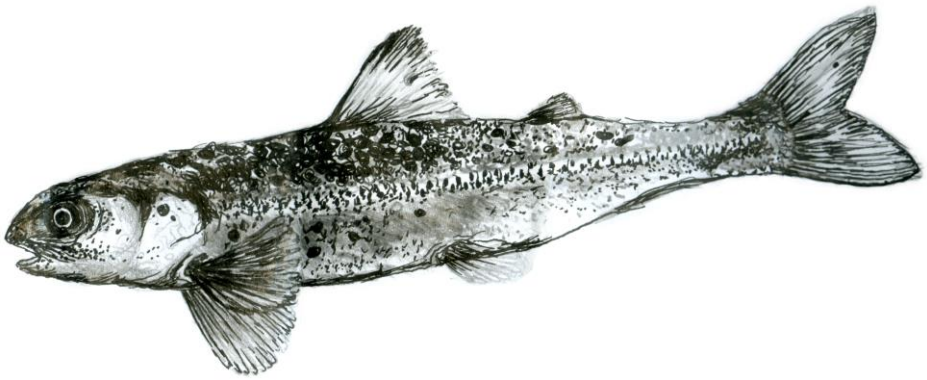
The effect of low temperatures and photoperiods on growth in Atlantic salmon (*Salmo salar*)



Thesis for fulfilment of the degree

Master of Science in Aquaculture biology

Inge Døskeland

Department of Biology

University of Bergen

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Front page drawing by Solveig Sanden Døskeland

Forord

Å arbeide med denne oppgaven har vert utviklende. Forsøket har krevd ulike typer ressurser fra det praktiske i konstruksjon av deler til karforsøket, styring av vannparameter og laboratoriearbeid, til faglige vurderinger og statistikk og analyse. Særlig takk til veilederne Sigurd Handeland, Albert Imsland og Sigurd Stefansson.

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Inge Døskeland

Contents

Abstract

This thesis examines the growth response of Atlantic salmon post-smolt (*Salmo salar*) in a factorial experiment with three temperatures and two light regimes. The aim of this study was to investigate under laboratory conditions the interaction between photoperiod and temperature in order to make recommendations on the use of additional cage light under low temperatures in Northern Norway.

The experimental part of the study was conducted at the High Technology Centre in Bergen in the period from October 15th 2013 until March 17th 2014.

1140 post-smolt (96 g SE ± 3.1) were distributed in six groups, and exposed to 4.3 (4), 6.5 (6) and 9.3 (9) °C, and either natural light regime of Tromsø (LDN, N 69° 40`) or LDN 24:0. Each group consisted of two replicate tanks for a total of 12 tanks. Subsets of 20 fish in each replicate, approximately 240 fish in total, were individually tagged to follow individual growth responses.

Growth was measured as increase in weight and fork length from the start of the experiment to four time points including the end of the experiment at day 145. Feed intake was monitored during the last 4 weeks of the trial period. Blood glucose, Na^+, HCO_3^-, CO_2 partial pressure, dorsal fin area, heart weight, liver weight and gill tissue were also sampled or measured in order to identify physiological and welfare effects of photoperiod and temperature treatments. Samples for measurement of filet quality were also taken (by Dr. Bjørn Roth, NOFIMA Stavanger) and are partly presented in this thesis.

The fish exposed to low temperature and natural light regime (4LDN) had a significantly lower growth (26 % less in overall SGR) than the 4LL group, corresponding to the effect of approx. 1.2 °C temperature increase. Fish in the 6 °C and 9 °C groups did not show any significant growth benifit of continuous light (LL). Compared to the 4LDN group, the 4LL group showed overall higher condition factor, higher total feed conversion efficiency, lower levels of blood Na^+ and lower hepato-somatic and cardio-somatic indexes. A negative correlation between growth rate and filet hardness was observed, but no direct correlation between temperature and light was shown.

1. Introduction

Background

The Atlantic salmon (*Salmo salar*) aquaculture industry has a particularly important role in Norway. The industry produced in 2013 a total of 1.2 billion tons of fish at a value of 37.5 billon NOK (NDF, 2013). Historically the industry was primarily located in the western and central parts of Norway (Hovland & Møller, 2010). To better utilize available area for an increasing production, more activity has been localized at high latitudes in Northern Norway above the Arctic Circle. Fish farming in high latitude areas may give shorter growth seasons and longer production cycles (Koskela, Pirhonen, & Jobling, 1997). Long, cold and dark periods are common in wintertime, and Northern Norwegian salmon farming is carried out under a yearly cycle of "midnight sun" in summer and midday moon in dark winters. In southern Norway slaughtering may start in early summer due to good winter growth, while this is less profitable in the north where one is more dependent on a longer production time in order to regain lost winter growth (Bjorn Roth et al., 2005). These sub-optimal production conditions in that northern region are particularly related to photoperiod and temperature. Because salmon are ectothermic, ambient temperature has a controlling effect on their rate of growth and feed consumption (Klemetsen et al., 2003). In the Atlantic salmon smolt industry, manipulation of environmental parameters such as photoperiod and temperature (in land based facilities) are commonly used to enhance growth in order to attain market size as quickly as possible (S. Handeland & Stefansson, 2001). This study is part of the "Nordlys" project (Regional research fund North Norway) aiming at development of new production protocols for optimization of quality and production of salmon in Northern Norway. Present study examines growth rate, feed conversion, filet quality, allometry of selected organs and selected physiological welfare parameters.

Growth mechanisms

Effects of temperature

Temperature is the central controlling factor for growth, and will boost metabolic rates and hence increase the efficiency of food energy transformation to net biomass development (Brett & Groves, 1979; Elliott, 1982; A. K. Imsland & Jonassen, 2001; Jøsrgensen, Johansen, & Jobling, 1997; Pörtner et al., 2001; Van Ham et al., 2003). Higher temperatures will increase oxygen consumption due to higher metabolism and increased activity (Groot, 2010; A. K. Imsland, A. Folkvord, & S. O. Stefansson, 1995; Jonassen et al., 2000). In fish, growth

usually increases proportionally to the increase of water temperature, until an optimum temperature is reached (Austreng, Storebakken, & Åsgård, 1987; Brett & Groves, 1979; Forsberg, 1995). Temperature optima (Topt) for growth will differ with species, age and size (McCauly, 1979) and specific growth rate (SGR, % day^{-1}) increases with temperature until reaching maximum growth (Nytrø et al., 2014). In Atlantic salmon, Handeland, A. K. Imsland, and S. O. Stefansson (2008) suggest an optimum temperature for growth of 12.8 °C for 70–150 g and 14.0 °C for 150–300 g post-smolts. Below the optimum temperature, growth rate approximates the linear equation: G=m+nT were T is water temperature and m and n are coefficients (M Jobling, 1983; Ricker, 1979). Temperature for optimal feed conversion is generally below optimum temperature for SGR. After optimal feed conversion is reached, an increase in appetite will still result in increased growth until maximum SGR is achieved (S. O. Handeland, Björnsson, Arnesen, & Stefansson, 2003). At low temperatures relevant to this study, both growth and appetite decrease and eventually cease (Brett & Groves, 1979; Elliott, 1991; M Jobling & Baardvik, 1994). The relative influence of temperature on the smaller fish as used in this experiment is also greater than that on larger fish (Glencross & Felsing, 2006).

Effects of photoperiod

Numerous studies have shown effects of light as both a modulator of growth, a timer of development (zeitgeber) and a growth stimulator in fresh and seawater (Boeuf & Le Bail, 1999; Bromage, Porter, & Randall, 2001; Handeland et al., 2008; S. O. Handeland, Porter, Björnsson, & Stefansson, 2003; Stephen D McCormick & Saunders, 1987). The growth enhancing effect of continuous light (LL) has been reported for *Salmo salar* (Sigurd O. Handeland et al., 2003; Krakenes, Hansen, Stefansson, & Taranger, 1991; S. D. McCormick, Moriyama, & Bjornsson, 2000; Stefansson et al., 1991).

Stefansson et al. (1991) and Taranger et al. (1999) discuss that continuous light increased growth rates in seawater. Positive growth effects of light have also been shown in marine fish, for example turbot *Scophthalmus maximus* (A. K. Imsland, Folkvord, Jónsdóttir, & Stefansson, 1997), Atlantic cod, *Gadus morhua* (Otterlei, Nyhammer, Folkvord, & Stefansson, 1999) and Atlantic halibut, *Hippoglossus hippoglossus* (Simensen, Jonassen, Imsland, & Stefansson, 2000). Furthermore the effect of continuous light on growth and inhibition of sexual maturation has been comprehensively investigated (Boeuf & Le Bail, 1999; Porter, Duncan, Handeland, Stefansson, & Bromage, 2001). Due to these demonstrated effects of photoperiod, it is particularly interesting in this study to identify the extent to which

light can compensate for the growth disadvantages associated with rearing in low temperatures during the posts-molt sweater phase.

Temperature and photoperiod interactions

There is a paucity of literature studying the effect of interactions between temperature and photoperiod at the post-smolt stage of Atlantic salmon in seawater. However, (A. Imsland, Handeland, & Stefansson, 2014) reported a growth-enhancement in fresh water of photoperiod treatment alone for LL corresponding to a 4.5 ℃ increase in water temperature. Kråkenes, Hansen, Stefansson, and Taranger (1991) observed increase in growth rate in groups (one year old, 1 + smolts of Atlantic salmon) subjected to additional continuous light in sea water and suggest this may be caused by a direct photo-stimulation of growth as well as an alteration of seasonal growth patterns. It was therefore a task for the present experiment to expand knowledge towards lower temperatures in combination with different photoperiods. While a positive relationship between day length and temperature on growth has been reported in Atlantic salmon in freshwater (Solbakken, Hansen, & Stefansson, 1994), it appears that there is a less pronounced seasonal light and temperature adaption on growth in several marine species (Hallaråker, Folkvord, & Stefansson, 1995). The interactive effects of temperature and photoperiod can cause a shift in the optimum temperature for growth when the photoperiod is altered for Atlantic turbot (A. K. Imsland & Jonassen, 2001). This may be explained by the relatively stable temperature regime in the ocean, thus reducing the selective pressure for such adaptations (A. K. Imsland & Jonassen, 2001). Further, the growth-promoting effect of continuous light has been shown to be inversely related to temperature for turbot (A. K. Imsland, A. Folkvord, & S. Stefansson, 1995) and Atlantic halibut (Norberg, Weltzien, Karlsen, & Holm, 2001). It was therefore a task for this experiment to expand knowledge towards lower temperatures in combination with different photoperiods.

Seasonal effects on filet quality

Salmon filet is the main end product in Norwegian fish farming, but growth as such is not enough if quality is compromised. Flesh quality is a complex set of characters involving factors such as texture, chemical composition, color and fat content (Fauconneau, Alami-Durante, Laroche, Marcel, & Vallot, 1995). Firmness in relation to fiber size and distribution is a major factor influencing acceptability of raw fish products and is therefore important for characteristics like hardness of fish flesh (Veland & Torrissen, 1999). In teleost fish, muscle growth is characterized by its high plasticity, and may be altered by a wide range of environmental and endogenous signals (Larsen, Imsland, Lohne, Pittman, & Foss, 2011). The

influence of temperature on muscle texture hardness has been studied in Atlantic salmon and is known to decrease during summer months (Espe et al., 2004; Bjorn Roth et al., 2005). The impact of temperature and light on these mechanisms depends on the affected life stages, as reviewed by Rowlerson and Veggetti (2001). The effect of season may overshadow endogenous rhythms and affect quality (Bjorn Roth et al., 2005). Ian A Johnston et al. (2003) studied Atlantic salmon during their first sea winter and found significantly higher numbers of fast muscle fibers and a shift in the distribution of fiber diameter in groups reared at continuous light compared with groups reared at natural daylight at the same temperature, while no effect on hypertrophy was found. These authors added that an effect of continuous light on muscle fiber recruitment was obtained only during a discrete seasonal window of decreasing day length, and that these effects may be enhanced or inhibited by changing the timing of light treatment. It is therefore interesting to consider how muscle hardness as an expression of fillet quality, is affected by different light regimes at sub optimal temperatures.

Physiological and welfare indicators

Abrupt changes in blood parameters linked to hydro-mineral balance, acidity and metabolism might indicate changes in fish physiology and welfare, and therefore have implications for growth. In ectothermic animals ambient temperature variations directly influence cellular biochemistry and thus the physiology of the organism (Barton, 2002). Physical and chemical influences such as temperature, feeding regime and oxygen levels/water flow may disturb equilibrium and homeostatic state in fish in relation to stress (Hosfeld, Hammer, Handeland, Fivelstad, & Stefansson, 2009). Stress related factors may disrupt the hydro-mineral balance and can be assessed by measuring blood ion (sodium and potassium) levels (Sakamoto, McCormick, & Hirano, 1993). In salmonids, development of seawater tolerance is correlated with higher activity of the enzyme gill Na^+, K^+ -ATPase, the primary enzyme for excretion of plasma Na^+ and Cl^- (Stephen D McCormick, Saunders, Henderson, & Harmon, 1987). High circulating blood sodium in sea-water may indicate reduced ability to maintain homeostasis and suggest an osmoregulatory challenge to newly smoltified salmon (Cnaani, McLean, & Hallerman, 2013). Furthermore Polakof, Panserat, Soengas, and Moon (2012) and Cnaani et al. (2013) describe a variety of physiological and environmental conditions where glycemic changes clearly indicate the sensitivity of blood glucose levels in fish species. Major increases in glycaemia are induced during seasonal osmoregulatory changes, the presence of different stressors, and shifts in dietary composition (Polakof et al., 2012). Glucose levels have been shown to be a typical secondary stress response (after plasma cortisol) (Bonga, 1997). Acid-

base disturbances (pH) in fish occur under stressful environmental conditions such as abrupt temperature changes, hypercapnia, hypoxia etc. (Morris, 1989). The bicarbonate buffering system is an important buffer system in the acid-base homeostasis. In this system carbon dioxide (CO_2) combines with water (H_2O) to form carbonic acid (H_2CO_3), which in turn rapidly dissociates to form hydrogen ions (H^+) and bicarbonate (HCO_3^-) (Wikimedia Foundation, 2015).

Differential growth rates of in example heart and liver in relation to body weight, and dorsal fin index, may give additional information on rearing conditions influencing stocking density welfare between treatment groups (Hosfeld et al., 2009; Person-Le Ruyet & Le Bayon, 2009; Pettersen et al., 2014).

Monitoring of selected blood parameters and organ indexes throughout present experiment may in sum be seen as an indicator of fish welfare and homeostasic challenges induced by the experimental conditions and adaption to low sea temperatures.

Objectives

The aim of this study was to study the combined effect of two photoperiod regimes, continuous light (LL) and simulated natural photoperiod (LDN, Tromsø) at low temperatures (4. 6 and 9 °C) on growth, feeding parameters, selected organ indexes and blood physiology in post smolt (size interval 85-250 g) Atlantic salmon. The experiment can be seen as a direct follow-up of A. Imsland et al. (2014) (reporting a growth-enhancement effect of LL treatment in FW corresponding to a 4.5 °C increase in temperature for smolt/post-smolt ranging from approx. 15 to 500 g), by investigating if corresponding results are also valid for sub-optimal SW temperatures, fish size outside the maturation window, and high contrast photoperiod (LDN, Tromsø). Furthermore the aim was to monitor selected blood physiological responses (hydro-mineral, acid-base and metabolic status) as indicators of fish welfare. Flesh samples were also taken by NOFIMA Stavanger to uncover possible differences in filet quality between treatment groups based on muscle hardness.

The experiment was based on the following alternative hypotheses:

HA1: Growth will be stimulated by LL photoperiod at low temperatures in seawater

HA2: Welfare indicators (blood parameters, organ indexes, feed uptake) will differ between LL/LDN photoperiods and high and low temperatures

HA3: Filet quality (muscle hardness) will be affected by the combined photoperiods and temperatures

Where H0 being that temperature and photoperiod have no significant effect on the parameters above.

2 Material and methods

Fish stock

Atlantic salmon smolt arrived at Bergen High Technology Centre, Bergen, Norway on October 15th 2013 (n= 1140). Mean length of the fish was 20.2 cm (standard error of mean, SE 0.2) and mean weight 96.0 g (SE 3.1), total biomass 98.0 kg, and the fish were distributed among 12 400 l tanks. The origin of the batch was the commercial hatchery Sjøtroll Havbruk located in municipality of Fitjar, location Kjærelva. Before arrival the fish were kept in fresh water at 13.6 °C and continuous light (LL) (APPENDIX I: Fish stock and rearing conditions, hatchery data sheet).

Experimental setup

Tank setup and initial handling

The experiment was carried out at the BIO lab at the Bergen High Technology Centre (BHTC) room 11 and 12 from October 15th 2013 to March 17th 2014. All tanks were thoroughly cleaned and supplied with a flow of 5 l min^{-1} freshwater before fish handover. Tank circulation was provided through a perforated PVC riser tube positioned similarly and parallel to tank wall in all tanks for optimum circulation and self-cleaning effect. Tank flow was measured with precise timing of two 4 liter samples and adjusted during the whole experiment to compensate for increase in biomass. Similarity between tank setups was sought in order to avoid consequences for growth and development. (Millidine, Armstrong, & Metcalfe, 2006).

On arrival, the fish were randomly distributed with 94 -95 fish into 12 1-meter square gray fiberglass tanks (Blia tanks, Askøy, Norway), containing approx. 400 l each in room 11 and 12 (APPENDIX I, fig. II). Tanks were supplied with freshwater at 9.4 °C (ambient temperature). Initial photoperiod was simulated natural photoperiod for Bergen (LDN N 60° 25`). The fish were gradually transferred to natural saltwater approx. 32‰ during week 42. Flow rate was adjusted to 8 l min^{-1}.

Tagging procedure

On October 16th a selection of 240 representative fish, 20 from each tank, were identified for individual tagging (Floy Tag Inc., Seattle USA). Prior to tagging and measurements, the fish were anaesthetized with Metacain (3 ml l^{-1} stock solution, Argent Laboratories, Redmond

USA). Tags were inserted beneath dorsal fin. Precise individual weight and length measurements were carried out. After tagging fish were placed in an intermediate tank for recovery before being put back in the experimental tanks. Tagged fish were evenly distributed in all 12 tanks with no significant size difference in tagged individuals (Appendix II. TABLE XVI). No fish were lost during tagging (Tab. 3.1.)

TABLE 2.1. Overview biometric condition at start of experiment

Initial biometric data tagged fish				
Number of fish N	Total biomass kg	Mean weight g	Mean length cm	Condition-factor
240	**20.68**	**86.2 SE ± 3.1**	**20.2 SE ± 0.2**	**1.05 SE ± 0.01**

Temperature management

Water system input temperature was automatically adjusted and logged through the Visual Vigo system provided by Sterner Aquatech AS (Oslo, Norway) and managed by ILAB Bergen. Individual tank temperatures were established on September 21st through three header tanks, two in room 11 and one in room 12. The header tank in room 12 supplied tank no. 11 and 12. The tanks in room 11 were divided in two separate chambers allowing two different temperatures. Mixing of water from the two header tanks was necessary to achieve the third temperature (6, 4 °C) in tanks 5 and 6 (APPENDIX I fig. I).

All groups were replicated. Temperatures are rounded to nearest degree (4, 6 and 9 °C) for further discussion and results in this thesis, and referred to as 4LDN, 4LL, 6LDN, 6LL, 9LDN and 9LL.

TABLE 2.2. Overview measured tank temperatures (°C) through experiment.

Temperatures (°C) through experiment						
	4LDN	4LL	6LDN	6LL	9LDN	9LL
Mean °C	4.5	4.4	6.5	6.7	9.3	9.4
N tot	95	95	95	95	95	95
Means ±SD	1.05	1.06	0.70	0.65	0.40	0.38
Min °C	4.1	4.1	5.4	5.7	8.5	8.8
Max °C	9.5	9.5	9.5	9.4	11.1	11.1

Oxygen

Input water oxygen saturation was managed and logged continually in OxyGuard software, supplied by Sterner Aquatech. In order to control oxygen levels, a feedback loop was set up in room 11, continuously monitoring levels and supplying extra oxygen in all four header tanks

based on sensory data from one tank at each temperature. This system was not available in room 12 were adjustments were manually administrated.

TABLE 2.3 Overview measured tank oxygen saturation % O_2 through experiment.

Oxygen saturation % O2 through experiment						
	4LDN	4LL	6LDN	6LL	9LDN	9LL
Mean %	82.1	81.9	81.4	77.3	83.8	82.1
N tot	80	80	80	80	80	80
Means ±SD	4.3	4.2	4.8	8.9	5.0	4.4
Min %	75.0	73.5	71.5	63.5	73.5	70.0
Max %	94.0	91.5	93.0	101.0	93.0	95.5

Light

Final photoperiod was set for all tanks September 21st. Six tanks, two for each temperature, were adjusted to continuous light (LL), and the other six tanks were adjusted to LDN, natural light period for Tromsø (N 69° 40`) (Tab. 2.4.). Light output, dimming and shut off were controlled through the Visual Vigo software supplied by Sterner Aquatech.

Individual tank light was supplied by one halogen lamp (12V35W Hidoa Lite Spot 6500) positioned in center of the tank lid. Each lamp was cleaned weekly in order to prevent salt buildup and possible light output reduction. Room light was also turned off during nighttime in order to prevent stray light in LDN tanks. Actual light input in tanks containing fish was measured in all tanks at the end of the experiment March 17th, 2014 by using a submerged photo meter (I-COR LI-193SA Spherical Quantum sensor) (TABLE 4.2.).

TABLE 2.4. In tank light mesurement. µmol m^{-2} s^{-1} light output measured March 17th. Sensor placed at bottom of each tank containing fish. Measurement was carried out by selecting the highest value of twenty measurements during a 20 second period in each tank.

Light measurement												
Tank no.	1	2	3	4	5	6	7	8	9	10	11	12
µmol m^{-2} s^{-1}	**13.60**	**13.40**	**14.90**	**16.80**	**17.75**	**12.01**	**18.10**	**15.27**	**22.94**	**18.50**	**7.40**	**20.20**

Feeding procedures

The fish were feed with standard commercially available feed pellets from EWOS AS (Florø, Norway) "SMOLT 30" thought the experiment.

Feed was delivered by automatic screw feeders (Arvo-Tec Oy, Finland) during daytime. These were calibrated and tested at regular intervals during the experiment. Timing, calibration and amount of feed were programmed in Visual Vigo software. Amount of feed was adjusted according to biomass development, temperature and visual inspection in order to always feed approx. 10% in surplus and apparent satiation. Feed was only administrated during light period. Initially all groups were feed 100 g (divided in 3 daily intervals).

Feed consumption was measured manually and administrated in the period from January 29th to February 19th (Table APPENDIX. VIII). Specific amount of feed was delivered twice a day between 08:00-09:00 and 14:00-15:00. Feed was measured using a calibrated feeding cup for each of the three temperature groups. During this part of the experiment (22 days) the 9 °C groups were fed 329 g, the 6 °C groups 212 g and the 4 °C groups 111 g day^{-1}, corresponding to approx. 2%, 1.5% and 1% of biomass, respectively.

Waste feed was collected one hour after each feeding (i.e. at 10:00 and 16:00) by sieving flush water collected from bottom of tank, through tubing, and into purpose build flow through collection boxes. Excess feed was gently removed from sieve and poured into individual bowls for drying and subsequent weight measurements (Sartorius BC 1500 S, Goettingen, Germany). Excess water was drained from bowls and samples were dried to constant weight in drying oven for 24 hours in order to establish dry feed weight. Collection was carried out within short time (approx. 1 hour) after morning and afternoon feeding in order to avoid crushed and dissolved pellets.

Daily routines

Daily, in interval from 10:00 to 13:00

- In tank temperatures were logged manually using a calibrated (± 0.1 °C) OxyGuard Handy Gamma (Blokken, Denmark) and checked against the Visual Vigo system.
- In tank oxygen levels were measured manually using OxyGuard Handy Gamma (Blokken, Denmark) and checked against the Visual Vigo system.
- System oxygen sensor membranes were cleaned.
- Temperature and flow adjustments were carried out manually using room inlet mixing panel and/or tank inlet valves on tank lids.
- Header tank level and flow were inspected and adjusted manually.

- All fish were visual inspected for welfare and behavior. Dead or seriously injured fish were removed.
- Excess feed was cleaned by tank flushing. Flushing was carried out twice a day during period of feed collection.

Sampling routines

Initial sampling of fish status from the same batch and delivery of smolt was carried out by Bergen University College (BUC) 13 days after arrival (September 28th) (ref. Camilla Hosfeld, BUC and Sara Calabrese, Marine Harvest ASA). Methods and materials used for this baseline sampling were exact replicate of protocol used for the rest of the experiment. Since biometric and blood and tissue sampling were not performed at same intervals, days from start (Tx) are related to first sampling.

The experiment established a schedule with two separate sampling procedures: the biometric measurement part (length, weight of tagged fish and total biomass) and the blood sample, fin measurement and tissue collecting part.

Weight and length sampling of tagged fish and biomass measurement (biometric data) started September 16th 2013 (T0: day 0), and then in interval T1: day 42, T2: day 83, T3: day 124 and ended at T4: day 145, March 17th 2014.

Blood and tissue collecting schedule started September 28th 2013 (T0: day 0), and then in interval T1: day 30, T2: day 71 and ended at T3: day 113 February 18th 2014.

Sampling procedures

Biometric sampling

Tagged fish were selected consecutively from tank no. 1 room 11 to tank no. 12 room 12.

- Water level in fish stock tank was reduced in order to transfer fish. Fish sieved into smaller tank in order to select tagged individuals.
- Groups of four tagged fish anaesthetized (Metacain, 0.05 g l^{-1}, exposure time 30–45 s)
- Visual inspection of fish in order to identify possible injuries
- Weight and length measurement

- Total biomass weight measured for each tank including tagged fish
- Tagged fish returned to original stock tank

Blood and tissue sampling

Prior to sampling all Eppendorf tubes (a total of 1296 during experiment) were color coded and numbered.

Sampling was carried out following this routine at each of the measuring point (Fig. 2.1)

- A random sample of 6 untagged fish were removed from each tank, anaesthetized (Metacain) and killed by a blow to the head
- Blood were collected into heparinized syringes from the caudal peduncle
- Blood sample was split in two parts ("A" = yellow sample and "B" = blue sample) (Fig. 2.1)
- "A" blood sample was used for immediate i-STAT 1 (http://www.abbottpointofcare.com/Customer-Info-Center/User-Documentation.aspx) analysis. The i-STAT was used with single use cartridges (Abbott i-Stat EC8+) for in vitro quantification analyses in whole blood. The unit was calibrated and tested before each sampling. Measured blood components were: Na^+, Cl^-, Glucose, hematocrit, pH level, partial pressure of CO_2 (pCO_2), bicarbonate HCO_3^- and hemoglobin. In this thesis Na^+, Glucose, pCO_2 and bicarbonate HCO_3^- were used. The i-STAT instrument is intended for human blood samples with respect to temperature and blood physiological properties. Measured values will therefore not be absolutely correct, but are expected to give adequate relative accuracy between groups of fish.
- "B" blood samples were put in Eppendorf tubes, put on ice, and centrifuged (pre cooled centrifuge at 4 °C, 4000 rpm). Plasma was frozen at minus 80 °C for possible later investigations.
- Heart and liver were removed by scalpel and weighted using calibrated weight (Sartorius BC 1500 S, Goettingen, Germany). Data from end of experiment, (day 113), was chosen to fully leverage delayed growth.
- One slice of the liver and second anterior left gill arch were cut off and fixed in RNAlater (Life Technologies, by Thermo Fisher CA, USA) for possible later investigations. Gill sample was cut by scissor and split in two parts and fixed in two different Eppendorf tubes stored in ice filled polystyrene boxes (approx. 4 °C). One

sample for RNAlater was refrigerated and set for storage in freezer at minus 80 °C after 24 hours. Second gill sample was SEI buffer fixed and stored directly in -80 C.

- Fork length (to nearest 0.1 cm) and weight (to nearest 0.1 g) of the were measured by using calibrated measure board and weight (Sartorius BC 1500 S, Goettingen, Germany)
- Both height and length of dorsal fin were measured using an analog slide caliper.

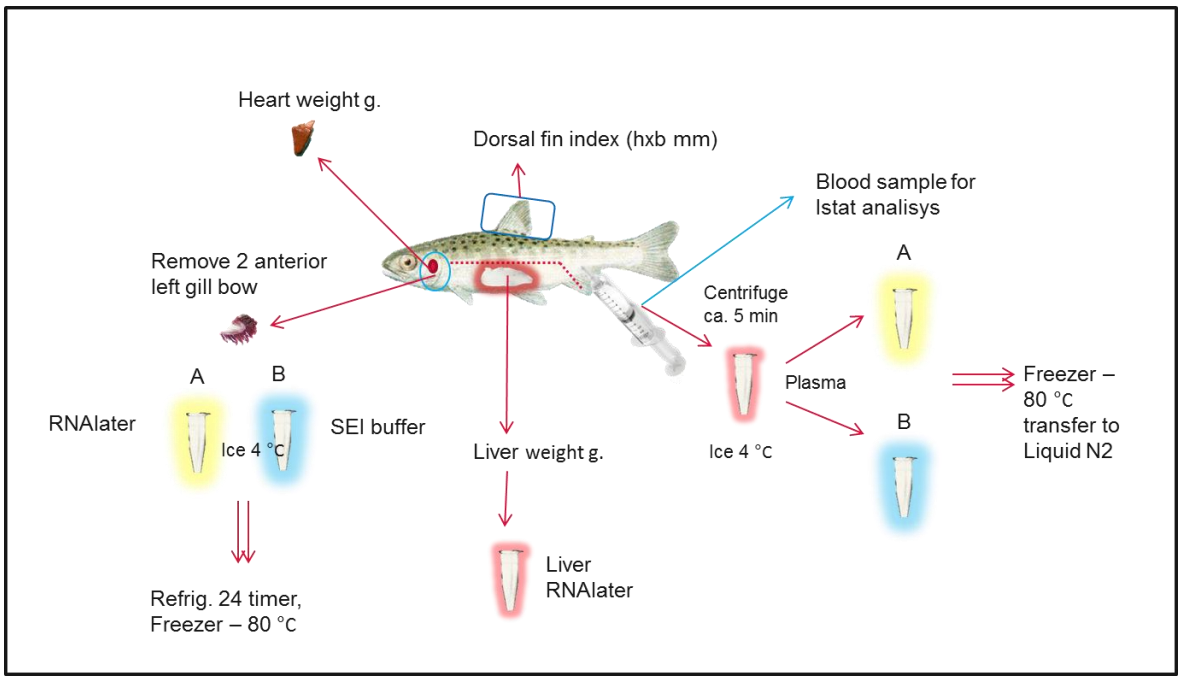

FIGURE 2.1. Setup for tissue and blood sampling

Analytical methods

Texture hardness

The filet samples were collected, filet texture and properties were measured and this method was described by Dr. Bjørn Roth, Nofima Processing Technology, Stavanger. On March 10th information on hardness, breaking strength and profile were obtained using a Texture Analyzer (TA-XT®-plus Texture Analyzer, Stable Micro Systems, Surrey, UK) with a load cell of 25 kg. A flat-ended cylinder (12.5 mm) was used as test probe. Seven days after collection a puncture test was assessed in 2 locations on the Norwegian quality cut (NQC, NS 1975) directly on the fillets (skin on) transverse to the muscle fiber orientation. The probe was programmed to penetrate 80 % into the initial fillet height and max forces were recorded in addition to forces at 20, 40 and 60 % compression (B. Roth, Oines, S., Rotabakk, B.T., Birkeland, S., 2008). The speed of the probe was set to 1 mms^{-1}. The fracture (fracturability)

was defined by the peak force occurring before fracturing, and hardness (N) as the highest force recorded during the first compression cycle (Bourne, 1977).

Growth and biomass calculations

The condition factor (CF) was calculated as

$CF=100\ WL^{-3}$

Where W is the weight (g) and L is the length (cm) of the fish.

Specific growth rate (SGR) was calculated according to the formula of Houde and Schekter (1981):

$$SGR = (e^{g} - 1)100$$

Were g is the instantaneous growth coefficient defined as $g = (\ln W_2 - \ln W_1)\ (t_2 - t_1)^{-1}$ and W_2 and W_1 are mean wet weights for individually tagged fish in g at days t_2 and t_1.

Feed consumption (FC) was calculated by using the formula:

$$FC = b/((W_2 + W_1)/2)$$

Were W_2 is fish weight at end of experiment, W_1 is fish weight at start and b is dry weight feed eaten.

Feed conversion efficiency (FCE) was calculated by using the formula:

$$FCE = (W_2 - W_1)/b$$

Were W_2 is fish weight at end of experiment, W_1 is fish weight at start and b is dry weight feed eaten.

Cardio-somatic index (CSI) and/or hepato somatic index (HSI) were calculated by using the formula:

$$HSI = (LW * BW)/100$$
$$CSI = (HW * BW)/100$$

Were LW is liver weight, HW is heart weight and BW is body weight in g

Dorsal fin index was calculated by using the formula:

$$FI = ((b*h)/2)/(L*100)$$

Were b is length at dorsal fin base and h is dorsal fin height and L is fork length in mm.

Statistical methods

All statistical analyses were conducted using the STATISTICA™ software ("STATISTICA," 2013). Before statistical analysis, normality of distributions was examined by using Kolmogorov Smirnov test (J. Zar, 1984). Homogeneity of variances among the different groups was tested using the Levenes test (Brown & Forsythe, 1974). Possible differences between replicates were tested with one way ANOVA and replicates combined in case of non-significant ANOVAs. The effects of different temperature and photoperiod combinations on growth, blood chemistry and organ indexes were analyzed by applying a two-way factorial ANOVA (J. Zar, 1984). To locate differences among treatments and time periods for each parameter, significant ANOVAs were followed by a Student-Newman-Keuls (SNK) multiple comparison post hoc test (J. Zar, 1984). A linear regression was used to test the relationship between filet texture hardness (y) and SGR (period 1 – 4) (x). A significance level of $\alpha=0.05$ was used if not otherwise stated. All data in tables and figures are given as means ± standard error of mean (SEM).

Results of statistical tests and data for all FIGURES are shown in APPENDIX II.

3 Results

Mortality

Mortality was low during the experiment. A total of 7 tagged dead fish were removed. In addition 4 of the non-tagged fish died (TABLE 3.1). This totals 11 dead fishes during the experiment period. Fish were removed in order to maintain fish welfare based on fin abrasion and "looser" tendencies. There were no systematic tendencies in mortality related to temperature and photoperiods.

TABLE 3.1. Total mortality for tagged and untagged fish during experimental period.

			Day no.	*0-83*	*83-124*	*124-145*	*Cause*
Tank no.	*Group*	*Tag no.*					
1	*9LDN*	*55008*		*X*			*Not identified*
7	*4LDN*	*46649*				*X*	*Removed 1 feb*
7	*4LDN*	*46652*				*X*	*Removed 22 jan*
8	*4LL*	*46621*				*X*	*Not identified*
8	*4LL*	*46639*				*X*	*Removed 28 jan*
10	*9LL*	*55085*			*X*		*Removed 28 dec*
10	*9LL*	*55098*				*X*	*Removed 22 jan*
2	*9LL*				*X*		*Removed* 27 dec
7	*4LDN*			*X*			*Removed* 4 nov
7	*4LDN*				*X*		*Removed* 28 dec
12	*6LL*				*X*		*Removed* 30 nov
SUM				*2*	*4*	*5*	

Biometric results

Weight

Initial mean weights ranged between 82.6 g to 89.4 g at start and did not vary between the experimental groups (Fig. 3.1.). After 83 days, the two 4 ℃ groups had significantly lower mean weight than the two 9 ℃ groups and the 6LDN group (SNK post hoc test, $P<0.05$), and the 6LDN group had significantly higher weight than the 6LL group ($P < 0.05$). At day 124, both 4 ℃ groups had a significantly lower weight than all other groups ($P < 0.05$). There was a significant effect of photoperiod at 4℃ from day 83 until end of experiment at day 145 (two-way factorial ANOVA, $P < 0.05$). At day 145, all temperature groups had a significantly different mean weight (two-way factorial ANOVA, $P < 0.05$). Only the 4 ℃ group had a positive significant effect of the LL photoperiod ($P< 0.05$).

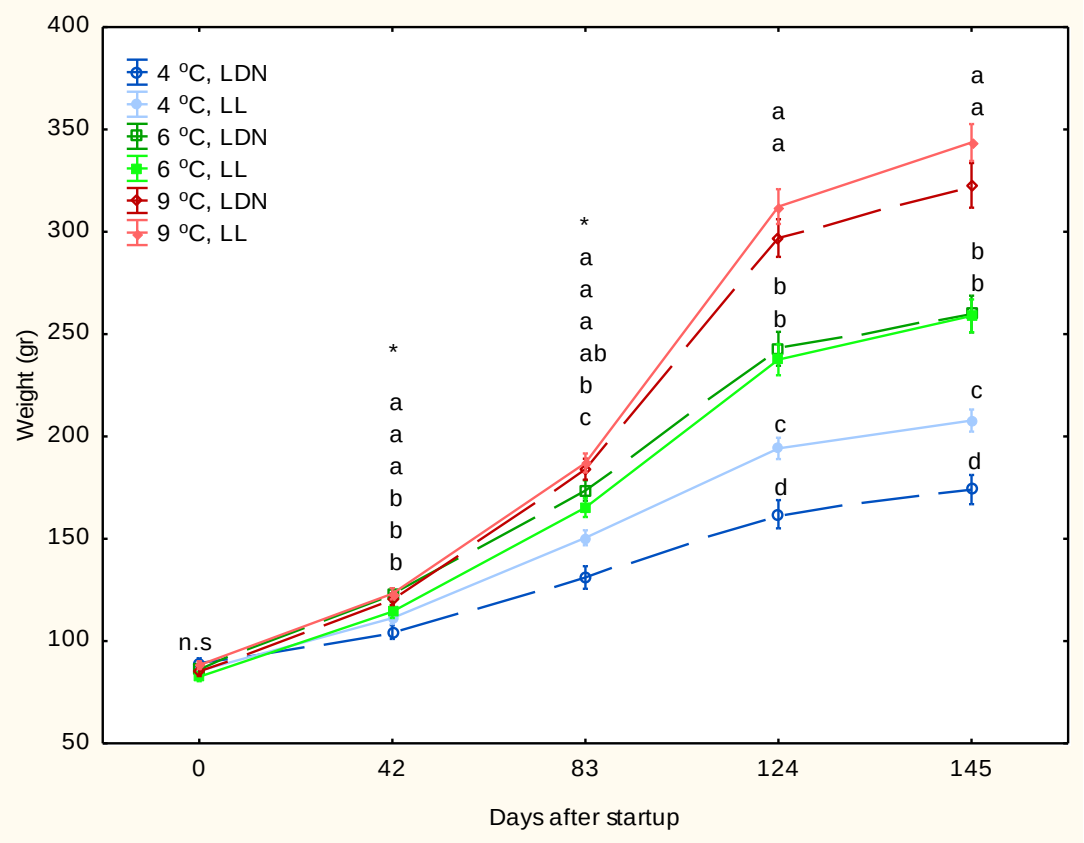

FIGURE 3.1. *Weight development of PIT tagged juvenile Atlantic salmon reared at two photoperiods (LDN = simulated natural photoperiod for Tromsø and LL= continuous light) and three temperatures (4, 6 and 9 °C). The three temperature groups and two light regimes are separated by color, symbol and line type. Broken line = LDN, solid line = LL. Blue line = 4 °C and circle symbol, green line = 6 °C and square symbol and red line = 9 °C and diamond symbol. Vertical whiskers indicate standard error of mean (SEM). Different letters represent significant differences between temperature and light groups between treatments (SNK P<0.05).ns = non-significant.* = significant interaction (Two-way crossed ANOVA P<0.05 between photoperiod and temperature.*

Length

No significant length differences were seen between groups at start of the experiment (Fig. 3.2.). At day 42, the two 4 °C groups had significant shorter length than the 9 °C groups and the 6LDN group (SNK post hoc test, $P < 0.05$), whereas the 6LL group did not show different length development than any other group ($P < 0.05$). At day 83, both 4 °C groups had shorter length than all other groups. At the same day, the length of the 4LDN group was lower than the 4LL group and the 9 °C groups were significantly longer than the 6LL group ($P < 0.05$). At day 124, all temperature groups show significantly different length (two-way factorial ANOVA, $P < 0.05$). The 4LDN group was significantly shorter than the 4LL group (SNK post hoc test, $P < 0.05$) from day 83 onwards. Length of the 4LDN group was significantly affected by photoperiod from day 83 to 145 ($P < 0.05$). No further changes were seen throughout the study.

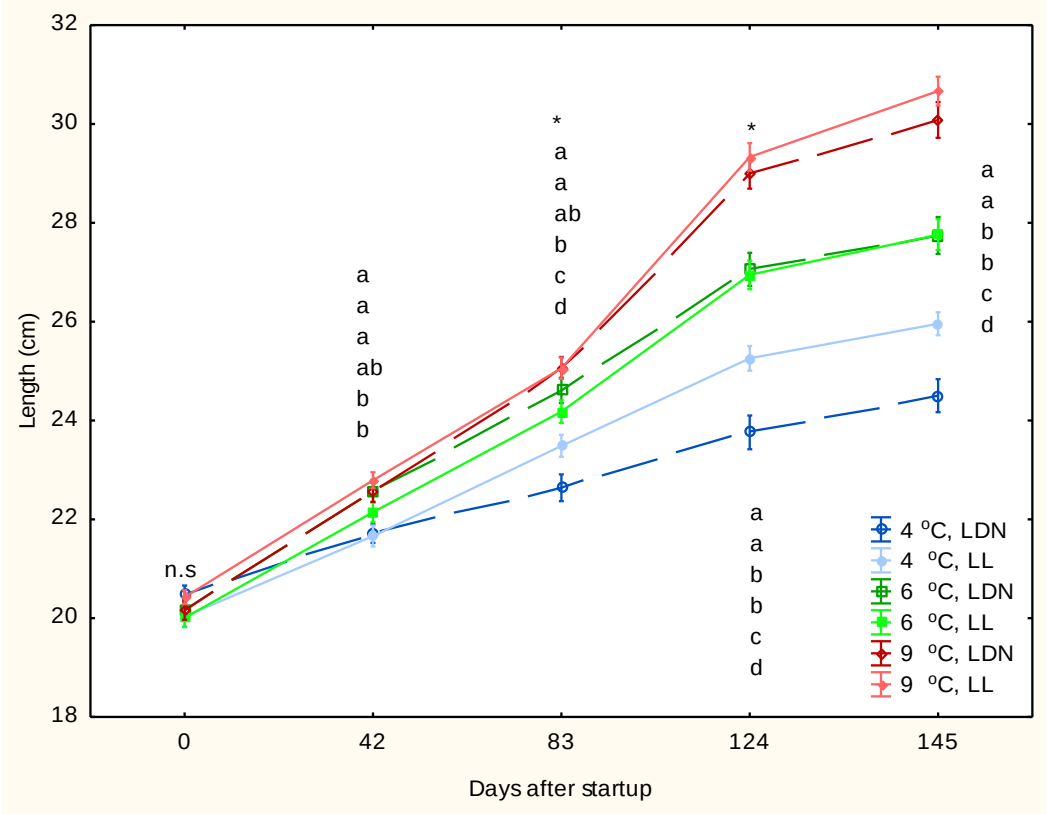

FIGURE 3.2. *Length development of PIT tagged juvenile Atlantic salmon reared at two photoperiods (LDN = simulated natural photoperiod for Tromsø and LL= continuous light) and three temperatures (4, 6 and 9 °C). The three temperature groups and two light regimes are separated by color, symbol and line type. Broken line = LDN, solid line = LL. Blue line = 4 °C and circle symbol, green line = 6 °C and square symbol and red line = 9 °C and diamond symbol. Vertical whiskers indicates standard error of mean (SEM). Different letters represent significant differences between temperature and light groups between treatments (SNK P<0.05).ns = non-significant. * = significant interaction (Two-way crossed ANOVA P<0.05 between photoperiod and temperature.*

Condition factor

There were no initial differences in condition factor (CF) between any groups (Fig. 3.3.). At day 42, the 4LL group had significantly higher CF than all other groups (SNK post hoc test, $P< 0.05$). In contrast, the 4LDN group showed a lower CF than the 6LL group and 9 °C groups ($P<0.05$). Further, at day 83 there were no significant differences between groups except the 4LDN group which was significantly lower than all other groups ($P < 0.05$). An overall significant increase in CF was observed in all groups between days 42 to 124 (two-way factorial ANOVA, $P < 0.05$). At day 124 and 145, CF leveled out and there were no significant differences between groups.

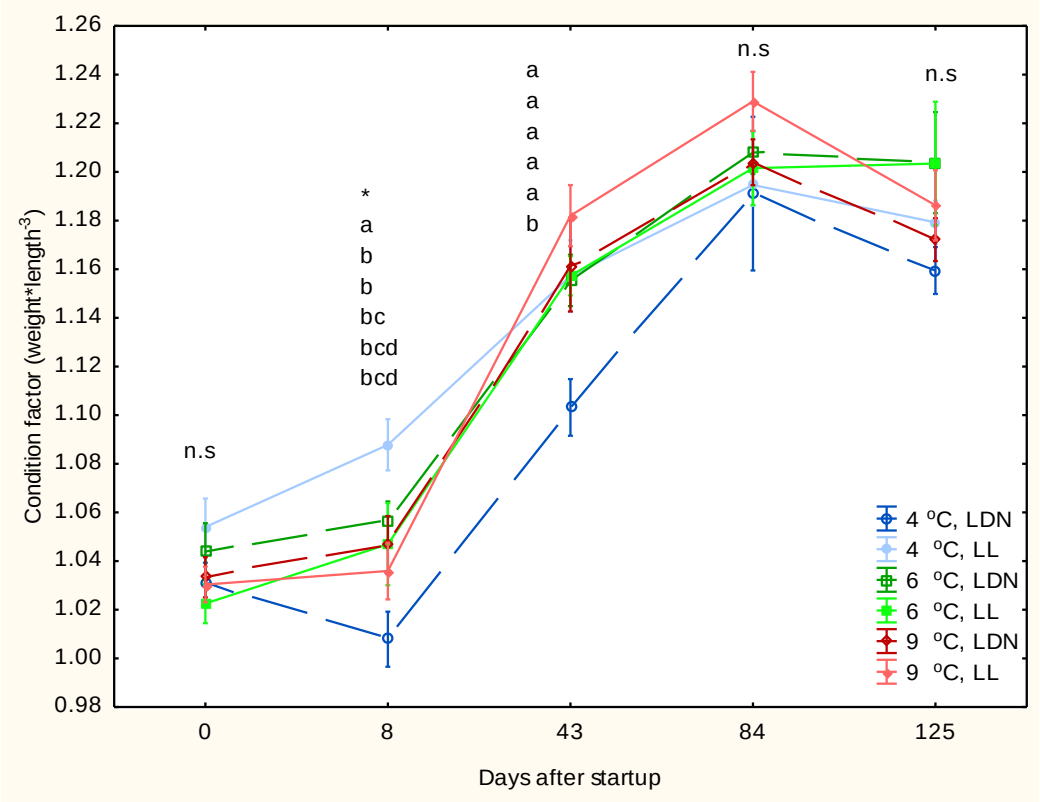

Figure 3.3. *Condition factor development of PIT tagged juvenile Atlantic salmon reared at two photoperiods (LDN = simulated natural photoperiod for Tromsø and LL= continuous light) and three temperatures (4, 6 and 9 °C). The three temperature groups and two light regimes are separated by color, symbol and line type. Broken line = LDN, solid line = LL. Blue line = 4 °C and circle symbol, green line = 6 °C and square symbol and red line = 9 °C and diamond symbol. Vertical whiskers indicates standard error of mean (SEM). Different letters represent significant differences between temperature and light groups between treatments (SNK P<0.05).ns = non-significant. * = significant interaction (Two-way crossed ANOVA P<0.05 between photoperiod and temperature.*

Specific growth rate (SGR)

In the first period, from day 0 to day 42, the 4LDN group had significantly lower SGR than all other groups (SNK post hoc test, $P< 0.05$), (Fig. 3.4.). The 4LL group had a significantly higher growth rate than the 4LDN group ($P < 0.05$), but was also lower compared to both the 6 and 9 °C groups ($P < 0.05$). Significant effect of photoperiod was only seen in the low temperature 4 °C group (two-way factorial ANOVA, $P < 0.05$). Highest growth rate at 1.25 % day^{-1} was observed for fish between approximately 250 – 300 g in the 9LL group between day 83 to 124. The lowest growth rate was observed in the 4LL group with approximately 0.5% day^{-1} in the last period from day 124 to 145 of the experiment.

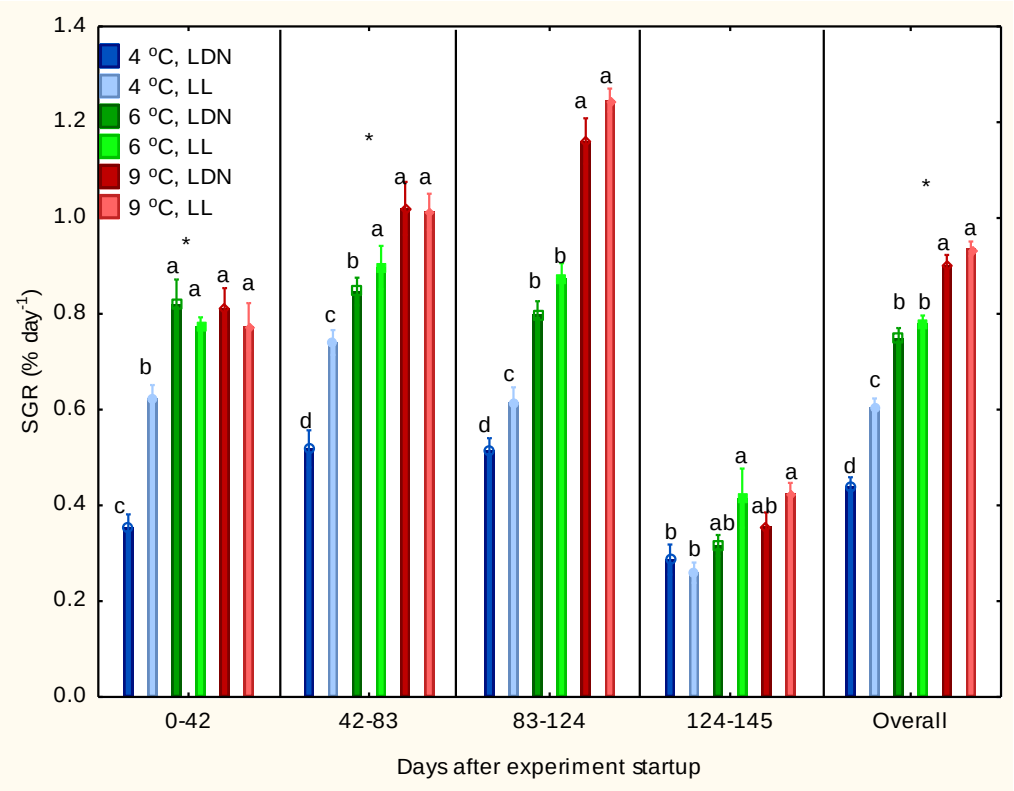

Figure 3.4. *Specific growth rate development of PIT tagged juvenile Atlantic salmon reared at two photoperiods (LDN = simulated natural photoperiod for Tromsø and LL= continuous light) and three temperatures (4, 6 and 9 °C). The three temperature groups and two light regimes are separated by bar color and box symbol. Heavy colour = LDN, light colour = LL. Blue bar = 4 °C and circle symbol, green bar = 6 °C and square symbol* ***and*** *red bar = 9 °C and diamond symbol. Vertical whiskers indicates standard error of mean (SEM). Different letters represent significant differences between temperature and light groups between treatments (SNK P<0.05). * = significant interaction (Two-way crossed ANOVA P<0.05 between photoperiod and temperature.*

From day 42 to 83, the two 6 °C groups had a significantly lower growth rate than the two 9 °C groups (SNK post hoc test, P<0.05). The 4 °C group showed 77.1% growth enhancing effect of continuous light (LL) between day 0 to 42, versus only 36.4 % for the experiment period overall.

The 4 °C groups were still the only groups showing a significant difference in growth rate related to photoperiod (two way factorial ANOVA, $P < 0.05$). In the third period, day 83 to 124, there was a significant effect of temperature for all groups, whereas effect of photoperiod was only seen at 4°C ($P < 0.05$). In the last period, from day 124 to 145, there was a significant reduction in growth rate for all groups (SNK post hoc test, $P< 0.05$). In this period none of the groups had an effect of photoperiod. Overall, for the whole project period and for each time interval, the interaction effect of photoperiod and temperature was seen for all groups (two way factorial ANOVA, $P < 0.05$). The effect of photoperiod alone was only seen at 4°C ($P < 0.05$).

Feed consumption (FC) and feed conversion ratio (FCE)

Feed consumption

The figures for daily feed consumption show stepwise increase in values for the 4, 6 and 9 °C groups (Fig. 3.5.). During the sampling period, between January 8th and February 19th 2014 (42 days), total feed consumption increased with temperature and was 0.15, 0.13, 0.24, 0.23, 0.38, and 0.38 in the 4LDN, 4LL, 6LDN, 6LL, 9LDN and 9LL respectively. There was no clear indication of photoperiod effect.

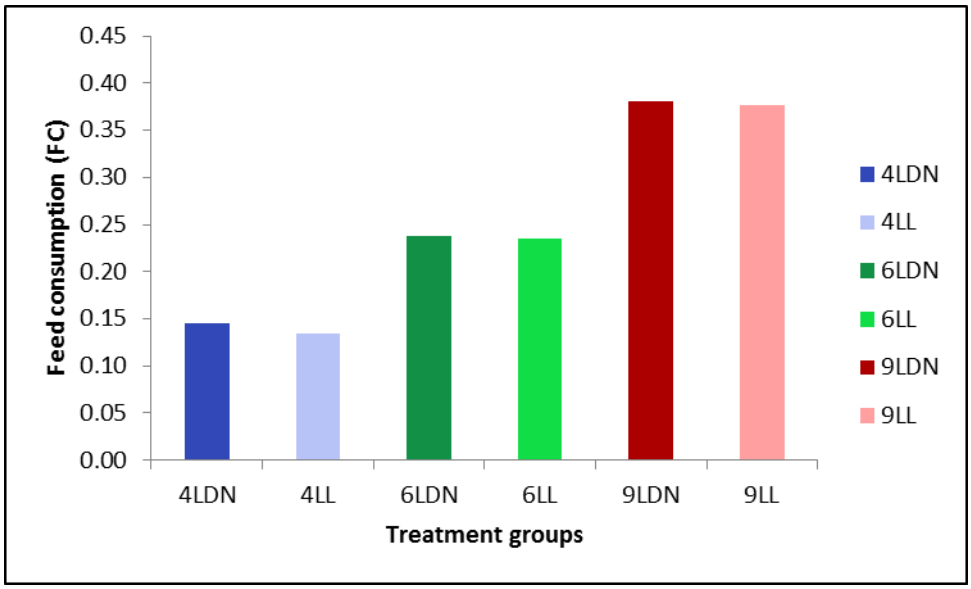

FIGURE 3.5. *Feed consumption, for 42 days between January 18th to February 19th 2014 juvenile Atlantic salmon reared at two different photoperiods (LDN= simulated natural photoperiod for Tromsø and LL= continuous light) at three temperatures (4, 6 and 9 °C). The three temperature groups and two light regimes are separated by color.*

Feed conversion efficiency

Feed conversion efficiency values indicate a marked difference between the 4LL and the 4LDN groups (Fig. 3.6.). During the sampling period, between January 8th to February 19th 2014 (42 days), feed conversion efficiency was 0.83, 1.21, 0.84, 0.89, 0.64 and 0.66 in the 4LDN, 4LL, 6LDN, 6LL, 9LDN and 9LL respectively.

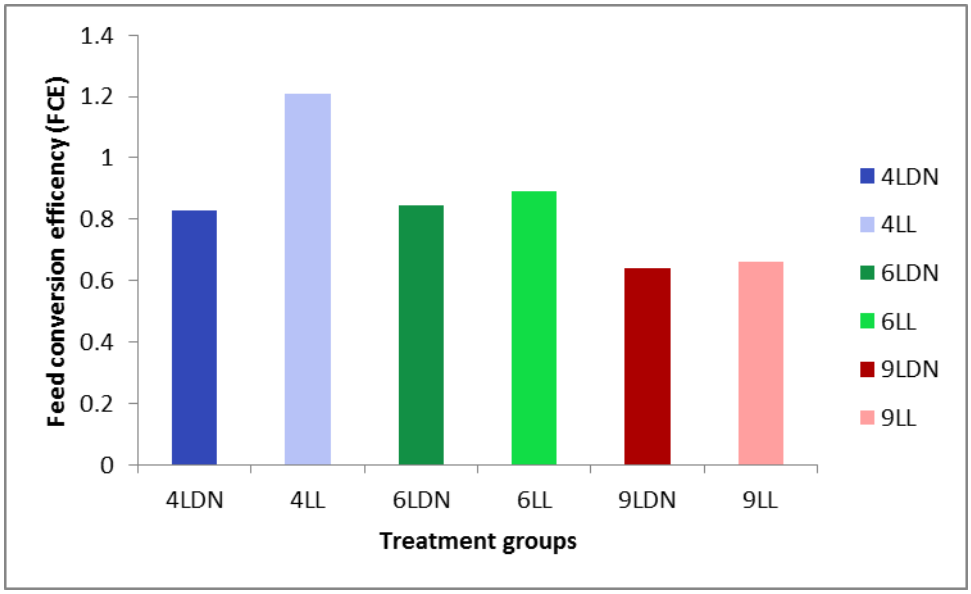

FIGURE 3.6. *Feed conversion efficiency (FCE) 42 days between January 8th to February 19th 2014. Juvenile Atlantic salmon reared at two different photoperiods (LDN= simulated natural photoperiod for Tromsø and LL= continuous light) at three temperatures (4, 6 and 9 °C). The three temperature groups and two light regimes are separated by color.*

Blood chemistry

At the start of the experiment, photoperiod and temperature regimes were not established, and therefore no significant differences between groups for any of the measured parameters were present at day 0.

Blood glucose

After 30 days, significantly lower plasma glucose levels were seen in the two 4 °C groups (SNK post hoc test, $P < 0.05$) (Fig. 3.7.), but no differences were seen after that. All groups except the 4LDN group had a significant rise in plasma glucose from start of experiment until day 30 ($P < 0.05$). From day 30 to 71 the two 4 °C groups had a significant increase, while the two 9 °C groups had a significant decline in values ($P < 0.05$). From day 71 to day 113 all groups displayed declining glucose levels, although only significant for the 6 °C groups and the 9LDN group ($P < 0.05$).

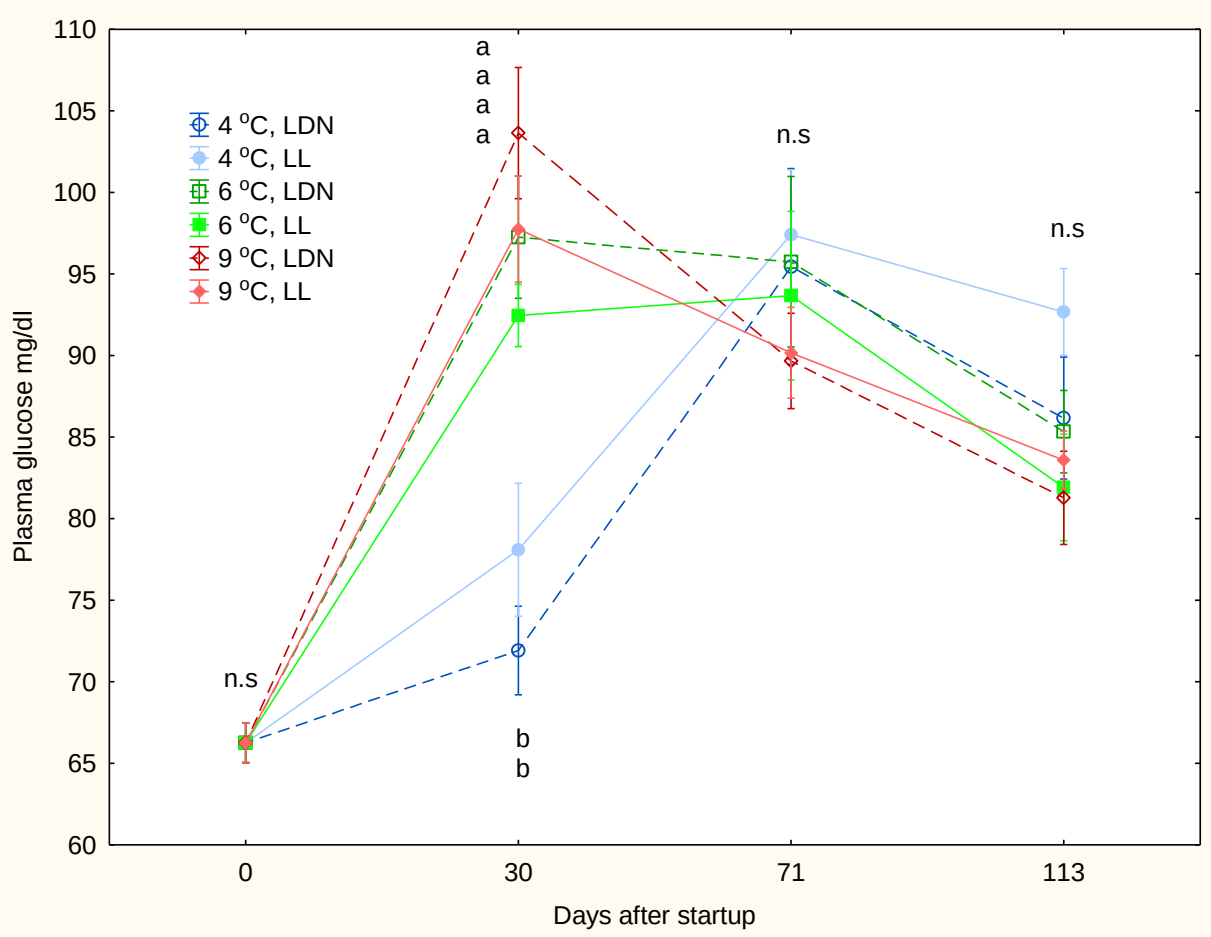

FIGURE 3.7. *Blood glucose levels of selected juvenile Atlantic salmon reared at two different photoperiods (LDN= simulated natural photoperiod for Tromsø and LL= continuous light) at three temperatures (4, 6 and 9 °C). The three temperature groups and two light regimes are separated by color, symbol and line type. Broken line = LDN, solid line = LL. Blue line = 4 °C and circle symbol, green line = 6 °C and square symbol and red line = 9 °C and diamond symbol. Vertical whiskers indicates standard error of mean (SEM). Different letters represent significant differences between temperature and light groups between treatments (SNK P<0.05). ns = non-significant.*

Blood sodium ions (Na^+)

No significant differences between groups were found at day 30 (SNK post hoc test, $P < 0.05$), (Fig. 3.8.). At day 71, the two 4 °C groups had significantly higher blood sodium ion levels compared to the other groups ($P < 0.05$). At day 113, the 4LDN group had higher levels than all other groups ($P<0.05$).

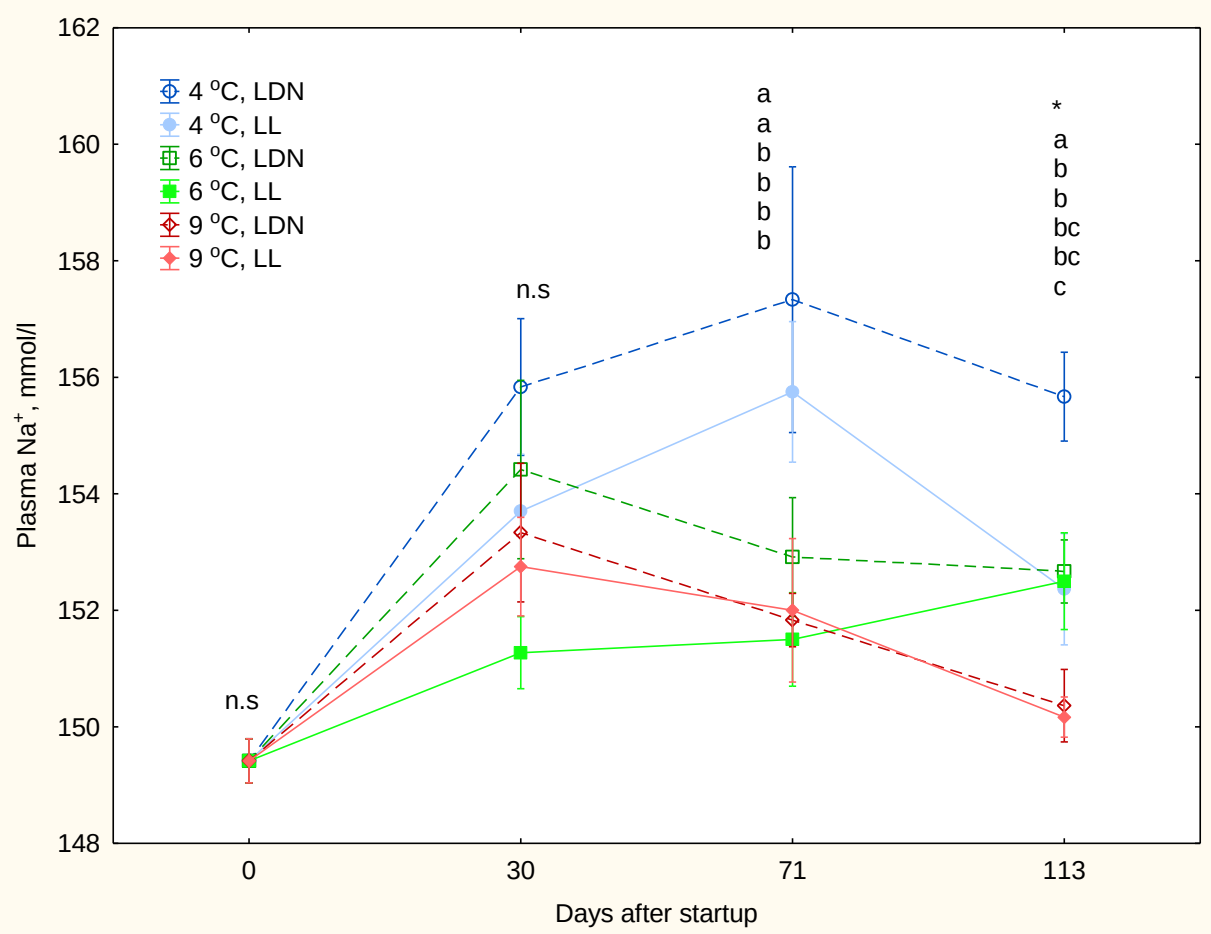

FIGURE 3.8. *Blood Sodium ion (Na^+) levels of selected juvenile Atlantic salmon reared at two different photoperiods (LDN= simulated natural photoperiod for Tromsø and LL= continuous light) at three temperatures (4, 6 and 9 °C). The three temperature groups and two light regimes are separated by color, symbol and line type. Broken line = LDN, solid line = LL. Blue line = 4 °C and circle symbol, green line = 6 °C and square symbol and red line = 9 °C and diamond symbol. Vertical whiskers indicates standard error of mean (SEM). Different letters represent significant differences between temperature and light groups between treatments (SNK P<0.05). ns = non-significant. * = significant interaction (Two-way crossed ANOVA P<0.05) between photoperiod and temperature.*

Blood HCO_3^-

At day 30 of the experiment, there was a significant difference in plasma HCO_3^- levels between the 9LL group and the 6LL group (SNK post hoc test, $P < 0.05$), (Fig. 3.9.). At day 71, the 9LDN group showed a significantly higher plasma HCO_3^- levels than all groups except the 9LL group ($P < 0.05$). At day 113, the 9LL and 9LDN groups showed significantly

higher values than all other groups ($P < 0.05$). The 6LL group had a significant reduction in plasma HCO_3^- level from start of the experiment until day 30 (two-way factorial ANOVA, $P < 0.05$). There was also a significant reduction for the 9LDN and 4LL groups from day 71 to day 113 ($P < 0.05$).

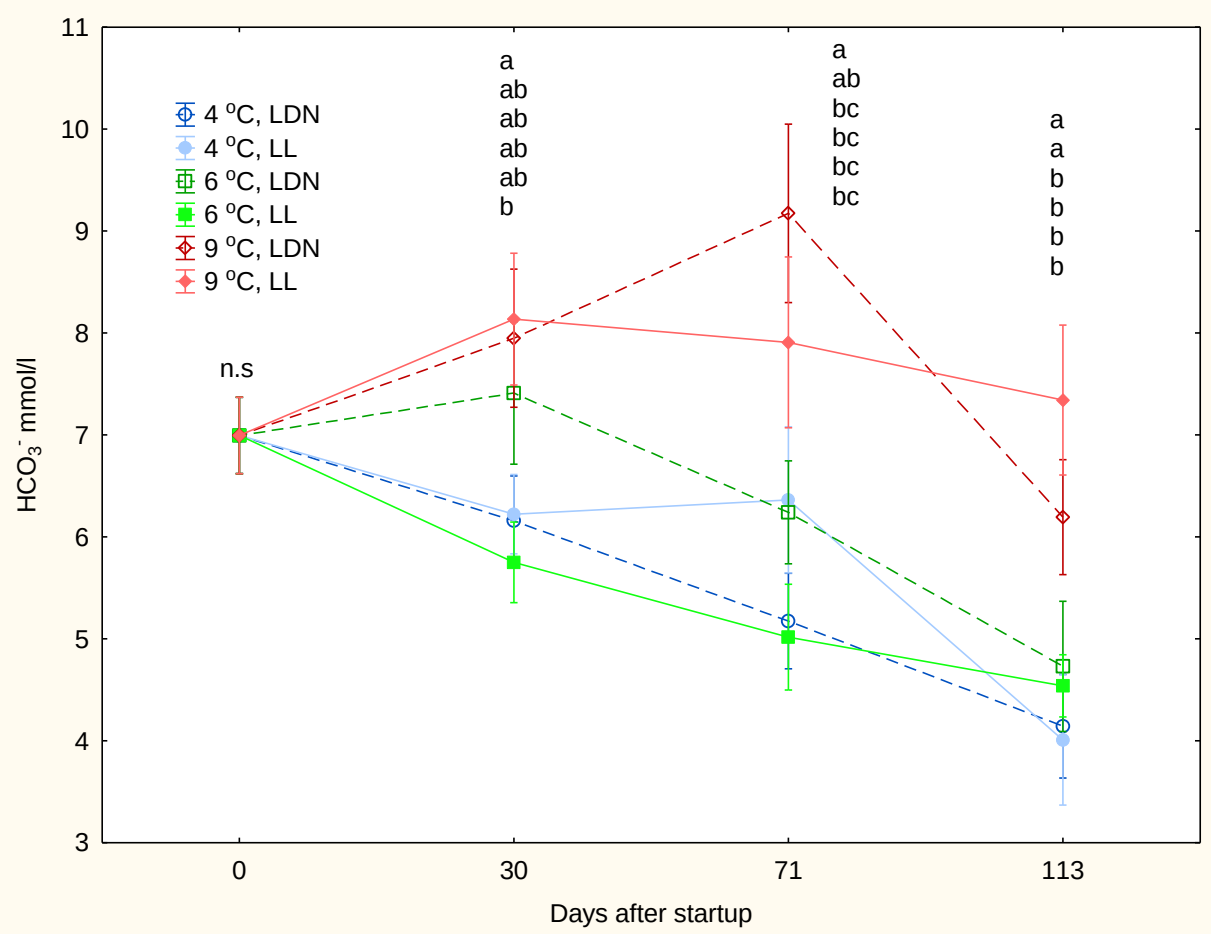

Figure 3.9. *Blood HCO_3^- levels of selected juvenile Atlantic salmon reared at two different photoperiods (LDN= simulated natural photoperiod for Tromsø and LL= continuous light) at three temperatures (4, 6 and 9 °C). The three temperature groups and two light regimes are separated by color, symbol and line type. Broken line = LDN. solid line = LL. Blue line = 4 °C and circle symbol, green line = 6 °C and square symbol and red line = 9 °C and diamond symbol. Vertical whiskers indicates standard error of mean (SEM). Different letters represent significant differences between temperature and light groups between treatments (SNK P<0.05). ns = non-significant.*

Blood CO_2 partial pressure (pCO_2)

During the experimental period, the two 9 ℃ groups had significantly higher levels than all other groups (SNK post hoc test, $P < 0.05$), (Fig. 3.10). Further, there was a significant reduction in blood pCO_2 for the 4 ℃ groups and the 6LL group from day 0 to 71 ($P < 0.05$). For the rest of the experiment all groups had a pCO2 reduction. This trend was significant for the 9 °C groups and the 4LL group from day 71 to day 113 ($P < 0.05$).

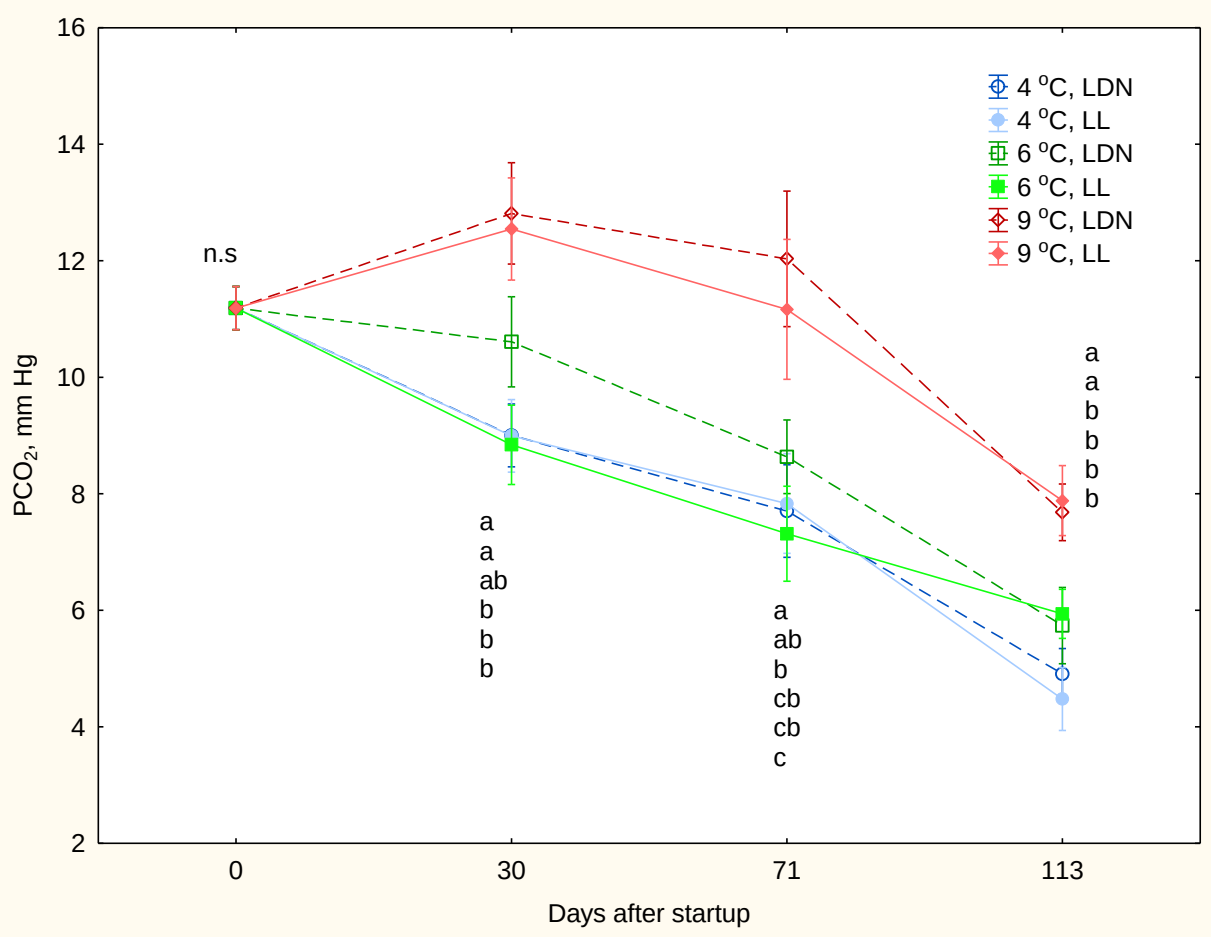

Figure 3.10. *Blood CO_2 partial pressure of selected juvenile Atlantic salmon reared at two different photoperiods (LDN= simulated natural photoperiod Tromsø and LL= continuous light) at three temperatures (4. 6 and 9 °C). The three temperature groups and two light regimes are separated by color, symbol and line type. Broken line = LDN, solid line = LL. Blue line = 4 °C and circle symbol. green line = 6 ℃ and square symbol and red line = 9 ℃ and diamond symbol. Vertical whiskers indicates standard error of mean (SEM). Different letters represent significant differences between temperature and light groups between treatments (SNK P<0.05).ns = non-significant.*

Indexes

Hepato-somatic index

At day 113, mean observed hepato-somatic indexes were 1.75, 1.47, 1.29, 1.36, 1.29 and 1.32 in the 4LDN, 4LL, 6LDN, 6LL, 9LDN and 9LL respectively (SNK post hoc test, $P < 0.05$), (Fig. 3.11.). Overall, the 4LDN group showed a significantly higher hepato-somatic index than all other groups ($P < 0.05$).

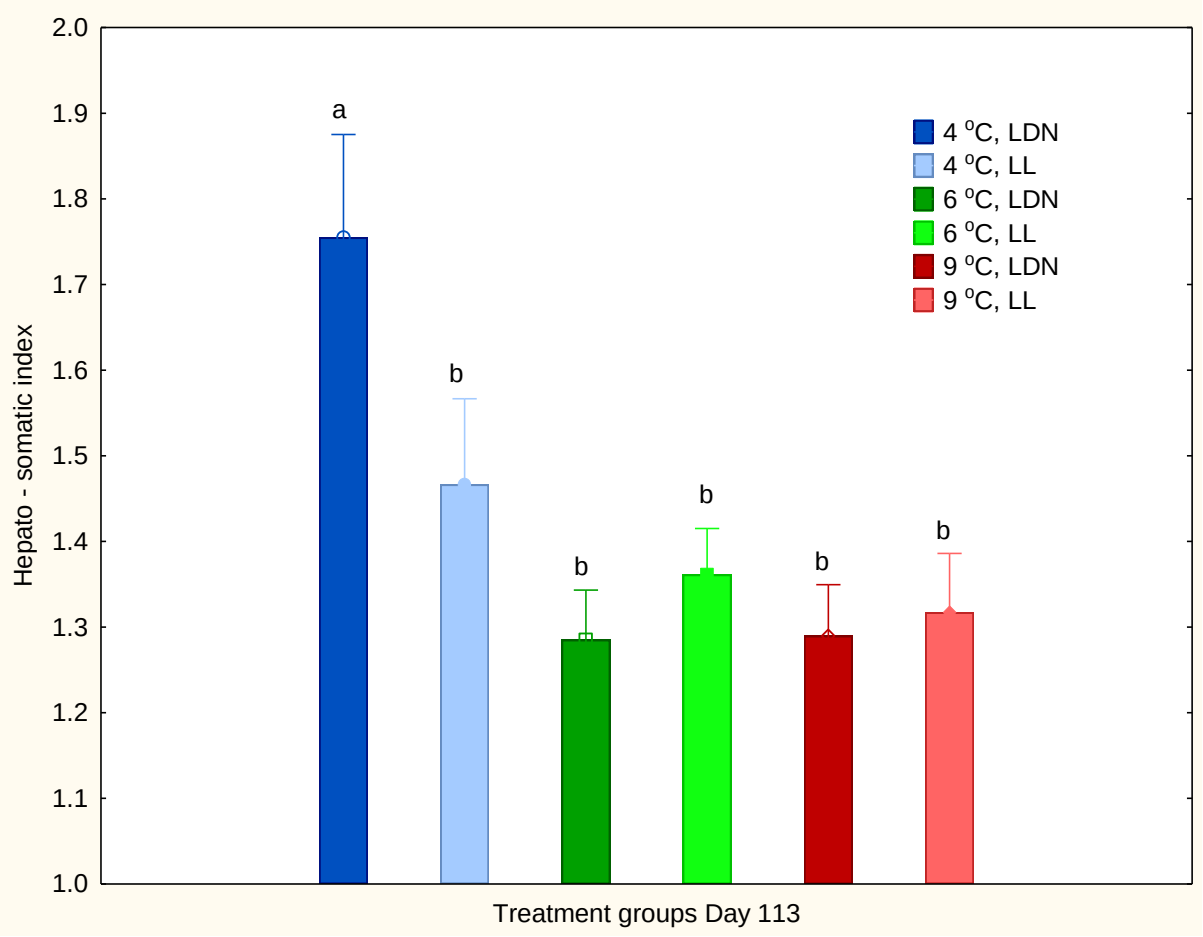

Figure 3.11. *Hepato-somatic index of sampled juvenile Atlantic salmon reared at two different photoperiods (LDN= simulated natural photoperiod Tromsø and LL= continuous light) at three temperatures (4, 6 and 9 °C at day 113 of the experiment). The three temperature groups and two light regimes are separated by color. Blue bar = 4 °C, green line = 6 °C and red line = 9 °C and diamond symbol. Heavy color = LDN and light color = LL. Vertical whiskers indicates standard error of mean (SEM). Different letters represent significant differences between temperature and light groups between treatments (SNK P<0.05).*

Cardio-somatic index

At day 113, mean observed cardio-somatic indexes were 0.15, 0.14, 0.13, 0.14, 0.14 and 0.13 in the 4LDN, 4LL, 6LDN, 6LL, 9LDN and 9LL respectively (SNK post hoc test, $P < 0.05$), (Fig. 3.12.). The 4LDN group had a significantly higher cardio-somatic index than all other groups ($P < 0.05$).

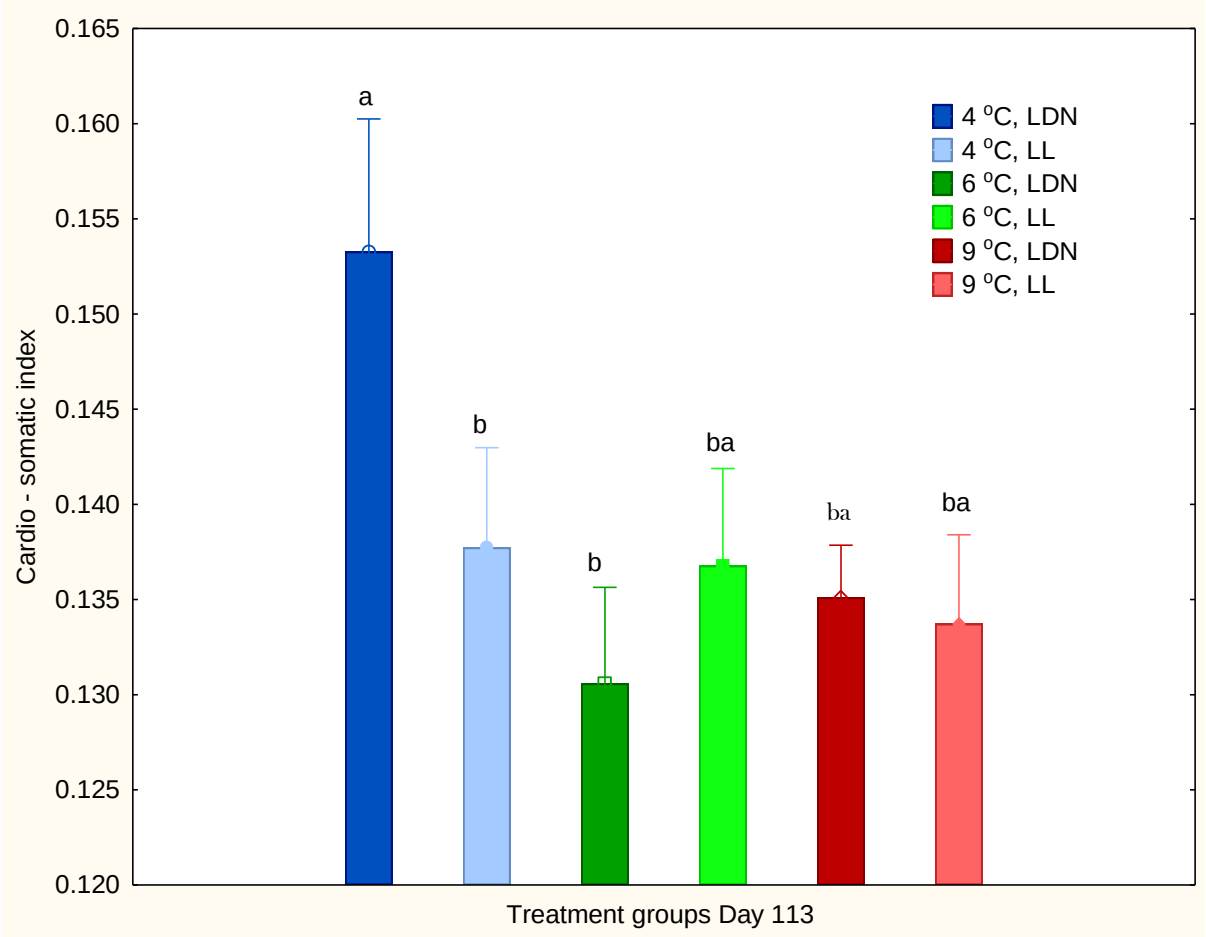

Figure 3.12. *Cardio-somatic index of sampled juvenile Atlantic salmon reared at two different photoperiods (LDN= simulated natural photoperiod Tromsø and LL= continuous light) at three temperatures (4, 6 and 9 °C at day 113 of the experiment). The three temperature groups and two light regimes are separated by color. Blue bar = 4 °C, green line = 6 °C and red line = 9 °C and diamond symbol. Heavy color = LDN and light color = LL. Vertical whiskers indicates standard error of mean (SEM). Different letters represent significant differences between temperature and light groups between treatments (SNK P<0.05).*

Dorsal fin index

At day 113, mean observed dorsal fin-indexes were 0.27, 0.30, 0.30, 0.31, 0.30 and 0.27 in the 4LDN, 4LL, 6LDN, 6LL, 9LDN and 9LL respectively (Fig. 3.13.). No significant differences between temperature or photoperiod groups were seen.

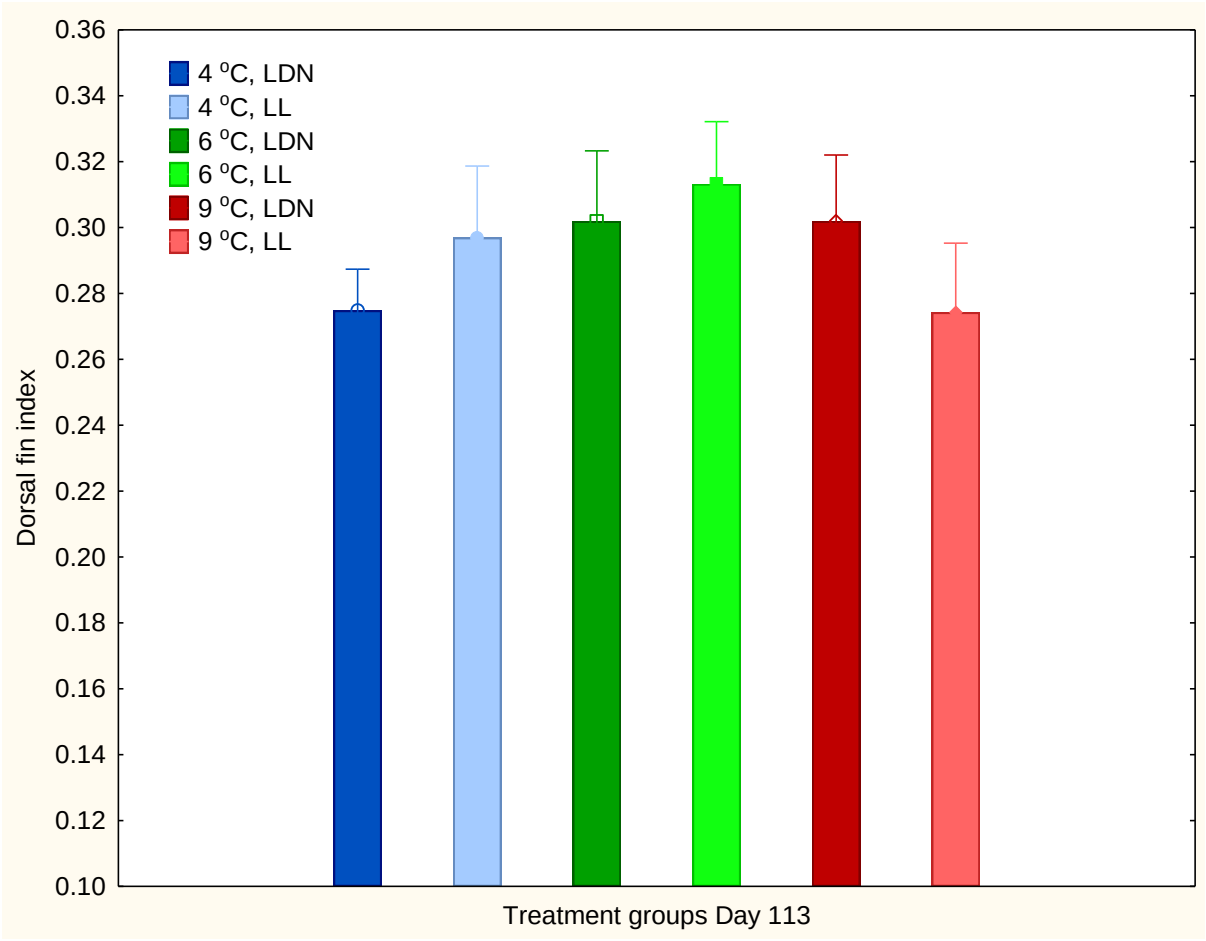

FIGURE 3.13. *Dorsal fin index of sampled juvenile Atlantic salmon reared at two different photoperiods (LDN= simulated natural photoperiod for Tromsø and LL= continuous light) at three temperatures (4, 6 and 9 °C at day 113 of the experiment). The three temperature groups and two light regimes are separated by color. Blue bar = 4 °C, green line = 6 °C and red line = 9 °C and diamond symbol. Heavy color = LDN and light color = LL. Vertical whiskers indicates standard error of mean (SEM).*

Filet quality

Hardness vs SGR

There was an overall significant correlation between fillet hardness and SGR, all temperature groups included (linear regression, $P < 0.001$, $R^2 = 0.38$), (Fig. 3.14).

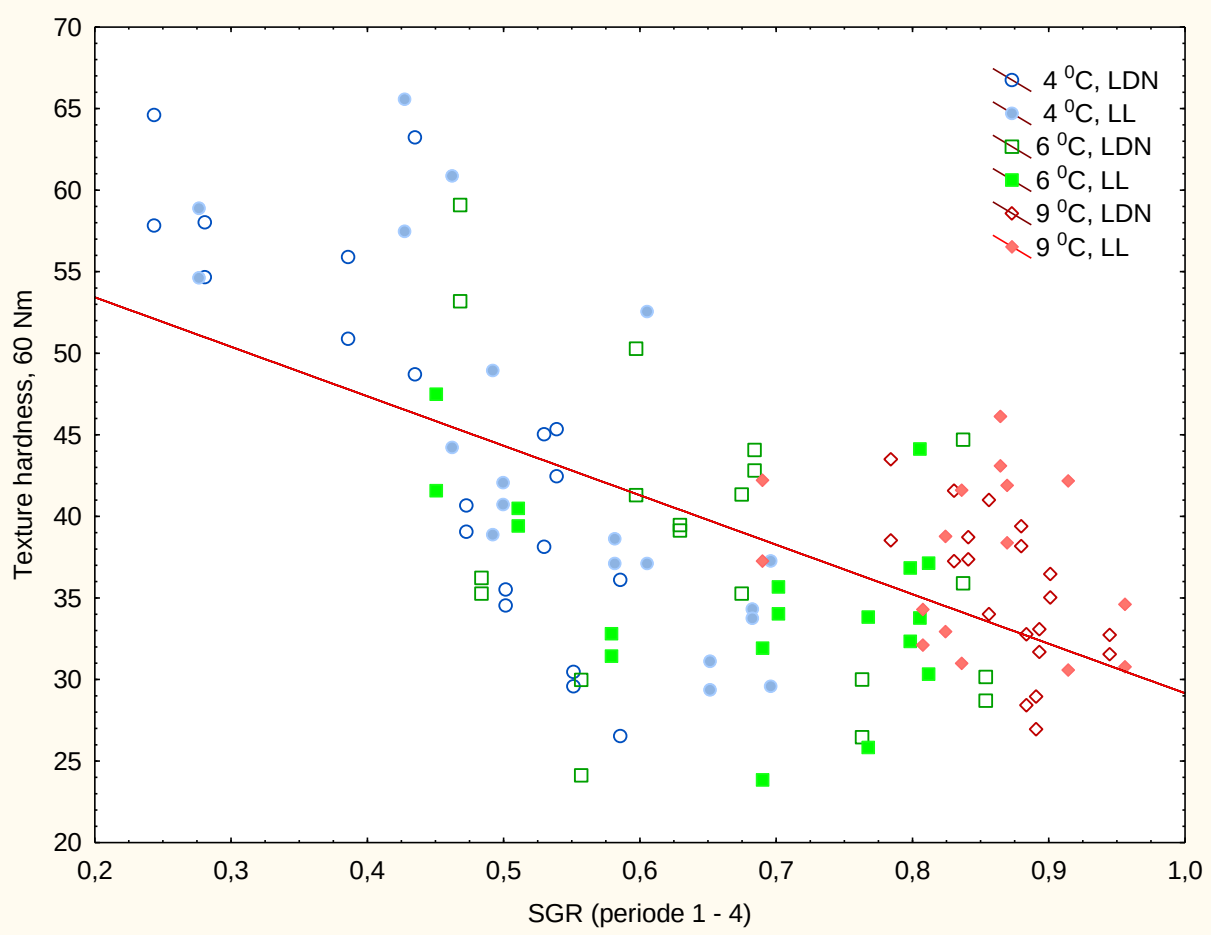

FIGURE 3.14. *Texture hardness of PIT tagged juvenile Atlantic salmon reared at two different photoperiods (LDN= simulated natural photoperiod for Tromsø and LL= continuous light) at three temperatures (4, 6 and 9 °C). The three temperature groups and two light regimes are separated by color and box symbol. Open symbol = LDN. closed symbol = LL. Blue = 4 °C and circle symbol, green = 6 °C and square symbol and red = 9 °C and diamond symbol. (by Dr. Bjørn Roth, Nofima Processing Technology, Stavanger).*

4 Discussion

Relevance for aquaculture

Atlantic salmon is known to sense and respond to a range of environmental variables within sea-cages, including light, temperature, salinity, dissolved oxygen, water currents and chemical treatments used during production (Handeland et al., 2008; Oppedal, Dempster, & Stien, 2011). Water temperature as one of these factors plays the most important role in teleost fish development in general because it can modulate all physiological processes and endocrine regulations (Taranger et al., 2010). The optimum temperature for growth of Atlantic salmon in seawater ranges from 14–18 ℃ (weight approx. 1.5 kg) (M. Jobling, 1981; Johansson, Ruohonen, Juell, & Oppedal, 2009), and between 11-14° C for 70-350 g post-smolt Atlantic salmon (Handeland, Imsland et al. 2008) indicating the temperature range in our experiment being well below the optimal, representing conditions typically found in Northern Norway.

A clear and expected positive development for the standard biometric parameters, growth rate, length and weight development over time was observed overall, and in particular in the first and middle part of the experiment until approximately day 124. One of the important findings in present experiment was the significant positive growth effects of photoperiod between the LDN and LL groups at 4 ℃. Furthermore, the highest overall growth rate at 1.25 % day^{-1} for fish in between approximately 250 – 300 g in the 9LL group was observed between day 83 to 124. The lowest growth was observed in the 4LL group with 0.51% day^{-1} in the last period from day 124 to 145 of the experiment.

The observed growth enhancing effect of additional light in cold water might prove important in order to either optimize rearing conditions in open seawater cage aquaculture, or save energy costs related to reduced heating in closed circuit facilities. Unpublished results from the second part of the "Nordlys" project (A. K. Imsland) indicate that a comparable positive growth effect, approx. 20% gain in growth below 4 ℃, may also be obtained in full scale commercial open sea cages by applying continuous light in sea cages from October to March. Both these experiments illustrate the great plasticity and influence of external factors controlling growth in Atlantic salmon, and indicate a rationale for further development and adaption of aquaculture and research on Atlantic salmon in sub optimal climate regions. This is especially relevant in a context were the aquaculture industry is adapting contained and

closed circuit solutions which enable both temperature and light control. In example, the greater energy intensity of land based systems, is the primary source of their increased environmental life cycle impacts compared to open sea pen systems (Ayer & Tyedmers, 2009). To "substitute energy used for heating with light" at low temperatures could therefore be a viable way of optimizing environmental performance of land-based recirculating systems (RAS) in order to obtain higher life cycle environmental impacts or higher profitability for future aquaculture.

Growth rate and sub optimal temperature regimes

There was only significant difference for the LL group over the LDN group at 4 ° C. This observed overall 36.4 % elevated effect on SGR of photoperiod treatment corresponds to approx. 1.2 °C increase in water temperature according to Fig. 4.1. adapted from (S. O. Handeland et al., 2003). This is in accordance with (Boeuf & Le Bail, 1999) stating that Atlantic salmon is very sensitive to light manipulation also in seawater. There are surprisingly few studies specifically evaluating the post-smolt stage in sea water, relevant for direct comparison. A. Imsland et al. (2014) reported a growth enhancing effect of continuous light for Atlantic salmon in fresh water corresponding to a 4.5 °C increase in temperature in an experiment investigating both smolt and post-smolt at 8.3 and 12.7 °C. Due to the present experiment having a simpler setup with few variables, sea water only, and relatively low temperatures were maturation was not expected (Hutchings & Jones, 1998), one can assume that the positive growth effect is even more reliable and stronger associated with photoperiod alone.

Although there has been significant progress in breeding since Austreng et al. (1987) estimated growth rates for Atlantic salmon, the study is relevant because it reviews a quite similar temperature range. Growth rates in fresh water were reported being approx. 0.4 % day^{-1} for the 4 °C group, 0.8 for the 6 C° group and 1.5 for the 9 C° group. Data from present experiment indicates growth rates for the first three periods from day 0 to day 124 for the 4 C° LL group to be in the high 0.6 % day^{-1} range, and in the start of the experiment from day 42 to 83 being 0.7 day^{-1}, approaching growth rates close to expected values for smolts kept at 6 C° according to (Austreng et al., 1987). These comparisons must however be used with caution due to different developmental effects given that the fish are in different ontogenetic phases related to smoltification and seawater adaptation. A. Imsland et al. (2014) present data on pre- and post-smolt Atlantic salmon, which further indicate that the temperature effect is modulated by photoperiod treatment as demonstrated by the LL groups having higher overall

growth rate. The positive growth development observed in present experiment, related to increase in temperature, is expected and in accordance with the overall increase in metabolism with higher temperatures. These effects are reviewed in Jonsson, Forseth, Jensen, and Næsje (2001) which specifically mention elevated growth related to temperature in combination with nutritional status. This is in accordance with the present findings which indicate low condition factor and glucose levels at 4 °C and especially for the LDN group. In this experiment, it was not a specific goal, or part of the hypothesis to define an optimum temperature for growth (ToptG). Anyhow, the result from present experiment is that overall growth was clearly highest in the 9 °C group. S. O. Handeland et al. (2003) combines earlier published growth data for Norwegian farmed Atlantic salmon strains (Arnesen, Johnsen, Mortensen, & Jobling, 1998; S. Handeland, Berge, Björnsson, & Stefansson, 1998; S. O. Handeland, Berge, Bjornsson, Lie, & Stefansson, 2000) and provided a plot indicating approximate growth ratios (% day^{-1}) relating to the temperature steps used in present experiment being 0.28 for 4 °C group, 0.55 for the 6 °C group and 0.87 for the 9 °C group (Fig. 4.1). This indicates that present data follow Sigurd O. Handeland et al. (2003), but at a higher growth rate, especially for the 4 and 6 °C groups, and in particular for the LL groups. None of the groups in Sigurd O. Handeland et al. (2003) included fish treated with continuous light, hence present data add to the understanding of environmental control of post-smolt growth.

In addition to factors clearly supporting the hypotheses, there are some data giving less clear indications. Firstly, the photoperiodic effect for the 4 °C group was not observed until after approximately one month of the trial period. This may be because the fish are generally in an adaptation phase to new conditions in this first period due to gradually being transferred to seawater. Furthermore, a clear decline in growth for all groups during the last period of the experiment was observed. This dilutes the photoperiodic effect on growth rate observed during most of the experiment. This may partly be explained by the light period for the LDN groups getting increasingly longer in spring and therefore reduces the relative differences between the two groups. An additional explanation may be that the overall biomass development in the tanks may have given negative effect on growth due to crowding and distress. However data on the dorsal fin indexes did not indicate fin erosion due to overstocking. Furthermore total biomass weight was measured at T4: day 124 to approximately 60 g l^{-1} which is far below stress threshold levels reported by Hosfeld et al. (2009) and Kjartansson, Fivelstad, Thomassen, and Smith (1988). In spite of these possible

contradictions, both weight development and length development figures, displays significantly higher values for 4LDN group compared to 4LL group also in the last period.

Effect of photoperiod on growth rate

The periodic growth rate results further underline the declining effect of photoperiod from start to end of the project. The 4 ℃ group in the early period from day 0 to 42, showed 77,1 % higher growth effect for the LL group, versus only 36,4 % for the whole experiment period from day 0 to day 145. Late in the experiment between day 83 and 124, there were no differences between photoperiod groups, except for the 4 ℃ group. In the final period from day 124 to day 145 no systematic growth differences between photoperiod was observed. Handeland et al. (2003) found photoperiod to enhance growth through stimulation of food intake. Several other authors (Boeuf & Le Bail, 1999; Hansen, Stefansson, & Taranger, 1992; Krakenes et al., 1991; Stephen D McCormick et al., 1987) also demonstrated a substantial improvement of post-smolt growth related to light stimuli in sea water.

A. Imsland et al. (2014) reported a significant positive SGR effect of photoperiod also for the 12 ℃ LL and 8 ℃ LL (approx. 30%) groups. This discrepancy in response to light between seawater and freshwater is interesting, but challenging to explain. Explanation may be related to differences in photoperiod for the two experiments related to length of experiment versus timing related to spring and summer season. The A. Imsland et al. (2014) trials were conducted for 11 months and had a sea water phase in a high light output phase from May to July. Also the LDN period was correlated to Lønningdal (60 °N) giving less overall contrast between LL and LDN. On the other hand our experiment was carried out in winter conditions from October until March with photoperiod Tromsø (69 °N), in total giving a relatively weaker LDN day light signal due to very large difference in light output between LL and LDN. There is a change in daylight over the experiment period from zero (until mid-January) to approximately 9 hours daylight at the end of experiment (APPENDIX I, FIGURE II). This may further indicate that the coldest groups in this thesis are able to exploit even short periods of continues light and that this group specifically benefits in setups with major contrasts between LL and LDN lighting.

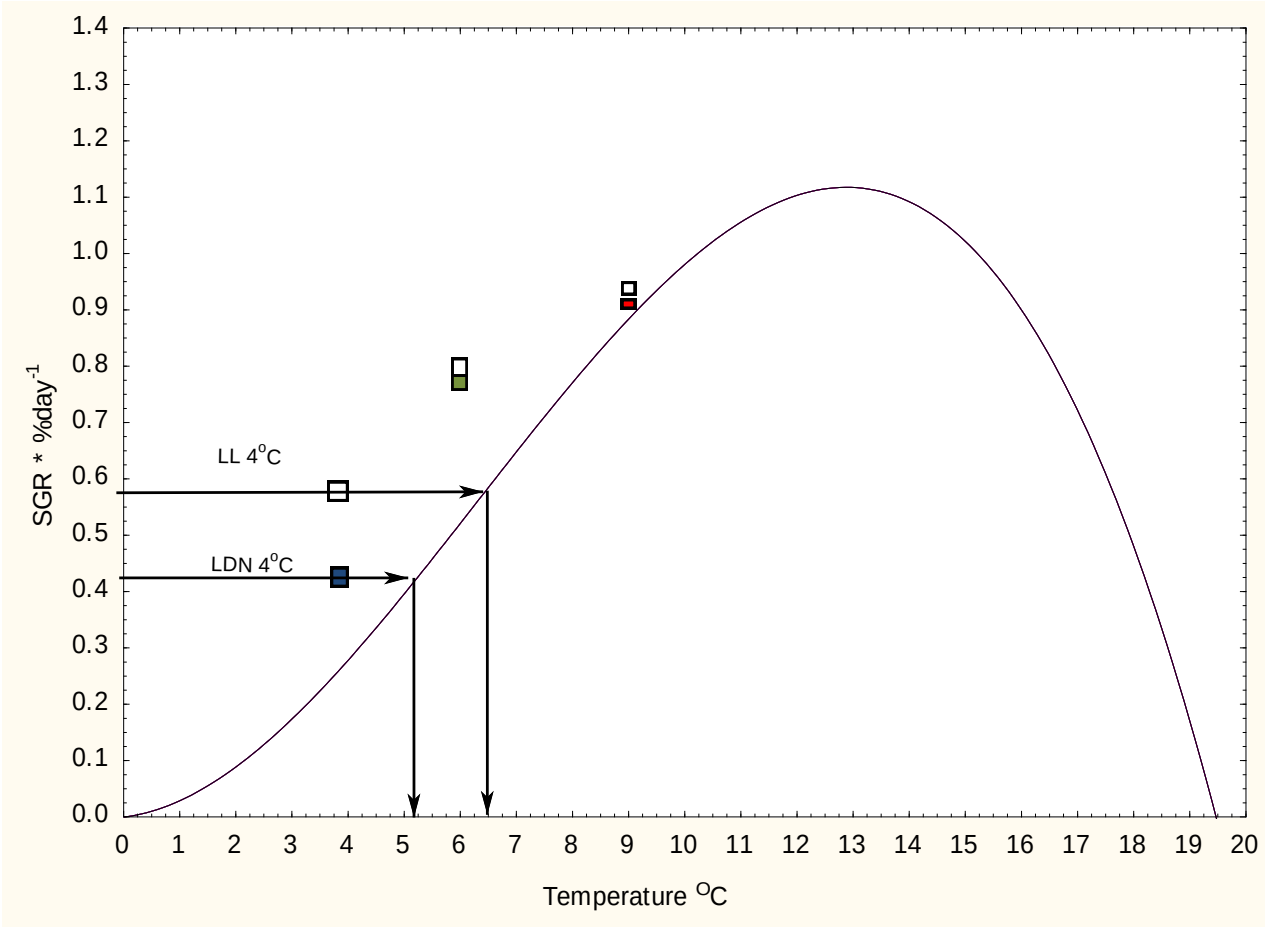

FIGURE 4.1. Changes in growth rate (approximate overall SGR) with temperature from present experiment plotted against figure adapted from (S. O. Handeland et al., 2003). SGR of PIT tagged juvenile Atlantic salmon reared at to different photoperiods (LDN= simulated natural photoperiod for Tromsø and LL= continuous light) at three different temperatures (4, 6 and 9 °C). The three temperature groups and two light regimes are separated by color and box symbol. Open symbol = LL, closed symbol = LDN. Blue = 4 °C, green = 6 °C and red = 9 °C. The line represents a third order polynomial fit to the SGR data reviewed by (S. O. Handeland et al., 2003). Arrows indicate temperature gain (approx. 1,2 °C) by using continuous light for the 4 °C group.

Condition factor in relation to growth

Parallel to decreased growth rates, metabolic compromises (i.e. due to feed intake, temperature and photoperiod related stress) may also result in decreased condition factor. Bone growth will likely continue whereas muscle mass and lipid deposits are more reflective of energy stores for the fish (Weatherley, Gill, & Casselman, 1987). Thus fish would continue to grow in length (bone mass) without the complementary increase in bulk (i.e. muscle, lipid and organ mass) (Danley, 2001; Haugen et al., 2006). In accordance with this the 4LDN group has a lower CF at all time intervals, and has maintained its length development, but gained relatively less weight, resulting in a longer slimmer fish. In total changes in CF indicate an S – shaped curve indicating more length or decreased weight gain in the start and at the end of the experiment, and a high growth phase in the middle of the experiment. The

stable phase in the in beginning of experiment may indicate an acclimation phase due to transition to seawater and general acclimation to rearing setup (Arnesen et al., 2003). Especially from start of experiment from day 0 to 42, the 4LL group has increasing CF while the 4LDN group has decreasing values. This corresponds with generally lower feed conversion efficiency for the 4LDN group. Alne, Oehme, Thomassen, Terjesen, and Rorvik (2011) report that low-performing periods coincide with reduced fat and energy retention, low levels of muscle fat and poor CF. Peterson and Harmon (2005) propose that percent muscle lipid increases linearly with CF indicating that the 4LDN fish generates less stored energy. This indicates that the "cold and dark" 4LDN group is challenged with more marginal rearing conditions. This is in agreement with Sarkar et al. (2013) stating that fatness, or well-being of fish, is based on the assumption that heavier fish of a given length are in better condition. In the period from day 83 to 124 the 9LL group has the highest CF indicating healthy growth and good rearing conditions. At the end of the experiment there are only minor differences between groups corresponding to the equalization trend seen weight, fork length and SGR parameters.

Feed conversion efficiency (FCE)

FCE ranged from 0.64 to 1.21 but showed no clear effects of photoperiod between any of the groups except for the 4 °C group. The continuous light 4 °C group had 46% higher values compared to 4LDN, while the 6 °C and 9 °C groups only have respectively 6% and 3% difference in FCE between photoperiod groups at each temperature. This indicates that only the cold group benefits from use of artificial light in regard to better utilization of feed. Moreover, it is only negligible differences in total feed consumption between photoperiods at each temperature step. Handeland et al. (2008) showed FCE values at approx. 0.5 at 6 °C for post smolt in weight class between 170 to 300 g. Compared to our results these are in a somewhat lower range than this experiment being approximately 0.8. The choice of method limited the range and resolution of these results. Due to design of the experiment setup it was only possible to obtain data for the last period in the experiment. Even so there was a stepwise increase in feed consumption between the temperature groups which is natural in relation to increased metabolism of the warm 9 and 6 °C groups compared to the 4 °C group. This is in accordance with (Handeland et al., 2008) showing feed intake and stomach evacuation rates tightly linked to temperature, and variation in optimum temperature for growth in juvenile Atlantic salmon smolts with decreased temperature for feed conversion efficiency as the fish

grow bigger (Arnesen et al., 2003; S. O. Handeland, A. K. Imsland, & S. O. Stefansson, 2008).

Blood glucose

Blood glucose levels are in line with Hosfeld et al. (2009) and are often used as an indicator of nonspecific stress (Hunn & Greer, 1991). A change in metabolism in conjunction with reduced feed uptake may affect these levels. Both the LL and LDN 4 °C groups are affected at first part of experiment indicating tendency for temperature related stress. This finding is well in accordance with the growth and feed uptake parameters for the 4 °C group at this point of the experiment.

Blood sodium

Only small absolute variations in circulating blood sodium levels were observed indicating that the hydro mineral balance was maintained during the experiment across the different temperature and light groups. Even so there was a clear increase for all groups from start of experiment until day 30. High circulating blood sodium in sea water could be a result of stress related to reduced ability to maintain homeostasis (Cnaani et al., 2013), and may be indicative of transition from freshwater to saltwater (Deane & Woo, 2009). Being an anadromous species Atlantic salmon will have an opposite hydro mineral balance challenge in fresh water compared to sea water. After seawater adaptation, sodium levels will stabilize on higher natural seawater related level (Arnesen et al., 1998). Moreover a non-significant difference at the end of the experiment was seen were the 4LL and LDN groups have higher values after 30 days, and in particular and significant after 145 days, were 4LDN group in addition shows effect of photoperiod being significantly higher than 4LL group. This might be an indication that this group is challenged by ion regulatory stress related to osmoregulation due to low temperature (Stephen D McCormick et al., 1987).

Blood pCO2 and HCO3-

In line with Hosfeld et al. (2009), blood pCO_2 and HCO_3^- values showed small variations during the experiment. However, in the present study higher levels of both parameters generally correlated with higher rearing temperatures. The 9LL and LDN groups showed generally higher values than all other groups due to higher activity and increased metabolism (S. O. Handeland et al., 2014). Blood CO_2 partial pressure is normally correlated to ambient water pCO_2 plus approx. 1-3 mmHg (Ultsch, 1996). CO_2 partial pressure was mostly in the

range 5 - 13 mmHg indicating corresponding tank levels below 10mmHg. Safe blood pCO_2 level in Norwegian smolt production based on experiments performed between 4 and 10 ℃ is 15 mmHg (Fivelstad et al., 2015).

Organ indexes and filet quality

Regarding the liver, only the index for the 4LDN group differed clearly from all the other groups by being higher. Even so the relative difference between 4 ℃ photoperiods, although significant, was only 3%. It is generally known that energy demanding processes such as growth, deplete the level of energy in the liver, and thereby lower its relative weight (Kryvi, 1992). Also the cardio-somatic index showed effect of photoperiod between the same groups. Large organ relative to body size indicates less than optimal rearing condition due to organ growth being more stable and more easily affected by growth inhibitory external factors (Rosenfeld, Van Leeuwen, Richards, & Allen, 2015).

No significant differences in fin index between groups were observed. Person-Le Ruyet and Le Bayon (2009), Jones, Noble, Damsgard, and Pearce (2011), Brockmark, Neregård, Bohlin, Björnsson, and Johnsson (2007) assess density effects on dorsal fin damage. Atlantic salmon at reduced density had less-damaged fins than those reard at standard density. This indicates that all groups had quite equal conditions in regard to fin abrasion and stocking density at day 145.

The flesh quality of fish is influenced by season (Espe et al., 2004; Hagen, Solberg, Sirnes, & Johnston, 2007) and is therefore an obvious and relevant parameter in commercial aquaculture. It was not performed a specific analysis for each temperature group, only regression between filet hardness and growth rate. The analysis of fillet quality gave indications of reduced filet hardness with increasing growth rate in accordance with I. A. Johnston (1999) and Rasmussen (2001). Results on turbot showed that softness of the flesh was mainly influenced by factors associated with growth, such as season and photoperiod (B. Roth et al., 2010). In line with present findings Morkore and Rorvik (2001) investigated product quality of farmed Atlantic salmon for hardness, and found highest values during the winter period. There are not sufficient data in this experiment to conclude whether the two different photoperiods in this experiment have a similar effect, but results from A. K. Imsland et al. (2009) on Atlantic halibut suggest photoperiod regimes only have a minor effect on

flesh-quality, whereas a significant seasonal effect was seen with a tendency towards lower hardness in summer time compared to winter.

Synoptic discussion and concluding remarks

In this experiment the concurrences of several parameters were seen that could implicate that post-smolt held at low temperatures, and with a short natural light period, experience stress, leading to reduced growth. The main finding was the increased growth of the 4LL group compared to the 4LDN group corresponding to approx. 1.2 ℃ increase in water temperature. The experiment shows the 4 ℃ and 9 ℃ degrees groups as outer point on each end of the axis, in that they for the most important measured growth and quality related parameters show either high or low values. Essentially the explanation is that the 9 ℃ group being closer to the Atlantic salmon growth optimum for this size of salmon. It is thoroughly shown in literature that temperature has a regulatory growth effect, and that optimum for Atlantic salmon for this size range of post smolts is at approximately 13 ℃ (12.8 ℃ for 70–150 g to 14.0 ℃ for 150–300 g post-smolts) (Bromage et al., 2001; S. O. Handeland et al., 2008; Sigurd O. Handeland et al., 2003). Furthermore, it was shown that Atlantic salmon is particularly sensitive to photoperiod manipulation and management (S. O. Handeland et al., 2013). The precise mechanism of these effects are not entirely clear (Stefansson et al., 2007), and Atlantic salmon differs from several other species in that light plays a key role for ontogenetic shifts (Boeuf & Le Bail, 1999; Stephen D McCormick et al., 1987). In these rapidly changing phases the fish does not exhibit as linear growth patterns as for the post-smolt phase weight class which is the topic of this study (approx. 80-350 g) (Thorpe, Mangel, Metcalfe, & Huntingford, 1998). To get a clearer picture of the mechanisms the experiment was designed in order to include a number of relevant parameters. The measured values for these parameters further underline that the cold 4 °C groups, and especially the 4LDN group, may experience a growth inhibiting stress.

Firstly, blood level values of glucose showed significantly lower values for 4LDN group throughout the experiment. This suggests that these fish have lower nutritional status and general lowered metabolism. Glucose plays a key role in muscle metabolism (Hemre, Mommsen, & Krogdahl, 2002; Polakof et al., 2012). It is therefore a possible relationship to data from the same fish group also having a 46% lower feed conversion ratio compared with the fish that received continuous artificial light. Sodium ion concentration in the blood for 4LDN group showed high values throughout the experiment. This indicates that the same fish groups also have greater challenges in adapting to sea water and are confronted with grater osmotic disturbance than the other groups (S. O. Handeland et al., 2000). This is an indication of stress which may be related to marginal light exposure and generally lower fitness

(Leonardi & Klempau, 2003). Although pCO_2 and HCO_3^- levels showed no significant effects of selected photoperiods, the general trend was towards higher values at 9 °C. This coincides with the presumption that fish in 4 °C group may have a stress induced acid - base disturbance.

Finally, the hepato-somatic and cardio-somatic indexes also may indicate that growth slowed down in 4LDN group. In particular, liver weight development is considered to be relatively constant and less influenced by external growth regulatory mechanisms (Aas, Klemetsen, Einum, & Skurdal, 2011), but also weight sensitive to demanding metabolic processes related to the high temperature groups (Kryvi, 1992). In this experiment, the fish reared at 4LDN had higher relative weight of these organs. Effect of photoperiod treatment on the cardio-somatic index was also seen.

Although the results of filet quality measurement (fillet hardness) were based on a relatively simple experimental set-up at one point at the end of the experiment, the results show that the quality in terms of hardness is lower for 9 °C group. This could be due to the rapid growth phases for the medium and high temperature groups related to muscle tissue becoming looser to allow growth (I. A. Johnston, 1999). Skeletal muscle may have a higher growth rate than that of the whole body indicated by the observed increase in condition factor seen in this experiment (Fauconneau et al., 1995).

5 Conclusions

This study suggests that post smolt in size range approx. 75 – 400 g stocked in seawater at low temperatures, and exposed to continuous light (LL 4 ℃), grow significantly faster (27%) than smolt reared with natural photoperiod for Tromsø. These findings may have consequences for optimization of commercial production. Feed conversion rate was 34% lower for the 4LDN group compared to the 4LL group. The 9 ℃ and 6 ℃ groups generally showed higher values for growth and good adaptability to both light regimes. No significant differences in weight, length or growth rate development was observed for either 6 ℃ or 9 ℃ groups with regard to photoperiod.

The cold group receiving extra artificial light, 4LL, revealed the following positive growth tendencies.; Significantly higher weight and length growth from day 83 until end of experiment, higher specific growth rate from day 0 to day 124 and higher condition factor from day 42 to day 83. Thus, HA1 can be accepted. Furthermore significant higher levels of blood Na^+ at day 113, 3%, higher hepato-somatic index compared to the 4LL group and 14% higher cardio-somatic index are all significant responses compared to the 4LL group. Thus, HA2 can be accepted.

Filet hardness, as secondary product quality indicator, showed a significant decrease in fillet hardness between treatment groups with increasing growth rate. Although it is shown negative correlation between growth rate and filet hardness, it is not shown direct correlation between temperature and light. Hypothesis H3 can therefore neither be confirmed nor rejected.

Future perspectives

Although the results indicate a growth potential of about 27% through the use of artificial light at low temperatures, it is even more interesting whether this gain can be realized outside the laboratory. Ongoing studies from the "Nordlys" project at the Lerøy Aurora AS facilities has already to a large extent demonstrated a similar effect at temperatures below 4 ℃ (A.K. Imsland pers. comm.). Under natural growth conditions there are in addition a number of external factors that may affect growth, and to a larger extent, than the light and temperature factors which were subject for this experiment (in example genetic differences, disease status, oxygen levels etc.). Further investigation of hormone levels and analysis of the different tissue samples collected in this experiment would also contribute to a more thorough explanation of

the mechanisms of the photoperiod effect specially related to the seawater post-smolt phase. The difference in growth response to light between seawater and freshwater at higher temperatures is interesting and needs further investigations. This is particularly important because it is a much smaller selection of literature for the post-smolt sea water phase compared to freshwater investigations.

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Appendix I

Discussion of Materials and Methods

Generally the experiment was carried out within the technical limits of available infrastructure in the given facilities. Possible sources of error were monitored and evaluated on an ongoing basis, and corrective actions were performed within practical limits.

We assume possible measurement technique errors related to measured blood physiology values (i-STAT) would be in terms of absolute values and not to relative differences between groups. Iversen, Finstad, McKinley, and Eliassen (2003) concluded that portable instruments for measuring blood glucose and lactate, could be used as a relative measure to evaluate responses to stressors. Furthermore Hunn and Greer (1991) reported that anesthesia resulted in no major changes in selected blood chemistry characteristics; hematocrit, plasma glucose and chloride levels, and osmolality, and that Atlantic salmon exhibited a limited stress response to netting, indicated by minor changes in plasma glucose concentrations. Harter, Shartau, Brauner, and Farrell (2014) suggests that the i-STAT analyzer tool used in this thesis is an appropriate tool for assessing the acid–base status of blood in rainbow trout. The accuracy of i-STAT measurements of plasma Na^+ concentration, pCO_2, HCO_3^- and pO_2 were dependent on the measured range and associated with a high measurement error at those values typically expected for rainbow trout. Due to ISTAT being a tool developed for human blood at 37 ^{0}C, necessary calibrations were performed. In order to compensate for other possible artificial effects, present experiment focused on a high degree of standardization. All fish samples were taken within 2 minutes and analyzed within 5 minutes.

Technically it was challenging to adjust the 6 °C group precisely between the 4 °C and 9 °C groups. The experimental facilities at ILAB were not equipped with thermostatically controlled regulating systems. Temperature correction of the 6 °C group was especially challenging because obtaining this third temperature step only could be achieved by mixing water from two separate header tanks, using the less precise flow adjustment valves located on the individual fish tank. This may have given this group more stress due to frequent minor adjustments and fluctuations. It must also be emphasized that 6LL group due to space considerations were kept in a separate room from the other groups. This group was chosen because we wanted to have the best possible, and most similar, conditions for the 4 °C and 9 °C groups in the same room, in order that the experiment should be as precise as possible in

regard to the results for the low and top end of the temperature scale. In room 12 there were other experiments on going giving generally less setup control. Differences between the two rooms may be an additional source of error related to light and temperature control. In aqua room 12 there were no automatic oxygen monitoring- and supply setup, which may have affected the 6LL group in particular.

As illustrated in Fig. 2.4., varying light intensity between tanks was observed. There was variation during experiment due to need for adjustments and building up of salt deposits. Boeuf and Le Bail (1999) states that light intensity does not have clear effect for growth stimulation and that day length appears much more important.

For technical, economical and practical reasons it was chosen a manual and work intensive method for feed collection. At the beginning of this part of the experiment it was assumed that the automatic feeders delivered a precise defined amount of feed pellets for each of the three temperature groups, and within the same time interval each day. When verifying the actual output, it turned out less accurate than desirable, in spite of repeated calibrations. This is due to the feeding system being designed for larger volumes used in commercial hatcheries. We therefore switched to completely manual feeding by measuring precise amounts of feed pellets in calibrated measuring cups two times a day for each temperature interval. In addition, it turned out that the collection of waste feed was demanding. In the start of the experiment it was discovered that some of the collected pellets were sandwiched between the sieve screen/net and the collection tank, and therefore not measured. This was corrected by use of silicone glue. Because of these calibrations of equipment at the start of the feed collection, we chose to disregard data collected during this period. Another possible source of error in the chosen setup is that waste feed pellets dissolve in the fish tanks, and may be destroyed mechanically in the collection process. This particularly applies to the 9 °C groups due to the effect of higher temperatures on decomposing. It is therefore strongly recommended to use more robust nondestructive techniques for further studies.

Small dorsal fin size, speed of operation, and partly worn and slippery fins, made height measurement for dorsal fin area index calculation challenging. Even so the results show small differences in mean values, but greater variation, and no systematic differences between the groups were recorded.

Thorstad, Rikardsen, Alp, and Okland (2013) mention that the catch, handling and tagging procedures should have minimal effects on the fish in order not to measure artifacts not

related to the intensions of the experiment. Furthermore Atlantic salmon is a good experimental model because it exhibits few changes in blood chemistry in response to routine sampling methods (Hunn & Greer, 1991). In this experiment standard procedures and careful handling were applied and there were no visual signs indicating welfare challenges or less growth for PIT tagged fish.

More details

Fish stock and rearing conditions

Information from hatchery data sheet September 30th 2013 (PHARMAQ Analytiq, Høyteknologisenteret i Bergen)

"ATPase activity at a high level transition. Increase in ATPase activity since last sampling. Variation up to smolt level (40%). An improvement in smolt index (3.4) since the previous sampling, but this is still a bit low. Nice decrease in condition factor down to good level. Positive correlation between ATPase activity and weight (0.36) and between ATPase activity and smolt index (0.31), may indicate that fish group is still in progress. Estimated number degree days with 24: 0 light is now approximately 400. Fish Group considered being seawater skilled and in beginning of the smolt window. Fish Group is ready for release in accordance with the plan."

Experiment setup, figures and illustrations

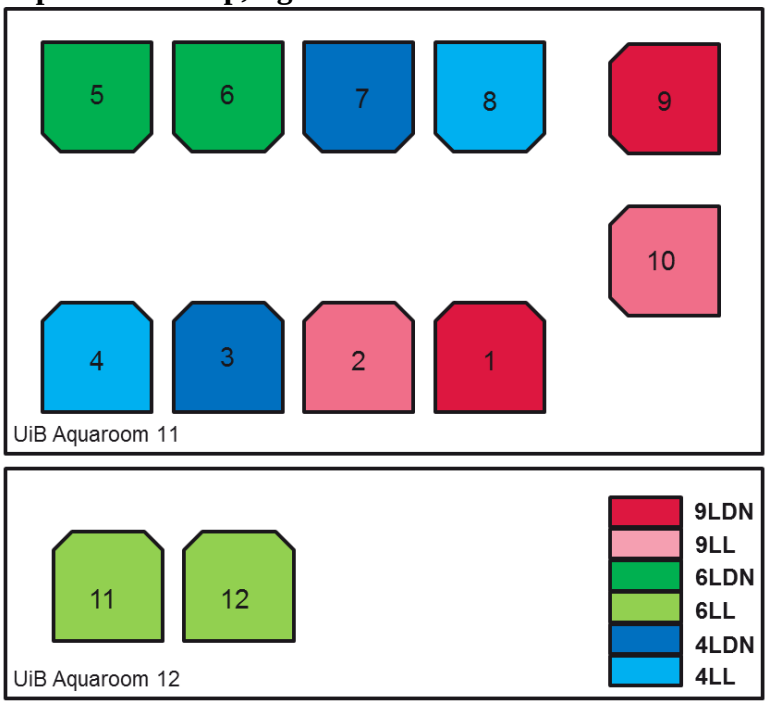

FIGURE I. Experimental setup

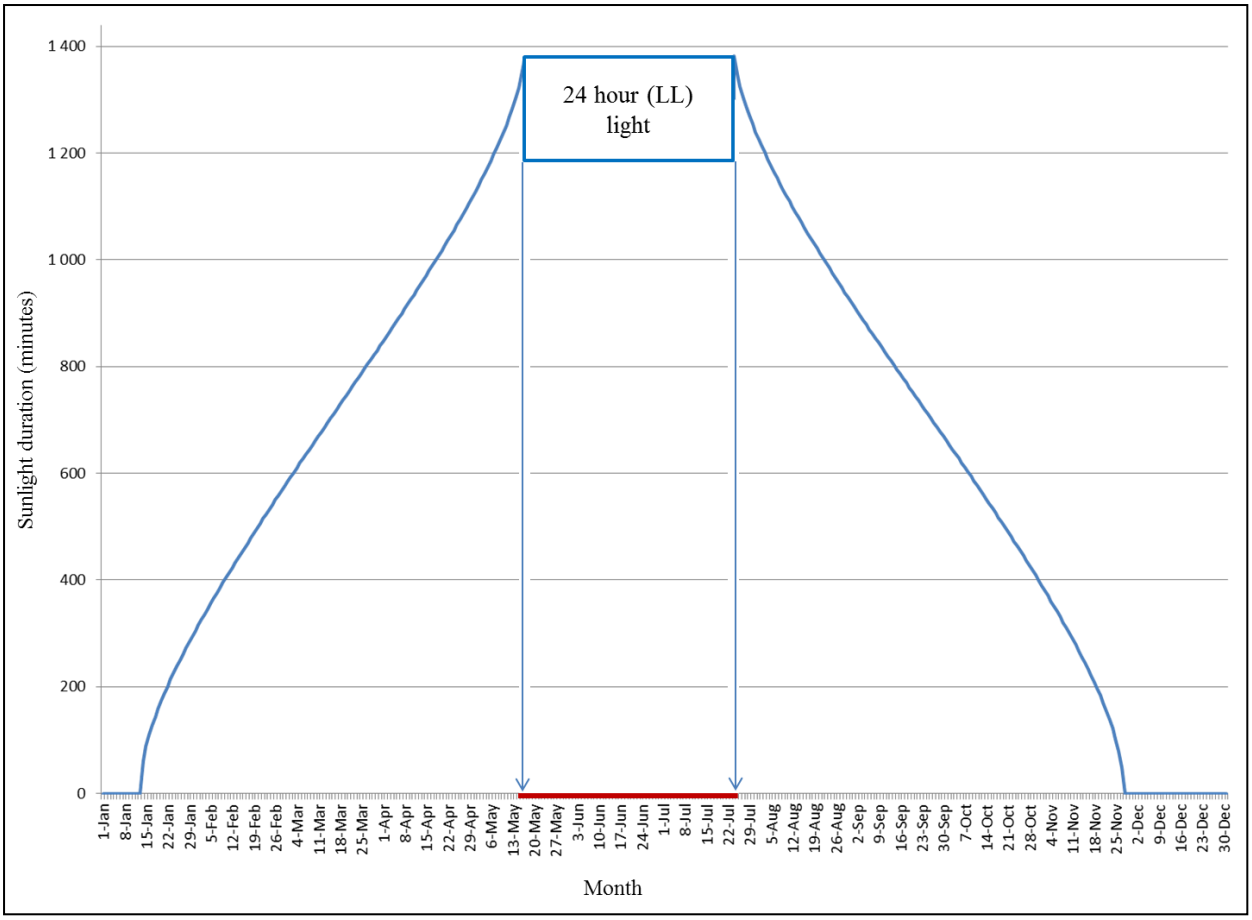

FIGURE II Light regime Tromsø (N 69° 40`) sunlight duration minutes (NOAA, 2010).

Appendix II

Descriptive statistics

Experimental conditions

TABLE I. Descriptive statistics based on daily temperature measurements in all tanks. (2*LDN4/LL4= constant 4°C , 2*LDN6/LL6= constant 6°C , 2*LDN9/LL9= constant 9°C). Means, total number of observations (N), standard deviation (SD), standard error (SE), minimum and maximum are included in the TABLE.

Temperature							
	Replicate tank no.	Means	N	SD	SE	Min	Max
4LDN	Tank 3	4.5	95	1.04	0.11	4.1	9.5
4LDN	Tank 7	4.4	95	1.06	0.11	4	9.5
4LL	Tank 8	4.4	95	1.06	0.11	4.1	9.5
4LL	Tank 4	4.4	95	1.06	0.11	4.1	9.5
6LDN	Tank 5	6.4	95	0.69	0.07	5.5	9.5
6LDN	Tank 6	6.5	95	0.71	0.07	5.3	9.5
6LL	Tank 11	6.7	95	0.65	0.07	5.5	9.4
6LL	Tank 12	6.7	95	0.64	0.07	5.9	9.4
9LDN	Tank 1	9.7	95	0.57	0.06	8.9	12.6
9LDN	Tank 9	9.0	95	0.24	0.02	8	9.5
9LL	Tank 2	9.6	95	0.56	0.06	8.9	12.6
9LL	Tank 10	9.1	95	0.20	0.02	8.7	9.6

TABLE II. Descriptive statistics based on daily measurements of oxygen saturation in all tanks. Means, total number of observations (N), standard deviation (SD), standard error (SE), minimum and maximum are included in the TABLE

Oxygen saturation							
	Replicate tank no.	Means	N	SD	SE	Min	Max
4LDN	Tank 3	81.5	80	3.93	0.44	75	93
4LDN	Tank 7	82.7	80	4.68	0.52	75	95
4LL	Tank 8	81.9	80	4.18	0.47	73	91
4LL	Tank 4	81.9	80	4.27	0.48	74	92
6LDN	Tank 5	80.6	80	4.72	0.53	74	93
6LDN	Tank 6	82.2	80	4.83	0.54	69	93
6LL	Tank 11	77.8	80	8.61	0.96	64	101
6LL	Tank 12	76.9	80	9.14	1.02	63	101
9LDN	Tank 1	83.7	80	4.83	0.54	73	93
9LDN	Tank 9	84.0	80	5.09	0.57	74	93
9LL	Tank 2	82.4	80	4.71	0.53	70	99
9LL	Tank 10	81.7	80	4.04	0.45	70	92

TABLE III. Descriptive statistics based on measurements of weight for all fish (Treatment+Replicate) at T0 (day 0), T1 (day 42), T2 (day 83), T3 (day 124) and T4 (day 145) Means, total number of observations (N), standard deviation (SD), standard error (SE) minimum and maximum are included in the TABLE

Weight g. all								
Treatment	Replicate	Period	Means	N	SD	SE	Min	Max
4LDN	a	T0	96.6	20	13.0	2.9	73.1	135.9
4LDN	a	T1	119.5	20	17.7	4.0	97.4	173.4
4LDN	a	T2	160.2	20	60.7	5.0	124.4	224.2
4LDN	a	T3	197.1	20	29.2	6.5	141.6	271.6
4LDN	a	T4	204.1	20	32.3	7.2	143.1	281.2
4LDN	b	T0	82.1	20	11.4	2.5	94.1	100.6
4LDN	b	T1	89.9	20	11.9	2.7	70.1	110.9
4LDN	b	T2	102.1	20	16.5	3.7	124.4	189.4
4LDN	b	T3	127.7	20	19.6	4.4	90.7	162.0
4LDN	b	T4	140.7	18	25.0	5.9	94.1	180.6
4LL	a	T0	84.3	20	13.9	3.1	64.3	104.8
4LL	a	T1	113.4	20	16.3	3.7	84.7	141.0
4LL	a	T2	155.5	20	20.1	4.5	111.2	187.4
4LL	a	T3	201.6	20	29.2	6.5	137.3	249.6
4LL	a	T4	213.5	20	34.2	7.6	143.4	278.9
4LL	b	T0	87.2	20	18.7	4.2	56.3	128.9
4LL	b	T1	109.3	20	19.5	4.4	68.6	144.9
4LL	b	T2	145.5	20	24.4	5.5	90.9	196.8
4LL	b	T3	186.3	19	34.9	8.0	105.0	254.6
4LL	b	T4	200.8	19	31.0	7.1	155.0	267.7
6LDN	a	T0	87.2	20	13.1	2.9	71.6	122.6
6LDN	a	T1	124.4	20	18.4	4.1	97.0	162.4
6LDN	a	T2	168.6	20	26.4	5.9	113.5	213.9
6LDN	a	T3	239.2	20	48.6	10.9	157.5	326.8
6LDN	a	T4	259.3	20	53.1	11.9	180.4	360.5
6LDN	b	T0	84.9	20	18.2	4.1	55.5	137.4
6LDN	b	T1	120.5	20	23.8	5.3	72.9	183.0
6LDN	b	T2	178.6	20	35.8	8.0	109.9	254.5
6LDN	b	T3	246.5	20	56.8	12.7	131.3	349.8
6LDN	b	T4	260.3	20	61.6	13.8	135.5	360.9
6LL	a	T0	80.3	20	15.4	3.4	54.5	104.7
6LL	a	T1	111.7	20	21.2	4.7	68.5	148.7
6LL	a	T2	158.6	20	30.4	6.8	108.4	222.5
6LL	a	T3	223.4	20	49.7	11.1	158.5	339.5
6LL	a	T4	248.3	20	50.9	11.4	165.9	342.1
6LL	b	T0	84.8	20	11.9	2.7	65.2	106.2
6LL	b	T1	117.1	20	18.6	4.2	84.6	148.2
6LL	b	T2	171.9	20	27.6	6.2	116.1	225.9

Weight g. all								
Treatment	Replicate	Period	Means	N	SD	SE	Min	Max
6LL	b	T3	251.6	20	43.8	9.8	164.6	323.8
6LL	b	T4	269.6	20	49.3	11.0	170.4	346.2
9LDN	a	T0	88.8	19	16.4	3.8	63.1	121.0
9LDN	a	T1	123.2	12	17.2	5.0	98.6	155.5
9LDN	a	T2	193.0	19	28.4	6.5	145.7	261.5
9LDN	a	T3	327.8	19	51.4	11.8	249.7	443.6
9LDN	a	T4	370.3	14	51.0	13.6	304.4	492.8
9LDN	b	T0	81.7	20	10.9	2.4	59.4	104.5
9LDN	b	T1	119.7	20	14.3	3.2	93.2	143.9
9LDN	b	T2	175.4	20	33.5	7.5	101.1	229.5
9LDN	b	T3	267.7	20	47.1	10.5	163.2	354.4
9LDN	b	T4	289.4	20	49.4	11.0	183.1	382.3
9LL	a	T0	86.4	20	11.0	2.5	61.7	109.3
9LL	a	T1	122.1	20	19.1	4.3	87.9	159.9
9LL	a	T2	187.4	20	33.3	7.5	130.6	262.2
9LL	a	T3	320.9	20	62.2	13.9	220.9	455.4
9LL	a	T4	350.1	20	65.0	14.5	238.1	488.8
9LL	b	T0	90.1	20	12.7	2.8	67.9	117.7
9LL	b	T1	114.5	41	19.7	3.1	68.5	148.7
9LL	b	T2	186.7	20	25.9	5.8	133.5	243.5
9LL	b	T3	303.7	20	43.7	9.8	231.6	409.7
9LL	b	T4	336.5	18	43.8	10.3	255.6	443.6

Response variables

TABLE IV. Descriptive statistics based on measurements of weight at T0 (day 0), T1 (day 42), T2 (day 83), T3 (day 124) and T4 (day 145). Means, total number of observations (N), standard deviation (SD) and standard error (SE) for each group are included in the TABLE.

	Weight T0				Weight T1				Weight T2				Weight T3				Weight T4			
Treatment	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
4LDN	89.4	40	12.2	2.7	104.7	40.0	14.8	3.3	88.8	40.0	12.0	2.7	111.5	35	17.1	3.5	137.9	40	20.8	4.7
4LL	85.8	40	16.3	3.6	111.4	40.0	17.9	4.0	150.5	40.0	22.3	5.0	193.9	39	32.1	7.3	207.2	20	32.6	7.4
6LDN	86.1	40	15.7	3.5	122.5	40.0	21.1	4.7	173.6	40.0	31.1	7.0	242.8	40	52.7	11.8	259.8	40	57.3	12.8
6LL	82.6	40	13.6	3.0	114.4	40.0	19.9	4.5	165.3	40.0	29.0	6.5	237.5	40	46.8	10.5	258.9	40	50.1	11.2
9LDN	85.3	40	13.6	3.1	121.5	32	15.8	4.1	184.2	40.0	30.9	7.0	297.7	39	49.2	11.2	329.8	34	50.2	12.3
9LL	88.2	40	11.9	2.7	118.3	31	19.4	3.7	187.0	40.0	29.6	6.6	312.3	40	53.0	11.8	343.3	38	54.4	12.4

TABLE V. Descriptive statistics based on measurements of length at T0 (day 0), T1 (day 42), T2 (day 83), T3 (day 84) and T4 (day 145). Means, total number of observations (N), standard deviation (SD) and standard error (SE) for each group are included in the TABLE.

	Length T0				Length T1				Length T2				Length T3				Length T4			
Treatment	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
4LDN	20.5	40	1.2	0.2	21.7	40	1.2	0.2	22.6	40	1.7	0.3	23.8	40	2.2	0.3	24.5	38	2.1	0.3
4LL	20.1	40	1.4	0.2	21.7	40	1.3	0.2	23.5	40	1.4	0.2	25.3	39	1.6	0.2	25.9	39	1.4	0.2
6LDN	20.2	40	1.4	0.2	22.6	40	1.4	0.2	24.6	40	1.6	0.3	27.1	40	2.1	0.3	27.7	40	2.4	0.4
6LL	20.0	40	1.1	0.2	22.1	40	1.2	0.2	24.2	40	1.4	0.2	26.9	40	1.8	0.3	27.8	40	2.0	0.3
9LDN	20.2	39	1.2	0.2	22.5	39	1.2	0.2	25.1	39	1.4	0.2	29.0	39	1.9	0.3	30.1	34	2.1	0.4
9LL	20.4	40	0.9	0.1	22.8	40	1.0	0.2	25.1	40	1.4	0.2	29.3	40	1.8	0.3	30.7	38	1.8	0.3

TABLE VI. Descriptive statistics based on calculated SGR from T0-T1 (day 0-42), T1-T2 (day 42-83), T2-T3 (day 83-124), T3-T4 (day 124-145) and Overall T0- T4 (day 0-145). Means, total number of observations (N),standard deviation (SD) and standard error (SE) for each group are included in the TABLE.

	SGR T0- 1				SGR T1- 2				SGR T2- 3				SGR T3- 4				Overall T0- 4			
Treatment	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
4LDN	0.35	40	0.18	0.03	0.52	40	0.25	0.04	0.51	40	0.16	0.03	0.29	38	0.19	0.03	0.44	38	0.12	0.02
4LL	0.62	39	0.18	0.03	0.74	39	0.16	0.03	0.61	39	0.20	0.03	0.26	38	0.13	0.02	0.60	38	0.12	0.02
6LDN	0.82	40	0.32	0.05	0.85	40	0.18	0.03	0.80	40	0.18	0.03	0.32	40	0.14	0.02	0.75	40	0.13	0.02
6LL	0.77	40	0.13	0.02	0.87	40	0.31	0.05	0.87	40	0.20	0.03	0.42	40	0.39	0.06	0.78	40	0.11	0.02

	SGR T0- 1				SGR T1- 2				SGR T2- 3				SGR T3- 4				Overall T0- 4			
9LDN	0.73	19	0.26	0.06	1.15	19	0.18	0.04	1.29	19	0.13	0.03	0.32	14	0.16	0.04	0.90	34	0.13	0.02
9LL	0.78	19	0.24	0.05	1.03	19	0.30	0.07	1.32	19	0.10	0.02	0.42	19	0.15	0.03	0.94	38	0.09	0.02

TABLE VII. Descriptive statistics based on measurements of CF at T0 (day 0), T1 (day 42), T2 (day 83), T3 (day 84) and T4 (day 125). Means, total number of observations (N), standard deviation (SD) and standard error (SE) for each group are included in the TABLE.

	CF T0				CF T1				CF T2				CF T3				CF T4			
Treatment	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
4LDN	1.03	40	0.05	0.01	1.01	40	0.07	0.01	1.10	40	0.07	0.01	1.19	40	0.20	0.03	1.16	38	0.06	0.01
4LL	1.05	40	0.07	0.01	1.09	40	0.07	0.01	1.16	40	0.09	0.01	1.19	39	0.05	0.01	1.18	39	0.05	0.01
6LDN	1.04	40	0.07	0.01	1.06	40	0.05	0.01	1.16	40	0.07	0.01	1.21	40	0.06	0.01	1.20	40	0.13	0.02
6LL	1.02	40	0.05	0.01	1.05	40	0.11	0.02	1.16	40	0.05	0.01	1.20	40	0.10	0.02	1.20	40	0.16	0.03
9LDN	1.03	39	0.05	0.01	1.05	39	0.07	0.01	1.16	39	0.12	0.02	1.20	39	0.06	0.01	1.17	34	0.05	0.01
9LL	1.03	40	0.05	0.01	1.04	40	0.07	0.01	1.18	40	0.08	0.01	1.23	40	0.08	0.01	1.19	38	0.09	0.01

TABLE VIII. Descriptive statistics based on calculated FCR, FCE and FC from 42 days.

Feed conversion efficiency (FCE), Feed conversion (FC)			
Gr	Replicate	FCE	FC
4LD	a	1.11	0.13
4LD	b	0.60	0.16
4LL	a	1.20	0.14
4LL	b	1.22	0.13
6LD	a	0.82	0.24
6LD	b	0.87	0.24
6LL	a	0.86	0.24
6LL	b	0.92	0.23
9LD	a	0.63	0.41
9LD	b	0.65	0.35
9LL	a	0.67	0.38
9LL	b	0.66	0.37

TABLE IX. Descriptive statistics based on measurements of blood Na^+ at T0 (day 0), T1 (day 30), T2 (day 71) and T3 (day 113). Means, total number of observations (N), standard deviation (SD) and standard error (SE) for each group are included in the TABLE.

	Na^+ T0				Na^+ T1				Na^+ T2				Na^+ T3			
Treatment	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
4LDN	149.42	12	1.31	0.38	155.83	12	4.06	1.17	157.33	12	7.90	2.28	155.67	12	2.64	0.76
4LL	149.42	12	1.31	0.38	153.70	10	3.09	0.98	155.75	12	4.18	1.21	152.36	11	3.17	0.96
6LDN	149.42	12	1.31	0.38	154.42	12	5.30	1.53	152.92	12	3.53	1.02	152.67	12	1.87	0.54
6LL	149.42	12	1.31	0.38	151.27	11	2.05	0.62	151.50	12	2.78	0.80	152.50	12	2.88	0.83
9LDN	149.42	12	1.31	0.38	153.33	12	4.12	1.19	151.83	12	1.59	0.46	150.36	11	2.06	0.62
9LL	149.42	12	1.31	0.38	152.75	12	2.93	0.84	152.00	12	4.26	1.23	150.17	12	1.19	0.34

TABLE X. Descriptive statistics based on measurements of blood Glucose at T0 (day 0), T1 (day 30), T2 (day 71) and T3 (day 113). Means, total number of observations (N), standard deviation (SD) and standard error (SE) for each group are included in the TABLE.

	Glu T0				Glu T0				Glu T0				Glu T0			
Treatment	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
4LDN	66.25	12	4.20	1.21	71.92	12	9.39	2.71	95.45	11	19.92	6.01	86.17	12	12.94	3.74
4LL	66.25	12	4.20	1.21	78.09	11	13.52	4.08	97.42	12	13.77	3.97	92.67	12	9.22	2.66
6LDN	66.25	12	4.20	1.21	97.25	12	12.98	3.75	94.65	12	17.70	5.11	85.33	12	8.75	2.53
6LL	66.25	12	4.20	1.21	92.45	11	6.33	1.91	93.67	12	17.92	5.17	81.92	12	11.33	3.27
9LDN	66.25	12	4.20	1.21	103.64	11	13.34	4.02	89.67	12	10.13	2.92	81.27	11	9.49	2.86
9LL	66.25	12	4.20	1.21	97.75	12	11.26	3.25	90.17	12	9.65	2.78	83.58	12	6.19	1.79

TABLE XI. Descriptive statistics based on measurements of blood pCO_2 at T0 (day 0), T1 (day 30), T2 (day 71) and T3 (day 113). Means, total number of observations (N), standard deviation (SD) and standard error (SE) for each group are included in the TABLE.

	pCO_2 T0				pCO_2 T1				pCO_2 T3				pCO_2 T4			
Treatment	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
4LDN	11.19	12	1.29	0.37	9.00	12	1.86	0.54	7.70	11	2.63	0.79	4.91	11	1.44	0.43
4LL	11.19	12	1.29	0.37	8.99	11	2.07	0.62	7.83	10	2.70	0.85	4.48	11	1.79	0.54
6LDN	11.19	12	1.29	0.37	10.61	12	2.68	0.77	8.63	11	2.10	0.63	5.74	10	2.07	0.66
6LL	11.19	12	1.29	0.37	8.84	11	2.26	0.68	7.31	11	2.70	0.82	5.94	11	1.40	0.42
9LDN	11.19	12	1.29	0.37	12.81	12	3.01	0.87	12.03	12	4.03	1.16	7.68	11	1.61	0.49

	pCO_2 T0				pCO_2 T1				pCO_2 T3				pCO_2 T4			
9LL	11.19	12	1.29	0.37	12.54	12	3.04	0.88	11.17	12	4.16	1.20	7.88	12	2.08	0.60

TABLE XII. Descriptive statistics based on measurements of blood HCO_3^- at T0 (day 0), T1 (day 30), T2 (day 71) and T3 (day 113). Means, total number of observations (N), standard deviation (SD) and standard error (SE) for each group are included in the TABLE.

	HCO_3^- T0				HCO_3^- T1				HCO_3^- T2				HCO_3^- T3			
Treatment	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE	Means	N	SD	SE
4LDN	7.00	12	1.30	0.37	6.16	12	1.52	0.44	5.17	11	1.55	0.47	4.14	11	1.69	0.51
4LL	7.00	12	1.30	0.37	6.22	11	1.29	0.39	6.36	10	2.26	0.72	4.01	11	2.12	0.64
6LDN	7.00	12	1.30	0.37	7.41	12	2.42	0.70	6.24	11	1.67	0.50	4.73	10	2.02	0.64
6LL	7.00	12	1.30	0.37	5.75	11	1.31	0.39	5.02	11	1.72	0.52	4.54	11	1.01	0.30
9LDN	7.00	12	1.30	0.37	7.95	12	2.35	0.68	9.17	12	3.03	0.88	6.19	11	1.87	0.56
9LL	7.00	12	1.30	0.37	8.14	12	2.24	0.65	7.91	12	2.90	0.84	7.34	12	2.55	0.73

TABLE XIII. Descriptive statistics based on measurements of Dorsal fin index T3 (day 113). Means, total number of observations (N), standard deviation (SD) and standard error (SE) for each group are included in the TABLE.

Dorsal fin index						
Treatment	Means	n	SD	SE	Min	Max
4LDN	0.27	12	0.04	0.01	0.21	0.35
4LL	0.30	12	0.08	0.02	0.19	0.42
6LDN	0.30	12	0.07	0.02	0.16	0.41
6LL	0.31	12	0.07	0.02	0.19	0.40
9LDN	0.30	12	0.07	0.02	0.19	0.39
9LL	0.27	12	0.07	0.02	0.14	0.40

TABLE XIV. Descriptive statistics based on measurements of Hepato - somatic index T3 (day 113). Means, total number of observations (N), standard deviation (SD) and standard error (SE) for each group are included in the TABLE

Hepato - somatic index						
Treatment	Means	n	SD	SE	Min	Max
4LDN	1.75	12	0.42	0.12	1.19	2.71
4LL	1.47	12	0.35	0.10	1.07	2.06
6LDN	1.29	12	0.20	0.06	0.88	1.60
6LL	1.36	12	0.19	0.05	1.13	1.74
9LDN	1.29	12	0.21	0.06	1.05	1.76
9LL	1.32	12	0.24	0.07	1.09	1.75

TABLE XV. Descriptive statistics based on measurements of Cardio - somatic index T3 (day 113). Means, total number of observations (N), standard deviation (SD) and standard error (SE) for each group are included in the TABLE

Cardio - somatic index						
Treatment	Means	n	SD	SE	Min	Max
4LDN	0.15	12	0.02	0.01	0.12	0.22
4LL	0.14	12	0.02	0.01	0.11	0.17
6LDN	0.13	12	0.02	0.01	0.11	0.18
6LL	0.14	12	0.02	0.01	0.11	0.17
9LDN	0.14	12	0.01	0.00	0.13	0.15
9LL	0.13	12	0.02	0.00	0.11	0.16

ANOVA

Two-way factorial ANOVA

Weight

TABLE XVI. Test results from two- way factorial ANOVA on weight data from T0 (day 0). .

Weight T0 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1765333.53	1	1765333.53	8297.911	<0.001
Temperature	405.90	2	202.95	0.954	0.387
Photoperiod	117.18	1	117.18	0.551	0.459
Temperature*Photoperiod	593.93	2	296.97	1.396	0.250
Error	49356.68	232	212.74		

TABLE XVII. Test results from two- way factorial ANOVA on weight data from T1 (day 42). .

Weight T1 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	3202105.06	1	3202105.06	8652.736	<0.001
Temperature	8364.69	2	4182.34	11.302	<0.001
Photoperiod	14.77	1	14.77	0.040	0.842
Temperature*Photoperiod	2353.35	2	1176.67	3.180	0.043
Error	85855.90	232	370.07		

TABLE XVIII. Test results from two- way factorial ANOVA on weight data from T2 (day 83). .

Weight T2 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	6496620.26	1	6496620.26	7090.973	<0.001
Temperature	81096.75	2	40548.37	44.258	<0.001
Photoperiod	1316.70	1	1316.70	1.437	0.232
Temperature*Photoperiod	7718.49	2	3859.24	4.212	0.016
Error	212554.18	232	916.18		

TABLE XIX. Test results from two- way factorial ANOVA on weight data from T3 (day 84). .

Weight T3 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	13816608.34	1	13816608.34	5823.203	<0.001
Temperature	632849.45	2	316424.72	133.362	<0.001
Photoperiod	11786.99	1	11786.99	4.968	0.027
Temperature*Photoperiod	13985.03	2	6992.52	2.947	0.054
Error	550462.22	232	2372.68		

TABLE XX. Test results from two- way factorial ANOVA on weight data from T4 (day 145). .

Weight T4 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	15502843.73	1	15502843.73	5866.536	<0.001
Temperature	747194.17	2	373597.08	141.375	<0.001
Photoperiod	18293.69	1	18293.69	6.923	0.009
Temperature*Photoperiod	11985.07	2	5992.54	2.268	0.106
Error	586654.79	222	2642.59		

Length

TABLE XXI. Test results from two- way factorial ANOVA on length data from T0 (day 0). .

Length T0 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	97193.34	1	97193.34	67680.000	<0.001
Temperature	1.89	2	0.95	0.659	0.518
Photoperiod	0.71	1	0.71	0.491	0.484
Temperature*Photoperiod	5.08	2	2.54	1.769	0.173
Error	333.17	232	1.44		

TABLE XXII. Test results from two- way factorial ANOVA on length data from T1 (day 42). .

Length T1 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	117684.9	1	117684.9	76532.314	<0.001
Temperature	39.9	2	20.0	12.978	<0.001
Photoperiod	0.4	1	0.4	0.242	0.623
Temperature*Photoperiod	4.6	2	2.3	1.488	0.228
Error	356.7	232	1.5		

TABLE XXIII. Test results from two- way factorial ANOVA on length data from T2 (day83). .

Length T2 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	139053.3	1	139053.3	62411.739	<0.001
Temperature	163.6	2	81.8	36.704	<0.001
Photoperiod	1.1	1	1.1	0.500	0.480
Temperature*Photoperiod	16.8	2	8.4	3.760	0.025
Error	516.9	232	2.2		

TABLE XXIV. Test results from two- way factorial ANOVA on length data from T3 (day 124). .

Length T3 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	172112.6	1	172112.6	47245.434	<0.001
Temperature	858.4	2	429.2	117.811	<0.001
Photoperiod	19.5	1	19.5	5.346	0.022
Temperature*Photoperiod	27.5	2	13.7	3.774	0.024
Error	845.2	232	3.6		

TABLE XXV. Test results from two- way factorial ANOVA on length data from T4 (day 145). .

Length T4 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	175507.9	1	175507.9	44712.360	<0.001
Temperature	976.8	2	488.4	124.429	<0.001
Photoperiod	26.6	1	26.6	6.789	0.010
Temperature*Photoperiod	20.4	2	10.2	2.604	0.076
Error	871.4	222	3.9		

Condition Factor (CF)

TABLE XXVI. Test results from two- way factorial ANOVA on CF data from T0 (day 0). .

CF T0 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	255.3677	1	255.4	73550.248	<0.001
Temp	0.0055	2	0.0	0.787	0.457
Lys	0.0000	1	0.0	0.005	0.943
Temp*Lys	0.0194	2	0.0	2.793	0.063
Error	0.8055	232	0.0		

TABLE XXVII. Test results from two- way factorial ANOVA on CF data from T1 (day 42). .

CF T1 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	256.2410	1	256.2	58220.334	<0.001
Temp	0.0243	2	0.0	2.765	0.065
Lys	0.0002	1	0.0	0.037	0.848
Temp*Lys	0.2108	2	0.1	23.952	<0.001
Error	1.0211	232	0.0		

TABLE XXVIII. Test results from two- way factorial ANOVA on CF data from T2 (day83). .

CF T2 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	316.2137	1	316.2	46533.324	<0.001
Temp	0.0696	2	0.0	5.122	0.007
Lys	0.0387	1	0.0	5.700	0.018
Temp*Lys	0.0277	2	0.0	2.037	0.133
Error	1.5765	232	0.0		

TABLE XXIX. Test results from two- way factorial ANOVA on CF data from T3 (day 124). .

CF T3 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	345.3576	1	345.4	31905.807	<0.001
Temp	0.0220	2	0.0	1.018	0.363
Lys	0.0033	1	0.0	0.301	0.584
Temp*Lys	0.0102	2	0.0	0.472	0.624
Error	2.5112	232	0.0		

TABLE XXX. Test results from two- way factorial ANOVA on length data from T4 (day 145). .

CF T4 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	318.7184	1	318.7	30728.766	<0.001
Temp	0.0487	2	0.0	2.349	0.098
Lys	0.0071	1	0.0	0.688	0.408
Temp*Lys	0.0042	2	0.0	0.204	0.815
Error	2.3026	222	0.0		

SGR

TABLE XXXI. Test results from two- way factorial ANOVA on SGR data from T1-T2. .

SGR 1-2 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	114.0561	1	114.1	1938.820	<0.001
Temperature	4.9459	2	2.5	42.037	<0.001
Photoperiod	0.2234	1	0.2	3.798	0.053
Temperature*Photoperiod	1.2907	2	0.6	10.970	<0.001
Error	13.6480	232	0.1		

TABLE XXXII. Test results from two- way factorial ANOVA on SGR data from T2-T3. .

SGR 2-3 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	166.0921	1	166.1	2541.303	<0.001
Temperature	6.0110	2	3.0	45.986	<0.001
Photoperiod	0.3898	1	0.4	5.963	0.015
Temperature*Photoperiod	0.6028	2	0.3	4.612	0.011
Error	15.1628	232	0.1		

TABLE XXXIII. Test results from two- way factorial ANOVA on SGR data from T3-T4. .

SGR 3-4 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	26.76126	1	26.8	571.096	<0.001
Temperature	0.57283	2	0.3	6.112	0.003
Photoperiod	0.12821	1	0.1	2.736	0.100
Temperature*Photoperiod	0.17046	2	0.1	1.819	0.165
Error	10.40281	222	0.0		

TABLE XXXIV. Test results from two- way factorial ANOVA on SGR data from T1-T5. .

SGR 1-5 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	122.7440	1	122.7	8818.291	<0.001
Temperature	5.9334	2	3.0	213.136	<0.001
Photoperiod	0.3343	1	0.3	24.014	<0.001
Temperature*Photoperiod	0.2262	2	0.1	8.124	<0.001
Error	3.0901	222	0.0		

***Na*$^+$**

TABLE XXXV. Test results from two- way factorial ANOVA on Na^+ data T0. .

Na^+ T0 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	6566031	1	6566031.3	497304.400	<0.001
Temperature	323	2	161.3	12.215	<0.001
Photoperiod	75	1	75.1	5.685	0.018
Temperature*Photoperiod	32	2	15.9	1.206	0.301
Error	3591	272	13.2		

TABLE XXXVI. Test results from two- way factorial ANOVA on Na^+ data T1. .

Na^+ T1 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1619108	1	1619107.5	113365.010	<0.001
Temperature	50	2	24.9	1.746	0.183
Photoperiod	66	1	65.5	4.587	0.036
Temperature*Photoperiod	20	2	9.8	0.685	0.508
Error	900	63	14.3		

TABLE XXXVII. Test results from two- way factorial ANOVA on Na^+ data T2. .

Na^+ T2 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1697710	1	1697710.2	84342.397	<0.001
Temperature	322	2	161.0	7.999	0.001
Photoperiod	16	1	16.1	0.798	0.375
Temperature*Photoperiod	11	2	5.6	0.278	0.758
Error	1329	66	20.1		

TABLE XXXVIII. Test results from two- way factorial ANOVA on Na^+ data T3. .

Na^+ T3 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1640368	1	1640367.7	286889.439	<0.001
Temperature	163	2	81.6	14.279	<0.001
Photoperiod	24	1	23.6	4.119	0.046
Temperature*Photoperiod	41	2	20.3	3.552	0.034
Error	372	65	5.7		

Glucose

TABLE XXXIX. Test results from two- way factorial ANOVA on glucose data T0. .

Glu T0 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1985405	1	1985405.2	7569.773	<0.001
Temperature	565	2	282.7	1.078	0.342
Photoperiod	9	1	9.0	0.034	0.853
Temperature*Photoperiod	556	2	278.0	1.060	0.348
Error	71340	272	262.3		

TABLE XL. Test results from two- way factorial ANOVA on glucose data T1. .

Glu T1 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	560115.4	1	560115.4	4288.890	<0.001
Temperature	8325.7	2	4162.9	31.876	<0.001
Photoperiod	38.9	1	38.9	0.298	0.587
Temperature*Photoperiod	510.7	2	255.4	1.955	0.150
Error	8227.6	63	130.6		

TABLE XLI. Test results from two- way factorial ANOVA on glucose data T2. .

Glu T2 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	622528.3	1	622528.3	2636.990	<0.001
Temperature	539.5	2	269.8	1.143	0.325
Photoperiod	0.3	1	0.3	0.001	0.973
Temperature*Photoperiod	49.5	2	24.7	0.105	0.901
Error	15344.9	65	236.1		

TABLE XLII. Test results from two- way factorial ANOVA on glucose data T3. .

	Glu T3 Two- way factorial ANOVA				
Effect	SS	DF	MS	F	p
Intercept	519127.8	1	519127.8	5294.293	<0.001
Temperature	701.8	2	350.9	3.579	0.033
Photoperiod	78.3	1	78.3	0.799	0.375
Temperature*Photoperiod	250.9	2	125.5	1.280	0.285
Error	6471.6	66	98.1		

pCO_2

TABLE XLIII. Test results from two- way factorial ANOVA on pCO2 data T1. .

pCO_2 T1 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	7656.209	1	7656.2	1188.345	<0.001
Temperature	179.428	2	89.7	13.925	<0.001
Photoperiod	8.111	1	8.1	1.259	0.266
Temperature*Photoperiod	10.412	2	5.2	0.808	0.450
Error	412.336	64	6.4		

TABLE XLIV. Test results from two- way factorial ANOVA on pCO_2 data T2. .

pCO_2 T2 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	5543.237	1	5543.2	544.599	<0.001
Temperature	214.057	2	107.0	10.515	<0.001
Photoperiod	7.849	1	7.8	0.771	0.383
Temperature*Photoperiod	5.878	2	2.9	0.289	0.750
Error	620.893	61	10.2		

TABLE XLV. Test results from two- way factorial ANOVA on pCO_2 data T3. .

pCO_2 T3 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	2459.527	1	2459.5	783.761	<0.001
Temperature	111.812	2	55.9	17.815	<0.001
Photoperiod	0.092	1	0.1	0.029	0.865
Temperature*Photoperiod	2.287	2	1.1	0.364	0.696
Error	191.425	61	3.1		

HCO_3^-

TABLE XLVI. Test results from two- way factorial ANOVA on HCO_3^- data T1. .

HCO_3^- T1 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	3363.760	1	3363.8	899.643	<0.001
Temperature	45.005	2	22.5	6.018	0.004
Photoperiod	3.866	1	3.9	1.034	0.313
Temperature*Photoperiod	12.372	2	6.2	1.654	0.199
Error	239.296	64	3.7		

TABLE XLVII. Test results from two- way factorial ANOVA on HCO_3^- data T2. .

HCO_3^- T2 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	2947.918	1	2947.9	558.965	<0.001
Temperature	124.728	2	62.4	11.825	<0.001
Photoperiod	3.142	1	3.1	0.596	0.443
Temperature*Photoperiod	21.326	2	10.7	2.022	0.141
Error	321.707	61	5.3		

TABLE XLVIII. Test results from two- way factorial ANOVA on HCO_3^- data T3. .

HCO_3^- T3 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1774.214	1	1774.2	477.100	<0.001
Temperature	92.030	2	46.0	12.374	<0.001
Photoperiod	1.454	1	1.5	0.391	0.534
Temperature*Photoperiod	6.182	2	3.1	0.831	0.440
Error	226.843	61	3.7		

TABLE XLIX. Test results from two- way factorial ANOVA on Dorsal fin index data T3. .

Dorsal fin index T3 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	6,216	1	6,2	1352,812	0,000
Temperature	0,007	2	0,0	0,745	0,478
Photoperiod	0,000	1	0,0	0,014	0,905
Temperature*Photoperiod	0,008	2	0,0	0,897	0,413
Error	0,303	66	0,0		

TABLE L. Test results from two- way factorial ANOVA on Hepato - somatic index data T3. .

Hepato - somatic index T3 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	143,647	1	143,6	1839,468	0,000
Temperature	1,420	2	0,7	9,090	0,000
Photoperiod	0,068	1	0,1	0,875	0,353
Temperature*Photoperiod	0,470	2	0,2	3,008	0,056
Error	5,154	66	0,1		

TABLE LI. Test results from two- way factorial ANOVA on Cardio - somatic index data T3. .

Cardio - somatic index T3 Two- way factorial ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1,368	1	1,4	4350,459	0,000
Temperature	0,002	2	0,0	3,345	0,041
Photoperiod	0,000	1	0,0	0,734	0,395
Temperature*Photoperiod	0,001	2	0,0	2,322	0,106
Error	0,021	66	0,0		

One-way ANOVA

Weight

TABLE LII. Test results from one- way ANOVA on calculated weight data 4LDN from T0-T4 (day 0-145).

Weight Overall 4LDN (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	3455793	1	3455793	3035.983	<0.001
Time	206656	4	51664	45.388	<0.001
Error	219688	193	1138		

TABLE LIII. Test results from one- way ANOVA on calculated weight data 4LL from T0-T4 (day 0-145).

Weight Overall 4LL (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	4353875	1	4353875	6644.207	<0.001
Time	423699	4	105925	161.646	<0.001
Error	123850	189	655		

TABLE LIV. Test results from one- way ANOVA on calculated weight data 6LDN from T0-T4 (day 0-145).

Weight Overall 6LDN (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	6262710	1	6262710	4097.863	<0.001
Time	897726	4	224431	146.852	<0.001
Error	298016	195	1528		

TABLE LV. Test results from one- way ANOVA on calculated weight data 6LL from T0-T4 (day 0-145).

Weight Overall 6LL (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	5898924	1	5898924	4640.669	<0.001
Time	928664	4	232166	182.644	<0.001
Error	247872	195	1271		

TABLE LVI. Test results from one- way ANOVA on calculated weight data 9LDN from T0-T4 (day 0-145).

Weight Overall 9LDN (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	7716859	1	7716859	4505.791	<0.001
Time	1649437	4	412359	240.772	<0.001
Error	316841	185	1713		

TABLE LVII. Test results from one- way ANOVA on calculated weight data 9LL from T0-T4 (day 0-145).

Weight Overall 9LL (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	8800121	1	8800121	6095.886	<0.001
Time	2013298	4	503325	348.655	<0.001
Error	278618	193	1444		

Length

TABLE LVIII. Test results from one- way ANOVA on calculated length data 4LDN from T0-T4 (day 0-145).

Length Overall 4LDN (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	101243.8	1	101243.8	34741.016	<0.001
Time	403.7	4	100.9	34.630	<0.001
Error	562.4	193	2.9		

TABLE LVIX. Test results from one- way ANOVA on calculated length data 4LL from T0-T4 (day 0-145).

Length Overall 4LL (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	105126.6	1	105126.6	52118.029	<0.001
Time	940.1	4	235.0	116.520	<0.001
Error	381.2	189	2.0		

TABLE LX. Test results from one- way ANOVA on calculated length data 6LDN from T0-T4 (day 0-145).

Length Overall 6LDN (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	119371.8	1	119371.8	35927.908	<0.001
Time	1582.9	4	395.7	119.103	<0.001
Error	647.9	195	3.3		

TABLE. LXI. Test results from one- way ANOVA on calculated length data 6LL from T0-T4 (day 0-145).

Length Overall 6LL (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	117205.5	1	117205.5	48062.649	<0.001
Time	1680.9	4	420.2	172.322	<0.001
Error	475.5	195	2.4		

TABLE LXII. Test results from one- way ANOVA on calculated length data 9LDN from T0-T4 (day 0-145).

Length Overall 9LDN (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	121926.8	1	121926.8	48258.929	<0.001
Tid	2642.3	4	660.6	261.460	<0.001
Error	467.4	185	2.5		

TABLE LXIII. Test results from one- way ANOVA on calculated length data 9LL from T0-T4 (day 0-145).

Weight Overall 9LL (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	130256.0	1	130256.0	64645.356	<0.001
Tid	2931.3	4	732.8	363.693	<0.001
Error	388.9	193	2.0		

Condition Factor (CF)

TABLE LXIV. Test results from one- way ANOVA on calculated CF data 4LDN from T0-T4 (day 0-145).

CF Overall 4LDN (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	238.8618	1	238.9	20966.097	<0.001
Time	0.9934	4	0.2	21.799	<0.001
Error	2.1988	193	0.0		

TABLE LXV. Test results from one- way ANOVA on calculated CF data 4LL from T0-T4 (day 0-145).

CF Overall 4LLL (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	249.7063	1	249.7063	51933.94	0.00
Time	0.5752	4	0.1438	29.91	0.00
Error	0.9087	189	0.0048		

TABLE LXVI. Test results from one- way ANOVA on calculated CF data 6LDN from T0-T4 (day 0-145).

CF Overall 6LDN (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	256.9246	1	256.9246	38702.09	0.00
Time	0.9994	4	0.2499	37.64	0.00
Error	1.2945	195	0.0066		

TABLE LXVII. Test results from one- way ANOVA on calculated CF data 6LL from T0-T4 (day 0-145).

CF Overall 6LL (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	253.7371	1	253.7371	24553.26	0.00
Time	1.1853	4	0.2963	28.67	0.00
Error	2.0152	195	0.0103		

TABLE LXVIII. Test results from one- way ANOVA on calculated CF data 9LDN from T0-T4 (day 0-145).

CF Overall 9LDN (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	239.1087	1	239.1087	41910.38	0.00
Time	0.9362	4	0.2341	41.03	0.00
Error	1.0555	185	0.0057		

TABLE LXIX. Test results from one- way ANOVA on calculated CF data 9LL from T0-T4 (day 0-145).

CF Overall 9LL (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	253.9367	1	253.9367	45923.02	0.00
Time	1.3704	4	0.3426	61.96	0.00
Error	1.0672	193	0.0055		

Sodium ion Na^+

TABLE LXX. Test results from one- way ANOVA on calculated Na^+ data 4LDN from T0-T4 (day 0-113).

Na^+ Overall 4LDN (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1146699	1	1146699.2	52343.492	<0.001
Time	444	3	148.0	6.754	0.001
Error	964	44	21.9		

TABLE LXXI. Test results from one- way ANOVA on calculated Na^+ data 4LL from T0-T4 (day 0-113).

Na^+ Overall 4LL (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1044821	1	1044820.5	107683.096	<0.001
Time	252	3	84.0	8.656	<0.001
Error	398	41	9.7		

TABLE LXXII. Test results from one- way ANOVA on calculated Na^+ data 6LDN from T0-T4 (day 0-113).

Na^+ Overall 6LDN (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1135248	1	1135247.8	100172.699	<0.001
Time	158	3	52.8	4.660	0.006
Error	510	45	11.3		

TABLE LXXIII. Test results from one- way ANOVA on calculated Na^+ data 6LL from T0-T4 (day 0-113).

Na^+ Overall 6LL (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1072571	1	1072571.2	194520.688	<0.001
Time	60	3	19.8	3.599	0.021
Error	237	43	5.5		

TABLE LXXIV. Test results from one- way ANOVA on calculated Na^+ data 9LDN from T0-T4 (day 0-113).

Na^+ Overall 9LDN (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1073485	1	1073485.1	167369.909	<0.001
Time	105	3	35.0	5.464	0.003
Error	276	43	6.4		

TABLE LXXV. Test results from one- way ANOVA on calculated Na+ data 9LL from T0-T4 (day 0-113).

Na^+ Overall 9LL (T0-T4) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1095656	1	1095656.3	146605.814	<0.001
Time	87	3	28.9	3.873	0.015
Error	329	44	7.5		

Glucose

TABLE LXXVI. Test results from one- way ANOVA on calculated glucose data 4LDN from T0-T4 (day 0-113).

Glu Overall 4LDN (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	299975.2	1	299975.2	1849.691	<0.001
Time	6129.5	3	2043.2	12.599	<0.001
Error	6973.6	43	162.2		

TABLE LXXVII. Test results from one- way ANOVA on calculated glucose data 4LL from T0-T4 (day 0-113).

Glu Overall 4LL (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	328062.8	1	328062.8	2797.426	<0.001
Time	7222.7	3	2407.6	20.529	<0.001
Error	5042.7	43	117.3		

TABLE LXXVIII. Test results from one- way ANOVA on calculated glucose data 6LDN from T0-T4 (day 0-113).

Glu Overall 6LDN (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	361362.1	1	361362.1	2457.139	<0.001
Time	7370.5	3	2456.8	16.706	<0.001
Error	6618.0	45	147.1		

TABLE LXXIX. Test results from one- way ANOVA on calculated glucose data 6LL from T0-T4 (day 0-113).

Glu Overall 6LL (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	327795.3	1	327795.3	2544.920	<0.001
Time	5722.5	3	1907.5	14.809	<0.001
Error	5538.6	43	128.8		

TABLE LXXX. Test results from one- way ANOVA on calculated glucose data 9LDN from T0-T4 (day 0-113).

Glu Overall 9LDN (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	333335.0	1	333335.0	3496.832	<0.001
Time	8452.8	3	2817.6	29.558	<0.001
Error	4003.6	42	95.3		

TABLE LXXXI. Test results from one- way ANOVA on calculated glucose data 9LL from T0-T4 (day 0-113).

Glu Overall 9LL (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	342225.2	1	342225.2	4964.555	<0.001
Time	6498.7	3	2166.2	31.425	<0.001
Error	3033.1	44	68.9		

pCO_2

TABLE LXXXII. Test results from one- way ANOVA on calculated pCO_2 data 4LDN from T0-T4 (day 0-113).

pCO_2 4LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		0.008	<0.001	<0.001
T1	0.008		0.103	<0.001
T2	<0.001	0.103		0.001
T3	<0.001	<0.001	0.001	

TABLE LXXXIII. Test results from one- way ANOVA on calculated pCO_2 data 4LL from T0-T4 (day 0-113).

pCO_2 4LL T0 - T3				
Tid	T0	T1	T2	T3
T0		0.014	0.001	<0.001
T1	0.014		0.179	<0.001
T2	0.001	0.179		<0.001
T3	<0.001	<0.001	<0.001	

TABLE LXXXIV. Test results from one- way ANOVA on calculated pCO_2 data 6LDN from T0-T4 (day 0-113).

pCO_2 6LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		0.515	0.016	<0.001
T1	0.515		0.030	<0.001
T2	0.016	0.030		0.001
T3	<0.001	<0.001	0.001	

TABLE LXXXV. Test results from one- way ANOVA on calculated pCO_2 data 6LL from T0-T4 (day 0-113).

pCO_2 6LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		0.008	<0.001	<0.001
T1	0.008		0.076	0.004
T2	<0.001	0.076		0.108
T3	<0.001	0.004	0.108	

TABLE LXXXVI. Test results from one- way ANOVA on calculated pCO_2 data 9LDN from T0-T4 (day 0-113).

pCO_2 9LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		0.331	0.459	0.004
T1	0.331		0.494	<0.001
T2	0.459	0.494		0.001
T3	0.004	<0.001	0.001	

TABLE LXXXVII. Test results from one- way ANOVA on calculated pCO2 data 9LL from T0-T4 (day 0-113).

pCO_2 9LL T0 - T3				
Tid	T0	T1	T2	T3
T0		0.250	0.985	0.018
T1	0.250		0.469	0.001
T2	0.985	0.469		0.007
T3	0.018	0.001	0.007	

HCO_3^-

TABLE LXXXVIII. Test results from one- way ANOVA on calculated HCO_3^- data 4LDN from T0-T4 (day 0-113).

HCO_3^- Overall 4LDN (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1449.029	1	1449.0	630.308	<0.001
Time	52.283	3	17.4	7.581	<0.001
Error	96.555	42	2.3		

TABLE LXXXIX. Test results from one- way ANOVA on calculated HCO_3^- data 4LL from T0-T4 (day 0-113).

HCO_3^- Overall 4LL (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1523.810	1	1523.8	482.773	<0.001
Time	57.048	3	19.0	6.025	0.002
Error	126.255	40	3.2		

TABLE XC. Test results from one- way ANOVA on calculated HCO_3^- data 6LDN from T0-T4 (day 0-113).

HCO_3^- Overall 6LDN (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1838.863	1	1838.9	522.163	<0.001
Time	49.777	3	16.6	4.712	0.006
Error	147.908	42	3.5		

TABLE XCI. Test results from one- way ANOVA on calculated HCO_3^- data 6LL from T0-T4 (day 0-113).

HCO_3^- Overall 6LL (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	1396.617	1	1396.6	758.890	<0.001
Time	39.742	3	13.2	7.198	0.001
Error	75.454	41	1.8		

TABLE XCII. Test results from one- way ANOVA on calculated HCO_3^- data 9LDN from T0-T4 (day 0-113).

HCO_3^- Overall 9LDN (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	2695.108	1	2695.1	538.521	<0.001
Time	57.333	3	19.1	3.819	0.016
Error	215.200	43	5.0		

TABLE XCIII. Test results from one- way ANOVA on calculated HCO3- data 9LL from T0-T4 (day 0-113).

HCO_3^- Overall 9LL (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	2768.879	1	2768.9	512.740	<0.001
Time	9.769	3	3.3	0.603	0.617
Error	237.607	44	5.4		

SNK test

Weight by treatments

TABLE XCIV. p-values from SNK test, testing for differences in weight between treatments at T1 (day 42) .

Weight T1						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.114	<0.001	0.054	0.001	<0.001
4LL	0.114		0.046	0.464	0.086	0.047
6LDN	<0.001	0.046		0.149	0.632	0.879
6LL	0.054	0.464	0.149		0.165	0.181
9LDN	0.001	0.086	0.632	0.165		0.803
9LL	<0.001	0.047	0.879	0.181	0.803	

TABLE XCV. p-values from SNK test, testing for differences in weight between treatments at T2 (day 83) .

Weight T1						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.004	<0.001	<0.001	<0.001	<0.001
4LL	0.004		0.002	0.030	<0.001	<0.001
6LDN	<0.001	0.002		0.220	0.127	0.119
6LL	<0.001	0.030	0.220		0.016	0.007
9LDN	<0.001	<0.001	0.127	0.016		0.653
9LL	<0.001	<0.001	0.119	0.007	0.653	

TABLE XCVI. p-values from SNK test, testing for differences in weight between treatments at T3 (day 124) .

Weight T2						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.003	<0.001	<0.001	<0.001	<0.001
4LL	0.003		<0.001	<0.001	<0.001	<0.001
6LDN	<0.001	<0.001		0.629	<0.001	<0.001
6LL	<0.001	<0.001	0.629		<0.001	<0.001
9LDN	<0.001	<0.001	<0.001	<0.001		0.160
9LL	<0.001	<0.001	<0.001	<0.001	0.160	

TABLE XCVII. p-values from SNK test, testing for differences in weight between treatments at T4 (day 145) .

Weight T3						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.004	<0.001	<0.001	<0.001	<0.001
4LL	0.004		<0.001	<0.001	<0.001	<0.001
6LDN	<0.001	<0.001		0.941	<0.001	<0.001
6LL	<0.001	<0.001	0.941		<0.001	<0.001
9LDN	<0.001	<0.001	<0.001	<0.001		0.076
9LL	<0.001	<0.001	<0.001	<0.001	0.076	

Weight by time

TABLE XCVIII. p-values from SNK test, testing for differences in weight for 4LDN between time periods.

Weight 4LDN T0 - T5 (Mean weight)					
Tid	T0 (89.230)	T1 (104.41)	T2 (131.07)	T3 (161.98)	T4 (174.01)
T0		0.045	<0.001	<0.001	<0.001
T1	0.045		<0.001	<0.001	<0.001
T2	<0.001	<0.001		<0.001	<0.001
T3	<0.001	<0.001	<0.001		0.113
T4	<0.001	<0.001	<0.001	0.113	

TABLE XCIX. p-values from SNK test, testing for differences in weight for 4LL between time periods.

Weight 4LL T0 - T5 (Mean weight)					
Tid	T0 (85.477)	T1 (111.24)	T2 (150.47)	T3 (194.13)	T4 (207.77)
T0		<0.001	<0.001	<0.001	<0.001
T1	<0.001		<0.001	<0.001	<0.001
T2	<0.001	<0.001		<0.001	<0.001
T3	<0.001	<0.001	<0.001		0.019
T4	<0.001	<0.001	<0.001	0.019	

TABLE C. p-values from SNK test, testing for differences in weight for 6LDN between time periods.

Weight 6LDN T0 - T5 (Mean weight)					
Tid	T0 (86.087)	T1 (122.46)	T2 (173.60)	T3 (242.83)	T4 (259.81)
T0		<0.001	<0.001	<0.001	<0.001
T1	<0.001		<0.001	<0.001	<0.001
T2	<0.001	<0.001		<0.001	<0.001
T3	<0.001	<0.001	<0.001		0.052
T4	<0.001	<0.001	<0.001	0.052	

TABLE CI. p-values from SNK test, testing for differences in weight for 6LL between time periods.

Weight 6LL T0 - T5 (Mean weight)					
Tid	T0 (82.561)	T1 (114.40)	T2 (165.26)	T3 (237.54)	T4 (258.94)
T0		<0.001	<0.001	<0.001	<0.001
T1	<0.001		<0.001	<0.001	<0.001
T2	<0.001	<0.001		<0.001	<0.001
T3	<0.001	<0.001	<0.001		0.007
T4	<0.001	<0.001	<0.001	0.007	

TABLE CII. p-values from SNK test, testing for differences in weight for 9LDN between time periods.

Weight 9LDN T0 - T5 (Mean weight)					
Tid	T0 (85.179)	T1 (120.39)	T2 (183.96)	T3 (296.95)	T4 (322.69)
T0		<0.001	<0.001	<0.001	<0.001
T1	<0.001		<0.001	<0.001	<0.001
T2	<0.001	<0.001		<0.001	<0.001
T3	<0.001	<0.001	<0.001		0.007
T4	<0.001	<0.001	<0.001	0.007	

TABLE CIII. p-values from SNK test, testing for differences in weight for 9LL between time periods.

Weight 9LL T0 - T5 (Mean weight)					
Tid	T0 (88.247)	T1 (123.11)	T2 (187.02)	T3 (312.32)	T4 (343.63)
T0		<0.001	<0.001	<0.001	<0.001
T1	<0.001		<0.001	<0.001	<0.001
T2	<0.001	<0.001		<0.001	<0.001
T3	<0.001	<0.001	<0.001		<0.001
T4	<0.001	<0.001	<0.001	<0.001	

Length by treatments

TABLE CIV. p-values from SNK test, testing for differences in length between treatments at T1 (day 42) .

Length T1						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.862	0.010	0.118	0.008	0.001
4LL	0.862		0.009	0.192	0.008	0.001
6LDN	0.010	0.009		0.266	0.910	0.445
6LL	0.118	0.192	0.266		0.150	0.094
9LDN	0.008	0.008	0.910	0.150		0.656
9LL	0.001	0.001	0.445	0.094	0.656	

TABLE CV. p-values from SNK test, testing for differences in length between treatments at T2 (day 83).

Length T2						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.012	<0.001	<0.001	<0.001	<0.001
4LL	0.012		0.002	0.039	<0.001	<0.001
6LDN	<0.001	0.002		0.202	0.357	0.179
6LL	<0.001	0.039	0.202		0.041	0.024
9LDN	<0.001	<0.001	0.357	0.041		0.978
9LL	<0.001	<0.001	0.179	0.024	0.978	

TABLE CVI. p-values from SNK test, testing for differences in length between treatments at T3 (day 84) .

Length T3						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		<0.001	<0.001	<0.001	<0.001	<0.001
4LL	<0.001		<0.001	<0.001	<0.001	<0.001
6LDN	<0.001	<0.001		0.793	<0.001	<0.001
6LL	<0.001	<0.001	0.793		<0.001	<0.001
9LDN	<0.001	<0.001	<0.001	<0.001		0.441
9LL	<0.001	<0.001	<0.001	<0.001	0.441	

TABLE CVII. p-values from SNK test, testing for differences in length between treatments at T4 (day 145).

Length T4						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.001	<0.001	<0.001	<0.001	<0.001
4LL	0.001		<0.001	<0.001	<0.001	<0.001
6LDN	<0.001	<0.001		0.974	<0.001	<0.001
6LL	<0.001	<0.001	0.974		<0.001	<0.001
9LDN	<0.001	<0.001	<0.001	<0.001		0.200
9LL	<0.001	<0.001	<0.001	<0.001	0.200	

Length by time

TABLE CVIII. p-values from SNK test, testing for differences in length for 4LDN between time periods.

Length 4LDN T0 – T4 (Mean length cm)					
Tid	T0 (20.475)	T1 (21.710)	T2 (22.640)	T3 (23.760)	T4 (24.502)
T0		0.001	<0.001	<0.001	<0.001
T1	0.001		0.015	<0.001	<0.001
T2	<0.001	0.015		0.004	<0.001
T3	<0.001	<0.001	0.004		0.053
T4	<0.001	<0.001	<0.001	0.053	

TABLE CIX. p-values from SNK test, testing for differences in weight for 4LL between time periods.

Length 4LLL T0 – T4 (Mean length cm)					
Tid	T0 (20.033)	T1 (21.662)	T2 (23.487)	T3 (25.259)	T4 (25.958)
T0		<0.001	<0.001	<0.001	<0.001
T1	<0.001		<0.001	<0.001	<0.001
T2	<0.001	<0.001		<0.001	<0.001
T3	<0.001	<0.001	<0.001		0.030
T4	<0.001	<0.001	<0.001	0.030	

TABLE CX. p-values from SNK test, testing for differences in weight for 6LDN between time periods.

Length 6LDN T0 – T4 (Mean length cm)					
Tid	T0 (20.164)	T1 (22.577)	T2 (24.607)	T3 (27.060)	T4 (27.745)
T0		<0.001	<0.001	<0.001	<0.001
T1	<0.001		<0.001	<0.001	<0.001
T2	<0.001	<0.001		<0.001	<0.001
T3	<0.001	<0.001	<0.001		0.093
T4	<0.001	<0.001	<0.001	0.093	

TABLE CXI. p-values from SNK test, testing for differences in weight for 6LL between time periods.

Length 6LL T0 – T4 (Mean length cm)					
Tid	T0 (20.008)	T1 (22.145)	T2 (24.180)	T3 (26.948)	T4 (27.760)
T0		<0.001	<0.001	<0.001	<0.001
T1	<0.001		<0.001	<0.001	<0.001
T2	<0.001	<0.001		<0.001	<0.001
T3	<0.001	<0.001	<0.001		0.020
T4	<0.001	<0.001	<0.001	0.020	

TABLE CXII. p-values from SNK test, testing for differences in weight for 9LDN between time periods.

Length 9LDN T0 – T4 (Mean length cm)					
Tid	T0 (20.154)	T1 (22.546)	T2 (25.067)	T3 (29.003)	T4 (30.082)
T0		<0.001	<0.001	<0.001	<0.001
T1	<0.001		<0.001	<0.001	<0.001
T2	<0.001	<0.001		<0.001	<0.001
T3	<0.001	<0.001	<0.001		0.003
T4	<0.001	<0.001	<0.001	0.003	

TABLE CXIII. p-values from SNK test, testing for differences in weight for 9LL between time periods.

Length 9LL T0 – T4 (Mean length cm)					
Tid	T0 (20.425)	T1 (22.790)	T2 (25.058)	T3 (29.333)	T4 (30.666)
T0		<0.001	<0.001	<0.001	<0.001
T1	<0.001		<0.001	<0.001	<0.001
T2	<0.001	<0.001		<0.001	<0.001
T3	<0.001	<0.001	<0.001		<0.001
T4	<0.001	<0.001	<0.001	<0.001	

Condition Factor (CF) by treatments

TABLE CXIV. p-values from SNK test, testing for differences in CF between treatments at T1 (day 42) .

CF T1						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		<0.001	0.007	0.279	0.025	0.060
4LL	<0.001		0.033	<0.001	0.016	0.003
6LDN	0.007	0.033		<0.001	0.526	0.367
6LL	0.279	<0.001	<0.001		0.001	0.009
9LDN	0.025	0.016	0.526	0.001		0.474
9LL	0.060	0.003	0.367	0.009	0.474	

TABLE CXV. p-values from SNK test. testing for differences in CF between treatments at T2 (day 83) .

CF T2						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.010	0.005	0.018	0.014	<0.001
4LL	0.010		0.920	0.997	0.972	0.538
6LDN	0.005	0.920		0.994	0.988	0.602
6LL	0.018	0.997	0.994		0.823	0.375
9LDN	0.014	0.972	0.988	0.823		0.266
9LL	<0.001	0.538	0.602	0.375	0.266	

Condition Factor by time

TABLE CXVI. p-values from SNK test. testing for differences in CF for 4LDN between time periods.

CF 4LDN T0 - T5 (Mean CF)					
Tid	T0 (1.03)	T1 (1.00)	T2 (1.10)	T3 (1.19)	T4 (1.16)
T0		0.329	0.003	<0.001	<0.001
T1	0.329		<0.001	<0.001	<0.001
T2	0.003	<0.001		0.001	0.019
T3	<0.001	<0.001	0.001		0.187
T4	<0.001	<0.001	0.019	0.187	

TABLE CXVII. p-values from SNK test, testing for differences in CF for 4LL between time periods.

CF 4LL T0 - T5 (Mean CF)					
Tid	T0 (1.05)	T1 (1.09)	T2 (1.16)	T3 (1.19)	T4 (1.18)
T0		0.032	<0.001	<0.001	<0.001
T1	0.032		<0.001	<0.001	<0.001
T2	<0.001	<0.001		0.046	0.161
T3	<0.001	<0.001	0.046		0.329
T4	<0.001	<0.001	0.161	0.329	

TABLE CXVIII. p-values from SNK test, testing for differences in CF for 6LDN between time periods.

CF 6LDN T0 - T5 (Mean CF)					
Tid	T0 (1.04)	T1 (1.06)	T2 (1.16)	T3 (1.20)	T4 (1.20)
T0		0.504	<0.001	<0.001	<0.001
T1	0.504		<0.001	<0.001	<0.001
T2	<0.001	<0.001		0.011	0.008
T3	<0.001	<0.001	0.011		0.820
T4	<0.001	<0.001	0.008	0.820	

TABLE CXIX. p-values from SNK test, testing for differences in CF for 6LL between time periods.

CF 6LL T0 - T5 (Mean CF)					
Tid	T0 (1.02)	T1 (1.05)	T2 (1.16)	T3 (1.20)	T4 (1.20)
T0		0.283	<0.001	<0.001	<0.001
T1	0.283		<0.001	<0.001	<0.001
T2	<0.001	<0.001		0.051	0.105
T3	<0.001	<0.001	0.051		0.933
T4	<0.001	<0.001	0.105	0.933	

TABLE CXX. p-values from SNK test, testing for differences in CF for 9LDN between time periods.

CF 9LDN T0 - T5 (Mean CF)					
Tid	T0 (1.03)	T1 (1.05)	T2 (1.16)	T3 (1.20)	T4 (1.17)
T0		0.445	<0.001	<0.001	<0.001
T1	0.445		<0.001	<0.001	<0.001
T2	<0.001	<0.001		0.038	0.536
T3	<0.001	<0.001	0.038		0.067
T4	<0.001	<0.001	0.536	0.067	

TABLE CXXI. p-values from SNK test, testing for differences in CF for 9LL between time periods.

CF 9LL T0 - T5 (Mean CF)					
Tid	T0 (1.03)	T1 (1.04)	T2 (1.18)	T3 (1.23)	T4 (1.19)
T0		0.739	<0.001	<0.001	<0.001
T1	0.739		<0.001	<0.001	<0.001
T2	<0.001	<0.001		0.014	0.800
T3	<0.001	<0.001	0.014		0.011
T4	<0.001	<0.001	0.800	0.011	

SGR period by treatment

TABLE CXXII. p-values from SNK test, testing for differences in SGR 1-2 between treatments.

SGR 1-2						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		<0.001	<0.001	<0.001	<0.001	<0.001
4LL	<0.001		0.003	0.016	0.003	0.006
6LDN	<0.001	0.003		0.656	0.863	0.818
6LL	<0.001	0.016	0.656		0.483	1.000
9LDN	<0.001	0.003	0.863	0.483		0.762
9LL	<0.001	0.006	0.818	1.000	0.762	

TABLE CXXIII. p-values from SNK test, testing for differences in SGR 2-3 between treatments.

SGR 2-3						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		<0.001	<0.001	<0.001	<0.001	<0.001
4LL	<0.001		0.064	0.051	<0.001	<0.001
6LDN	<0.001	0.064		0.630	0.013	0.010
6LL	<0.001	0.051	0.630		0.030	0.015
9LDN	<0.001	<0.001	0.013	0.030		0.904
9LL	<0.001	<0.001	0.010	0.015	0.904	

TABLE CXXIV. p-values from SNK test, testing for differences in SGR 3-4 between treatments.

SGR 3-4						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.578	0.558	0.049	0.360	0.042
4LL	0.578		0.489	0.015	0.219	0.010
6LDN	0.558	0.489		0.114	0.436	0.121
6LL	0.049	0.015	0.114		0.225	0.828
9LDN	0.360	0.219	0.436	0.225		0.326
9LL	0.042	0.010	0.121	0.828	0.326	

TABLE CXXV. p-values from SNK test, testing for differences in SGR 1-5 between treatments.

SGR1-5						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		<0.001	<0.001	<0.001	<0.001	<0.001
4LL	<0.001		<0.001	<0.001	<0.001	<0.001
6LDN	<0.001	<0.001		0.271	<0.001	<0.001
6LL	<0.001	<0.001	0.271		<0.001	<0.001
9LDN	<0.001	<0.001	<0.001	<0.001		0.202
9LL	<0.001	<0.001	<0.001	<0.001	0.202	

Sodium ion (Na^+) by treatment

TABLE CXXVI. p-values from SNK test, testing for differences in Na^+ between treatments at T1.

Na^+ T1						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.373	0.373	0.057	0.396	0.302
4LL	0.373		0.652	0.422	0.817	0.820
6LDN	0.373	0.652		0.283	0.773	0.718
6LL	0.057	0.422	0.283		0.398	0.353
9LDN	0.396	0.817	0.773	0.398		0.713
9LL	0.302	0.820	0.718	0.353	0.713	

TABLE CXXVII. p-values from SNK test, testing for differences in Na^+ between treatments at T2.

Na^+ T2						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.391	0.048	0.026	0.030	0.025
4LL	0.391		0.127	0.152	0.152	0.109
6LDN	0.048	0.127		0.866	0.825	0.619
6LL	0.026	0.152	0.866		0.856	0.960
9LDN	0.030	0.152	0.825	0.856		0.928
9LL	0.025	0.109	0.619	0.960	0.928	

Sodium ion (Na^+) by period

TABLE CXXVIII. p-values from Na^+ test, testing for differences in Na+for 4LDN between time periods.

Na^+ 4LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		0.005	0.001	0.002
T1	0.005		0.437	0.931
T2	0.001	0.437		0.661
T3	0.002	0.931	0.661	

TABLE CXXIX. p-values from Na^+ test, testing for differences in Na+for 4LL between time periods.

Na^+ 4LL T0 - T3				
Tid	T0	T1	T2	T3
T0		0.006	<0.001	0.031
T1	0.006		0.127	0.316
T2	<0.001	0.127		0.036
T3	0.031	0.316	0.036	

TABLE CXXX. p-values from Na^+ test, testing for differences in Na+for 6LDN between time periods.

Na^+ 6LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		0.004	0.035	0.030
T1	0.004		0.276	0.331
T2	0.035	0.276		0.740
T3	0.030	0.331	0.740	

TABLE CXXXI. p-values from Na+ test, testing for differences in Na+for 6LL between time periods.

Na^+ 6LL T0 - T3				
Tid	T0	T1	T2	T3
T0		0.062	0.092	0.014
T1	0.062		0.816	0.422
T2	0.092	0.816		0.308
T3	0.014	0.422	0.308	

TABLE CXXXII. p-values from Na+ test, testing for differences in Na+for 9LDN between time periods.

Na^+ 9LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		0.003	0.065	0.370
T1	0.003		0.159	0.019
T2	0.065	0.159		0.167
T3	0.370	0.019	0.167	

TABLE CXXXIII. p-values from Na^+ test, testing for differences in Na+for 9LL between time periods.

Na^+ 9LL T0 - T3				
Tid	T0	T1	T2	T3
T0		0.023	0.064	0.505
T1	0.023		0.505	0.064
T2	0.064	0.505		0.108
T3	0.505	0.064	0.108	

Glucose by treatment

TABLE CXXXIV. p-values from SNK test, testing for differences in Na+ between treatments at T1.

Glu T1						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.200	<0.001	<0.001	<0.001	<0.001
4LL	0.200		0.001	0.004	<0.001	0.001
6LDN	<0.001	0.001		0.319	0.379	0.917
6LL	<0.001	0.004	0.319		0.099	0.512
9LDN	<0.001	<0.001	0.379	0.099		0.222
9LL	<0.001	0.001	0.917	0.512	0.222	

TABLE CXXXV. p-values from SNK test, testing for differences in Na+ between treatments at T2.

Glu T1						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.948	0.963	0.778	0.797	0.682
4LL	0.948		0.793	0.934	0.822	0.781
6LDN	0.963	0.793		0.942	0.871	0.813
6LL	0.778	0.934	0.942		0.803	0.582
9LDN	0.797	0.822	0.871	0.803		0.937
9LL	0.682	0.781	0.813	0.582	0.937	

TABLE CXXXVI. p-values from SNK test, testing for differences in Na^+ between treatments at T3.

Glu T1						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.113	0.675	0.721	0.746	0.800
4LL	0.113		0.114	0.072	0.068	0.122
6LDN	0.675	0.114		0.805	0.860	0.829
6LL	0.721	0.072	0.805		0.874	0.682
9LDN	0.746	0.068	0.860	0.874		0.836
9LL	0.800	0.122	0.829	0.682	0.836	

Glucose by period

TABLE CXXXVII. p-values from Glu test, testing for differences in Glu for 4LDN between time periods.

Glu 4LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		0.287	<0.001	0.001
T1	0.287		<0.001	0.010
T2	<0.001	<0.001		0.085
T3	0.001	0.010	0.085	

TABLE CXXXVIII. p-values from Glu test, testing for differences in Glu for 4LL between time periods.

Glu 4LL T0 - T3				
Tid	T0	T1	T2	T3
T0		0.011	<0.001	<0.001
T1	0.011		<0.001	0.002
T2	<0.001	<0.001		0.294
T3	<0.001	0.002	0.294	

TABLE CXXXIX. p-values from Glu test, testing for differences in Glu for 6LDN between time periods.

Glu 6LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		<0.001	<0.001	0.001
T1	<0.001		0.761	0.032
T2	<0.001	0.761		0.026
T3	0.001	0.032	0.026	

TABLE CXL. p-values from Glu test, testing for differences in Glu for 6LL between time periods.

Glu 6LL T0 - T3				
Tid	T0	T1	T2	T3
T0		<0.001	<0.001	0.002
T1	<0.001		0.797	0.030
T2	<0.001	0.797		0.042
T3	0.002	0.030	0.042	

TABLE CXLI. p-values from Glu test, testing for differences in Glu for 9LDN between time periods.

Glu 9LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		<0.001	<0.001	0.001
T1	<0.001		0.002	<0.001
T2	<0.001	0.002		0.046
T3	0.001	<0.001	0.046	

TABLE CXLII. p-values from Glu test, testing for differences in Glu for 9LL between time periods.

Glu 9LL T0 - T3				
Tid	T0	T1	T2	T3
T0		<0.001	<0.001	<0.001
T1	<0.001		0.030	0.001
T2	<0.001	0.030		0.059
T3	<0.001	0.001	0.059	

pCO_2 by treatment

TABLE CXLIII. p-values from SNK test, testing for differences in pCO_2 between treatments at T1.

pCO_2 T1						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.995	0.132	0.987	0.003	0.004
4LL	0.995		0.282	0.884	0.005	0.007
6LDN	0.132	0.282		0.342	0.099	0.071
6LL	0.987	0.884	0.342		0.005	0.007
9LDN	0.003	0.005	0.099	0.005		0.799
9LL	0.004	0.007	0.071	0.007	0.799	

TABLE CXLIV. p-values from SNK test, testing for differences in pCO_2 between treatments at T2.

pCO_2 T2						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.925	0.771	0.775	0.018	0.061
4LL	0.925		0.555	0.923	0.015	0.043
6LDN	0.771	0.555		0.764	0.039	0.066
6LL	0.775	0.923	0.764		0.011	0.046
9LDN	0.018	0.015	0.039	0.011		0.524
9LL	0.061	0.043	0.066	0.046	0.524	

TABLE CXLV. p-values from SNK test, testing for differences in pCO_2 between treatments at T3.

pCO_2 T3						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.567	0.444	0.362	0.003	0.002
4LL	0.567		0.376	0.219	0.001	<0.001
6LDN	0.444	0.376		0.549	0.013	0.012
6LL	0.362	0.219	0.549		0.024	0.032
9LDN	0.003	0.001	0.013	0.024		0.789
9LL	0.002	<0.001	0.012	0.032	0.789	

pCO_2 by period

TABLE CXLVI. p-values from pCO_2 test, testing for differences in pCO_2 for 4LDN between time periods.

pCO_2 Overall 4LDN (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	3087.355	1.000	3087.4	884.143	<0.001
Time	236.380	3.000	78.8	22.565	<0.001
Error	146.661	42.000	3.5		

TABLE CXLVII. *p*-values from pCO_2 test, testing for differences in pCO_2 for 4LL between time periods.

pCO_2 Overall 4LL (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	2890.965	1.000	2891.0	729.258	<0.001
Time	267.880	3.000	89.3	22.525	<0.001
Error	158.570	40.000	4.0		

TABLE CXLVIII. *p*-values from pCO_2 test, testing for differences in pCO_2 for 6LDN between time periods.

pCO_2 Overall 6LDN (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	3701.636	1.000	3701.6	832.401	<0.001
Time	225.591	3.000	75.2	16.910	<0.001
Error	186.771	42.000	4.4		

TABLE CXLIX. *p*-values from pCO_2 test, testing for differences in pCO_2 for 6LL between time periods.

pCO_2 Overall 6LL (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	3110.550	1.000	3110.6	788.076	<0.001
Time	174.979	3.000	58.3	14.777	<0.001
Error	161.828	41.000	3.9		

TABLE CL. *p*-values from pCO_2 test, testing for differences in pCO_2 for 9LDN between time periods.

pCO_2 Overall 9LDN (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	5604.744	1.000	5604.7	747.604	<0.001
Time	173.792	3.000	57.9	7.727	<0.001
Error	322.369	43.000	7.5		

TABLE CLI. *p*-values from pCO_2test, testing for differences in pCO_2 for 9LL between time periods.

pCO_2 Overall 9LL (T0-T3) One- way ANOVA					
Effect	SS	DF	MS	F	p
Intercept	5490.059	1.000	5490.1	675.430	<0.001
Time	141.491	3.000	47.2	5.802	0.002
Error	357.643	44.000	8.1		

HCO3 by treatment

TABLE CLII. *p*-values from SNK test, testing for differences in HCO_3^- between treatments at T1.

HCO_3^- T1						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.937	0.269	0.612	0.125	0.111
4LL	0.937		0.143	0.826	0.087	0.090
6LDN	0.269	0.143		0.173	0.505	0.640
6LL	0.612	0.826	0.173		0.058	0.045
9LDN	0.125	0.087	0.505	0.058		0.817
9LL	0.111	0.090	0.640	0.045	0.817	

TABLE CLIII. *p*-values from SNK test, testing for differences in HCO_3^- between treatments at T2.

HCO_3^- T2						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.446	0.278	0.872	0.001	0.033
4LL	0.446		0.901	0.515	0.015	0.118
6LDN	0.278	0.901		0.425	0.019	0.209
6LL	0.872	0.515	0.425		0.001	0.034
9LDN	0.001	0.015	0.019	0.001		0.199
9LL	0.033	0.118	0.209	0.034	0.199	

TABLE CLIV. *p*-values from SNK test, testing for differences in HCO_3^- between treatments at T3.

HCO_3^- T3						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.869	0.798	0.630	0.068	0.002
4LL	0.869		0.851	0.793	0.069	0.002
6LDN	0.798	0.851		0.875	0.067	0.005
6LL	0.630	0.793	0.875		0.114	0.006
9LDN	0.068	0.069	0.067	0.114		0.165
9LL	0.002	0.002	0.005	0.006	0.165	

HCO_3^- by period

TABLE CLVI. *p*-values from HCO_3^- test, testing for differences in HCO_3^- for 4LDN between time periods.

HCO_3^- 4LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		0.194	0.017	<0.001
T1	0.194		0.128	0.008
T2	0.017	0.128		0.111
T3	<0.001	0.008	0.111	

TABLE CLVII. *p*-values from HCO_3^- test, testing for differences in HCO_3^- for 4LL between time periods.

HCO_3^- 4LL T0 - T3				
Tid	T0	T1	T2	T3
T0		0.194	0.017	<0.001
T1	0.194		0.128	0.008
T2	0.017	0.128		0.111
T3	<0.001	0.008	0.111	

TABLE CLVIII. *p*-values from HCO_3^- test, testing for differences in HCO_3^- for 6LDN between time periods.

HCO_3^- 6LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		0.598	0.341	0.013
T1	0.598		0.304	0.006
T2	0.341	0.304		0.051
T3	0.013	0.006	0.051	

TABLE CLIX. *p*-values from HCO_3^- test, testing for differences in HCO_3^- for 6LL between time periods.

HCO_3^- 6LL T0 - T3				
Tid	T0	T1	T2	T3
T0		0.035	0.004	0.001
T1	0.035		0.208	0.099
T2	0.004	0.208		0.408
T3	0.001	0.099	0.408	

TABLE CLX. *p*-values from HCO_3^- test, testing for differences in HCO_3^- for 9LDN between time periods.

HCO_3^- 9LDN T0 - T3				
Tid	T0	T1	T2	T3
T0		0.308	0.058	0.390
T1	0.308		0.192	0.151
T2	0.058	0.192		0.012
T3	0.390	0.151	0.012	

TABLE CLXI. *p*-values from HCO_3^- test, testing for differences in HCO_3^- for 9LL between time periods.

HCO_3^- 9LL T0 - T3				
Tid	T0	T1	T2	T3
T0		0.629	0.605	0.717
T1	0.629		0.811	0.682
T2	0.605	0.811		0.554
T3	0.717	0.682	0.554	

Organ indexes

TABLE CLXIII. *p*-values from SNK test, testing for differences in Dorsal fin index between treatments at T3.

Dorsal fin index						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.427	0.593	0.637	0.761	0.984
4LL	0.427		0.857	0.935	0.982	0.692
6LDN	0.593	0.857		0.912	0.999	0.750
6LL	0.637	0.935	0.912		0.684	0.721
9LDN	0.761	0.982	0.999	0.684		0.854
9LL	0.984	0.692	0.750	0.721	0.854	

TABLE CLXIV. *p*-values from SNK test, testing for differences in Hepato – somatic index between treatments at T3.

Hepato - somatic index						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.014	0.002	0.003	0.001	0.002
4LL	0.014		0.509	0.359	0.415	0.395
6LDN	0.002	0.509		0.909	0.967	0.957
6LL	0.003	0.359	0.909		0.807	0.701
9LDN	0.001	0.415	0.967	0.807		0.811
9LL	0.002	0.395	0.957	0.701	0.811	

TABLE CLXV. *p*-values from SNK test, testing for differences in Cardio – somatic index between treatments at T3.

Cardio - somatic index						
Treatment	4LDN	4LL	6LDN	6LL	9LDN	9LL
4LDN		0.036	0.030	0.066	0.068	0.065
4LL	0.036		0.860	0.896	0.931	0.945
6LDN	0.030	0.860		0.828	0.807	0.667
6LL	0.066	0.896	0.828		0.819	0.906
9LDN	0.068	0.931	0.807	0.819		0.848
9LL	0.065	0.945	0.667	0.906	0.848	

Levene´s test for homogenity of variance

TABLE CLXVI. Test results from Levene's test performed on observations of all response variables. for each sampling date. or period.

Levene´s test for homogenity of variance Growth variables				
Variable	MS Effect	MS Error	F	p
Weight T0	69.55	76.96	0.90	0.479
Weight T1	31.59	131.45	0.24	0.944
Weight T2	533.67	339.55	1.57	0.169
Weight T3	533.67	339.55	1.57	0.169
Weight T4	2363.35	917.45	2.58	0.027
Length T1	0.57	0.52	1.11	0.358
Length T2	0.33	0.56	0.59	0.710
Length T3	1.00	0.83	1.20	0.311
Length T4	1.00	0.83	1.20	0.311
Length T5	3.04	1.36	2.23	0.052
CF T1	0.00	0.00	2.35	0.042
CF T2	0.01	0.00	2.47	0.034
CF T3	0.01	0.00	2.22	0.053
CF T4	0.01	0.00	2.22	0.053
CF T5	0.01	0.01	1.24	0.293
SGR 1	0.01	0.00	1.25	0.286
SGR 2	0.17	0.02	8.41	<0.001
SGR 3	0.10	0.03	3.51	0.004
SGR 4	0.10	0.03	3.51	0.004
SGR Overall	0.05	0.03	1.77	0.121
Dorsal fin index	0.00	0.00	0.73	0.602
Hepato somatic index	0.08	0.02	3.25	0.011
Cardio somatic index	0.00	0.00	1.05	0.398

Levene´s test for homogenity of variance Blood variables by treatment				
Variable	MS Effect	MS Error	F	p
Na^+T0	0.00	0.66	0.00	1.000
Na^+ T1	5.67	5.10	1.11	0.363
Na^+ T2	5.45	4.74	1.15	0.344
Na^+ T3	62.77	34.57	1.82	0.123
Glu T0	0.00	5.84	0.00	1.000
Glu T1	46.13	35.59	1.30	0.277
Glu T2	100.61	81.57	1.23	0.305
Glu T3	10.12	3.78	2.68	0.030
pCO_2 T0	0.00	0.36	0.00	1.000
pCO_2 T1	1.60	1.88	0.85	0.519
pCO_2 T2	7.58	2.86	2.65	0.032
pCO_2 T3	1.07	0.96	1.11	0.363

Levene´s test for homogenity of variance Blood variables by treatment				
HCO_3^- T0	0.00	0.51	0.00	1.000
HCO_3^- T1	1.88	1.09	1.72	0.143
HCO_3^- T2	4.00	1.38	2.90	0.021
HCO_3^- T3	1.97	1.12	1.76	0.135

Levene´s test for homogenity of variance Blood variables by period				
Variable	MS Effect	MS Error	F	p
Na^+ 4LDN	14.78	2.45	6.02	0.002
Na^+ 4LL	9.36	2.98	3.14	0.036
Na^+ 6LDN	16.07	5.46	2.94	0.044
Na^+ 6LL	3.43	2.27	1.51	0.227
Na^+ 9LDN	7.29	1.33	5.48	0.003
Na^+ 9LL	9.11	3.82	2.38	0.082
Glu 4LDN	213.20	61.28	3.48	0.024
Glu 4LL	91.61	26.73	3.43	0.026
Glu 6LDN	271.39	41.18	6.59	0.001
Glu 6LL	223.65	51.94	4.31	0.010
Glu 9LDN	113.47	29.11	3.90	0.015
Glu 9LL	78.22	21.79	3.59	0.021
pCO_2 4LDN	3.08	0.84	3.67	0.020
pCO_2 4LL	1.67	1.24	1.35	0.273
pCO_2 6LDN	2.82	1.26	2.24	0.097
pCO_2 6LL	3.65	1.15	3.18	0.034
pCO_2 9LDN	10.20	2.22	4.60	0.007
pCO_2 9LL	15.46	2.16	7.17	0.001
HCO_3^- 4LDN	0.18	0.68	0.27	0.848
HCO_3^- 4LL	2.63	0.69	3.81	0.017
HCO_3^- 6LDN	2.12	0.98	2.16	0.107
HCO_3^- 6LL	0.65	0.57	1.14	0.344
HCO_3^- 9LDN	4.49	1.43	3.14	0.035
HCO_3^- 9LL	3.77	1.67	2.26	0.095