Effects of an advanced spring temperature increase on parr-smolt transformation in Arctic Charr *(Salvelinus alpinus L.)*

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Abstract

Anadromous Arctic charr (*Salvelinus alpinus* (L.)) was investigated to reveal whether an advanced vernal increase in freshwater temperature results in an advanced parr-smolt transformation, measured as an increase in hypoosmoregulatory ability. First generation hatchery reared anadromous Arctic charr from Talvik Research station (69° N. Finmark, northern Norway) was reared under a short day regime (L:D 8:16) until 7 April when continuous light was applied. One group was held on ambient temperature, whereas a second group were given elevated temperature (6°C) from 27 March until the ambient temperature reached 6°C in early June. At regular intervals fish were sampled from freshwater to obtain samples for measuring gill Na^+K^+ATPase activity, plasma osmolality, plasma chloride concentrations and morphological data. In addition 24h seawater tests were performed at the same dates as freshwater samplings, and blood samples for plasma osmolality and plasma chloride concentrations analysis were obtained in order to investigate the ability to hypoosmoregulate in full strength seawater. The experimental fish reached full smolt status at the time of seaward migration of hatchery reared Arctic charr released into the Hals watercourse (69°N) from Talvik Research Station. Full hypoosmoregulatory ability coincided with high Na^+K^+ATPase activity. No advance in hypoosmoregulatory ability was seen in the fish held on advanced elevated temperature, indicating that the development of hypoosmoregulatory ability in Arctic charr is mainly initiated by changes in photoperiod. The results indicate that a seawater temperature increase in early spring due to global warming may lead to a mismatch between optimal feeding conditions and seawater migration of Arctic charr. In the prospects of an air temperature increase in Norway of 2,5 to 4° C during the next decade, more research is needed for elucidating if, and how much a future global warming may affect the northernmost distributed freshwater fish.

Key words: Arctic charr; Temperature; Na^+K^+ATPase; Global warming.

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1.0 Introduction

1.1 Arctic charr biology and distribution

The Arctic Charr (*Salvelinus alpinus* (L.)) is found throughout circumpolar areas, and is the northernmost distributed freshwater fish (Johnson 1980). Both resident and anadromous (sea-migratory) forms can be found, with the latter being restricted to the northernmost part (north of 65°N) of the species distribution area (Nordeng 1961). To circumvent low food availability in the freshwater habitats, the anadromous Arctic charr undergo a yearly seasonal feeding migration to the sea in early spring. Larvae is hatched in spring and undergo 2-6 years of growth in the watercourse (preferably a lake), before migrating to the sea (Rikardsen and Elliott 2000). Duration of the seawater residency is four to nine weeks after which they return to fresh water for overwintering, and the migration is spatially restricted to coastal areas near the watercourse (Nordeng 1977; Johnson 1980; Berg and Berg 1989). This short duration of seawater residency of the anadromous Arctic charr is in contrast to many other salmonids like the Atlantic Salmon (*Salmo salar* (L.)), which spends 1-5 years in the sea to feed before returning to the river for spawning (Klemetsen et al. 2003). However, during the short seawater residency Arctic charr has been reported to more than double their body weight (Mathisen and Berg 1968; Jorgensen et al. 1997; Rikardsen 2000).

Feeding migration occurs during a period with very low seawater temperatures, and the early part of this period accounts for a large part of the annual growth (Berg and Berg 1989; Rikardsen et al. 2000). It is presently not known why the feeding migration takes place at such an early date and low temperature, but it must be considered to be an adaptive strategy. It is, for example, not known whether the seawater temperatures later in the summer are too high for an optimum performance of this cold-water adapted species. The high growth in cold water facilitates aquaculture in circumpolar areas, and this is performed with success in Scandinavia, Iceland, Canada, Greenland and Scotland (Reynolds 1994; Heasman and Black 1998).

1.2 Parr-smolt transformation

The parr-smolt transformation or smoltification process of the Arctic charr comprises a series of pre-adaptory biochemical, physiological, and behavioural changes. Also repeat migrants of anadromous Arctic charr undergo a re-smoltification process before they migrate to the sea which include a re-development of seawater tolerance and migratory behaviour (Aas-Hansen et al. 2005). The fulfilment of the smoltification process is pivotal for optimal performance (growth and survival) in seawater. Several studies have demonstrated underdeveloped hypoosmoregulatory ability in first time migrating small sized Arctic charr (Dempson 1993; Arnesen et al. 1995; Lysfjord and Staurnes 1998; Nilssen and Gulseth 1998), but no explanations for this phenomena is currently available. However, it is well known that some populations may spend most of their seawater residence in estuarine brackish water before moving into coastal sea (McCart 1980), and it may be speculated that these populations do not develop full seawater tolerance before they migrate (Dempson 1993; Arnesen et al. 1995; Lysfjord and Staurnes 1998; Nilssen and Gulseth 1998). Fish from the Hals watercourse which was used in the present study experience, however, salinities above 25 ‰ when entering seawater (Audun Rikardsen, Norwegian college of fishery science, University of Tromsø, Tromsø, Norway, Unpublished data) and displays a full strength hypoosmoregulatory ability prior to seawater entry (Jørgensen et al. in press).

Morphological changes related to smoltification in Arctic charr are less pronounced than in Atlantic salmon (*Salmo salar* L.) (Hoar 1988) and brown trout (*Salmo trutta* L.), but the parr is still distinguishable from the anadromous individuals, although difficult in some populations (Damsgard 1991; Arnesen et al. 1995). The Arctic charr parr has a silvery colour on the ventral side and the dorsal side is dark brown-green with lateral finger marks. The silvery smolt colour is obtained by the deposit of the light reflecting pigments guanine and hypoxanthine in the scales and the outer dermis prior to seawater migration (Markert and Vanstone 1966; Johnston and Eales 1967). During the smoltification process the dorsal side of the fish and the fin margins darkens, while the outer part of the fins brightens (Damsgard 1991). Ventral side coloration lightens and become more silvery when seawater migration takes place (Damsgard 1991)

Physiological and behavioural changes during parr-smolt transformation are closely related, and in other salmonids a shift in the relationship between body length and body weight is seen accompanying the smoltification process. Unlike Atlantic salmon the Arctic charr does not seem to have a pronounced decrease in condition factor accompanying the smoltification process (Eliassen

et al. 1998; Jorgensen and Arnesen 2002; Strand et al. in press), a finding that is in accordance with the common opinion that the Arctic charr establish a pelagic behaviour in the lake long time before seaward migration.

Body fluids of freshwater fish are hyper-osmotic in relation to the surrounding water. As a result of the difference in osmolality, a net loss of ions through osmosis over the skin and gill epithelia and a net uptake of water across the gill epithelia occurs (Folmar and Dickhoff 1980). These processes are counteracted by the excretion of large amounts of hypo-osmotic urine and active uptake of ions from the surrounding water. The retention of ions from feed and water uptake takes place primarily in the intestine and the kidney by absorption and reabsorption respectively, whereas an active uptake of ions takes place in the gill (Folmar and Dickhoff 1980, 1981).

When descending to saltwater, anadromous salmonids will experience the opposite situation, i.e. they enter a hyper-osmotic environment. A higher concentration of ions in the surrounding environment will cause a net loss of water across skin and gill epithelia due to osmosis (Folmar and Dickhoff 1980, 1981). The water loss is compensated by drinking of seawater, and the production of small amounts of highly concentrated urine. The drinking of seawater causes an extensive load of ions, which have to be actively secreted via the gills and kidney.

An important mechanism in the salmonid hypoosmoregulatory apparatus is the enzyme Na^+K^+ATPase located in the chloride cells in the gill filament (McCormick 1990). This protein constitute an important part of the gill salt extrusion machinery that excrete of Na^+ and Cl^- in seawater fish (Payan et al. 1984; McCormick et al. 1996). During smoltification a recruitment and size increase of chloride cells, accompanied by an increase in Na^+K^+ATPase activity, occurs (McCormick 2001). In fully smoltified salmonids, plasma osmolality and chloride concentrations rapidly stabilize at values lower than 350 mOsm kg^{-1} and 150 mmol l^{-1} after seawater entry (Strand et al. in press)

1.3 Endogenous rhythms and entrainment

A cyclical nature of several features in the salmonid life cycle has been shown in both onchorynchus and salmo species (Hoar 1988). An endogenous rhythm of growth and silvering have been shown in Baltic salmon kept under constant light and temperature conditions (Eriksson and Lundqvist 1982). A characteristic of endogenous annual rhythms is, however, that they are out of phase with the 356

day annual cycle, therefore these rhythms has to be face adjusted ("entrained") by a "zeitgeber" i.e. an environmental cue which provide reliable calendar information (Duston and Saunders 1990). For example, the vernal increase in gill Na^+K^+ATPase activity seems to be dependent on an increasing daylength (Boeuf and Falcòn 2001), and photoperiod is generally accepted as the most important (if not the only) environmental cue for the stimulation and entrainment of the smoltification process (McCormick et al. 1996).

The stimulation and entrainment of the parr-smolt transformation in first time migrating Arctic charr is stated to be mainly dependent upon increasing daylengths in spring, which also implicates a short day (winter signal) prior to smolting (Johnsen et al. 2000). In addition to this well established effects of photoperiod within the salmonids, increasing vernal temperature has also been stated as an environmental cue that may both stimulate smolt development and determine the rate of smolt development (McCormick et al. 1996; Handeland et al. 2004). Controversy exists upon whether temperature is a rate controlling factor, or if temperature alone can be a trigger for the parr-smolt transformation process. Considering the prospects of global warming in the near future, an earlier increase in vernal water temperature can be expected (Working group II contribution to the intergovermental Panel on Climate Change 2007). Early increase in water temperature yields earlier optimal conditions for seawater migration at high latitudes (McCormick et al. 1996; Working group II contribution to the intergovernmental Panel on Climate Change 2007), but if the pre-adaptory smoltification process is mainly stimulated by photoperiod, a mismatch between optimal conditions and migration may be hypothesized to occur.

1.4 Objective

Current information points to the photoperiod as the most important entrainment cue (zeitgeber) for the smoltification process in salmonids, whereas temperature may have an effect as a rate determining factor. Based on the lack of knowledge on the effect of water temperature on the smoltification process in anadromous Arctic charr, the present investigation was undertaken to address if, and to what extent, elevated water temperature in spring affect the timing of the smoltification process in this species. The results of the study will hopefully reveal information about the mechanisms underlying the timing of the smoltification process in anadromous Arctic charr and provide information on the adaptive value of these mechanisms in the scenario of a global warming.

2.0 Materials and methods

2.1 Experimental fish and rearing conditions

The fish investigated in this study was first generation, one year old offspring of wild anadromous Arctic charr from the Hals watercourse in Talvik, Finmark (70° N, 22°E). Eggs were hatched at Talvik Research Station in January 2005, and the fry was held on elevated temperature (7°C-12°C) until 4 August, 2005. A light regime of L (hours of daylight) :D (hours of darkness) 0:24 was maintained until Early December 2005, when a continuous light regime was initiated gradually. From late September 2005 onwards the fish were held on a short day (L:D 10:14) photoperiod. Start feeding was commenced on 12 February 2005, and commercial dry feed pellets intended for cod larvae was used (Nutra Gemma Micro 0,3 mm to 1,0 mm, Skretting AS, Stavanger). The fish was transferred to grow-out facilities in primo July, and the feed was changed to dry-feed pellets from 1,2 mm to 3,0 mm intended for salmonids (Nutra Olympic, Skretting AS, Stavanger).

Sorting of the fish had been routinely performed four times before the start of the experiment, in mid March, mid May, mid June and late November 2005, and the largest of these fish were used in the experiment. On March 6 (2005), 10 fish was sampled in freshwater for blood and gill samples, and 10 fish was exposed to a 24 hour seawater challenge test (SWT). The individuals exposed to the SWT were sampled on March 7 as described below. On March 8, 424 fish (average weight 66 g) were transported to Kårvik research station in Tromsø. One fish died during transportation, due to accidental encounter with the landing net.

2.2 Experimental design

The main experiment was carried out at the Aquaculture Research Station, in Kårvik, Tromsø (69°N) in the period March 8 to July 5, 2006. The fish was randomly distributed among four circular freshwater filled tanks (500 litre, 50 cm water depth), with a circumferential flow. Water supply was calculated according to the fish's oxygen needs (minimum 90% oxygen saturation) and varied between 7 l/min (April) and 9 l/min (July). Two of these tanks were thereafter held at an ambient water temperature until the end of the experiment, whereas water temperature in the other two tanks was gradually elevated to 6°C between March 27 and March 31. The elevated groups were maintained at 6°C until the natural water temperature increased above this level in June (figure 2.1). After June 13 the two temperature groups experienced the same water temperature until the end of the experiment.

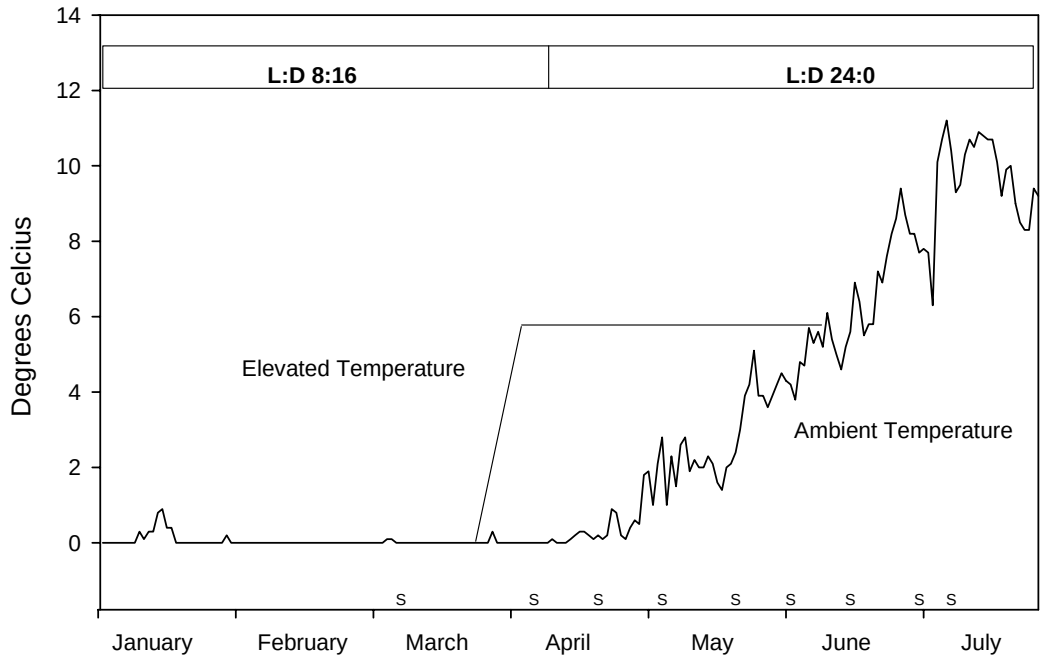


Figure 2.1: Ambient and elevated freshwater temperature during the course of the experiment. Horizontal bar denotes light regimes (Light: Darkness h: h). S symbols the various sampling dates.

Light regime was controlled by automatic timers without a twilight period. In Kårvik the fish was held on short days LD: 8:16 until April 7 after which daylength was gradually elevated to continuous light L:D 24:0 within 5 days (Figure 2.1). The fish were fed commercial dry pellets intended for salmon (Skretting 3.0 mm, Stavanger) according to predicted growth rates (Skretting, Stavanger) by automatic disc feeders during the light hours of the day.

Fish was tattooed according to tank belonging to be able to perform SWTs with mixed replicates and temperature treatments in the same tank. The tattoo was performed with Alcian Blue staining dye (BDH Chemicals Ltd. UK) injected with a Pan Jet needleless injector (Whright Dental Dundee, UK), and treatment/replicate identification was done by placing the tattoo on four distinctly different positions on the fish's abdomen.

2.2.1 Samplings

At regular intervals (for dates: see table 2.1), six fish from each tank were transferred to a 24 hours SWT (see below). After 24 hours, fish from the SWT were sampled first, where after 24 fish from the freshwater tanks (n= 6 per tank) were sampled. Before sampling, the fish were killed by an overdose of benzocaine (150ppm), mixed into either saltwater or freshwater for the fish sampled after SWT fish or from freshwater, respectively. Blood samples for plasma osmolality and plasma chloride concentration analysis were taken immediately after the fish was dead and kept on ice before centrifugation. Thereafter gill samples for analysis of Na^+K^+ATPase activity were obtained and immediately frozen in liquid nitrogen. Finally, fork length (nearest 0.1cm), weight (nearest 0.1 gram) and silvering colour were registered.

The silvering colour was recorded by the use of an index developed by Birt and Green (1986) and modified by Sigholt et al. (1998), were 1 is fully parr and 4 is fully developed morphological characteristics of a silvery smolt (Appendix 2).

In addition to the 24 h SWT, one group of fish were in June exposed to a prolonged (3 days and 7 days) seawater exposure in June (Table X).

Table 2.1

Sampling dates and type of sampling

Location	Sampling	Freshwater	Seawater Challenge Test
Talvik	1	07.03.2006	06-07.03.2006
Kårvik	2	05.04.2006	04-05.04.2006
Kårvik	3	19.04.2006	18-19.04.2006
Kårvik	4	03.05.2006	02-03.05.2006
Kårvik	5	19.05.2006	18-19.05 2006
Kårvik	6	31.05.2006	30-31.05.2006
Kårvik	7	13.06.2006	12-13.06.2006
Kårvik	8		12-15.06.2006*
Kårvik	9		12-19.06.2006**
Kårvik	10	28.06.2006	27-28.06.2006
Kårvik	11	05.07.2006	04-05.07.2006

*3 (72h) Day Seawater Challenge test

**7 (168) Day Seawater Challenge test

2.2.2 Seawater Challenge test

The seawater challenge test (Blackburn and Clarke 1987) was performed by transferring 6 fish from each tank, to a circular green 500 litre tank with full strength seawater (32-33‰) for 24 hours. During the tests they were held under continuous light and at a water temperature of 6 °C, without being fed. At the end of the test the fish were killed and blood samples obtained within 15 minutes. In order to evaluate long term survival and hypoosmoregulatory ability a 3 (72 h), and 7 (168 h) day SWT were performed. Conditions with regard to light, temperature and feeding were the same as those for the 24 hour SWTs.

2.2.3 Plasma samples

Blood samples were taken from the caudal vein with lithium heparinized vacutainers (BD Vacutainer, 2ml 13x75mm, BD diagnostics, Preanalytical systems). The samples were kept on ice for a maximum of fifteen minutes. Plasma was then separated by centrifugation (Jouan CR 4.22) for 10 minutes at 3500 rpm (2380 X *g*) and kept at -80°C until analysis.

2.2.4 Gill filament samples

Gill samples for analyzing Na^+K^+ATP-ase activity were taken from the second gill arch on the right side of each fish. Two filaments of 2-3mm length was obtained from each fish and immersed in 1ml ice cold SEI solution (150mM sucrose, 10 mM Na2EDTA and 50mM Imidazol, pH 7,3) (McCormick 1993) in 2ml eppendorf tubes. To prevent problems during defrosting, the eppendorf tube lids were punctured with a syringe. The tubes were frozen in liquid nitrogen in an upright position to ensure that the samples were frozen within the SEI buffer, and stored at -80°C until analysis.

2.3 Analyses

2.3.1 Osmolality and plasma chloride concentration

All samples were analyzed for osmolality and chloride concentration. Samples were defrosted on ice and vortexed (Heidolph Reax 2000) for thirty seconds. Osmolality was measured with an osmometer (Fiske® Associates, Norwood, Massachusetts USA), which was calibrated with a standard (290 mOsm kg^{-1}). Two parallels of each sample were analysed, and when variance exceeded 5 mOsm kg^{-1} a third sample was analysed.

Parallel with the osmolality analysis, chloride concentration was measured by titration (Chloride Analyzer 925, KEBO Lab, Corning). The apparatus was calibrated by testing it with a 100 mmol $liter^{-1}$ chloride standard solution (Bayer Multical™) prior to analyses, and at an interval of ten plasma samples to insure accuracy. Two parallels of each sample was analysed, and when variance exceeded 3 mmol $liter^{-1}$ a third sample was analysed.

2.3.2 Na^+K^+ATPase activity in gill filament samples

The analyses of Na^+K^+ATPase activity were performed according to a method described by McCormick (1993). Activity of Na^+K^+ATPase enzyme is expressed as the rate of oubain sensitive enzymatic hydrolysis of ATP to ADP+ $P_{i,}$ given a certain amount of protein in the sample. The hydrolysis of ATP is enzymatically coupled to the oxidation of NADH according to equation 2.1 (McCormick 1993).

Equation 2.1: The equation illustrates the complete reaction during the analysis. The amount of oxidized NADH, is determined spectrophotometricaly, as a decrease in optical density (OD) value in the sample.

$$(1)\ ATP + H_2O \xleftarrow{ATPase} ADP + P_i$$

$$(2)\ ADP + Phosphoenylpyruvat \xrightarrow{Pyruvat-kinase} Pyruvat + ATP$$

$$(3)\ Pyruvat + NADH + H^+ \xrightarrow{Lactate-dehydrogenase} Lactat + NAD^+$$

One mole of hydrolysed ATP equals one mole of NAD^+ which can be directly read by observing Optical Density (OD) in a microplate reader at 340 nm. Decreased content of NADH in the sample equals decreased OD value, and this corresponds to the activity of the Na^+K^+ATPase enzyme in the sample. A standard curve of known ADP concentrations was made to be able to relate spectrophotometric readings of samples to known concentrations of ADP, and thus the activity of the Na^+K^+ATP-ase enzyme in the samples. By adding the toxic compound Oubain, one can inhibit the Na^+K^+ATPase enzyme in two out of the four replicates from each fish in order to calculate the Oubain sensitive activity of the enzyme in the sample. The Oubain sensitive activity is calculated by subtracting the inhibited values from the non-inhibited values and then relating the activity value to the standard curve and the sample protein content. The final analysis result describes specific activity expressed as μmole ADP^{-1}mg $protein^{-1}hour^{-1}$.

The analysis procedures were performed on ice or in a temperature regulated centrifuge (<4˚C>0˚C), unless otherwise stated. Prior to sample analysis, a standard curve describing hydrolysis of ATP to ADP was constructed with IB-buffer and ADP standard (appendix 1) in 0,5ml eppendorf tubes labelled according to concentration (0, 5, 10, and 20 nmol ADP $10\mu l^{-1}$) of ADP. Ten μl of every standard (0, 5, 10, and 20) was applied in triplicate to a 96 well microplate with a pipette. AM-medium and salt-solution (appendix 1) is mixed in a plastic container designed for multipipettes.

Using a multipipette, 200 µl of AM-salt solution is distributed to each of the microplate wells. The microplate was immediately read in a microplate reader (Spectramax plus 384 , Molecular Devices Corp., Palo Alto, California, USA) at 340nm, 25°C for 10 minutes, and a total of 21 readings was performed, with one every 30 seconds. The results were expressed as the ratio of $NADH^+$ that was oxidized to NAD^+. Soft max pro 5.0 files (Molecular Devices Corp., Palo Alto, California, USA) files were exported to Microsoft excel and optical density (OD) values were plotted against known concentrations. The standard curve was utilized in the calculation of NA^+K^+ATPase enzyme activity in the gill biopsies.

Gill filament samples were kept in eppendorf tubes and when defrosted, added 25ml of SEID (appendix 1), and homogenized with a Pellet pestle motor (Kontes, New Jersey, USA), for 15 seconds. To avoid cross-contamination between samples, the Pellet pestle motor was wiped off with facial tissues between samples. The samples were then centrifuged (Rotanta 460 R, Herrich zentrifuge) at 3800 rpm (3228,8 x *g*) for 2 minutes. A microplate was placed on ice with a protective layer of aluminium foil in between to avoid water stains from the ice biasing the OD read. An amount of 10µl of the supernatant was applied to a microplate in four replicates. The remaining pellet of the sample was frozen for later protein content analysis. Two of the replicates for each sample was added 200µl of AM-Salt solution (appendix 1), and the remaining two replicates was added 200µl of AM-Oubain salt solution (appendix 1). The microplate was immediately placed in the microplate reader, no later than 30 minutes after homogenization (McCormick 1993), as enzyme activity starts to decrease subsequent to homogenization. Reading was performed equal to the standard curve (see above), the results were edited using soft max pro 5.0, and the file was exported to Microsoft excel for calculation of enzyme activity relative to protein content.

Analysis of the protein content in each gill sample was performed with BCA Protein Assay Reagent Kit (PIERCE, Rockford IL, USA) with bovine serum albumin as the standard. Samples were de-frosted and centrifuged for 2 minutes at 3800 rpm (3228,8 x *g*) (Rotanta 460 R, Herrich zentrifuge). A working solution was prepared by mixing 20 ml of reagent A with 400µl of reagent B in a plastic well suitable for multipipettes. The solution was mixed by sucking and adding the solution five times while moving the 1-5ml pipette back and forth in the plastic well. The Albumin protein standard (PIERCE, Rockford IL, USA) was diluted according to table 6.4 (appendix 1), and 10 µl was delivered in triplicate to a microwell plate placed on aluminium foil and ice. Using a multipipette, 200 µl of WR solution (PIERCE, Rockford IL, USA) was added to each well in the microplate, including the wells containing albumin standard. A 30 second shaking of the plate was

then performed in order to ensure a good mix of samples and WR solution. Incubation of the samples was performed in a 37°C heat cabinet for one hour, with aluminium foil wrapped around the microwell plate to avoid contamination. Samples were unwrapped and stored until room temperature, before reading at 540nm for 12 seconds in the plate reader.
The soft max pro document were edited by masking outliers, the file was then exported to Microsoft excel and outliers were removed. The protein content was calculated on the basis of data for the standard curve produced by the albumin standards. Calculation of the Na^+K^+ATPase activity was performed using Microsoft excel and according to equation 2.2.

Equation 2.2: Calculation of the Na^+K^+ATPase activity in gill filaments of Arctic charr.

$$(1)\ \frac{Reaction\ rate\ (mOd\ \mu l^{-1}\ \min^{-1})}{Slope\ (mOD\ nmol\ -1\ min\ -1\ )} = nmol\ ADP\ \mu l^{-1}\ min^{-1}$$

$$(2)\ \frac{Sample\ activiy\ (nmol\ ADP\ /\ \mu l^{-1}\ /\ \min^{-1})}{Sample\ protein\ content\ (\mu g\ protein\ /\ \mu l^{-1})} = nmolADP\ /\ mg\ protein^{-1}\ /\ \min^{-1}$$

$$(3)\ (nmol\ ADP\ /\ mg\ protein^{-1}\ /\ \min^{-1} \times 60\ \min/\ hour) \times 1000 = \mu molADP\ /\ mg\ protein^{-1}\ /\ hour^{-1}$$

2.3.3 Data treatment and statistical analyses

Morphometric data, as well as plasma osmolalities and chloride concentrations, are expressed as means ± standard error of mean (SEM), Gill Na^+K^+ATPase activity are expressed as medians ±95% confidence limits. Condition factor was calculated by using equation 2.3.

Equation 2.3

$$Condition\ Factor\ (CF) = \left(\frac{Weight\ \ in\ \ grams}{(Length\ in\ cm)^{-3}} \right) \times 100$$

Statistical Analysis was performed using Statistica 6.1 (Statsoft, inc Tulsa,OK,USA).
A parametric test (ANOVA general linear model (GLM)) test was used to reveal effects of time and temperature and their interaction on plasma chloride concentrations, plasma osmolality and morphometric data. In the SWT groups, length was included as a covariat in the ANOVA GLM test of effects of time and temperature on plasma osmolalities and chloride concentrations. A post-hoc (Tuckey HSD) test was used to reveal significant differences within and between temperature regimes.

Due to strong skewness in the gill Na^+K^+ATPase activity data, a non-parametric (Kruskal Wallis) overall median test was used to reveal possible effects of time and temperature. In cases of significant effects, a multiple comparison of mean ranks was used to reveal where differences occurred. A Mann-Whitney U test was used to test significant differences between the temperature regimes. Results were considered to be significant when probability level was lower than or equal to 0,05.

3.0 Results

No mortality was observed during the SWTs. Due to a mistake, gill filaments for Na^+K^+ATPase activity analysis was not sampled from the FW group on May 31. No effect of replicates within temperature regimes was recorded and the replicates are therefore pooled in the presentation of the data. The number of fish sampled from each treatment at each sampling date, and the number of analyses presented in the results, are given in table 3.1. Due to errors during the analysis of Na^+K^+ATPase activity, some groups and dates have few samples successfully analysed.

Table 3.1: Number of fish sampled from freshwater and seawater for morphometric (weight and length) and plasma ion (chloride concentration and osmolality), and Na^+K^+ATPase activity in fish held at ambient temperature (T_A) and elevated temperature (T_E) at the different sampling dates.

	Freshwater			**Seawater**
Sampling Date	Morphometry Plasma ion T_A/T_E	Na^+K^+ATPase T_A	Na^+K^+ATPase T_E	Morphometry Plasma ion T_A/T_E
07.03.2006	24	-	-	24
05.04.2006	24	11	8	24
19.04.2006	24	9	10	24
03.05.2006	24	11	12	24
19.05.2006	24	10	2	24
31.05.2006	24	7	8	24
13.06.2006	24	8	6	24
15.06.2006*	24	-	-	24
19.06.2006**	24	-	-	24
28.06.2006	24	12	12	24
05.07.2006	24	7	11	24

*3 Day Seawater Challenge test

**7 Day Seawater Challenge test

3.1 Weight, length and condition factor of fish sampled in freshwater

3.1.1 Weight

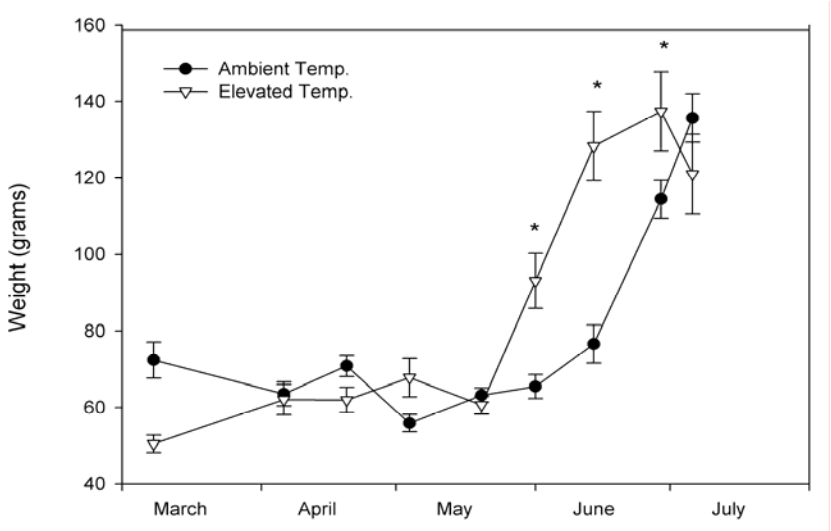


Figure 3.1: Body weight (mean ± SEM) of Arctic charr held in freshwater at ambient and elevated temperatures at the different sampling times during the course of the experiment. Asterixes denotes within date differences in weight between temperature treatments.

Weights of the fish sampled in freshwater were affected both by time (P<0,001) temperature treatment (P<0,001), and their interaction (P<0,001).Body weights remained relatively constant until mid-May, after which the weight of the fish in both temperature regimes increased approximately 2-fold until the end of the experiment in early July. During the period late May to mid June, weights of the fish in the elevated temperature group were higher than those of the fish held on ambient temperature.

3.1.2 Length

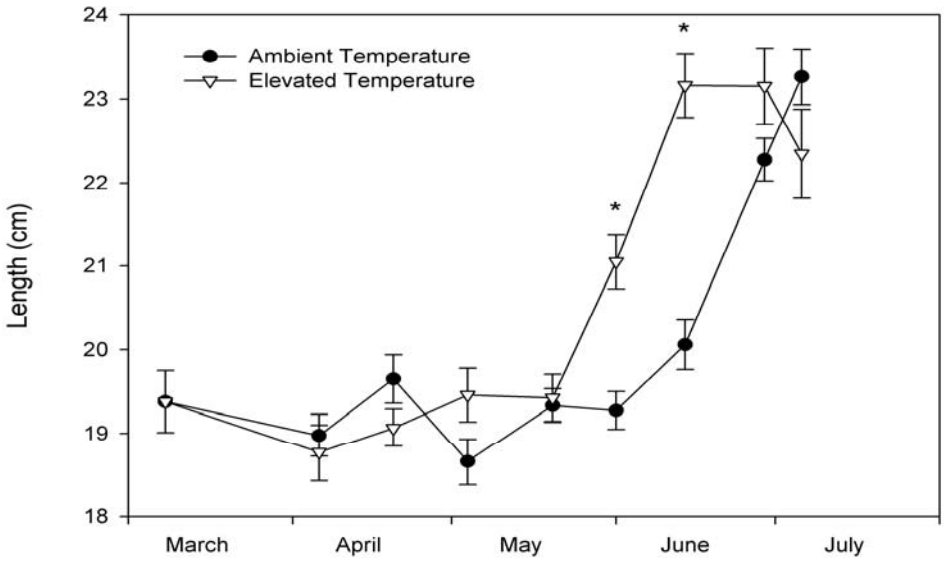


Figure 3.1.2: Body length (mean ±SEM) of Arctic charr held in freshwater at ambient and elevated temperatures at the different sampling times during the course of the experiment. Asterixes denote within date differences in length between temperature treatments.

There were effects of both time (P<0,001), temperature (P<0,001) and their interaction (P<0,001) on fish length. Body length remained relatively constant until mid May, after which the fish from both temperature groups increased in length. During the period from early June to mid June the fish in the elevated temperature group was longer than those in the ambient temperature group.

3.1.3 Condition Factor

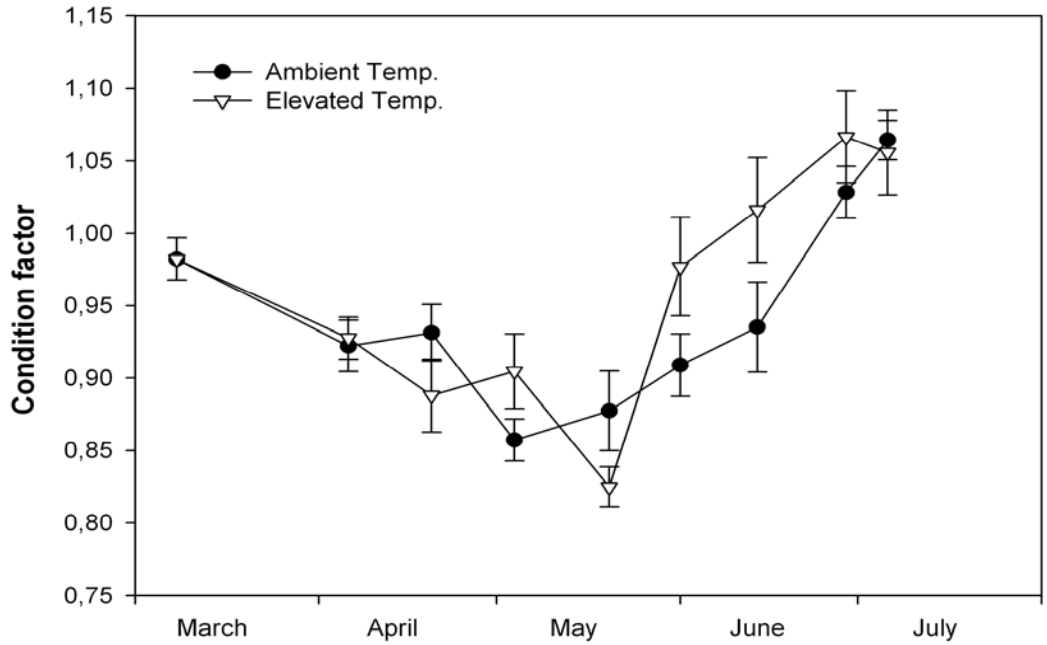


Figure 3.1.3: Condition factor (mean ± SEM) of Arctic charr held in freshwater at ambient and elevated temperatures at the different sampling times during the course of the experiment.

The condition factor of the fish held in freshwater showed was influenced by time ($F_{018,04}$; $P<0,001$) but not by temperature ($P>0,05$) or their interaction ($P>0,05$). There was a decrease in condition factor in both groups until mid May, after which condition factor increased in both groups until the end of the experiment in early July. There was no difference between temperature regimes at any of the sampling dates.

3.1.4 Morphometric data from fish exposed to SWT.

Table 3.1.2: Weight, length and condition factor (mean ± SEM) of Arctic charr exposed to the 24 h seawater challenge test at different dates. Asterisks denote significant differences between temperature treatments and groups within dates.

	Length		Weight		Condition Factor	
Date	T_A	T_E	T_A	T_E	T_A	T_E
06-07.03.2006	18,9 (0,35)	18,9 (0,35)	62,0 (3,86)	62,0 (3,86)	0,91 (0,02)	0,91 (0,02)
04-05.04.2006	18,5 (0,26)	19,1 (0,22)	56,6 (3,05)	60,7 (2,3)	0,88 (0,02)	0,87 (0,01)
18-19.04.2006	18,9 (0,24)	19,0 (0,30)	58,2 (2,87)	58,7 (2,6)	0,86 (0,01)	0,83 (0,02)
02-03.05.2006	19,2 (0,27)	20,1 (0,32)	62,3 (2,29)	75 (5,0)	0,86 (0,01)	0,89 (0,03)
18-19.05.2006	19,2 (0,35)	19,6 (0,43)	59,1 (3,14)	62,5 (5,3)	0,83 (0,01)	0,81 (0,04)
30-31.05.2006	20,0 (0,21)	21,3 (0,43)	79,5 (3,47)	84,1 (7,9)	0,92 (0,04)	0,84 (0,03)
12-13.06.2006	20,2 (0,45)*	22,0 (0,55)*	70,4 (5,4)	104,8 (11,6)	0,84 (0,03)	0,94 (0,04)
12-15.06.2006*	20,0 (0,31)	22,9 (0,46)	65,4 (4,07)	105 (9,7)	0,80 (0,03)	0,85 (0,04)
12-19.06.2006**	20,0 (0,28)	21,8 (0,42)	73,2 (4,71)	97,9 (7,4)	0,91 (0,03)	0,92 (0,03)
27-28.06.2006	21,1 (0,26)*	22,7 (0,37)*	88,9 (5,04)*	118,3 (5,0)*	0,94 (0,04)	1,02 (0,04)
04-05.07.2006	21,9 (0,39)*	23,5 (0,45)*	108,0 (9,16)*	139,4 (9,7)*	1,01 (0,04)	1,78 (0,72)

*=3 Day seawater test **=7 Day seawater test.

The length of fish exposed to SWT was influenced by time ($P<0,01$), temperature ($P<0,01$), and their interaction ($P<0,05$). There was no differences between temperature regimes until late May, were after the fish in the elevated temperature group were longer than those in the ambient temperature group.

The weight of the fish exposed to SWT was influenced by time ($P<0,01$), temperature ($P <0,01$) and their interaction ($P <0,01$). No differences were revealed between the temperature regimes until mid June, where after the fish from the elevated temperature group were larger than the fish from the ambient temperature group.

The condition factor of the fish exposed to SWT was influenced by time ($P<0,01$), but not by temperature ($P >0,05$) or their interaction ($P >0,05$). No differences were revealed between the temperature regimes.

3.1.5 Silvering

In the beginning of the experiment the fish had visible to slightly visible parr marks with a silvering index (SI[1]) of 1,5 (±SEM 0,17). From early to mid May the silvering index increased in all groups. A decrease was seen until the end of the experiment. However there was a tendency of less visible parr marks on the fish after exposure to seawater for 24 hours.

Table 3.1.3 Silvering index of the experimental fish, (silvering index is described in appendix 2).

	Freshwater		**Seawater Test**	
Date	T_A(±SEM)	T_E(±SEM)	T_A(±SEM)	T_E(±SEM)
07.03.2006	3,00(0,00)	3,00(0,00)	3,0 (0,00)	3,0(0,00)
05.04.2006	3,00(0,00)	3,00(0,00)	3,0 (0,00)	3,0(0,00)
19.04.2006	3,00(0,00)	3,00(0,00)	3,0 (0,00)	3,0(0,00)
03.05.2006	3,00(0,00)	3,08(0,08)	3,9 (0,10)	3,4(0,00)
19.05.2006	4,00(0,00)	3,83(0,11)	4,0(0,00)	3,8 (0,10)
31.05.2006	3,00(0,08)	3,00(0,00)	3,3(0,10)	3,8(0,10)
13.06.2006	4,00(0,00)	3,00(0,00)	3,2(0,10)	3,0(0,00)
28.06.2006	3,50(0,00)	3,50(0,00)	3,5(0,00)	3,5(0,00)
05.07.2006	4,00(0,00)	3,00(0,00)	3,5(0,00)	3,5(0,00)

Table 3.1.4 Silvering index from 1, 3, and 7 day tests

	Seawater Test	
Date	T_A(±SEM)	T_E(±SEM)
13.06.2006	3,17(0,11)	3,00(0,00)
15.06.2006	3,00(0,00)	3,00(0,00)
19.06.2006	3,50(0,00)	3,50(0,00)

3.2 Plasma osmolality and chloride concentrations

3.2.1 Plasma Chloride concentrations

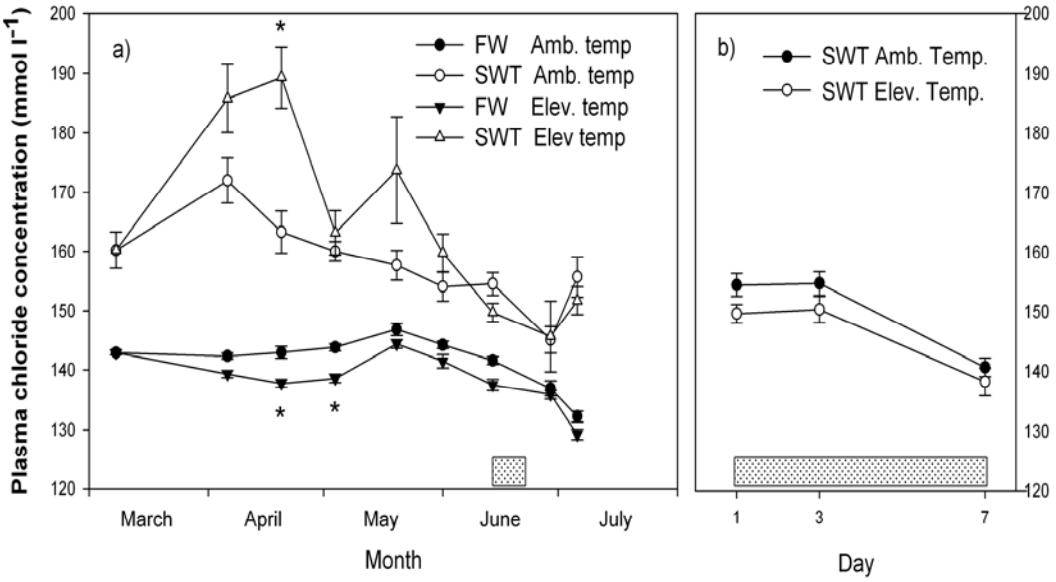


Figure 3.2.1: (a) Seasonal change in plasma chloride concentrations (means ± SEM) in Arctic charr held in freshwater (FW) and after a 24 h seawater challenge test (SWT) at two different temperature regimes. Asterixes denote significant differences in plasma chloride concentration between temperature regimes, within dates and salinities (b) Plasma chloride concentration from 1, 3, and 7 day SW sampled on 13, 15, 19 of June respectively. Patterned bars indicate time span of 1,3, and 7 day SW samplings in June. Asterixes denote significant differences in plasma chloride concentration between temperature regimes, within dates and salinities.

There were effects of time ($P<0,001$) and temperature ($P<0,001$), and their interaction ($P<0,001$) on plasma chloride concentration in the freshwater fish. In the FW elevated temperature group there was first a decrease in plasma chloride concentration until mid-April, then an increase until mid May, and thereafter a gradual decrease until the end of the experiment in July. In the ambient temperature group there was no change in the plasma chloride concentrations until June, after which there was a decrease. In April, May and mid June, plasma chloride concentration was lower in the elevated temperature group than in the ambient group.

In the seawater challenge test groups there were effects of time ($P<0,001$) temperature ($P<0,001$) and their interaction ($P<0,05$). No effect of length on plasma chloride concentration post SWT was found ($P>0,05$). In the ambient temperature group there was a decrease in post SWT plasma chloride concentrations from early April until late June. There were no significant differences between the other dates. Among the fish held at an elevated temperature, post SWT plasma chloride concentrations increased from March to April after which there was a gradual decrease until late

June. On April 19, post SWT plasma chloride concentrations were higher in the fish held at an elevated temperature than those held at ambient temperatures.

The 1, 3, and 7 day seawater test (figure 3.2.1 (b)) revealed overall effects of time (P<0,01) and temperature treatment (P<0,01), but not their interaction (P>0,05). Plasma chloride concentration was lowest on day 7 in both temperature regimes, but the post-hoc test revealed no significant differences between temperature regimes within dates. 3.2.2

3.2.2 Plasma Osmolality

In the freshwater fish there was effects of time (P<0,001) and temperature (P<0,001) on plasma osmolality in both temperature regimes. In both groups plasma osmolality decreased from March to June (Figure 3.2.2), after which there was an increase. In April and May plasma osmolality was higher in the ambient temperature group than in the elevated temperature group.

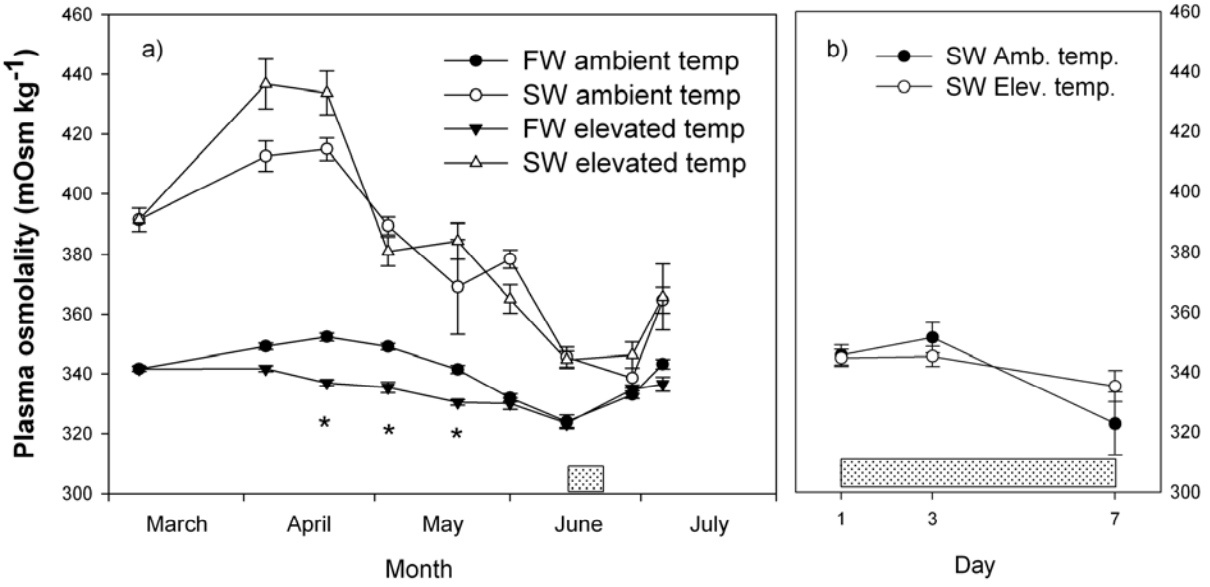


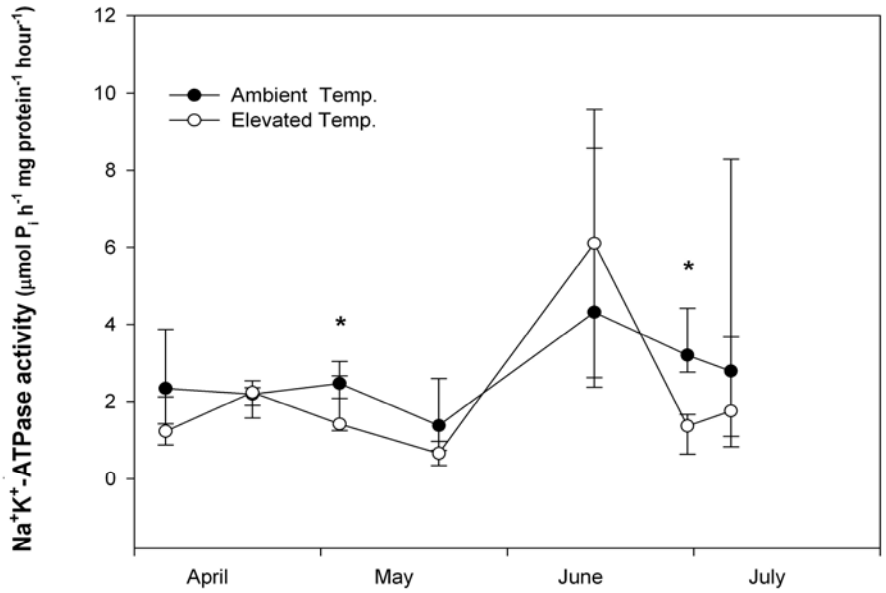
Figure 3.2.2: Seasonal change in plasma osmolality (means ± SEM) in Arctic charr held in freshwater (FW), and after 24h seawater challenge test (SW) at two different temperature regimes. (b) Plasma osmolality from 1, 3, and 7 day SW sampled on 13, 15, 19 of June respectively. Patterned bars indicate time span of 1,3, and 7 day SW samplings in June. Asterixes denote significant differences in plasma osmolalities between temperature regimes, within dates and salinities.

In the Seawater challenge test groups there was no effect of length (P>0,05) but effects of both time (P<0,001) and temperature (P=0,026) on plasma osmolality were revealed. There were no interaction effects of time and temperature on plasma osmolality after seawater tests. No differences

in plasma osmolality were found within dates between the temperature regimes. In the ambient temperature group there was a decrease in plasma osmolality between April and June. In the elevated temperature group there was an increase in plasma osmolality between March and April, after which there was a decrease from April to June.

The 1, 3, and 7 day seawater test (figure 3.2.2 (b)) revealed overall effects of time ($P<0,01$) but not by temperature treatment ($P>0,05$), or their interaction ($P>0,05$). Plasma osmolality was lowest on day 7 in the ambient temperature regime, and there were no differences revealed between temperature regimes within dates.

3.3 Gill Na^+K^+ATPase activity in freshwater fish.



Figur 3.4.1: Temporal changes in gill Na^+K^+ATPase activity (medians ± 95% confidence intervals) in Arctic charr held in freshwater subjected at two temperature regimes. Asterixes symbolize significant differences between temperature regimes.

Gill Na^+K^+ATPase activity was affected by time in both the ambient temperature regime ($P<0,001$), and in the elevated temperature group ($P<0,05$). For charr held on elevated temperature there were consistently low Na^+K^+ATPase activity until mid May, after which there was a transient increase during the first part of June. In the ambient temperature group there were generally small changes during the experiment period. The Na^+K^+ATPase level in mid June was, however, higher than the level in mid April and mid May. Na^+K^+ATPase levels at other dates were intermediate but not significantly different from the levels on other dates.

There was generally small difference in the Na^+K^+ATPase activities of the two temperature regimes. However the ambient temperature group had a higher Na^+K^+ATPase activity in early May than the elevated temperature group, whereas the opposite situation occurred in early July.

4.0 Discussion

The timing of peak hypoosmoregulary ability of the Arctic charr investigated in the present study coincided with timing of the seawater migration in mid- to late June of hatchery released smolts in the Hals watercourse (Frode Løvik, Talvik Research station, Talvik, Norway, pers. comm.), showing that the seaward migration coincide with the peak seawater tolerance in these fish. At the time of maximum hypoosmoregulatory ability the post SWT plasma osmolality and chloride concentration was below 350 mOsm and 150 mmol l^{-1} respectively, as reported from fully smoltified Atlantic salmon subjected to approximately the same SWT conditions (Strand et al. in press). Surprisingly an accumulated 350 day° difference between the two temperature regimes during the smoltification period affected neither the temporal development of the smoltification process nor the peak smolt quality, implying that photoperiod is the dominating environmental clue governing the smoltification process in the Arctic charr. Furthermore, it is clearly shown that temperature *per se* is not a zeitgeber for smoltification in the Arctic charr.

4.1 Morphological data

The fish used in the experiment were reared at high water temperatures and excess feeding prior to the experiment, and this ensured that they were above the threshold size reported for parr-smolt transforming Arctic charr, and thus smoltified when they were approximately 1,5 years old. This is 2,5 to 4,5 years earlier than the age at which wild anadromous Arctic charr undergo parr-smolt transformation in the northern parts of Norway (Rikardsen and Elliott 2000). Several studies has reported that increased body size correlate with increased hypoosmoregulatory ability for charr held in captivity (Arnesen et al. 1992; Staurnes et al. 1992; Johnsen et al. 2000). For free living sub-Arctic and high Arctic charr an increase in size has been shown to correlate positively to survival in SWT (Dempson 1993; Gulseth et al. 2001b). It could therefore not be excluded that the size difference seen between fish exposed to elevated temperatures and those held at an ambient temperature during spring in the present study could have influenced the development of their hypoosmoregulatory ability. The average size of the fish in the present study was, however, well above the minimum size of 60 grams (18 cm) that Staurnes et al. (1992) found to be necessary for a complete smoltification in sub-Arctic charr. Fish above this size responded well to photoperiod stimulation, displayed full hypoosmoregulatory ability, and no further increase in hypoosmoregulatory ability occurred with increasing size above 60 grams (Staurnes et al. 1992). This corresponds well to the lack of any influence of fish size on hypoosmoregulatory ability found

in the present study. Therefore our findings support the idea of a threshold size for parr-smolt transformation in Arctic charr, and explain, at least partly, why the peak hypoosmoregulatory ability was identical in the two temperature groups in the present study despite a relatively large difference in fish size. The results further indicate a smaller threshold size for a complete parr-smolt transformation in sub-arctic charr, than what is reported from captive high-Arctic charr (>25 cm) originating from Dieset river at Svalbard (Gulseth et al. 2001b).

Both increasing daylengths and temperature has been shown to stimulate appetite and growth in salmonids (Boeuf and Falcòn 2001). In the current study the increase in water temperature coincided with the increase in weight and length in both temperature regimes. Further evidence for a causal relationship between temperature and growth is provided by the advanced increase in growth in the elevated temperature group, corresponding to the advanced elevation of water temperature. The current growth data suggests that water temperature is a major regulator of appetite and growth and most likely appetite in parr-smolt transforming Arctic charr. It is important to note, however, that the fish held at elevated temperature started to grow at the same time as those held at ambient temperature (mid-May), more than one month after the increase in water temperature, emphasising the role of photoperiod as a permissive factor in the stimulation of appetite and growth (Boeuf and Falcòn 2001). The energy depletion during the overwintering in freshwater requires a rapid growth in seawater, which is reflected by of the intense feeding and growth seen during the first 2-3 weeks of the seawater residency of wild anadromous Arctic charr (Rikardsen et al. 2000). This is stated to be a compensatory growth mechanism which is characterised by hyperphagia, until the fish's growth trajectory is reached (Jobling 1994). The compensatory growth mechanism is suggested as an explanation to the short duration of the seawater stay, i.e. the fish migrates away from the feeding grounds when the potential for further growth is lost (Rikardsen et al. 2000).

A similar pattern of the condition factor development, i.e. a decrease until mid May and then an increase until the end of the experiment, has been seen in previous studies with parr-smolt transforming Arctic charr from the Hals watercourse (Jørgensen et al. in press). In addition Aas-Hansen et al (2003) observed an increase in condition factor during the parr-smolt transformation in a study of anadromous Arctic charr, originating form lake Vårfluesjøen at Svalbard. The observed increase in condition factor corresponds to a larger increase in weight than in length from mid May. This is particularly evident in the elevated temperature regime, which display a higher rate of weight gain from mid May than the fish held at ambient temperature.

Thus the Arctic charr observed in the elevated regime display an increasing weight approximately 6 weeks prior to the time of seawater entry, and there is a tendency of growth stagnation towards the end of the experimental period that is not seen in ambient temperature regime. According to Rikardsen et al. (2000) a further increase in weight is evident throughout the seawater residency. The weight increase during seawater residency is reported to be 100 % for wild post-smolts from Hals river, whereas the increase in length in comparison was only 14 % (Rikardsen et al. 2000), which leads to an increase in condition factor. A rapid increase in condition factor result in a body shape that is less suitable for long distance pelagic swimming and the ecological explanation for this may be a lesser need for optimum swimming performance due to a limited spatial distribution during the seawater residency of Arctic charr. In contrast, condition factor decrease in parr-smolt transforming Atlantic salmon (Strand et al. in press) and reflect the need for post-smolt of this species to perform long-distance rapid swimming away from the fjord immediately after seawater entry (Klemetsen et al. 2003)

A silvery appearance occurred from the beginning of the experiment, and this is in contrast to studies on Atlantic salmon that reports a parr like appearance until photoperiod and temperature increase in the spring (Sigholt et al. 1998). This difference between smolting Arctic charr and Atlantic salmon may be related to differences between these two species in behaviour prior to seaward migration. According to Klemetsen et al. (2003) Arctic charr normally spawn in lakes, and the parr also grow up in lakes. In contrast to the Atlantic salmon parr that grow up in rivers, the Arctic charr parr need to actively search for prey in the lake, and this could be an explanation for the more pelagic adapted camouflage early in the smoltification period. An increase in the silvering index was seen correlating with the initiation of the continuous light regime and elevating temperature in early to mid May. This corresponds with data from Atlantic salmon were an increase in daylength has a triggering effect on silvering, while water temperature is regulating the rate of silvering (Sigholt et al. 1998).

4.2 Development of hypoosmoregulatory ability

4.2.1 Plasma Osmolality and Chloride concentration

The observed decrease in plasma osmolality and plasma chloride concentrations in charr held in freshwater at both temperature regimes, coincided with the improvement of hypoosmoregulatory ability seen in the SWT groups. Similar results from studies with Arctic charr have previously been reported by Jørgensen and Arnesen (2002). It appears that changes in the osmoregulatory apparatus may cause osmotic disturbance in fish held in freshwater (McCormick and Saunders 1987), and this is supported by the net loss of transepithelial Na^+ of Atlantic salmon smolts retained in freshwater during the period of hypoosmoregulatory up-regulation (Primmet et al. 1988).

The fish that was exposed to SWTs displayed a preadaptory seasonal increase in seawater tolerance that was similar to what is reported for Atlantic salmon smolts with plasma osmolality and chloride concentrations below 350 mOsm and 150 mmol l^{-1} respectively, which can be considered as full hypoosmoregulatory ability (Strand et al. in press). A poorer hypoosmoregulatory ability was recorded in the elevated temperature group prior to the increase in hypoosmoregulatory ability, i.e. shortly after the increases in temperature. This may reflect an acclimation time of the Na^+K^+ATPase enzyme of 3 weeks, as recorded by a study from Tirri et al. (1978). The lowest values of plasma osmolality and plasma chloride concentrations were recorded in the 7 day seawater challenge test in mid June, indicating that a final improvement of hypoosmoregulatory ability takes place after exposure to seawater. Similar results were shown by Staurnes et al. (1994), who recorded plasma osmolality and chloride concentration values close to those in freshwater control groups in Arctic charr exposed to a 10 day seawater test in June (LD 24:0, Ambient Temp.). The recorded values from the 7 day SWT are also similar to the values after a 4 day SWT with wild first time migrants of Arctic charr from the Sila river in Norway (Gulseth et al. 2001a).

4.2.2 Gill Na^+K^+ ATPase activity

According to several studies (Staurnes et al. 1994; McCormick et al. 1995), there is a strong positive correlation between gill Na^+K^+ATPase activity and hypoosmoregulatory ability in Atlantic salmon, indicating a causative relationship between these two parameters in the development of seawater tolerance in general. Such a relationship between Na^+K^+ATPase activity and hypoosmoregulatory ability is, however, still uncertain in Arctic charr. Previous studies with Arctic charr have shown both a correlation (Arnesen et al. 1995; Eliassen et al. 1998; Lysfjord and Staurnes 1998), and no correlation (Johnsen et al. 2000). The lack of correlation was seen in a group of Arctic charr held on a constant temperature of 4° C until ambient temperature rose above this temperature in mid May, and a short daylength (L:D 4:20). The Arctic charr held on the short day regime were able to regulate plasma osmolality and plasma chloride to low levels (Approx. 360 mOsm and 160 mmol l^{-1}) after a 3 day SWT in early July, in spite of no increase in gill Na^+K^+ATPase activity (Johnsen et al. 2000). However, low levels of plasma chloride and plasma osmolality coincided with an increase in Na^+K^+ATPase in a group held on natural increasing daylengths. Based on these results, Johnsen et al. (2000) suggested that other mechanisms regulating salt balance might be acting during the development of hypoosmoregulatory ability in Arctic charr. Compensation of salt intrusion may be performed by an active pumping of salt out of the gill, or by an altered membrane permeability (Hochacka 1988).

In the present study maximum gill Na^+K^+ATPase activity occurred two weeks prior to full hypoosmoregulatory ability and this is in line with the findings in smolting Atlantic salmon parr (Steffensen 2006). However the sampling intervals in the period when maximum hypoosmoregulatory ability developed were so long that a putative closer correlation between hypoosmoregulatory ability and Na^+K^+ATPase activity could easily be missed. There were no significant difference in peak Na^+K^+ATPase between the ambient and the elevated temperature groups, and together with the data from analyses of plasma chloride concentrations and plasma osmolality this indicates that temperature has less influence on the development of hypoosmoregulatory ability in Arctic charr than what is the case in Atlantic salmon. Further, the results from the present study are in contrast to the results of Handeland (2004) which showed that approximately the same number of degree days from the onset of a smolt related increase in Na^+K^+ATPase activity is needed to reach peak gill Na^+K^+ATPase, between three temperature regimes (12°C, 8,9°C and ambient temperature, natural photoperiod.) in Atlantic salmon. Hence it would be expected that the advance in temperature should have produced an earlier peak in gill

Na^+K^+ATPase in the Arctic charr investigated in the present study. The results in the present study is also in contrast to the findings in other experiments with Atlantic salmon, in which it has been found that warmer rearing temperatures advance the peak in gill Na^+K^+ATPase activity is advanced.

However, the present data indicates that the increase in gill Na^+K^+ATPase activity was followed by a more rapid decrease in the elevated temperature group. This finding corresponds to the results in several studies with Atlantic salmon which found degree-days as the direct cause of loss of Na^+K^+ATPase activity (Handeland et al. 2004; Zydlewski et al. 2005). In a laboratory study of the downstream movements of smolting in Atlantic salmon, a group exposed to abrupt advanced elevated temperature and ambient photoperiod (42°30´N), displayed a shortened duration of downstream movements when compared to a group held on phase-delayed and slowly increasing temperature (Zydlewski et al. 2005). The pattern of migratory behaviour observed in Zydlewski et al.(2005) was similar to the pattern of Na^+K^+ATPase development, indicating that cumulated degree days is positively correlated to loss of hypoosmoregulatory capacity in Atlantic salmon (Zydlewski et al. 2005).

Viewed in the light of the shortened duration of the downstream movement and Na^+K^+ATPase activity seen in the advanced temperature regime in Zydlewski et al. (2005), it may be speculated that the duration of high Na^+K^+ATPase in Arctic charr may be shortened if freshwater temperatures increase earlier in spring. The current available information on gill Na^+K^+ATPase activity in Arctic charr indicate that upregulation of gill Na^+K^+ATPase enzyme activity is an important part of full development of hypoosmoregulatory ability in Arctic charr, similar to the Atlantic salmon (Jorgensen et al. in press). A possible effect of a previously elevated temperature on the loss of gill Na^+K^+ATPase activity in Arctic charr is difficult to explain, and more studies are needed to fully understand the role of temperature on the temporal changes in gill Na^+K^+ATPase.

4.3 Timing of the smoltification process

4.3.1 Photoperiod

Arnesen et al. (1992) showed that increasing photoperiod stimulated hypoosmoregulatory ability in Arctic charr held on 5-6 ° C water temperature. Growth hormone (GH) is shown to have an important role in mediating the photoperiod signal to stimulate physiological responses in smolting Atlantic salmon, and plasma GH levels shows a strong positive correlation with gill Na^+K^+ATPase

activity after an increase in daylength (McCormick et al. 2002). In the present study the response time elapsing from the onset of photoperiod increase until hypoosmoregulatory starts to increase was in line with McCormick et al. (1995), who reported that osmoregulatory responses started 2-3 weeks after a change in photoperiod. This delay was suggested to be due to the time needed for endocrine control mechanisms to cause measurable changes. In a previous study, Johnsen et al. (2000), exposed Arctic charr from the anadromous Hammerfest strain to an elevated temperature (4°C) from December until ambient temperature rose above this level in mid May. The fish were held under three different photoperiod regimes (L:D $_{Natural}$, L:D$_{24:0}$ and L:D$_{4:20}$) and the results from the repeated 3 day SWTs showed that photoperiod is an important cue for the timing of hypoosmoregulatory capacity in Arctic charr. This conclusion was based on the finding of a 6 week advance of the timing of increased hypoosmoregulatory ability in the LD$_{24:0}$ group, and a 6 week delay of low values of plasma chloride and plasma osmolality in the L:D$_{4:20}$ group, compared to the L:D$_{Natural}$ group. The response to photoperiod seems to occur in salmonids at both natural increases in photoperiod during spring, and when continuous light regimes is applied, provided that the fish has been subjected to a previous period of short days (Sigholt et al. 1998; Johnsen et al. 2000; Jørgensen and Arnesen 2002). Further support to the importance of photoperiod as a zeitgeber is given by the observation of a two week delay in timing of seawater migration of hatchery reared anadromous Arctic charr released in the Hals watercourse, compared to wild anadromous Arctic charr in the same watercourse (Jørgensen et al. in press). A plausible explanation for the delayed migration of hatchery reared smolts is that the increase in daylength in the hatchery light regime is ca. 3 weeks later than spring equinox (Even Jørgensen, Department of Aquatic BioSciences, Norwegian College of Fishery Science, pers. comm.).

4.3.2 Temperature

Higher water temperatures are linked to increased growth, and due to the size dependency of the smoltification process, it is expected that an overall increase in water temperatures will lead to lower age when smolting in wild anadromous Arctic charr (McCormick et al. 1996). This is supported for Arctic charr held in captivity by the present study and several others (Johnsen et al. 2000; Jørgensen et al. in press ; Strand et al. in press). Smolting at a lower age in wild anadromous Arctic charr is however not likely to occur due to the limited amount of nutrition in the freshwater habitats, although the effects of increased temperatures on the prey of Arctic charr has to be investigated for a fuller understanding of this relationship.

The effects of elevated temperature during spring can be expected to result in advanced smolting in spring due to a general Q_{10} effect on the rate of smoltification process (McCormick et al. 1996; Strand et al. in press). An advance in smolting due to increased temperature has been observed in several studies on Atlantic salmon (Solbakken et al. 1994; McCormick et al. 2000; Shrimpton et al. 2000). Surprisingly, such an effect of water temperature was not seen in the present study with Arctic charr. However, a missing relationship between increased hypoosmoregulatory ability and elevated temperatures has previously been shown in Atlantic salmon by Duston and Saunders (1995). The lack of difference between the elevated and the ambient temperature groups, could be due to a need for more degree-days to advance the parr-smolt transformation at higher temperatures as shown in Atlantic salmon by McCormick (1996). However, taken into account the magnitude of the differences (350° days) between the temperature regimes in the present study suggests that this can be ruled out.

On the other hand, low rearing temperatures (close to 0°C) until late April has been shown to inhibit smolting in Baltic salmon (*S. salar*) (Virtanen and Oikari 1984; Soivio et al. 1989), but this does not seem to be the case in Arctic charr (Jørgensen et al. in press), supporting the common opinion that Arctic charr is a more cold-water adapted species relative to Atlantic salmon. This is also supported by the findings of Arctic charr initiating smolting at low temperatures in the present study. When post SWT plasma osmolality and chloride concentrations started to decrease in the ambient temperature group in early April, the temperature was close to 0° C. When an additional increase in hypoosmoregulatory ability was seen in mid May, the temperatures were still low (approximately 2° C) in the ambient temperature group. The lack of an advance in on the rate of the parr-smolt transformation process when exposed to elevated temperature in the present study indicates that the rate of the process is not temperature dependent.

4.4 Future perspectives in a global warming scenario

According to data from several studies of north Norwegian sub-arctic charr, the timing of seawater residency seem to vary substantially between rivers and migration towards the sea has been reported to occur from early May to early July (Mathisen and Berg 1968; Berg and Berg 1989; Rikardsen et al. 1997; Johnsen et al. 2000; Aas-Hansen et al. 2003). The most important determinant of the timing of migration of anadromous Arctic charr within a watercourse is the size of the charr, as veteran migrants migrate much earlier than first-time migrants (Nordeng 1977). But conditions in the

watercourse (time of ice-breakup, discharge) and the coastal areas nearby would likely be of significance (Klemetsen et al. 2003). An important question is whether the anadromous Arctic charr is able to vary the timing of seaward migration so that it fits the optimal conditions for seawater residency and feeding based on adequate environmental stimuli, even if a temperature increase due to global warming occurs. The timing of peak smoltification in the present study coincided with the timing of migration of hatchery reared charr smolt from Talvik Research station (Frode Løvik, Talvik Research station, Talvik, Norway, pers. comm.). This shows that smolt indices (hypoosmoregulatory ability, gill Na^+K^+ATPase activity) are a reliable measure of the timing of the migration in Arctic charr. Therefore the finding that increased temperature did not results in an advanced timing of the smoltification process in the present study also indicate that Arctic charr migration will not take place earlier if the spring temperature increase occur earlier in the future. If that is the case, the strong dominance of photoperiod on the timing of the smoltification period (and migration) is maladaptive for the anadromous Arctic charr in a situation with future global warming.

4.4.1 Mismatch

If the spring increase in seawater temperature starts earlier as a result of climate change, it may be expected that the optimal feeding conditions in the sea for anadromous Arctic charr, also will occur earlier in spring. The most important period of weight gain in Arctic charr has been shown to be during the 2-3 first weeks of the short period of seawater residency, and this was mainly related to extensive feeding on *Calanus finmarchicus* and krill *Thysannoessa spp.* (Rikardsen et al. 2000). However zooplankton production in coastal areas is highly variable and the timing of onset may vary with temperature (Tande 1991; Tande and Slagstad 1992). *C. finmarchicus* is expected to be an important prey species for Arctic charr first time migrants with a fork length less than 400 mm (Rikardsen 2000). The importance or crustaceans is supported by Dempson et al. (2002) which found that crustaceans constitute the most important prey items for A. charr smaller than 300 mm fork length in northern Labrador Canada. *C. finmarchicus* is also an important prey for marine fish larva which again constitute an important part of the repeat migrating Arctic charr (fork length>400 mm) diet in Northern Norway (Rikardsen et al. 2007).

Viewed in the light that *Calanus finmarchicus* is only available for surface feeding during 4-8 weeks during spring an early summer and the large variability that may be related to temperature (Tande 1991) Arctic charr post-smolts, with their short seawater stay and dependency on crustacean zooplankton, may be considered more vulnerable to increased water temperatures that can lead to a

mismatch situation, opposed to other salmonids with a longer duration of seawater residency (Rikardsen et al. 2000). A mismatch between optimal feeding conditions at sea due to climate related advanced spring bloom of Crustaceans may occur, but future studies is needed to reveal effects of climate change on the spring bloom of crustaceans.

Another important issue is whether the optimal metabolism for compensatory growth in seawater comes earlier due to an advanced increase in spring water temperature, as it may seem from the present data. Seawater mortality is very high (50-85%) in first time migrating Arctic charr (Jensen and Berg 1977; Finstad and Heggberget 1993; Berg 1995; Finstad and Heggberget 1995; Rikardsen et al. 1997), and a disturbance of the seawater foraging may be assumed to increase the mortality further, which may have a detrimental effect on the population. Also, the effects of low weight gain at sea may lead to increased winter mortality for smaller individuals due to smaller lipid-storages (Finstad et al. 2004). In northern Labrador ,Canada, increased temperature has been shown have a detrimental effect to size at age in populations of anadromous Arctic charr (Power et al. 2000; Dempson et al. 2002), and this is most likely mediated through a lower abundance of prey. Another aspect of advanced increase in air and freshwater temperatures is the possibility of earlier loss of ice cover, which has been shown to have a negative effect on energy depletion during the spring phase in northern populations of Atlantic salmon that naturally experience ice cover (Finstad and Forseth 2006).

4.5 Conclusion

The fish showed full hypoosmoregulatory ability at the time of seaward migration of hatchery released smolts in the Hals watercourse and this is in line with the previous findings from this watercourse. The present study has demonstrated that, in the presence of correct photoperiod stimuli and with the fish being above threshold size, an early increase in water temperature does not trigger an advanced increase in hypoosmoregulatory capacity. The advanced increase in water temperature leads to a substantial increase in experienced cumulative temperature, which causes a shorter duration of maximum Na^+K^+ATPase activity in accordance with findings in Atlantic salmon. The perspective of global warming for the Arctic charr may therefore be that the duration of the smolt window is shortened. Viewed in light of the indications of a future mismatch between seawater migration and optimal feeding conditions presented, this may be detrimental to the population, although future studies is needed for a more detailed resolution of temperature effects on the seawater migration of anadromous Arctic charr.

The present study has shown that offspring of anadromous Arctic charr goes through a complete smoltification, including elevated gill Na^+K^+ATPase and improved hypoosmoregulatory ability. It has also been shown that the completion of the smoltification process occurred at the time when released smolts migrate to the ocean. Surprisingly, an almost 3 months advance in spring water temperature, accounting for a 350 day^o difference between the two temperature treatment groups, did not accelerate the temporal development of the smoltification process in the Arctic charr. This is in contrast to what is generally found in anadromous salmonids, and may be related to the fact that the smoltification process in anadromous Arctic charr naturally occurs at very low water temperatures. Photoperiod seems to be the dominating factor governing the smoltification process in anadromous Arctic charr. This may be maladaptive for the anadromous Arctic charr in a scenario of future global warming, since there may be a mismatch between the environmental conditions offering optimal feeding conditions for the charr in sea, and the signal triggering seaward migration (photoperiod).

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7.0 Appendix

Appendix 1: Solutions utilized in Na^+K^+ATP-ase assay.

SEI Buffer

26,76 g Sucrose

1,86 g Na_2EDTA/Titriplex III

1,70 g Imidazol

The Chemicals are dissolved in 475 ml dH_2O og justeres til 7,3 ved hjelp av HCL.. The volume is adjusted to 500 ml with H_2O

0,5% SEID

0,1g Natriumdeoksycholate is dissolved in 20 ml SEI buffer.

Imidazol buffer

3,404 g Imidazol is dissolved in 950 ml dH2O.

pH is adjusted to 7,5 with HCl

Volume is adjusted to 1000ml dH_2O

Salt-solution

5,52 g NaCl

1,07 g MgCl2·$6H_2O$

1,57 g KCl

Chemicals are dissolved in 500 ml Imidazol buffer.

PEP (Phosphoenolpyruvat)

0,491 g Phosphoenylpyruvate is dissolved in 100ml Imidazol buffer. The solution is distributed to 5 or 10ml test tubes and frozen.

Ouabain

0,362 g Ouabain is dissolved in 50 ml Imidazol buffer in boiling water bath inside a ventilating hood.

Na-Acetat buffer

0,767 Na-Acetat trihydrat is dissolved in 100 ml dH2O and pH is adjusted to 6,8.

ADP standard

0,0489 g ADP is dissolved in 25ml Na-Acetat buffer. Frozen at -80˚C for later use.

Dilution of ADP Standard

Table 6.1 Composition of ADP standard used in the making of the standard curve.

Concentration nmol $10\mu l^{-1}$	IB buffer (µl)	ADP standard (µl)
0	200	0
5	175	25
10	150	50
20	100	100

AM-medium

Table 6.2

Solutions	2 microplates
Startvolume Imidazol buffer (IB)	20ml
Pyruvat Kinase (PK)	24µl
Lactic Dehydrogenase (LDH)	31µl
NADH	5mg
Phosphoenolpyruvat (PEP)	5ml
Adenosin 5´triphosphate (ATP)	0,0145g
Final volume is adjusted with IB	35ml

A glass container is placed on ice and the start volume of IB is added.

PK and LDH is delivered to 2ml eppendorf tubes and centrifuged at 12000rpm for 8 minutes. The supernatant is removed and the pellet is re-suspendend in IB buffer.

Dissolve NADH in IB and added to the glass container.

Defrosted PEP in the glass container.

ATP is weighed out , IB is added to dissolve, and the solution is added to the glass container.

The solution is transferred to a graded cylinder, and the final volume is adjusted with IB.

Dilutions (AM and AM-O)

To 50 ml centrifuge tubes is labelled, and the AM-O tube is covered with aluminium foil due to Oubain light sensitivity. The solutions are mixed according to table 6.3.

Table 6.3 Dilutions of AM and AM-O

Solution	AM	AM-O
AM medium	17,5	17,5
Oubain		1,25
Imidazol Buffer (IB)	1,25	

Dilution of Protein Standard

Table 6.4: Dilutions of albumin protein standard in order to reveal the relationship between absorbance and protein content. Final concentrations of the diluted albumin standard is given to the right.

2mg/ml BSA Standard (µl)	dH2O (µl)	Protein Standard (µg/10µl)
0	100	0
25	75	5
50	50	10
100	0	20

Appendix 2: Smolt coloration index scale.

Table 6.5: Smolt coloration scale according to (Birt and Green 1986) and (Sigholt et al. 1998).

Scale	Description
1	Parr. No silvering, clearly visible parr marks
2	Parr. Slightly silvery colour. Visible parr marks.
3	Smoltlike Parr. Silvery colour and only slightly visible parr marks.
4	Smolt. Silvery Colour, Black fin margins. No visible parr marks.