of Assay diluents

Freeze in 50 µl aliquotes at -80°C

Secondary antibody:

Dilution 1/2000 in Assay diluents.

6.3 Overview: IGF-I analysis using the ELISA method

	Antibodies			Dilutions				Buffer		Time		
	Coating AB	Capture AB	Secondary AB	Coating AB	Standards	Capture AB	Substrate	+/- tween	Temp.	between steps	Schaking	Plate
Method 1	AB:1	AB:2	Sec AB:1	1/5000	2	1/10000	Substr. I	+	RT	90 min	Yes	1
Method 2	AB:1	AB:2	Sec AB:1	1/5000	2	1/10000	Substr. II	+	RT	90 min	Yes	1
Method 3	AB:2	AB:1	Sec AB:2	1/500	2	1/2000	Substr. I	+	RT	90 min	Yes	1
Method 4	AB:2	AB:1	Sec AB:2	1/500	2	1/2000	Substr. II	+	RT	90 min	Yes	1
Method 5	AB:1	AB:2	Sec AB:1	1/5000	2	1/10000	Substr. I	-	RT	90 min	Yes	1
Method 6	AB:1	AB:2	Sec AB:1	1/5000	2	1/10000	Substr. II	-	RT	90 min	Yes	1
Method 7	AB:2	AB:1	Sec AB:2	1/500	2	1/2000	Substr. I	-	RT	90 min	Yes	1
Method 8	AB:2	AB:1	Sec AB:2	1/500	2	1/2000	Substr. II	-	RT	90 min	Yes	1
Method 9	AB:1	AB:2	Sec AB:1	1/5000	1	1/10000	Substr. II	-	15°C	4 klst	No	1
Method 10	AB:1	AB:2	Sec AB:1	1/5000	1	1/10000	Substr. II	-	4°C	6 klst	No	1
Method 11	AB:1	AB:2	Sec AB:1	1/5000	1	1/10000	Substr. II	-	15°C	2 klst	Yes	1
Method 12	AB:2	AB:1	Sec AB:2	1/5000	1	1/2000	Substr. II	-	RT	90 min	No	2
Method 13	AB:1	AB:2	Sec AB:1	1/5000	1	1/10000	Substr. II	-	RT	90 min	No	2

AB:1 = IGF-I, anti-rat, monoclonal, Protein G purified mouse
AB:2 = IGF-I, anti-barramundi, polyclonal Protein G purified
Sec AB:1 = Antibody Enzyme Conjugate (anti-rabbit)
Sec AB:2 = Antibody Enzyme Conjugate (anti-mouse)

Substr. I = p-nitrophenyl phosphate
Substr. II = o-PD to 30% hydrogen peroxide solution, Na2HPO4 and citric acid

Buffer + tween = Tween in all buffers
Buffer - tween = Tween just in wash buffer

Standard:	ng/ml
1	100, 50, 25, 12.5, 6.25, 3.135, 1.56, 0.78
2	100, 0.08, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01

Plate:1	Greiner Microlon 600
Plate:2	Nunc

6.4 ELISA results

Sample	Treatment	Day nr.	D0 405	D0 405	OD405 Average	IGF-1 Calculated	IGF-1 Calcul. from * 7dilution	IGF-I RIA
124	0	63	0,723	0,766	0,745	1,02	7,12	7,56
126	0	63	0,851	0,927	0,889	1,22	8,51	12,53
132	0	63	0,922	0,751	0,837	1,14	8,00	9,87
140	0	63	1,144	0,934	1,039	1,42	9,94	9,24
144	0	63	0,691	0,632	0,662	0,90	6,33	6,30
145	0	63	0,705	0,616	0,661	0,90	6,32	11,69
146	0	63	0,942	0,922	0,932	1,27	8,92	8,47
152	1	63	0,886	0,793	0,840	1,15	8,03	17,36
156	1	63	0,792	0,794	0,793	1,08	7,59	19,18
159	1	63	0,667	0,634	0,651	0,89	6,22	5,81
160	1	63	0,771	0,679	0,725	0,99	6,94	8,61
164	1	63	0,649	0,556	0,603	0,82	5,76	6,93
169	1	63	0,710	0,593	0,652	0,89	6,23	8,89
171	1	63	0,834	0,768	0,801	1,09	7,66	9,38
237	1	99	0,688	0,655	0,672	0,92	6,42	1,75
245	1	126	0,859	0,834	0,847	1,16	8,10	6,72
246	1	126	0,581	0,532	0,557	0,76	5,32	6,58
247	1	126	0,592	0,594	0,593	0,81	5,67	8,68
249	1	126	0,662	0,759	0,711	0,97	6,80	4,97
255	1	126	0,711	0,867	0,789	1,08	7,55	7,28
259	1	126	0,753	0,775	0,764	1,04	7,31	7,07
260	1	126	0,829	0,710	0,770	1,05	7,36	7,84
263	1	126	1,143	0,833	0,988	1,35	9,45	6,86
268	1	126	0,896	0,943	0,920	1,26	8,80	6,23
269	1	126	0,746	0,767	0,757	1,03	7,24	6,72

270	1	126	0,683	0,944	0,814	1,11	7,78	7,77
276	0	126	0,971	0,863	0,917	1,25	8,77	13,23
277	0	126	0,667	0,719	0,693	0,95	6,63	5,88
281	0	126	0,570	0,610	0,590	0,81	5,64	6,86
282	0	126	0,907	0,735	0,821	1,12	7,85	5,74
286	0	126	0,595	0,769	0,682	0,93	6,53	7,42
290	0	126	0,815	0,791	0,803	1,10	7,68	6,86
294	0	126	0,715	0,774	0,745	1,02	7,12	8,12
300	0	126	0,480	0,596	0,538	0,74	5,15	6,02
301	1	156	0,876	0,805	0,841	1,15	8,04	9,17
302	1	156	0,808	0,845	0,827	1,13	7,91	8,68
305	1	156	0,669	0,679	0,674	0,92	6,45	5,81
306	1	156	0,810	0,798	0,804	1,10	7,69	6,44
307	1	156	0,535	0,726	0,631	0,86	6,03	7,35
309	1	156	0,718	0,580	0,649	0,89	6,21	5,67
310	1	156	0,884	0,713	0,799	1,09	7,64	6,30
311	1	156	0,821	0,650	0,736	1,01	7,04	9,10
312	1	156	0,645	0,798	0,722	0,99	6,90	7,21
314	1	156	0,592	0,804	0,698	0,95	6,68	7,14
316	1	156	0,736	0,716	0,726	0,99	6,95	6,02
324	1	156	0,644	0,645	0,645	0,88	6,17	5,39
326	1	156	0,664	0,609	0,637	0,87	6,09	6,23
328	1	156	0,687	0,908	0,798	1,09	7,63	9,24
330	0	156	0,529	0,653	0,591	0,81	5,65	6,93
333	0	156	0,690	0,854	0,772	1,06	7,39	6,02
334	0	156	0,712	0,617	0,665	0,91	6,36	6,23
339	0	156	0,617	0,687	0,652	0,89	6,24	6,37
347	0	156	0,751	0,824	0,788	1,08	7,53	6,44
350	0	156	0,691	0,629	0,660	0,90	6,31	10,36

6.5 Regression

Regression, ln W og ln L

Regression Statistics		
Multiple R	0,998527	
R Square	0,997056	skýringarhlutfall
Adjusted R Square	0,996923	
Standard Error	0,030191	staðalskekkja
Observations	24	

ANOVA	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	1	6,792305	6,792305	7451,924	2,42E-29
Residual	22	0,020053	0,000911		
Total	23	6,812358			

Aðhvarfs fervikasumma

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	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
Intercept	-5,5742	0,110605	-50,3974	3,16E-24	-5,80358	-5,34482	-5,80358	-5,34482
ln L(cm)	3,306767	0,038306	86,32453	2,42E-29	3,227325	3,386209	3,227325	3,386209

Regression with 1 dummy variable

Regression Statistics	
Multiple R	0,998629
R Square	0,99726
Adjusted R Square	0,996999
Standard Error	0,029815
Observations	24

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	2	6,79369	3,396845	3821,275	1,25E-27
Residual	21	0,018668	0,000889		
Total	23	6,812358			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
Intercept	-5,56662	0,109396	-50,8849	1,76E-23	-5,79413	-5,33912	-5,79413	-5,33912
ln L(cm)	3,306761	0,037829	87,41261	2,16E-28	3,228091	3,385432	3,228091	3,385432
dymbill	-0,01571	0,012586	-1,24827	0,225671	-0,04189	0,010463	-0,04189	0,010463

Regression with 3 dummy variables

Regression Statistics	
Multiple R	0,998734731
R Square	0,997471064
Adjusted R Square	0,996938656
Standard Error	0,030112088
Observations	24

ANOVA					*Significance*
	df	*SS*	*MS*	*F*	*F*
Regression	4	6,795129649	1,698782	1873,51	2,22897E-24
Residual	19	0,017228019	0,000907		
Total	23	6,812357668			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
Intercept	-5,533022784	0,13813416	-40,0554	8,09E-20	-5,822140993	-5,24390457	-5,82214099	-5,24390457
ln L(cm)	3,288870251	0,050473539	65,16029	8,35E-24	3,183227888	3,394512614	3,183227888	3,394512614
d1	0,020486067	0,014026631	1,460512	0,160489	-0,008872018	0,049844152	-0,00887202	0,049844152
d2	0,00666555	0,012331087	0,540548	0,5951	-0,019143721	0,03247482	-0,01914372	0,03247482
d3	-0,006310371	0,014737369	-0,42819	0,673329	-0,037156049	0,024535307	-0,03715605	0,024535307

6.6 Regression table

Day	Tank	Light	Weight	Length	Y	X	One	X	Three dummies		
nr.	nr	Control	(gr)	(cm)	ln W (gr)	ln L(cm)	dummy	ln L(cm)	d1	d2	d3
0	E-4	C	24,6	14,3	3,20	2,66	0	2,66	0	0	0
0	E-5	C	24,4	14,2	3,19	2,65	0	2,65	1	0	0
0	E-6	C	24,9	14,1	3,21	2,65	0	2,65	0	1	0
0	E-7	L	28,6	14,8	3,35	2,69	1	2,69	0	0	1
0	E-8	L	25,3	14,4	3,23	2,67	1	2,67	1	1	0
0	E-9	L	26,6	14,5	3,28	2,67	1	2,67	0	1	1
35	E-4	C	39,4	16,4	3,67	2,80	0	2,80	0	0	0
35	E-5	C	46,4	17,1	3,84	2,84	0	2,84	1	0	0
35	E-6	C	50,2	17,6	3,92	2,87	0	2,87	0	1	0
35	E-7	L	46,0	17,5	3,83	2,86	1	2,86	0	0	1
35	E-8	L	46,6	17,2	3,84	2,84	1	2,84	1	1	0
35	E-9	L	47,2	17,3	3,85	2,85	1	2,85	0	1	1
65	E-4	C	57,3	18,5	4,05	2,92	0	2,92	0	0	0
65	E-5	C	51,9	17,4	3,95	2,86	0	2,86	1	0	0
65	E-6	C	62,6	18,9	4,14	2,94	0	2,94	0	1	0
65	E-7	L	57,9	18,6	4,06	2,93	1	2,93	0	0	1
65	E-8	L	61,9	18,8	4,13	2,93	1	2,93	1	1	0
65	E-9	L	65,5	19,3	4,18	2,96	1	2,96	0	1	1
99	0	U-3	84,1	20,8	4,43	3,04	0	3,04	0	0	0
99	1	U-1	79,8	20,5	4,38	3,02	1	3,02	1	0	0

126	0	U-3	116,3	22,5	4,76	3,11	0	3,11	0	1	0
126	1	U-1	98,4	21,3	4,59	3,06	1	3,06	0	0	1
156	0	U-3	142,3	24,0	4,96	3,18	0	3,18	1	1	0
156	1	U-1	145,3	24,4	4,98	3,19	1	3,19	0	1	1

ln(W) = ln(k) + n *ln(L) + a1*d1 + a2*d2 + a3*d3 + a4*d4 + a5*d5 +a6*d6

lnC = α+B lnY+ε

lnW = lnk + n*lnL

lnW = Y axis og lnL = x axis

							Reg. with calculated factors				
				Reg. 1 dummy.				Reg. 3 dummies			
lnW = lnk + n*ln L	lnL= (lnW - lnk)/ n	W	L	ln W	ln L	W	L	ln W	ln L	W	L
3,22	2,65	24,93	14,21	3,22	2,66	25,11	14,27	3,10	2,66	22,12	14,27
3,20	2,65	24,58	14,18	3,21	2,65	24,76	14,21	3,10	2,66	22,27	14,30
3,19	2,66	24,21	14,26	3,19	2,65	24,39	14,15	3,07	2,65	21,64	14,17
3,33	2,70	27,96	14,87	3,32	2,69	27,73	14,70	3,20	2,69	24,60	14,75
3,24	2,66	25,66	14,34	3,24	2,66	25,44	14,33	3,15	2,68	23,38	14,52
3,27	2,68	26,19	14,55	3,26	2,67	25,97	14,42	3,15	2,67	23,23	14,49
3,68	2,80	39,61	16,39	3,69	2,80	39,90	16,42	3,55	2,80	34,85	16,42
3,82	2,85	45,65	17,22	3,83	2,84	45,98	17,14	3,71	2,85	40,89	17,24
3,91	2,87	49,83	17,64	3,92	2,87	50,19	17,60	3,78	2,87	43,95	17,63
3,89	2,84	48,80	17,18	3,88	2,86	48,40	17,40	3,75	2,86	42,51	17,45
3,83	2,85	46,13	17,24	3,82	2,84	45,75	17,11	3,73	2,85	41,59	17,33
3,85	2,85	47,03	17,30	3,84	2,85	46,64	17,21	3,72	2,85	41,26	17,29
4,07	2,91	58,71	18,35	4,08	2,92	59,14	18,49	3,94	2,92	51,28	18,49
3,88	2,88	48,34	17,81	3,89	2,86	48,70	17,44	3,77	2,86	43,26	17,55
4,15	2,94	63,24	18,85	4,15	2,94	63,70	18,91	4,02	2,94	55,53	18,95
4,10	2,91	60,30	18,41	4,09	2,92	59,80	18,55	3,96	2,92	52,32	18,60
4,13	2,93	61,97	18,79	4,12	2,93	61,46	18,71	4,02	2,94	55,57	18,95
4,21	2,95	67,36	19,11	4,20	2,95	66,80	19,18	4,07	2,96	58,72	19,28

4,46	3,03	86,75	20,61	4,47	3,04	87,39	20,81	4,34	3,04	76,82	20,94
4,41	3,01	82,68	20,29	4,41	3,02	82,26	20,43	4,29	3,03	72,79	20,60
4,72	3,12	112,03	22,74	4,73	3,11	112,85	22,48	4,59	3,12	98,75	22,62
4,54	3,07	93,93	21,62	4,54	3,06	93,37	21,23	4,41	3,06	82,51	21,41
4,94	3,19	139,16	24,17	4,94	3,18	140,18	24,00	4,81	3,18	122,18	24,16
4,99	3,19	146,24	24,32	4,98	3,19	145,24	24,26	4,85	3,20	127,42	24,47

6.7 Extraction of plasma

Parallel extracted and pure plasma in RIA, IGF-I.

Sample nr.	Pure plasma ng/ml	Sample RIA nr.	Extracted plasma ng/ml	+ dilution X 7	Extr/pure
318	5,14	95-96	1,20	8,4	1,634
328	29,50	85-86	1,32	9,24	0,313
315	out of range	71-72	1,09	7,63	#VALUE!
339	8,99	67-68	0,91	6,37	0,709
165	5,98	99-100	1,41	9,87	1,651
302	27,67	97-98	1,24	8,68	0,314
353	4,57	93-94	1,48	10,36	2,267
233	9,45	83-84	1,46	10,22	1,081
244	9,21	81-82	1,10	7,7	0,836
224	8,10	75-76	1,23	8,61	1,063

Extraction (1:7 dilution)

Sample	Sampling 1	Sample	Sampling 2	Sample	Sampling 3	Sample	Sampling 4	Sample	Sampling 5	Sample	Sampling 6
nr.	ng/ml	nr.	ng/ml	nr.	ng/ml	nr.	ng/ml	nr.	ng/ml	nr.	ng/ml
3	5,16	100	1,83	162	1,24	207	1,03	291	1,11	337	1,15
57	1,80	93	1,23	160	1,23	202	0,87	288	1,07	341	1,19
24	2,55	72	1,46	124	1,08	203	0,94	260	1,12	349	1,16
37	2,58	99	1,92	137	0,74	206	1,10	252	1,38	301	1,31
6	4,04	78	1,69	174	1,11	226	0,93	262	1,18	335	0,84
42	3,40	87	5,08	140	1,32	209	0,97	241	1,64	333	0,86
4	3,41	118	1,40	179	1,12	235	1,50	293	1,31	352	1,27
5	2,18	96	1,65	176	1,17	224	1,23	264	1,42	312	1,03
27	3,31	73	2,02	131	1,30	233	1,46	265	1,23	339	0,91
49	3,02	110	1,81	153	0,77	216	1,06	294	1,16	315	1,08
14	2,55	80	1,37	134	2,62	185	1,03	244	1,10	328	1,32
38	2,97	83	3,37	170	0,81	231	0,88	282	0,82	353	1,48
43	3,27	88	2,83	164	0,99	181	1,48	284	1,15	318	1,20
41	2,15	84	1,73	167	1,57	228	0,94	258	0,87	302	1,24
19	7,41	116	1,84	165	1,41	190	0,87	280	0,79	313	1,04
23	2,29	89	1,94	139	0,84	232	0,68	292	0,95	326	0,89
1	3,15	81	3,37	149	1,05	205	1,36	289	0,58	327	0,79
21	2,61	71	2,44	129	0,97	189	1,35	298	0,76	320	0,92

58	2,56	61	2,03	144	0,90	188	0,81	253	0,85	303	0,94
10	4,11	98	1,77	166	0,73	230	1,84	273	0,59	306	0,92
31	2,64	113	1,74	148	0,84	195	1,17	300	0,86	323	0,90
35	2,59	70	1,58	133	0,94	229	0,84	243	0,27	316	0,86
44	6,59	79	2,01	128	1,18	191	0,91	268	0,89	336	0,84
17	3,22	77	1,95	173	0,94	211	1,16	261	0,91	309	0,81
34	2,30	112	1,77	136	0,56	214	0,75	248	0,81	314	1,02
56	3,97	111	2,36	147	1,30	194	0,88	267	1,18	319	0,90
25	2,89	67	3,72	150	0,87	204	0,60	256	1,02	347	0,92
11	2,51	85	3,34	151	1,26	223	1,01	286	1,06	343	0,72
22	2,85	63	1,75	159	0,83	198	1,04	245	0,96	308	0,95
26	4,75	95	2,41	126	1,79	197	1,14	247	1,24	310	0,90
33	3,70	104	2,08	132	1,41	208	0,79	290	0,98	325	1,11
12	3,13	106	2,36	146	1,21	237	0,25	263	0,98	342	0,79
28	3,32	64	2,02	122	0,91	183	1,32	275	0,96	346	1,03
		97	4,70	171	1,34	182	0,76	249	0,71	307	1,05
		107	2,53	127	0,91	192	1,24	281	0,98	304	0,80
		120	2,36	161	1,43	238	1,05	251	0,85	329	0,97
		62	2,09	142	1,00	236	1,30	277	0,84	305	0,83
		90	3,00	121	0,90	239	1,22	266	1,46	324	0,77
		92	2,01	172	1,53	199	0,78	255	1,04	334	0,89
		115	2,93	138	0,91	184	1,21	269	0,96	332	0,95
		86	2,77	180	0,80	220	1,32	250	0,82	345	1,07

		94	1,99	163	1,28	240	1,54	287	1,09	321	0,93
		103	2,86	156	2,74	186	2,48	297	0,92	351	1,11
		75	4,45	177	0,91	210	1,50	242	1,14	330	0,99
		68	1,85	169	1,27	218	1,17	279	1,05	350	1,48
		76	2,08	157	1,33	200	1,35	246	0,94	311	1,30
		65	1,83	141	1,59	196	2,25	270	1,11	354	1,81
		119	1,85	154	1,67	212	1,92	257	1,26	338	2,66
		82	4,62	145	1,67	221	1,47	285	1,08	348	1,53
		74	2,11	168	1,85	222	1,77	272	1,14	317	1,66
		69	2,29	152	2,48	201	1,39	295	1,29	331	1,77
		114	5,89	175	2,31	234	1,77	271	2,07	344	1,97
		102	2,41	143	1,82	213	2,28	254	0,95	340	2,24
				178	1,76	227	1,61	276	1,89	322	1,64
				135	1,36	215	1,44	278	1,43		
				158	1,41	219	1,29	296	1,33		
				123	2,18			274	1,42		
				155	1,95			259	1,01		
				125	2,00			299	2,07		
								283	1,71		
Average	3,30		2,42		1,31		1,22		1,10		1,14
Max	7,41		5,89		2,74		2,48		2,07		2,66
Med	3,02		2,03		1,24		1,17		1,06		1,03
Min	1,80		1,23		0,56		0,25		0,27		0,72

6.8 IGF-I values (ng/ml) in Atlantic cod plasma samples.

Multiplication by the dilution factor (X7) = Total amount IGF-I in ng/ml

Sample nr.	Sampling 1 ng/ml	Sample nr.	Sampling 2 ng/ml	Sample nr.	Sampling 3 ng/ml	Sample nr.	Sampling 4 ng/ml	Sample nr.	Sampling 5 ng/ml	Sample nr.	Sampling 6 ng/ml
3	36,12	100	12,81	162	8,68	207	7,21	291	7,77	337	8,05
57	12,60	93	8,61	160	8,61	202	6,09	288	7,49	341	8,33
24	17,85	72	10,22	124	7,56	203	6,58	260	7,84	349	8,12
37	18,06	99	13,44	137	5,18	206	7,70	252	9,66	301	9,17
6	28,28	78	11,83	174	7,77	226	6,51	262	8,26	335	5,88
42	23,80	87	35,56	140	9,24	209	6,79	241	11,48	333	6,02
4	23,87	118	9,80	179	7,84	235	10,50	293	9,17	352	8,89
5	15,26	96	11,55	176	8,19	224	8,61	264	9,94	312	7,21
27	23,17	73	14,14	131	9,10	233	10,22	265	8,61	339	6,37
49	21,14	110	12,67	153	5,39	216	7,42	294	8,12	315	7,56
14	17,85	80	9,59	134	18,34	185	7,21	244	7,70	328	9,24
38	20,79	83	23,59	170	5,67	231	6,16	282	5,74	353	10,36
43	22,89	88	19,81	164	6,93	181	10,36	284	8,05	318	8,40
41	15,05	84	12,11	167	10,99	228	6,58	258	6,09	302	8,68
19	51,87	116	12,88	165	9,87	190	6,09	280	5,53	313	7,28
23	16,03	89	13,58	139	5,88	232	4,76	292	6,65	326	6,23
1	22,05	81	23,59	149	7,35	205	9,52	289	4,06	327	5,53
21	18,27	71	17,08	129	6,79	189	9,45	298	5,32	320	6,44
58	17,92	61	14,21	144	6,30	188	5,67	253	5,95	303	6,58

10	28,77	98	12,39	166	5,11	230	12,88	273	4,13	306	6,44
31	18,48	113	12,18	148	5,88	195	8,19	300	6,02	323	6,30
35	18,13	70	11,06	133	6,58	229	5,88	243	1,89	316	6,02
44	46,13	79	14,07	128	8,26	191	6,37	268	6,23	336	5,88
17	22,54	77	13,65	173	6,58	211	8,12	261	6,37	309	5,67
34	16,10	112	12,39	136	3,92	214	5,25	248	5,67	314	7,14
56	27,79	111	16,52	147	9,10	194	6,16	267	8,26	319	6,30
25	20,23	67	26,04	150	6,09	204	4,20	256	7,14	347	6,44
11	17,57	85	23,38	151	8,82	223	7,07	286	7,42	343	5,04
22	19,95	63	12,25	159	5,81	198	7,28	245	6,72	308	6,65
26	33,25	95	16,87	126	12,53	197	7,98	247	8,68	310	6,30
33	25,90	104	14,56	132	9,87	208	5,53	290	6,86	325	7,77
12	21,91	106	16,52	146	8,47	237	1,75	263	6,86	342	5,53
28	23,24	64	14,14	122	6,37	183	9,24	275	6,72	346	7,21
		97	32,90	171	9,38	182	5,32	249	4,97	307	7,35
		107	17,71	127	6,37	192	8,68	281	6,86	304	5,60
		120	16,52	161	10,01	238	7,35	251	5,95	329	6,79
		62	14,63	142	7,00	236	9,10	277	5,88	305	5,81
		90	21,00	121	6,30	239	8,54	266	10,22	324	5,39
		92	14,07	172	10,71	199	5,46	255	7,28	334	6,23
		115	20,51	138	6,37	184	8,47	269	6,72	332	6,65
		86	19,39	180	5,60	220	9,24	250	5,74	345	7,49
		94	13,93	163	8,96	240	10,78	287	7,63	321	6,51

		103	20,02	156	19,18	186	17,36	297	6,44	351	7,77
		75	31,15	177	6,37	210	10,50	242	7,98	330	6,93
		68	12,95	169	8,89	218	8,19	279	7,35	350	10,36
		76	14,56	157	9,31	200	9,45	246	6,58	311	9,10
		65	12,81	141	11,13	196	15,75	270	7,77	354	12,67
		119	12,95	154	11,69	212	13,44	257	8,82	338	18,62
		82	32,34	145	11,69	221	10,29	285	7,56	348	10,71
		74	14,77	168	12,95	222	12,39	272	7,98	317	11,62
		69	16,03	152	17,36	201	9,73	295	9,03	331	12,39
		114	41,23	175	16,17	234	12,39	271	14,49	344	13,79
		102	16,87	143	12,74	213	15,96	254	6,65	340	15,68
				178	12,32	227	11,27	276	13,23	322	11,48
				135	9,52	215	10,08	278	10,01		
				158	9,87	219	9,03	296	9,31		
				123	15,26			274	9,94		
				155	13,65			259	7,07		
				125	14,00			299	14,49		
								283	11,97		
Average	23,12		16,97		9,18		8,54		7,67		8,00
Max	51,87		41,23		19,18		17,36		14,49		18,62
Med	21,14		14,21		8,68		8,19		7,39		7,18
Min	12,60		8,61		3,92		1,75		1,89		5,04

6.9 **Poster** presented at Aquaculture Europe held in Istanbul, Turkey in 24-27. October 2007.

The effects of supplementary light on growth and IGF-I levels of Atlantic cod juveniles: The use of cold cathode light technology

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Introduction

Photoperiod is an environmental regulator of growth and sexual maturation in cod as well as other species of fish. The use of novel cold cathode light system (CCL), which emits a single wavelength with improved distribution pattern in water compared to standard light systems used in aquaculture, will allow for manipulation of key developmental processes in cod aquaculture. By starting light treatment at an early stage, while juveniles are still in hatchery tanks, it is possible that "light imprinting" occurs, making photoperiod regulation more effective after transfer to sea-cages.

In the part of the study presented here the focus is on the effects of light treatment on growth of Atlantic cod during the first year in its life.

Early maturation is one of the major problems in intensive farming of Atlantic cod resulting in a loss of somatic growth rate and eventually prolonged production cycle. Farming procedures aimed at delaying maturation and/or increasing the growth rate are therefore of great interest. Recent data indicate that the growth rate of cod can be increased by using light control which also was shown to improve product quality (Davie *et al.* 2007a, 2007b).

Aims of the Study

To elucidate the importance of photoperiod regulation from an early life stage of Atlantic cod to enhance growth and to investigate if plasma IGF-I is a good indicator of growth.

Materials and Methods

Light technology: The lighting system used in the experiment is based on a Cold Cathode Light Tubes (CCL) lighting technologies. This light system is characterized by lower running costs as compared to the standard metal halogen systems (40%-60% saving in energy) – the strategy is to use novel lighting technology to develop narrow bandwidth lighting systems, specifically targeting the wavelengths which are most efficient at penetrating the water column and, for which fish are more sensitive, thus resulting in the expected biological response in the fish.

Experimental setup: Juvenile Atlantic cod were kept in indoor flow-through tanks at the Marine Research Institute in Iceland.

Fish was divided equally and at a random manner into two treatment groups; 3 tanks using 24L CCL light, 3 control tanks (NL).

Biomass was calculated as 14 kg m^3.

At each sampling point, 30 fish from each treatment were measured for body weight (±0.1g) and fork length (±0.1cm), plasma was sampled, frozen and stored at -80°C for later analysis of IGF-I by RIA method

Acknowledgments

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Figure 1. At the Marine Institute hatchery station, fish were kept in indoor flow through tanks. Each light treated tank was equipped with CCL light tubes

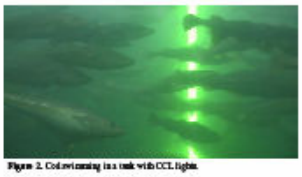

Figure 2. Cod swimming in a tank with CCL lights.

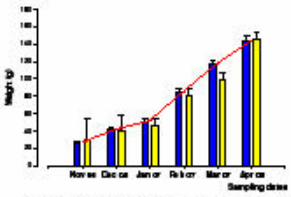

Figure 3. Weight of fish during the experiment. Blue bars represent control fish and yellow bars are light treated fish. Red line represents a growth model for Atlantic cod proposed by Bjornsson et al., (2007)

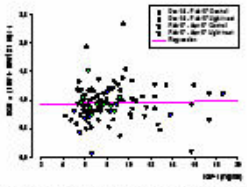

Figure 4. IGF1 was calculated for both control and light treated fish during two periods, from December 06 until February 07 and plasma IGF-I levels were measured for same individuals.

Results

From November 2006 to May 2007, fish grew from 25±15g to 145±25g.

There was no significant difference in weight of fish in the two treatment groups at any sampling dates. This indicates that this specific continuous light treatment (CCL) does not affect the growth pattern of Atlantic cod during the juvenile phase (Figure 3)

In order to further explore the functionality of the light-pituitary-GH-IGF-I axis of juvenile Atlantic cod, plasma IGF-I levels were analyzed, and which to our knowledge is the first time that IGF-I is measured in cod plasma.

Results show that there is a negative correlation between growth of fish and IGF-I concentration in plasma. There were however no difference observed between control fish and light treated fish (Figure 5 and 6).

Fish in this experiment is growing according to the growth model for Atlantic cod (Figure 3)

Figure 5. At sampling site, blood was sampled for assessment of insulin-like growth factor 1 (IGF-I) levels in order to correlate IGF-I levels with growth rate. Length and weight was measured and some fish were tagged for measuring the specific growth rate (SGR)

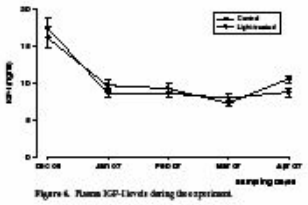

Figure 6. Plasma IGF-I levels during the experiment.

Conclusions

No significant difference in growth between light group and control group.

Growth of fish is according to the growth model for Atlantic cod.

IGF-I growth hormone in cod blood can be measured by the RIA method.