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Growth and Energy Utilization Under Different Temperature Regimes in Early Life-Stages of the Copepod *Calanus finmarchicus*

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Abstract

The calanoid copepod *Calanus finmarchicus* (Gunnerus) dominates the biomass and is considered a key species in the North Atlantic pelagic ecosystem. The species is found close to oil production fields along the Norwegian coast and may thus be exposed to discharges from petroleum-related activities. A negative effect on development and survival of the species may have major impact on the ecosystem structure and function. This has made *C. finmarchicus* a target species in large interdisciplinary studies and environmental monitoring. The early life stages represent a particular critical phase among the life stages in copepods and a combination of unfavourable temperatures and environmental contaminants may cause adverse effects on the development of the species. To determine to what extent the development of the early life stages of *C. finmarchicus* is affected by temperature, eggs and nauplii were subjected to 4 different temperatures (5.0, 7.5, 10.0 and 12.5 °C) in a series of laboratory experiment. Eggs were the starting point for each treatment. Measurements of oxygen consumption, dry weight and carbon-nitrogen analysis were done at each development stage including third-stage nauplii. Times of development were monitored. Longer development times were found with decreasing temperatures. Oxygen consumption increased with increasing temperature and increased with increasing stage of development. Considerable impacts of temperature on both carbon and nitrogen contents were found within all development stages. Repetitive lower contents were observed in nauplii treated at 12.5 °C compared to animals reared at lower temperatures. Significantly higher C and N values were found for the third naupliar stage, compared to earlier developmental stages for all treatment temperatures (except for animals developed at 5 °C).

KEY WORDS: *Calanus finmarchicus* • Temperature • Development • Growth

Sammendrag

Kopepoden *Calanus finmarchicus* (Gunnerus) eller "raudåte" er en av de viktigste artene i havets næringskjeder. Raudåta spiller en sentral rolle i økosystemet i de frie vannmassene i Nord-Atlanteren da den utgjør hovedmengden av den samlede biomassen. Raudåta har sitt kjerneområde i Norskehavet og er derfor utsatt for eksponering fra petroleum-relaterte aktiviteter. De unge stadiene av raudåta er den viktigste matressursen for larver av flere fiskeslag, og avgjørende for at yngel til våre viktigste fiskebestander skal overleve. En negativ effekt på utvikling og overlevelse av *C. finmarchicus* kan derfor medføre store konsekvenser på økosystemets funksjon og struktur. Dette har gjort at arten er tildelt en stor rolle i tverrfaglige studier og miljøovervåkning. De tidlige stadiene representerer en kritisk fase i livssyklusen til kopepoder og ugunstige temperaturer og miljøgifter kan medføre uønskede effekter på utviklingen til raudåta. For å avgjøre i hvilken grad utviklingen av de tidlige livsstadiene til *C. finmarchicus* påvirkes av temperatur, ble egg og nauplii eksponert for fire ulike temperaturer (5,0, 7,5, 10,0 og 12,5 °C) i en serie laboratorieeksperimenter. Egg var utgangspunkt i hver temperaturbehandling og målinger av oksygenforbruk, tørrvekt og karbon-nitrogen analyse ble gjort på de fire første livsstadiene, til og med nauplii 3 (N3). Utviklingstiden hos de ulike livsstadiene ble målt der lengre utviklingstid ble funnet med synkende temperatur. Oksygenforbruk økte med økende temperatur og økte med økende livsstadier. En betydelig påvirkning av temperatur på både karbon og nitrogen innhold ble funnet hos alle de undersøkte utviklingsstadiene. Lavere verdier ble funnet for dyr eksponert for 12,5 °C sammenliknet med dyr eksponert for lavere temperaturer. For alle temperaturbehandlinger ble signifikant høyere C og N verdier funnet i det tredje naupliistadiet, sammenlignet med tidligere utviklingsstadier (unntatt dyr utviklet ved 5 °C).

STIKKORD: *Calanus finmarchicus* • Temperatur • Utviklingstid • Vekst

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Abbreviations

µg	microgram
C	Carbon
C L^{-1}	Carbon per litre
DEB	Dynamic Energy Budget
DW	Dry weight
E	egg
GDP	Gross Domestic Product
$ind.^{-1}$	per individual
mg	milligram
N 1-3	Nauplii stages 1-3
N	Nitrogen
O_2	molecular oxygen
SD	Standard Deviation
Sm^3 o.e.	Standard cubic Meters of Oil Equivalents

1 Introduction

The marine calanoid copepod *Calanus finmarchicus* (Gunnerus) is considered a key component in the North Atlantic ecosystem (Planque & Batten, 2000). The zooplankton is one of the most abundant species of copepods (Conover, 1988) and regarded as an important trophic link between phytoplankton production and higher tropic levels (Runge, 1988). The importance of *C. finmarchicus* may be illustrated by its very large fraction of the biomass in the North Atlantic basin. In most areas the species is estimated to contribute to more than half of the total biomass (Planque & Batten, 2000). The pivotal role in the functioning of marine ecosystems, especially as prey for commercially exploited fish stocks (Harris et al., 2000), has made it a favoured target species in a wide range of interdisciplinary programs of laboratory, mesocosm, field and modelling studies. Studies seek to describe biological and physical factors affecting the abundance and population dynamics of the species (Campbell et al., 2001).

1.1 The biology of *Calanus finmarchicus*

The basic events in the life history of *C. finmarchicus* are spawning in early spring, development of a new generation up to the fifth copepodite stage (C5) during spring, occasionally early summer, before either descending to overwintering in deep water or maturing to produce a second generation (Conover, 1988; Miller & Tande, 1993). An illustration of the life cycle of *C. finmarchicus* is shown in Figure 1.1.

The eggs hatch as nauplii and there is a progression of 6 naupliar stages followed by 5 copepodite stages before turning into adulthood (Miller & Tande, 1993). The time taken for the whole life cycle depends partly on the temperature (Marshall & Orr, 1972). Spring and fall generations are somewhat prolonged, while summer generations take about a month to develop (Conover, 1988). The number of generations per year may vary in different parts of its range (Pasternak et al., 2004), but two generations are common in the Norwegian Sea and along the southern Norwegian coast (Wiborg, 1954).

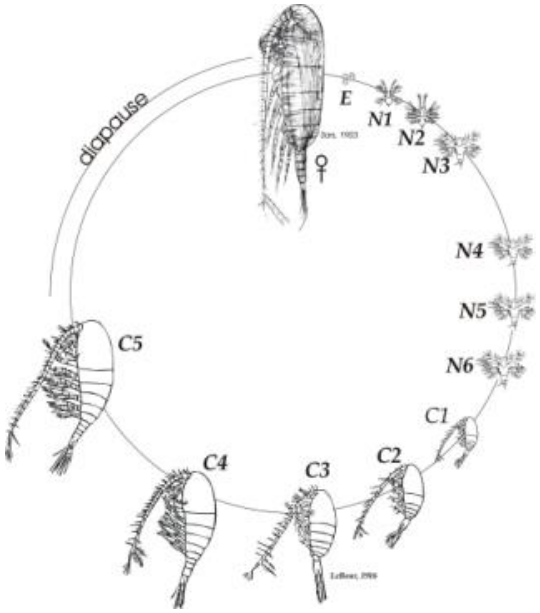

Fig. 1.1. The life cycle of *Calanus finmarchicus*. A full-grown female copepod spawns fertilized eggs. When eggs hatch, 6 naupliar stages (N1-N6) are followed by 5 copepodite stages (C1-C5) before reaching adult stage. The figure also illustrates relative body sizes and relative duration between each moult (Baumgartner, 2009).

During late summer and fall most stage 5 and some stage 4 copepodites migrate toward the seafloor and enter a period of dormancy (diapause). Vertical migration accompanied with reduced metabolism enables the species to successfully overcome the nutritional difficulties occurring in colder waters (Conover & Corner, 1968; Hirche, 1996). At the end of the winter the surviving copepodites complete their final moult to the adult stage, mate, and migrate to surface waters for feeding and egg laying (Conover, 1988).

1.1.1 Growth patterns and early naupliar development

According to Marshall and Orr (1972), the early naupliar stages of *C. finmarchicus* develop quickly and the two first nauplii stages are non-feeding stages. Recent times studies on naupliar development (Campbell et al., 2001; Harris et al., 2000; Hygum et al., 2000) has emphasised this prediction, where a negative, or near zero growth rates is observed in the non-feeding stages (egg, N1 and N2), whereas high growth-rates are observed in nauplii stage N3. The third naupliar stage has therefore been suggested as the first exogenously feeding stage in development of *C. finmarchicus* nauplii (Pedersen et al., 2014).

The shift in energy source, from maternal to exogenous food has indeed been reported to represent a critical transition within the early life stages of copepods (Takahashi & Ohno, 1996). The endogenous feeding stages (N1 and N2) are fully dependent on maternal resources and use the lipids provided with the egg as reserves to fuel development and respiration (Peterson, 2001). Thus, non-feeding stages must proceed quickly to the first feeding stages. Over-exploiting the maternal resources during the egg stage or the two first nauplii stages may result in energy shortage and eventually inhibit transition to active feeding.

1.1.2 Relation to the environment

The most important factors controlling stage durations in copepods are temperature and food quantity and quality (Cook et al., 2007). Some studies have concluded that food is rarely limiting for nauplii in nature (McLaren, 1978), and that nauplii of *C. finmarchicus* can obtain high growth and nearly maximal development rates at relatively low food levels (Hygum et al., 2000). A key assumption is that timing of the stage progression will be relatively insensitive to food availability above a fairly low threshold (Miller & Tande, 1993) and models using only temperature are therefore frequently used (Cook et al., 2007).

Research describing the development of marine copepods (Corkett, 1984; Corkett et al., 1986), has generally assumed the rule of equiproportional development, which states that each developmental stage occupies the same portion of time relative to other stages at any constant temperature if food is overabundant (Corkett, 1984; Tande, 1988). The study on growth and development rates done by Campbell et al. (2001) proved this assumption, where the relative duration of a given stage (i.e. stage duration/egg duration), was constant over all experimental temperatures. Stage durations however, vary between development stages. At a given temperature, the non-feeding stages (N1 and N2) were short in duration, while the first feeding stage (N3) lasted longer.

Results from several studies have shown that development time in *C. finmarchicus* decreases with increasing temperature (Campbell et al., 2001; Cook et al., 2007; Marshall & Orr, 1972). Campbell et al. (2001) also followed the method of Corkett et al. (1986), based upon parameters from the Belehradek (1935) temperature function.

The function predicts the influence of temperature on development times or stage durations under non-limiting food conditions.

Metabolism and growth are closely coupled in living organisms (Ikeda et al., 2001). The metabolic rate, the rate at which organisms transform energy and materials, is thus related to the sum of energy-demanding biological processes in the organism (Gillooly et al., 2001). Respiration and metabolic rates are generally linearly related under aerobic metabolism since all processes involved, consume oxygen as a reactant along with organic fuel (Reece et al., 2011). Respiration rate is frequently used as a proxy for metabolic rate in studies of pelagic copepods (Almeda et al., 2011; Ikeda et al., 2001; Maps et al., 2014).

Among the factors that regulate the success of copepod population, energetic balance is of prime importance. The metabolic budget may reveal important differences in the cost of maintenance and in the efficiency of food utilization (Almeda et al., 2011). Metabolism is described with remarkable consistency among metazoans, with relationship to temperature and body size (Maps et al., 2014). A study made on metabolic rates in early development stages of the marine copepod *Oithona davisae* (Ferrari) (Almeda et al., 2011), showed increased rates of respiration per animal with increasing body weight and temperature.

Since calanoid copepods first starts feeding at nauplii stage N3, it is crucial for the nauplii that the maternal resources suffice into the early feeding- and digestion activity in the first feeding-stage (N3). Rise in metabolism might lower the amount of energy left to support further development before they reach the first feeding stage (Pedersen et al., 2014). Further, retardation in the development may have consequences for the survival, since a delayed development can lead to animals staying in vulnerable stages for longer periods (Lopez, 1996). Combination of different types of stressors (e.g. rising temperature, environmental contaminants) may cause reduction in the development, related to the extra energy used for compensatory responses in an effort to maintain a normal internal environment (Pedersen et al., 2013). This may represent a substantial impact on the survival and development of the populations.

1.2 Oil production and produced water

After 40 years of offshore activities on the Norwegian continental shelf, oil and gas industry today account for about a quarter of total Norwegian GDP (NOG, 2014). In 2014, the total production of Norwegian petroleum was about 218.6 million standard cubic meters of oil equivalents (Sm^3oe) (Oljedirektoratet, 2015). Emissions from petroleum industry derive primarily from drilling and "produced water", i.e. water that accompanies oil and gas pumped from the reservoir to the surface (NOG, 2014). The water is treated to separate free oil before being released as operational discharges (Henderson et al., 1999). The total emissions from produced water on the Norwegian continental shelf reached a peak around 2007 and in 2013, a total of 1542 tonnes of dispersed oil was discharged to the sea with produced water (NOG, 2014)

The composition of produced water is complex and may contain a mixture of several thousand compounds, each potentially having its own environmental impact (Henderson et al., 1999). The discharges are the source that leads to the introduction of the greatest amount of oil and a number of other chemicals into the sea. Continuous efforts are therefore made to cut discharges in order to reduce environmental risks (RCN, 2012). The oil content in produced water is regulated by permit and the maximum allowed concentrations vary by region and nation (NRC, 2003). Since 2007, the OSPAR regulation has required that dispersed oil in produced water discharges shall not exceed an international performance standard of 30 mg/L (OSPAR, 2001).

1.2.1 Environmental impacts

Oil may kill or reduce the fitness of marine organisms, as well as disrupt the structure and function of marine communities and ecosystems. Determining long-term effects at low doses of oil exposure however, poses scientific challenges (NRC, 2003). Open and dynamic ecosystems on the continental shelf present a fundamental problem in identifying the environmental risk. Produced water discharges are usually dispersed and dilute rapidly if discharged into the ocean (NRC, 2003). Additionally, produced water from different production fields varies widely with regard to the content of environmentally hazardous substances (NOG, 2014; Oljedirektoratet, 2011). Because no produced waters are alike, region-specific studies are needed to address the risk

from the discharges (Lee et al., 2011; Oljedirektoratet, 2011). The relationship between cause and effects also need to be studied within a context of environmental factors that are important in shaping the ecosystem (NRC, 2003).

The level of activity on the Norwegian continental shelf still seems likely to remain high (NOG, 2014). Thus, knowledge about the effects and considerations of the impacts the oil and gas industry may have on the marine environment is important. It is necessary to coordinate the exploitation to avoid significant negative effects on the marine environment in the future (RCN, 2012)

1.2.2 Effects on *C. finmarchicus*

In order to understand how produced water may influence the pelagic production system, awareness regarding the effects on zooplankton is decisive (RCN, 2012). *C. finmarchicus* is found close to oilrigs and oil refineries along the Norwegian coast and is therefore likely to be affected by exposures to contaminants related to the oil industry (Hansen et al., 2007).

The impacts of produced waters on zooplankton have been extensively studied. Gamble et al. (1987) introduced a mesocosm experiment where natural assemblages of phytoplankton, zooplankton and larval fish were exposed to dilution of produced water. The concentrations were what would be expected within 0.5-1.0 km from platforms in the North Sea. A noticeable effect on copepods was detected, where early naupliar stages were sensitive to produced water and suffered high mortalities. Hansen et al. (2007, 2008, 2011) found that agents present in produced water can modify processes such as nutrient absorption, shell replacements, storage and conversion of fat, metabolism, as well as defence mechanism against toxicity and oxidative stress. However, varying lipid content and potential differences in metabolic activity in different developmental stages within the species should be considered when assessing the risk and impact on marine copepod populations´ exposure to oil compounds (Hansen et al., 2011).

1.3 Aim of study

The present study is a series of laboratory experiments carried out to reveal the energetic requirement for growth and development of the early stages of *C. finmarchicus* under different temperature regimes. Since growth and development involves energy demanding processes, the study investigates the potential effects of temperature. The main aim of the study is to determine the development times, growth and oxygen demand of eggs and the three first naupliar stages of *C. finmarchicus* reared in the laboratory at four different temperatures.

The species of study is selected due to its key position in the marine ecosystem. Eggs and nauplii of *C. finmarchicus* are a dominant sources of food for larval stages in commercially important fish stocks and a negative effect on the species can have a major impact on an ecosystem structure and function (Runge, 1988). Relatively few observations and little information are available on growth rates and development times of the naupliar stages of *C. finmarchicus* (Campbell et al., 2001).

The study is a part of the current research project ENERGYBAR where a modified dynamic energy budget (DEB) model for *C. finmarchicus* will be developed. The project aims to provide effect limits for potential long-term population effects of produced water that can be used in risk modeling and environmental monitoring.

2 Materials and methods

2.1 Experimental setup

All experiments were performed at the laboratories of NTNU Centre of Fisheries and Aquaculture and SINTEF Materials and chemistry at SeaLab in Trondheim, in the period between January and March 2015. The work was a part of the research project "ENERGYBAR" (Research Council of Norway, no. 225314).

2.1.1 Laboratory cultures of *C. finmarchicus*

The basis for the experiment was the continuous culture of C. *finmarchicus* maintained at the research facility at NTNU Sealab, described by Hansen et al. (2007). The animals used in this project were descendants of individuals collected in Trondheimsfjorden during autumn of 2004. The stock culture are maintained at 10 °C in 300 L tanks with filtered seawater and continuously fed with a mixture of the microalgae *Rhodomonas baltica* (Karsten), *Dunaliella tertiolecta* (Butcher) and *Isochrysis galbana* (Parke).

2.2 Experimental system

Four temperature experiments were performed, at 5.0, 7.5, 10.0 and 12.5 °C nominal values. The experiments were conducted in a newly developed flow-through microsystem, consisting of six 250 mL incubation chambers (Polyetylentereftalat (PET) bottles No.E907, NordicPack AS, Dilling, Norway), maintained in a vertical position in a rack (Fig. 2.1). Seawater was continuously added to each bottle using a peristaltic pump (mL/min). The set-up was placed in a temperature-controlled room adjusted to the chosen development temperature. To decrease temperature variations in the incubation chambers, the racks were immersed in water. Throughout the experimental period, the temperature was daily monitored using a thermometer (sensION156 Meter, P/N 5465069, Hach company, Colorado, USA). The actual measured temperatures in the incubation bottles were slightly lower than nominal values (Table 3.1). The individuals were constantly fed with the microalgae *R. baltica* at a concentration of 200 µg C L^{-1} preventing possible low nutritional access and reducing the energy cost for the nauplii in finding food.

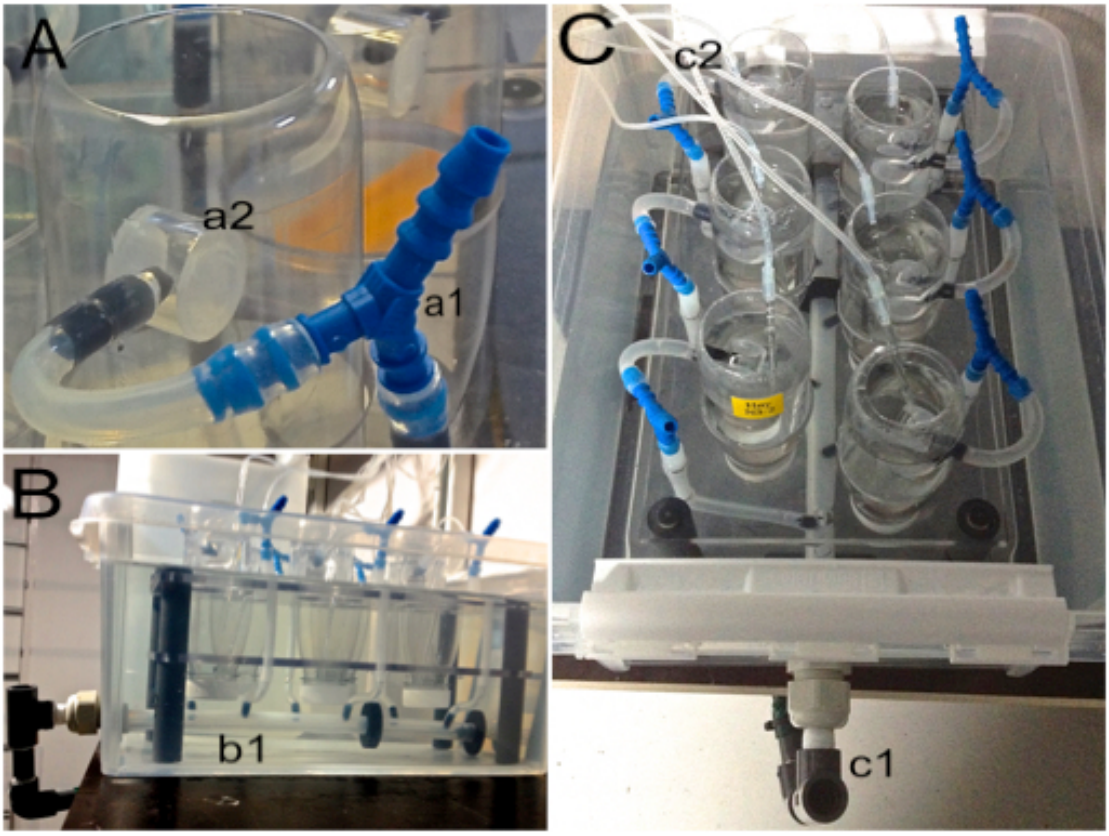

Fig. 2.1. Components in the experimental setup for rearing of *C. finmarchicus* at different temperatures. Close-up picture of an incubation chamber (A), with overflow (a1) to control the water level in the chambers and drain-sieve with mesh (a2) to prevent escapes of animals. The rack with six incubation chambers immersed in water bath is seen from a side view (B), with drain hose (b1) connected to the overflow from each chamber. Setup seen from above (C), with discharge pipe (c1) and tubes (c2) connected to the peristaltic pump.

2.3 Experimental procedure

Eggs from *C. finmarchicus* were the starting point in each experiment. Ovulating females were collected from the stock cultures and maintained in 50 L polyethylene tanks (Norplasta BeWi, Stjørdal, Norway) with filtered seawater at 10 °C and fed with *R. baltica* (200 µg C L^{-1}). For each temperature experiment, eggs were obtained after 12 hours incubation of approximately 400 ovulating females in clean tanks. A siphon with a sieve (300 µm) was used to collect eggs resting on the bottom of the tank. The eggs were transferred to a Petri dish and about 200 eggs were collected for each incubation chamber (18 chambers in total) using a customized glass Pasteur pipette with a narrow tip.

Eggs were raised under the selected temperature conditions until the desired development stage (N1, N2 and N3) was reached. The animals were subsequently sampled with six replicates at each development stage. The sample replicates were taken from six different incubation chambers.

2.3.1 Stage determination and development time

The animals were sampled roughly at midpoint of each development stage. Forty (egg-N2) and thirty (N3) individuals of the desired development stage were collected from the incubation chambers using a micropipette (10 µL, Eppendorf). Nauplii stages were determined on the basis of morphological characteristics using a stereo microscope (Leica M80 routine stereo microscope). Using a stage determination key with close up pictures and a short description of the most prominent characteristics (Researcher Iurgi Imanol Salaverria-Zabalegui, Department of Biology, NTNU) helped distinguishing between different developmental stages. Own pictures of eggs and nauplii with characteristics in each development stage are shown in Appendix C. The effect of temperature on development time from egg to a given stage was estimated by use of Belehradek (1935) temperature function as described in Campbell et al. (2001).

2.3.2 Oxygen consumption

Animals were transferred from the incubation bottles to temperature-adjusted micro chambers (100 µL) intended for sequential measurements of oxygen. The oxygen concentrations in the water were monitored using a fibre-optic oxygen meter (FireStingO2, PyroScience GmbH, Aachen, Germany). A fibre-optic oxygen sensor (Retractable oxygen microsensor, PyroScience, Item No. OXR230) monitored the continuous change in oxygen inside the micro chamber. Measurements were based on optical detection principles (REDFLASH technology) (del Alamo-Sanza et al., 2014) with an accuracy ± 0.01 mg/L and trace range close to 0 % O_2. The equipment was calibrated using a one-point calibration curve with air-saturated water according to the user manual.

Mounting the chamber cap onto a positive meniscus, squeezing out excess water and eliminating all air bubbles, was necessary for reliable results. The micro chambers were mounted onto a customized cooling block, made of aluminium. The block was

placed into a controlled water circulation bath inside a cooler unit (Fig. 2.2) and the chosen temperature was set. The oxygen content inside the micro-chambers was recorded at a minimum of 25 minutes or until a linear decrease in oxygen concentration was recorded for a sufficient time period. The temperature in the circulation bath was continuously logged throughout the measuring time.

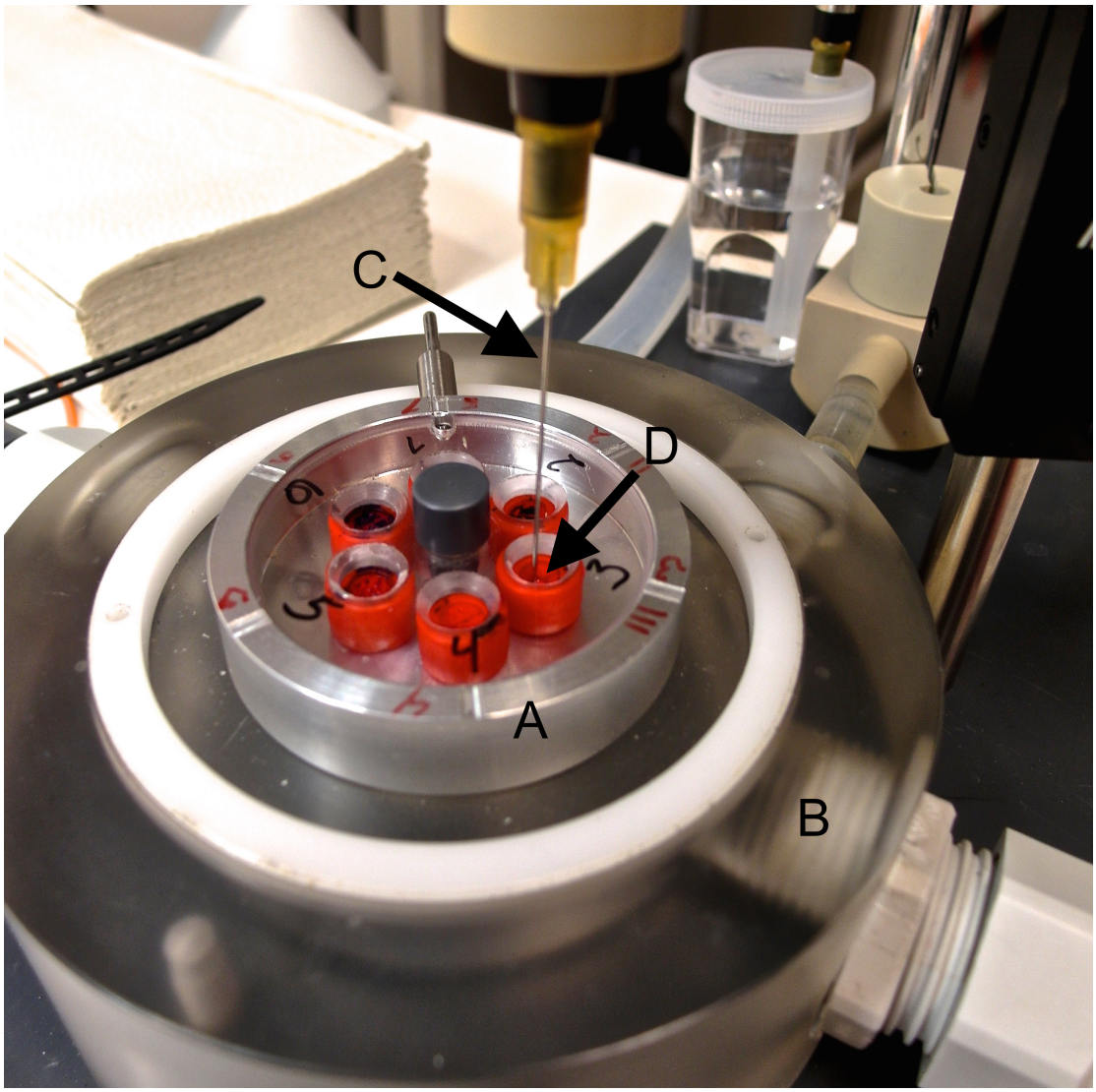

Fig. 2.2. Micro chambers placed on the cooling block (A). The block was surrounded by a water circulation bath (B) holding the chosen temperature. The sensor tip was completely retracted into the needle tip (C) when penetrating the chamber cap septum (D). A sliding step mechanism allowed the sensor tip to be moved out of the protective needle. The sensors were kept in a stable position using an adjustable tripod.

The oxygen consumption was determined from a last square regression of the measured oxygen concentration (mg L^{-1}) against time (min) according to the linear formula.

$$y = ax \quad [2.1]$$

where x equals a defined time fraction of the measuring period (in min), y is the difference in oxygen concentration over the same period (in mg L^{-1}), and a is the regression coefficient giving the oxygen reduction rate in the chamber in mg O_2 L^{-1} min^{-1}.

The regression coefficient was used to determine oxygen consumption (DW^{-1} h^{-1}) according to the formula:

$$O_2\ consumption\ (\mu g\ O_2\ DW^{-1}\ h^{-1}) = \frac{a*V*1000*60}{n} \quad [2.2]$$

where a is the regression coefficient, V is the volume of the chamber (L) and n is the number of animals in the chamber. Results were multiplied by 1000 to get the measured O_2 unit (mg L^{-1}) in µg L^{-1} and by 60 to convert the values to oxygen consumption per hour (h). After oxygen measurements, the animals were inspected for visible signs of damage that could have influenced the respiration rate before being counted under a stereo microscope. See Appendix B for details on the regression statistics.

2.3.3 Dry weight and CN analysis

All individuals from each micro-chamber were transferred to 5x9 mm pre-weighed tin capsules (Säntis Analytical AG, Teufen, Switzerland) placed inside a 96 well plate. To avoid formation of salt crystals, the animals were gently rinsed in an isotonic ammonium formate solution (CH_5NO_2, 0.5 M, Acros Organics, Geel, Belgium) before placed in the tin capsules. The samples were dried in a heating cabinet at 60°C for a minimum of 18 hours. The dry weight was determined by subtracting the weight of the empty tin capsules from the weight of the tin capsules containing the samples weighted on the same micro-scale weight (Mettler Toledo. Checked daily. At 10 mg

the acceptable deviation is 0.0008 mg). Egg and naupliar dry weights were determined according to equation 2.3 by dividing the weight of a sample on number of animals (n) added the tin capsule. Results were multiplied by 1000 to get the measured DW unit (mg) converted to µg.

$$\text{DW } (\mu\text{g ind.}^{-1}) = \frac{\text{sample (weight)}}{\text{n}} \qquad [2.3]$$

To determine the contents of nitrogen (N) and carbon (C) in the individuals, samples were analysed using an Elemental Combustion System CHNS-O (Costech ECS, model 4010, Costech International, Firenze, Italy) with a combustion temperature of 1020 °C. Carbon and nitrogen contents were determined with chromatography with acetanilide ($C_6H_5NH(COCH_3)$) as external standard. The number of individuals in each sample was adjucted according the size of the naupliar stages to account for the detection limit of the CN analyser (Appendix D). SINTEF Fisheries and Aquaculture conducted the analysis.

The C and N contents in each individual (µg ind.$^{-1}$) were determined in the same way as described by equation 2.3.

2.4 Statistics

Statistical analysis and generation of graphs were performed with the software *SigmaPlot 13.0*. Statistical comparisons of the different treatments were performed using One-way ANOVA, where level of significance was set at 0.05 for all tests. A Shapiro-Wilk-test was used to test for normality of data ($p < 0.05$), while a Brown-Forsythe test was used to test for equal variance ($p < 0.05$). In cases where significant differences were observed, paired contrasts were preformed to identify significant differences between development stages and temperature treatments. For equal group sizes, data with equal variance was followed by the Holm-Sidak-test, while a Tukey-test was performed if the equal variance data failed. For unequal group sizes Dunn´s test was used.

3 Results

3.1 Experimental conditions

Water temperature was measured daily throughout the development period for each temperature treatment (Table 3.1). The measured temperatures are used in the description of stage development times. To make later results more clear, nominal exposure temperatures are consistently used to describe growth and oxygen consumption.

Table 3.1. Measured water temperatures (mean±SD) for each treatment temperature. Temperatures in the incubation chambers rearing *C. finmarchicus* were measured daily throughout the developmental period.

Treatment temperature	Measured temperature (°C)
5.0 °C	4.1±0.4
7.5 °C	6.4±0.2
10.0 °C	9.2±0.2
12.5 °C	11.4±0.2

3.2 Stage durations and development

Developmental stages (N1 to N3) for *C. finmarchicus* are plotted against time (days) from eggs were incubated until the sampling of the selected stage took place (Fig. 3.1). Stage durations and developmental times decreased with increasing temperature. Time of development from egg to N3 decreased from 8.6 days at 4.1 °C to 3.2 days at 11.4 °C. More specific data on stage durations and developmental time in each treatment temperature are given in Appendix A.

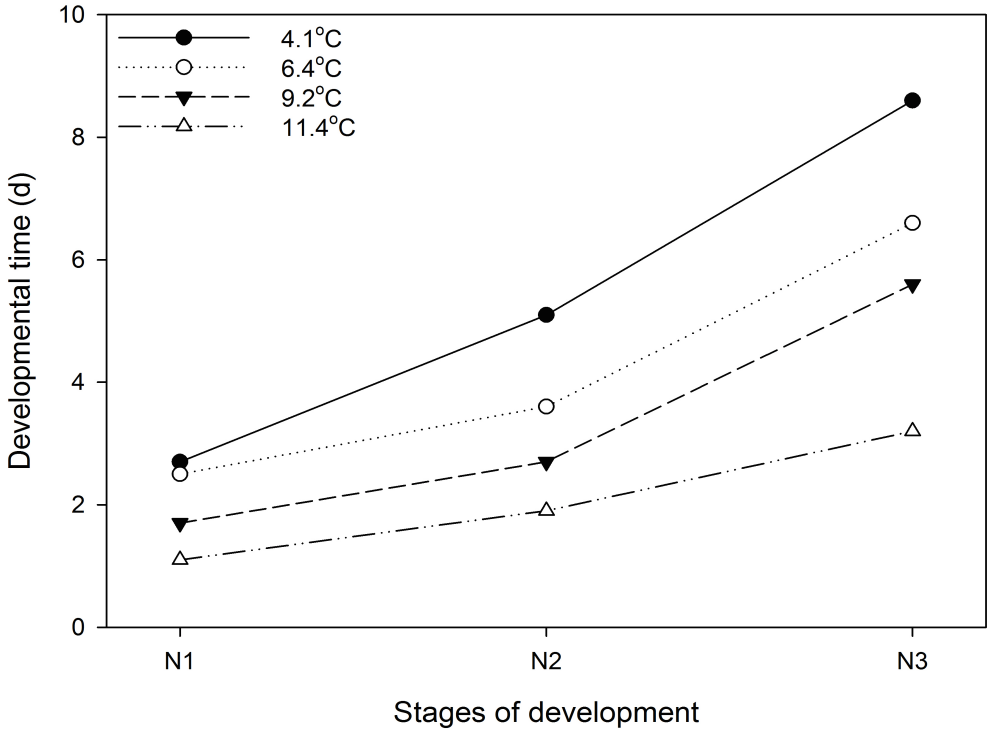

Fig. 3.1. Developmental times of *C. finmarchicus* nauplii at four different treatment temperatures. N1, N2 and N3 represent each naupliar stage. Development time equals the period from eggs were incubated (d±0.5) before a reached nauplii stage was sampled.

3.3 Growth and energy balance

3.3.1 Dry weight

At 10 °C, dry weight of stage N3 was significantly higher compared to the N1 stage ($p = 0.02$, Tukey, Fig 3.2). No significant difference in dry weight was found between development stages at temperature 5, 7.5 and 12.5 °C.

Within the developmental stages egg and N1, there was no significant difference in dry weight between the different temperature treatments. In contrast, a considerable effect of temperature on dry weight was detected at development stage N2 and N3. At development stage N3, dry weight of nauplii treated at 10 °C was significantly higher compared to nauplii reared at 5 °C ($p = 0.021$) and 12.5°C ($p = 0.028$), (Holm-Sidak). Dry weight of N2 nauplii at 12.5°C was significantly lower than dry weight of N2 nauplii treated at 7.5 °C ($p = 0.022$, Holm-Sidak).

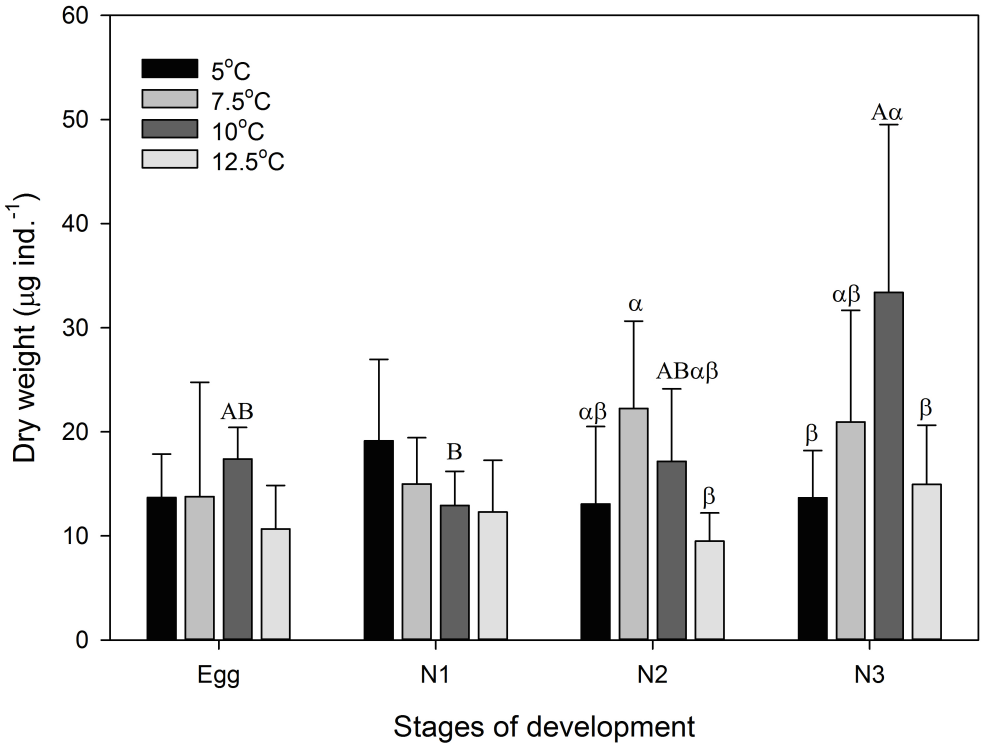

Fig. 3.2. Change in dry weight (μg ind.$^{-1}$) at different temperatures in developmental stage egg to N3 in *C. finmarchicus* (means±SD, n=6). Significant difference within a nauplii stage is indicated with Greek lowercase letters. Uppercase letters indicate significant difference between developmental stages within a certain temperature treatment (ANOVA, Tukey, Holm-Sidak, $p < 0.05$).

3.3.2 CN values

The carbon content increased sharply when nauplii reached development stage N3 in the two upper treatment temperatures (Fig. 3.3A). The increase in carbon content of N3 nauplii reared at 10 °C was significantly higher compared to all earlier development stages ($p < 0.001$, Holm-Sidak). For treatment 12.5 °C, nauplii stage N3 had a significantly higher content of carbon compared to eggs ($p = 0.05$) and stage N2 ($p = 0.015$), (Tukey). There was a minor change in average carbon content through the development period at the two lower temperatures. Only eggs reared at 7.5 °C had a significantly lower content of carbon compared to stage N3 ($p = 0.006$, Tukey). No significant effects of temperature were found between developmental stages in nauplii reared at 5 °C.

The carbon content in N3 nauplii reared at 10 °C was significantly higher compared to N3 nauplii reared at 5 °C and 12.5 °C ($p < 0.01$, Holm-Sidak). Significantly higher content of carbon in N3 nauplii reared at 10 °C, compared to N3 nauplii reared at 7.5 °C was also found ($p = 0.09$). Carbon content in N2 nauplii reared at 12.5 °C was significantly lower compared to nauplii reared at 7.5 °C ($p = 0.009$) and 10 °C ($p = 0.014$), (Holm-Sidak). No significant effect of temperature on carbon content was found within development stage N1. Eggs treated at 10 °C had a significantly higher content of carbon compared to eggs treated at 7.5 °C ($p = 0.001$) and 12.5 °C ($p = 0.002$), (Holm-Sidak).

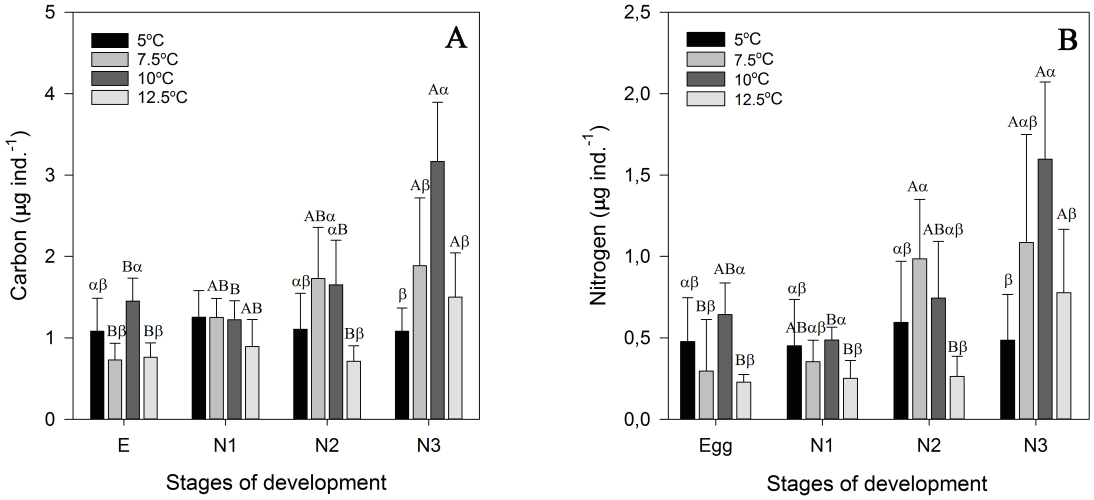

Fig. 3.3. Change in carbon (A) and nitrogen (B) content (μg ind.$^{-1}$) at different temperatures in development stage egg to N3 in *C. finmarchicus* (means±SD, n=6). Significant difference within a nauplii stage is indicated with Greek lowercase letters. Uppercase letters indicate significant difference between development stages within a certain temperature treatment. (ANOVA, Tukey, Holm-Sidak, Dunn´s, $p < 0.05$).

Development stage egg, N1 and N2 reared at 12.5 °C had a significantly lower content of nitrogen compared to the N3 stage ($p = 0.12$, 0.32, 0.36, respectively, Tukey, Fig. 3.3B). For nauplii reared at 10 °C, nitrogen content was significantly higher in stage N3 compared to N1 ($p = 0.03$, Tukey). At 7.5 °C, the nitrogen content of nauplii at stage N3 and N2 was significantly higher than in eggs reared at the same temperature ($p = 0.046$ and 0.036, respectively, Dunn´s). No significant difference in nitrogen content was found between developmental stages in animals reared at 5 °C.

A significantly higher content of nitrogen was detected in N3 nauplii reared at 10 °C compared to N3 nauplii reared at 5 °C and 12.5 °C (p = 0.04, 0.035, respectively, Holm-Sidak). Within development stage N2, nitrogen content in nauplii reared at 7.5 °C was significantly higher than in nauplii reared at 12.5 °C (p = 0.008, Holm-Sidak). In addition, N1 nauplii reared at 12.5 °C had a significantly lower nitrogen content compared to nauplii reared at 10 °C (p = 0.008, Dunn´s). Egg treated at 10 °C had a significantly higher content of nitrogen compared to egg treated at 7.5 °C (p = 0.026) and 12.5 °C (p = 0.044), (Dunn´s).

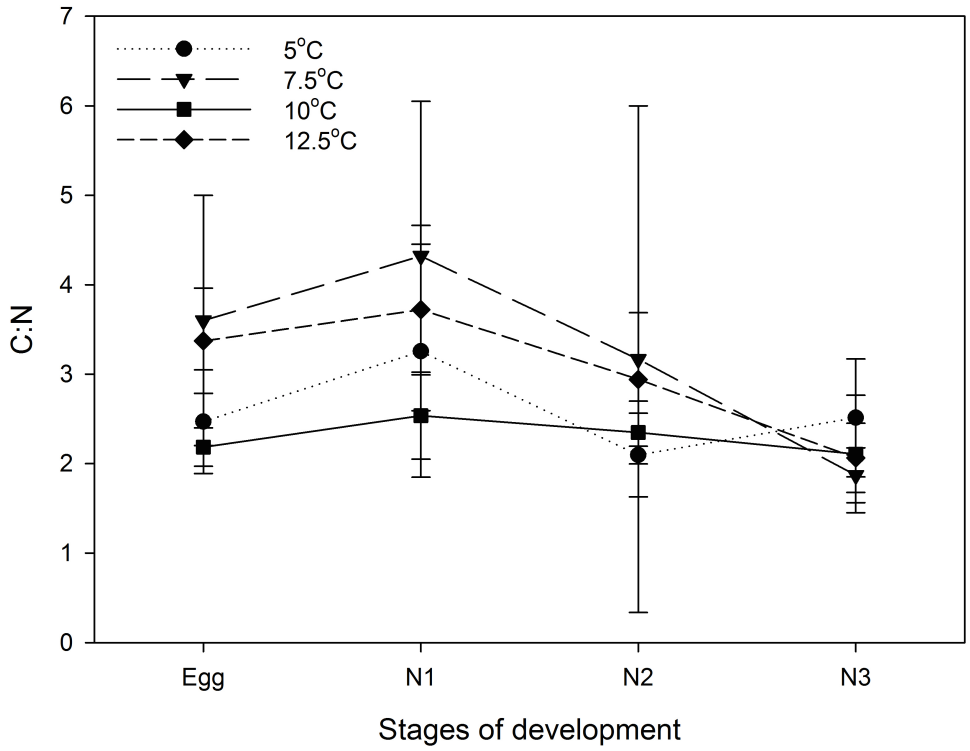

Fig. 3.4. CN ratio at development stage egg to N3 in *C. finmarchicus* (means±SD, n=6). Significant differences are stated in the text section (ANOVA, Tukey, Holm-Sidak, $p < 0.05$).

In general, the CN ratio tended to increase from egg to N1 before gradually declining from developmental stage N1 to N3 (Fig. 3.4). N1 nauplii treated at 7.5 °C, had a significantly higher CN ratio compared to N3 nauplii reared the same temperature (p = 0.022, Tukey). At 12.5 °C, significantly lower CN ratio was found in N3 nauplii compared to egg and N1 (p = 0.009, 0.001, respectively, Holm-Sidak).

3.4 Oxygen consumption

The oxygen consumption on dry weight basis (per mg) tended to increase when nauplii reached the N3 stage (Fig. 3.5). At 5 °C and 7.5 °C, oxygen consumption in the N3 stage was significantly higher compared to eggs reared at the same temperature (p = 0.008, 0.030, respectively, Dunn´s). Statistical analysis revealed no significantly difference in oxygen consumption between development stages at 10 °C and 12.5 °C.

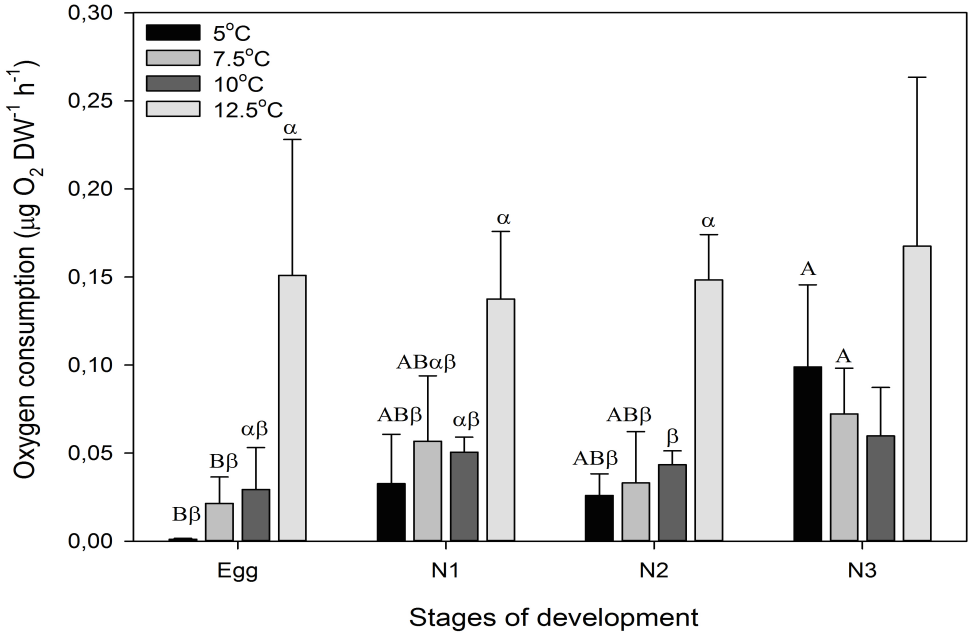

Fig. 3.5. Change in oxygen consumption (μg O_2 mg DW^{-1} h^{-1}) at different temperatures in developmental stage egg to N3 in *C. finmarchicus* (means±SD). Significant difference within a nauplii stage is indicated with Greek lowercase letters. Uppercase letters indicate significant difference between development stages within a certain temperature treatment (ANOVA, Holm-Sidak, Dunn´s, $p < 0.05$).

Within all development stages, a higher oxygen consumption was observed at 12.5 °C compared to animals reared at lower experimental temperatures. Oxygen consumption for eggs at 5 °C (p = 0.007) and 7.5 °C (p = 0.029), was significantly lower compared to eggs at 12.5 °C (Dunn´s). The respiration for nauplii within stage N1 was significant lower at 5 °C compared to 12.5 °C (p = 0.003, Dunn´s). The increased respiration for N2 nauplii at 12.5 °C was highly significant ($p < 0.001$, Holm-Sidak) compared to the respiration in N2 nauplii at lower temperatures. No significant difference was observed within development stage N3.

4 Discussion

The main objective of this study was to investigate the effect of temperature on the development of the earliest stages of *C. finmarchicus*. The results demonstrate the impact temperature poses on development time, metabolic rate and growth of the species. Oxygen consumption is in the present discussion used as a proxy for the rate of metabolism. The metabolic activities and treatment temperatures are also seen in connection to growth at the different development stages. Assessments regarding methodology and results are finally discussed in a separate section.

4.1 Development

The reduction in developmental time with increased temperature (Fig. 3.1) is consistent with earlier studies on stage durations in *C. finmarchicus* (Campbell et al., 2001; Cook et al., 2007; Corkett, 1984). The relative duration of a given stage was more or less constant over all temperature treatments, supporting the assumption of an equiproportional growth. For different species of *Calanus*, development rates are found not to be isochronal, i.e. stage durations are not of equal length (Campbell et al., 2001). Short durations of the two non-feeding stages, and prolonged first feeding stage are commonly observed. Since no further study on development after sampling of nauplii stage N3 was conducted in the present study, a precise estimate of the N3 stage duration time is not possible. However, the extended sampling period of N3 nauplii supports the assumption of a non-isochronal development rate in early life stages of *C. finmarchicus*. The prolonged duration of the first feeding stage (N3) is probably related to the time it takes for the nauplii to recuperate weight lost during the two first non-feeding stages (Marshall & Orr, 1972). Reduced metabolism along with increased development time is characteristic when copepods undergo dormancy in colder waters (Conover, 1988). Hence, a metabolic slow-down is a probable explanation of an extended development time for individuals reared at lower temperatures.

Greater dissimilarities in development time between different temperature treatment groups are found with increasing developmental stages (Fig. 3.1). Only 1.1 days differed between 12.5 °C and 5 °C within naupliar stage N1, while the corresponding difference was 5.4 days at development stage N3. The various development times at

different temperatures however, may be considered as a key adaption to the highly variable environment for *C. finmarchicus*. Miller et al. (1977) postulated that moulting in the genus *Acartia* is under control of a physiological process, where a sufficient level of resources is needed to induce moulting. Rise in respiration with increased temperatures might lower the amount of energy available to support development. Thus, animals are forced to moult quickly so the maternal resources suffice into the digestion activity in the first feeding stage. In contrast, the reduced development rates at lower temperatures may be a consequence of temperature effects on food species. Phytoplankton growth rates, although varying with species, generally increase with increasing temperature (Berges et al., 2002). Cook et al. (2007) observed a food-dependent naupliar development where *Calanus* nauplii developed more slowly past N3 at low food carbon concentrations compared to more saturated levels. Hence, when there might be no apparent source of nutrients available at low temperatures, moulting will involve a disadvantage, due to extra energetic costs. Reduced metabolic and development rates with decreased temperatures provides a longer survival for nauplii developing under unfavourable conditions. Cook et al. (2007) confirming this expectation, where decreased starved nauplii survival was found as temperature increased. However, experimental studies on *Calanus* spp. have indicated high mortalities at low temperatures (Tande, 1988). Death from low sea temperature is particularly prevalent in habitats subjected to thermal stress. Due to longer developmental time at lower temperatures, the period nauplii exist in the sensitive early naupliar stages will also be extended as discussed in section 4.2.

4.2 Metabolic rates

The metabolic rates, measured as oxygen consumption, tended to increase for animals exposed to higher temperatures (Fig. 3.5). Higher metabolic rate at 12.5°C was found within all development stages, with statistically significant difference within development stage eggs, N1 and N2. According to Stumpp et al. (2011) the resulting outcome of an increased metabolism will either be elevated or decreased performance, depending on whether or not the increased energy demand can be sustained through elevated energy intake (i.e. feeding). Since non-feeding stages only have a finite amount of energy available, any rise in respiration might lower the amount of energy left to support further development before they reach the first feeding stage (Pedersen et al., 2014). Hence, even though the energy content of the

maternal sack in the eggs and nauplii may provide enough energy for development, it might not be sufficient to cover the demand for additional energy due to suboptimal temperatures.

Low metabolism in endogenously feeding stages may provide a limited capacity to deal with disruptions influencing the internal conditions (homeostasis) (Pedersen et al., 2014). Nauplii with a reduced metabolic and development rate may therefore be less resistant to environmental stressors like components present in produced water. Delayed development may also have major impacts in an ecological setting. The survival of nauplii of *C. finmarchicus* depends on adaptation to often low and fluctuating food availability due to seasonally fluctuations in phytoplankton growth and availability (Hygum et al., 2000). *C. finmarchicus* is known to spawn in coincidence with the period of maximum phytoplankton bloom (Falk-Petersen et al., 2009). A development delay relative to the annual phytoplankton bloom is, according to Melle and Skjoldal (1998) regarded as an important reason for reduced survival of new generations. The combined effect of environmental stressors and changes in temperature may further enhance this negative ecological impact.

In the present experiment relatively similar metabolic rates are observed for eggs, N1 and N2 within a given treatment temperature. Increased oxygen consumption was on the other hand observed when nauplii reached stage N3. Significantly higher respiration rates in the third-stage nauplii compared to earlier development stages were found for the two lowest experimental temperatures. Results from the present study is supported by Pörtner et al. (2010), where the switch to exogenous feeding is known to raise the rate of respiration in marine larvae. Relating metabolism to the activities of the copepods may explain the increased metabolic demand for N3 nauplii. Prosser (1961) distinguish between; “standard”, “routine” and “active” metabolism, as described in Ikeda et al. (2001). In the present study, no measurements of swimming activity during the measurements of oxygen consumption could be done, but some activity could results in an intermediate between standard and routine metabolic rate in the non-feeding stages. The shift to exogenous feeding however, demands the N3 nauplii to start active feeding on algae in the surrounding water. Thus, swimming and feeding activity is likely to increase, corresponding to increased

metabolic demand. According to Maps et al. (2014), the metabolic demand in non-feeding stages may be half that of the active feeding stages.

Even though no significant difference in oxygen consumption was observed within stage N3, the expected response in metabolic demand is, as previously discussed, likely to increase with increasing temperatures (Fig. 3.5). The metabolism in the N3 stage, on the contrary, tended to decrease from 5 °C to 10 °C. According to Fuiman and Batty (1997), the physical effects of viscosity requires to be taken into account when studying the effect of temperature on the active metabolic rate of fish larvae. Copepod nauplii move in a medium dominated by viscosity (Borg et al., 2012). Since decreasing temperature causes an increase in viscosity (Sakshaug et al., 2009), cold water offers more resistance to swimming than warmer waters. A possible explanation for the higher metabolic rate at lower temperatures may be described by the higher energetic cost for the N3 nauplii to swim in water at lower temperatures.

To the authors' knowledge, no study describes whether there is an indirect effect of temperature on respiration in *Calanus*. Marshall and Orr (1972) however, describe an immediate decrease in respiration when cold-blooded animals are transferred to lower temperature. After being kept at a low temperature for some time, the respiration rises to a higher level. Similarly, when transferred from lower to higher temperature, their initially increased respiration falls gradually to a lower level. In our study, eggs were shortly after sampling at 10 °C, exposed to the experimental temperature for measurements of oxygen consumption. Transfer to "unnatural" temperature conditions may explain the negligible respiration rate for eggs at 5 °C and the highly significant increase in metabolic rate for eggs at 12.5 °C.

4.3 Growth of nauplii

There was no consistent difference in dry weight between different temperature treatments within the two first development stages. Significantly higher dry weights were, however, detected for developmental stage N2 and N3 at 7.5 °C and 10 °C, respectively (Fig. 3.2). Campbell et al. (2001) found body weights to be inversely related to temperature. The effect of temperature on dry weights was from our study inconclusive. However, dry weight within developmental stage N2 was significantly higher at 7.5 °C compared to nauplii developed at 12.5 °C. Comparing the duration of

the N3 stage in the present study with stage durations estimated by the previous described temperature function (Corkett et al., 1986), sampling of N3 nauplii at 10 °C were made approximately 0.5 days later, compared to other temperature treatments. This may have resulted in the larger growth of individuals found at the first feeding stage at 10 °C.

In the pre-feeding naupliar stages, no significant difference in carbon content was detected within a given treatment temperature (Fig. 3.3A). Carbon growth is in previous studies found to be zero or negative between hatched eggs and nauplii stage N2 (Campbell et al., 2001; Hygum et al., 2000). When nauplii are starving, they have to consume their own constitutive materials – protein and lipids, to obtain energy (Fernandez, 1979). Loss in C is known to be grater than loss in N, indicating that N is conserved while C-stores is used as energy for metabolism and morphological changes (Campbell et al., 2001).

Increased carbon content appeared, however, when nauplii reached the third developmental stage. The growth seemed to show an expected pattern at 10 °C and 12.5 °C, where a significantly increase in content of carbon were detected. This is likely to indicate the shift from maternal to exogenous energy consumption. On the contrary, at lower temperatures (5 °C and 7.5 °C) the increase in carbon content for N3 nauplii was limited. These results coincide with findings from Campbell et al. (2001), where the mean C-specific growth rates between N3 and C5 increased strongly with increasing temperature.

In the present study, decreasing CN ratios were found between nauplii stage N1 and N3 in all treatment temperatures, with significant differences for treatment 7.5 °C and 12.5 °C (Fig. 3.4). Changing energy storage conditions are reflected in changing CN ratios where increased C weight relative to N weight indicates an increase in lipid storage (Campbell et al., 2001). Decrease in CN ratios in the present results is assumed to indicate a lipid-dominated metabolism in the first naupliar stages of *C. finmarchicus.*

Except for treatment 7.5 °C, only minor changes and no significantly differences in N content were observed within a particular treatment in the non-feeding nauplii (Fig.

3.3B). Since N is not contained in stored lipids, N growth could be a proxy for structural growth (Campbell et al., 2001). During a crustacean moult cycle, the individual emerges from its old encasement with a thin exoskeleton that is stretched to its new volume by uptake of water (Skinner, 1985). Even though the volume of growth was not quantified, gain in body volume was observed from egg to N1 and from N1 to N2 (Fig. C1, Appendix). However, the increase in body volume is probably related to water intake and not somatic growth in the non-feeding stages (Hygum et al., 2000). According to Campbell et al. (2001), over the course of development the structural mass (N content) per unit volume is relatively constant. Increased N content was found in the third-stage nauplii. Significantly different values were found for temperatures 7.5 °C, 10 °C and 12.5 °C. Mean N growth rate is, as carbon, known to increase with increasing temperature (Campbell et al., 2001). Another notable effect is the significantly lower content of N at 12.5 °C within all developmental stages. According to Corner et al. (1967), 24 % of the nitrogen captured as food is used to build new tissue. The remaining larger fraction is lost as faecal pellets, moults and excreted end products of metabolism. Thus, an increased metabolism may cause reduction in nitrogen content.

4.4 Methodical reflections

The outermost treatment temperatures corresponded to an approximately 40 % temperature deviation, from which by Jonasdottir and Koski (2011) is characterized as preferred water temperatures for *C. finmarchicus* in the North Sea (7.5 - 9.0 °C). Seasonal variations in the sea temperature may expose copepods to different temperature levels throughout their life cycle. The possible significance of regular temperature changes however, has been little studied (Tande, 1988).

The study used the approach of laboratory *in vivo* experiments as tools to study the impacts of temperature on the development in early life stages of *C. finmarchicus*. Use of laboratory *in vivo* experiments permit close monitoring during the rearing of animals to the desired development stage. Laboratory rearing allows well-controlled environmental conditions (temperature, food, light), where the conditions could be maintained constant throughout the experimental period. Laboratory conditions may on the other hand induce stress on the individuals or a change in behaviour compared to that in natural environment (Harris et al., 2000). Yet, individuals used in the

present experiment are well adapted to laboratory conditions, after being maintained at the research facility over several years. Nonetheless, caution is required when comparing laboratory results to the field, considering that natural variability in the environment may not be possible to mimic in laboratory experiments.

It is important to keep in mind that results obtained from the experimental procedure could easily be affected by methodical and personal errors. The experimental outcome depended upon flawless handling of large quantities of small sized animals. Very tiny items and animals (approx. 200 μm) made mistakes inevitable and also difficult to conduct accurate measurements, resulting in high standard deviations for some samples. Animals may be stuck in pipettes or be absent in transfers between measurement devices. Hence, imprecise numbers of animals in each sample represent an uncertainty that may affect the results in the present study. Including a relatively large number of individuals in each parallel sample counteracted this variability. Additionally, formation of salt crystals in the samples may still have occurred, even though animals were rinsed in an ammonium formate solution. Since the weight of the animals is almost negligible, salt crystal formation will represent a considerably percentage of the total dry weight in a sample. The results must therefore be treated with caution as the values of both dry weight and CN was quite scattered. This also made the analysis more challenging and made it more difficult to make reliable correlation models.

Diel- and ontogentic vertical migration, as well as the diapause is characteristic energy-requiring behaviour in the life cycle of *C. finmarchicusis* (Mauchline, 1998). The absence of such energy demanding processes may affect the energy budget of the animals used in the experiment. To the authors' knowledge, no previous study has investigated whether the energy is saved, or if it will be available for other processes, such as the formation of lipid-rich eggs from egg-laying females. Besides, it is unknown whether there is a difference between cultured and free living *C. finmarchicus*. To verify both previous and future research done on cultured copepods, comparison studies regarding the possible difference in natural and cultured conditions is necessary.

4.5 Concluding remarks

The early naupliar stages of *C. finmarchicus* seems to be subject to a tuned trade-off between energy demanding processes as growth and development. The present study indicates that the metabolic rate is strongly influenced by temperature. Although this study did not provide significantly effects on growth, an increased metabolic rate is likely to cause energy depletion for non-feeding nauplii depending on their maternal resources. Even though effects of produced water on plankton communities is not fully known, exposure to environmental stressors as an additional effect might influence naupliar metabolism and development, besides growth. Since the study demonstrates how physiological processes are closely associated with temperature, present results also confirms that impacts of produced water have to be seen in a context of environmental factors like climate change. Eventually, the forthcoming results will hopefully benefit both the management and the industry in securing that the risk of environmental impacts from offshore petroleum activities are kept to a minimum.

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Appendix A

Table A1. Summary of temperature conditions and developmental time in all nauplii experiments conducted.

Development temperature (°C)	Sampling stage	Development time (days)	Stage durations (days)
4.14	N1	2.7	2.4
	N2	5.1	3.5
	N3	8.6	-
6.43	N1	2.5	1.1
	N2	3.6	3
	N3	6.6	-
9.23	N1	1.7	1
	N2	2.7	2.9
	N3	5.6	-
11.39	N1	1.1	0.8
	N2	1.9	1.3
	N3	3.2	-

Appendix B

Table B1. Regression statistics on oxygen consumption. Regression coefficients (mg O_2/L min^{-1}) with R-squared values for replicates (n) in all development stages and treatment temperatures.

Temp. (°C)	Stage of development	n	a (10^6)	R-squared
5	Egg	2	0.08	0.0029
	N1	5	1.35	0.58
	N2	6	1.40	0.41
	N3	5	5.79	0.90
7.5	Egg	6	1.50	0.67
	N1	5	3.35	0.82
	N2	6	3.23	0.80
	N3	5	5.91	0.81
10	Egg	6	4.14	0.91
	N1	5	3.44	0.85
	N2	5	3.92	0.83
	N3	5	8.52	0.93
12.5	Egg	6	9.91	0.96
	N1	6	8.13	0.94
	N2	6	9.09	0.98
	N3	5	12.86	0.94

Appendix C

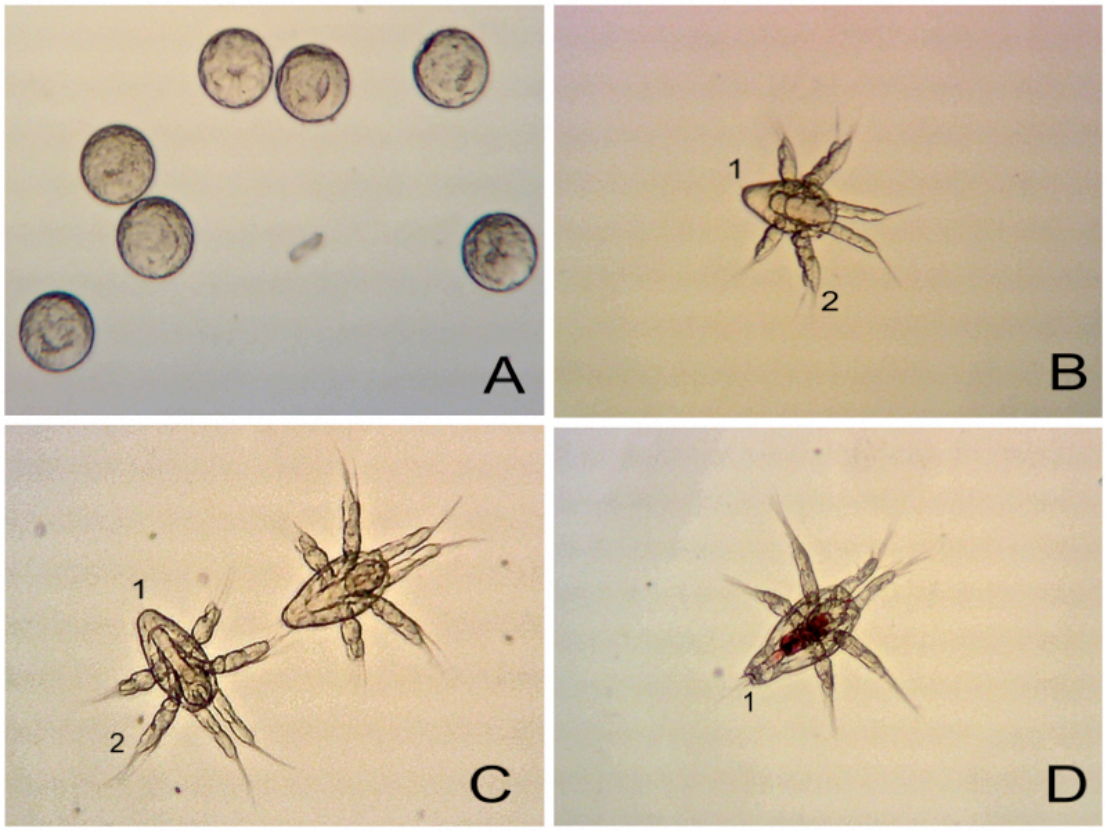


Fig. C1. Developmental stages in *C. finmarchicus*. Numbers indicate morphological characteristics on developmental stages. **A:** Eggs; diameter before hatching is about 145 μm (Marshall and Orr, 1955). **B**: N1; 2 thin, relatively short, widely separated spines on the posterior end of the body (1), 3 thin setae on the extremities on each antennule (2). **C**: N2; slightly more elongated body shape, 2 thin, long, closely gathered spines on the posterior end of the body (1), antennule structure similar to N1 (2). **D**: N3; 2 pairs of short spines and 1 pair of long spines on the posterior end of the body (1), red matter inside the gut (algae the animals has been feeding on) indicates start of exogenous feeding.

Appendix D

Analytical settings and standard curves for carbon and nitrogen analysis.

Elemental Analysis Software
www.costechanalytical.com

Calibration	: P_BURAAS_MARS_2015	By	: sd
Description	:		
Created	: 06.10.2000 14:41:57	Modified	: 01.06.2015 12:47:49

Calculation	: ESTD	Mode	: Calibrate
Calibrate	: Automatic	Recalibration Type	: Average
Change Response	: Enable	Weight	: 0,25
Update Reten. Time	: Enable	Search Criteria	: 0,00%

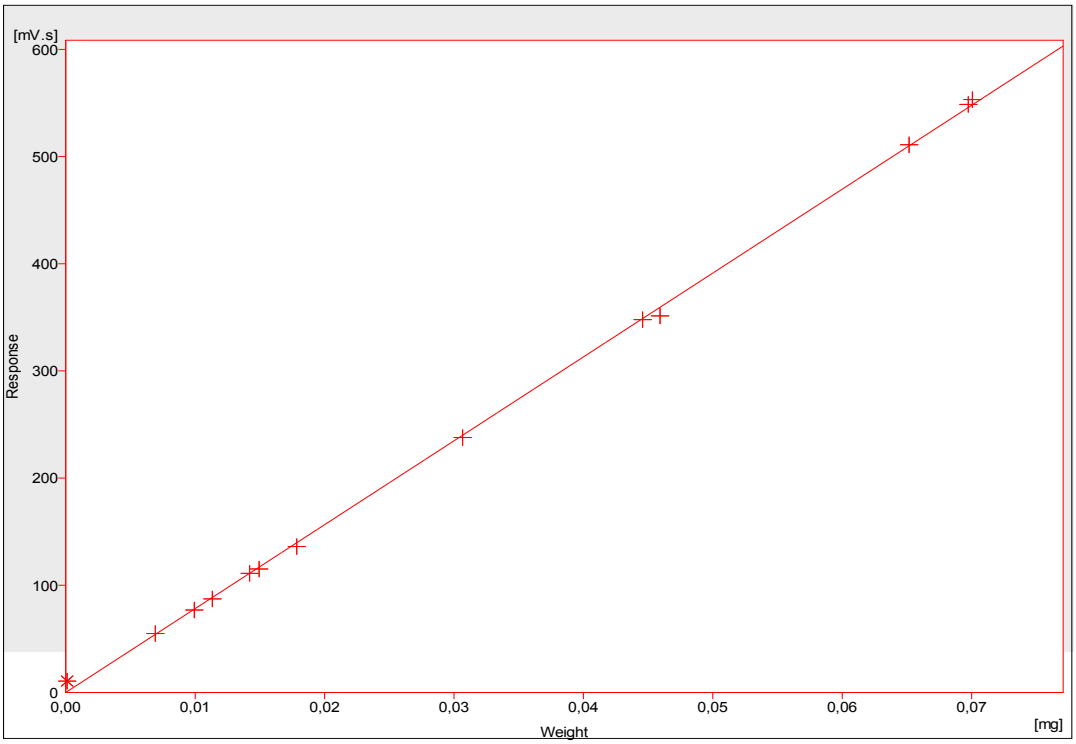
Calibration Summary Table (ESTD - P_BURAAS_MARS_2015 - Signal 1)

Used	Element Name	Reten.Time	Left Window	Right Window	Peak Type	Peak Color	LOD	LOQ	R B	Resp. Factor
☒	Nitrogen	1,683	0,500	0,500	Ordnr		0,000	0,000	A	0,0000
☒	Carbon	2,679	0,500	0,500	Ordnr		0,000	0,000	A	0,0000

Nitrogen - Signal 1 - 1,683 min.

Peak Type	: Ordnr
Left Window	: 0,5 min
Right Window	: 0,5 min
Response Base	: Area
Curve Fit Type	: Linear
Zero Type	: Curve from Zero
Weighting Method	: None
Subst. Equation	: Y = 7825,99684*X
Correlation Coef.	: 0,9997692
Residuum	: 3,95478 [mV.s]

	Response	Weight	Resp. Factor	Rec No.	Used	Deviation [%]
1	351,2833	0,04593	0,0001	1	☒	2,3151
2	237,7995	0,03067	0,0001	1	☒	0,9207
3	511,0011	0,06517	0,0001	1	☒	-0,1847
4	347,8127	0,04458	0,0001	1	☒	0,3056
5	87,2205	0,01132	0,0001	1	☒	1,6017
6	136,1087	0,01785	0,0001	1	☒	2,6358
7	54,9327	0,00692	0,0001	1	☒	-1,4073
8	111,1126	0,01420	0,0001	1	☒	0,0400
9	76,8976	0,00994	0,0001	1	☒	1,1126
10	115,1363	0,01494	0,0001	1	☒	1,5435
11	553,1479	0,07006	0,0001	1	☒	-0,8717
12	548,6497	0,06974	0,0001	1	☒	-0,5171
13	0,0000	0,00000	0,0000	0	☒	-
14	0,0000	0,00000	0,0000	0	☒	-
15	0,0000	0,00000	0,0000	0	☒	-
16	0,0000	0,00000	0,0000	0	☒	-
17	0,0000	0,00000	0,0000	0	☒	-
18	0,0000	0,00000	0,0000	0	☒	-
19	0,0000	0,00000	0,0000	0	☒	-
20	10,5499	0,00010	0,0000	2	☒	-92,5820



Carbon - Signal 1 - 2,679 min.

Peak Type : Ordnr

Left Window : 0,5 min

Right Window : 0,5 min

Response Base : Area

Curve Fit Type : Linear

Zero Type : Curve from Zero

Weighting Method : None

Subst. Equation : Y = 21546,4466*X

Correlation Coef. : 0,9999849

Residuum : 19,22013 [mV.s]

	Response	Weight	Resp. Factor	Rec No.	Used	Deviation [%]
1	6734,7431	0,31514	0,0000	1	☒	0,8233
2	4534,8004	0,21043	0,0000	1	☒	-0,0190
3	9632,2848	0,44723	0,0000	1	☒	0,0402
4	6583,4569	0,30590	0,0000	1	☒	0,1155
5	1664,9231	0,07770	0,0000	1	☒	0,5565
6	2630,1304	0,12249	0,0000	1	☒	0,3442
7	1017,6954	0,04749	0,0000	1	☒	0,5409
8	2106,2563	0,09746	0,0000	1	☒	-0,2965
9	1485,0830	0,06818	0,0000	1	☒	-1,0873
10	2207,0484	0,10251	0,0000	1	☒	0,0778
11	10367,2437	0,48078	0,0000	1	☒	-0,0782
12	10349,2819	0,47858	0,0000	1	☒	-0,3636
13	0,0000	0,00000	0,0000	0	☒	-
14	0,0000	0,00000	0,0000	0	☒	-
15	0,0000	0,00000	0,0000	0	☒	-
16	0,0000	0,00000	0,0000	0	☒	-
17	0,0000	0,00000	0,0000	0	☒	-
18	0,0000	0,00000	0,0000	0	☒	-
19	0,0000	0,00000	0,0000	0	☒	-
20	8,5906	0,00010	0,0000	1	☒	-74,9185