Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Simen Sæther

Effects of elevated water temperatures and microalgae diet on prosome size, fat sac development, egg production and hatching success in *Calanus finmarchicus*.

Master's thesis in Ocean Resources
Supervisor: Rolf Erik Olsen
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July 2021

Photo by: Dag Altin (NTNU)

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The Covid-19 pandemic have been a challenging period for most people. Despite delay and setbacks caused by the pandemic, we managed to complete the rearing experiment as well as the laboratory work.

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Simen Sæther

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Abstract

The calanoid copepod *Calanus finmarchicus* (Gunnerus) is a key species in the Norwegian and Barents Sea because of its abundance and importance for the energy transfer between primary producers and commercial fish species. Like other marine species, it is still unclear how climate change and rising ocean temperatures could affect the species in a future climate changed scenario. Some studies suggest a shift in the species biogeographical position and a general decrease in the worldwide biomass Others suggest an increase in the population, related to a more rapid hatching during the algae blooms in the spring, and that the species therefore will get an advantage over competing species.

The present study aimed to unveil the effect from elevated water temperatures and dietary change, focusing on prosome size, fat sac development, egg production and hatching success. *C. finmarchicus* was reared at three different temperatures (10, 12 and 14 °C) and with two different microalgae diets (*R. baltica* and *D. tertiolecta*) at a concentration of 200 C L^{-1}. Elevated water temperature affected the animals at a various degree. Prosome size as well as the fat sac size (both in general and relative to the prosome size) seem to increase significantly with temperature, though these results are contradicted by other studies. The explanation of these results may lie in the concentration of food available to the animals in the time before sampling and measuring. Animals fed *R. baltica* performed better than animals fed *D. tertiolecta* on several of the analysis, being both significantly longer and larger, as well as having significantly more lipids (both in general and relative to the prosome size). There were no major differences in the distribution of the major phospholipids 16:0, 22:6n-3 (DHA) and 20:5n-2 (EPA) in any of the treatment groups. The data on egg production and hatching did not show any statistical differences between the treatment groups (both diet and temperature), most likely because of high variety in data. However, animals fed *R. baltica* as well as animals maintained at lower temperatures showed trends of having a higher hatching success than those fed *D. tertiolecta* and those maintained at higher temperatures. It's encouraged to conduct similar experiments in bigger scale, with a wider temperature range, several more measurements over time, and a cultivation of *C. finmarchicus* over several generations.

KEY WORDS: *C. finmarchicus* • Temperature • Diet • Size • Fat sac • Eggs • Hatching

Sammendrag (Norwegian)

Raudåta (*Calanus finmarchicus,* Gunnerus) er en nøkkelart i Barents- og Norskehavet på grunn av sin tallrikhet og betydning knyttet til energioverføring mellom primærprodusenter og kommersielle fiskearter. Slik som mange andre marine dyrearter, er det fortsatt usikkerhet knyttet til hvordan klimaendringer og stigende havtemperaturer kan påvirke arten i et fremtidig klimaendret scenario. Noen studier tyder på et skifte i artens biogeografiske posisjon og en generell nedgang i artens biomasse på verdensbasis. Andre studier antyder en økning i bestanden, relatert til hurtigere klekking av egg under våroppblomstringen av planteplankton, og at arten derfor vil få en fordel ovenfor konkurrerende arter knyttet til mat.

Denne studien hadde et mål om å avdekke effekten av forhøyede vanntemperaturer og kostendringer, med et fokus på kroppsstørrelse (prosom), utvikling av fettsekk, eggproduksjon og klekkesuksess. *C. finmarchicus* ble oppdrettet under tre forskjellige temperaturregimer (10, 12 og 14 °C) og med to ulike microalgedietter (*R. baltica* og *D. tertiolecta*) med en konsentrasjon på 200 C L^{-1}. Forhøyet vanntemperatur påvirket dyrene i ulik grad. Størrelse på prosom så vel som størrelse på fettsekk (både generelt og i forhold til størrelse på prosom) ser ut til å øke betydelig med temperaturen, men resultatene motbevises i andre studier. Forklaringen kan ligge i konsentrasjonen av mikroalger i tiden før prøvetaking og måling av dyr. Dyr som ble fôret på *R. baltica* presterte bedre enn dyr fôret på *D. tertiolecta* på flere av analysene. De var betydelig lengre og større, og hadde i tillegg et betydelig høyere nivå av fett (både generelt sett og i forhold til størrelsen på prosom). Det var ingen store forskjeller i fordelingen av de sentrale fosfolipidene 16:0, 22:6n-3 (DHA) and 20:5n-2 (EPA) blant noen av behandlingsgruppene. Data om eggproduksjon og klekking viste ingen statistiske forskjeller mellom behandlingsgruppene (verken for algediet eller temperatur), mest sannsynlig på grunn av stor variasjon i data. Imidlertid viste både dyr som ble fôret på *R. baltica* så vel som dyr holdt på lave temperaturer en trend med å ha en større klekkesuksess enn de som ble fôret på *D. tertiolecta* og holdt på høyere temperaturer. Det oppfordres til å gjennomføre lignende studier i større skala, med en bredere temperaturskala, flere målinger over tid, og en oppdretting av *C. finmarchicus* over flere generasjoner.

NØKKELORD: *C. finmarchicus* • Temperatur • Diet • Størrelse • Fettsekk • Egg • Klekking

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Abbreviations

C (I-V)	-	Copepodite stage 1-5
C L^{-1}	-	Carbon per litre
D	-	*Dunaliella tertiolecta* (Butcher)
DW	-	Dry weight
EPR	-	Egg production rate
GC	-	Gas chromatograph (Lipid analysis)
HD	-	High-density
HS	-	Hatching success
MBSS	-	Metres below sea surface
mL	-	Millilitre (10^{-3}L)
MUFA	-	Monounsaturated fatty acids
N (I-VI)	-	Nauplii stage 1-6
PE	-	Polyethylene (Plastic)
pg	.	Picogram (10^{-12} g)
PP	-	Polypropylene (Plastic)
PUFA	-	Polyunsaturated fatty acids
R	-	*Rhodomonas baltica* (Karsten)
SAT	-	Saturated fatty acids (Saturated fat)
SD	-	Standard Deviation
TL	-	Total lipid
µg	-	Microgram (10^{-6}g)
µg C^{-1}	-	Microgram per Carbon
µL	-	Microliter (10^{-6}L)
µm	.	Micrometre (10^{-6}m)
WE	-	Wax ester (Lipid)

1. Introduction

Calanus finmarchicus (Gunnerus 1770) is a marine calanoid copepod found mainly in the North Atlantic Ocean (Marshall and Orr, 1972). The species is a herbivorous zooplankton, grazing on various species of phytoplankton (Meyer-Harms *et al.*, 1999) and is an important component in the marine food web. It is a main diet for several commercially exploited fish species in the North Atlantic, including Atlantic cod (*Gadus morhua*) (Runge, 1988) and herring (*Clupea harengus*) (Varpe, Fiksen and Slotte, 2005). Studies show that *C. finmarchicus* is one of the most abundant species of copepods in northern seas (Conover, 1988) and some studies even suggest that it contributes to more than half of the total biomass in the North Atlantic basin (Planque and Batten, 2000). Because of its abundance and central role in energy transfer between primary producers and fishes, *C. finmarchicus* is considered a key species in the Norwegian and Barents Sea (Planque and Batten, 2000; Runge, 1988). The motivation of this thesis is to better understand how climate change will affect growth, development, and survival of *C. finmarchicus*.

1.1 The biology of *Calanus finmarchicus*

C. finmarchicus goes through six nauplii stages (N-I to N-VI) and six copepodite stages (C-I to C-VI) during their lifetime, with the adult stage being the final (C-VI) (Marshall and Orr, 1972) (Figure 1.1.). In nature, overwintering *C. finmarchicus* spawn just before or during the spring bloom of phytoplankton, and the newly hatched nauplii develop almost in parallel with the microalgae (Melle *et al.*, 2014). Up to four generations of *C. finmarchicus* can spawn in one year (late-winter to late-autumn), depending on the latitude (Marshall and Orr, 1972). During the copepodite stages, the animals store high amounts of energy in form of lipids (wax esters) in a fat sac, peaking at stage CV (Miller *et al.*, 1998; Lee, Hagen and Kattner, 2006). The depot is utilized as energy to survive the winter (Maps, Record and Pershing, 2014), and to produce gonads during their final moulting (Hagen and Auel, 2001; Mayor *et al.*, 2009; Rey-Rassat *et al.*, 2002). The lipids also contain essential fatty acids which is important for the animal's growth and development. During the autumn, *C. finmarchicus* (mainly CV) descend to deeper waters layers, generally between 50-400 metres below sea surface (MBSS) (Marshall and Orr, 1972), although hibernating animals have been found at the dept of 1500 MBSS (Broms, 2019; Lee, Hagen and Kattner, 2006). Here the animals enter a non-feeding dormant stage (diapause), in which they will stay until late-winter/early-spring, dependent on

the latitude (Marshall and Orr, 1972; Hirche, 1996). They migrate towards the sea surface in time for the algae bloom, were the mature animals' breed and spawns a new generation, thus completing the one-year cycle (Marshall and Orr, 1972).

During diapause, *Calanus* spp. have a reduced metabolism, surviving only on the lipids deposited in the fat sac (Hirche, 1996; Maps, Record and Pershing, 2014). In addition, the excess lipids are used to produce gonads, and afterward eggs and sperm (Hagen, Kattner and Graeve, 1995; Marshall and Orr, 1972; Pasternak *et al.*, 2004). Rey-Rassat *et al.* (2002) proposed a WE value of 70 µg C^{-1} as a threshold for *C. finmarchicus* that were able to complete a diapause, although this value will vary between latitudes due to differences in water temperatures and therefore difference in energy requirement.

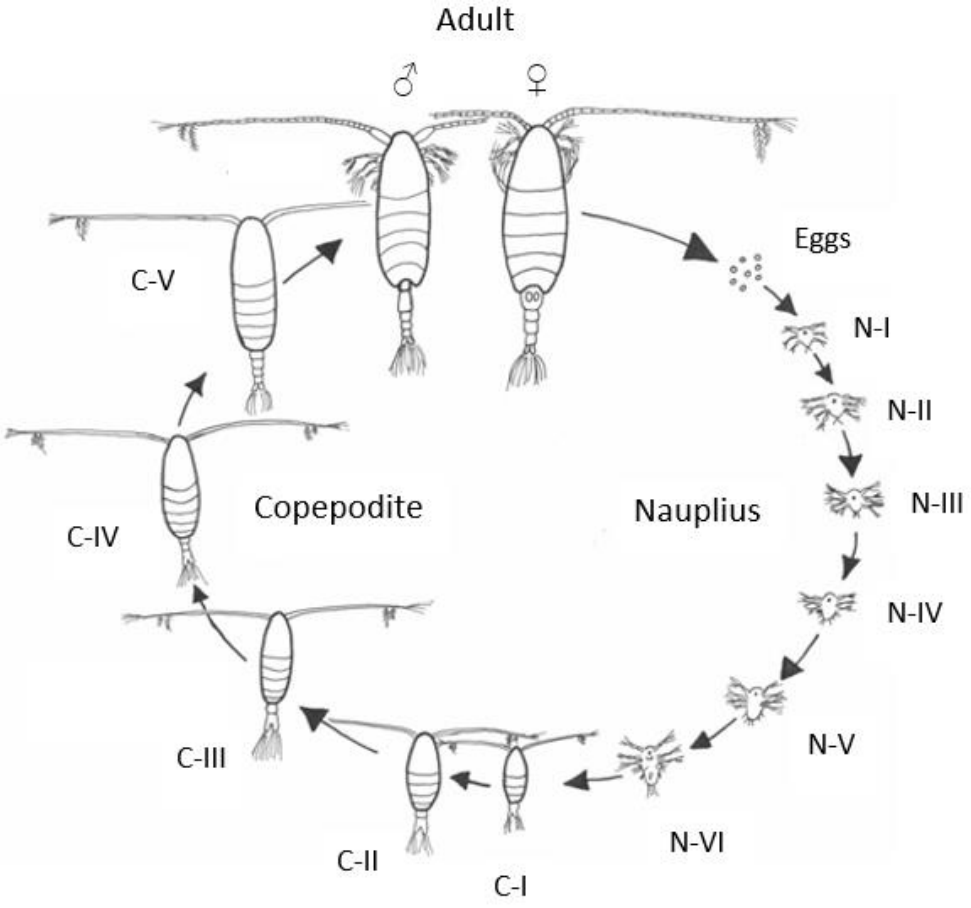

Figure 1.1. Illustration of the life cycle of the marine copepod *C. finmarchicus*. The species goes through 11 life stages after hatching (six nauplii stages and five copepodite stages) before reaching their mature adult stage. In nature, *C. finmarchicus* goes through a resting phase (diapause) during the winter months, before moulting into adulthood and completing their life cycle. The figure indicates relative body size difference between the life stages. Edited from Kvile (2015).

1.1.1 Feeding and fatty acids

C. finmarchicus feed on a vast variety of phytoplankton (e.g. diatoms, dinoflagellates, silicoflagellates and coccolithophores) depending on the season, biogeographic position and life stage of the animals (Marshall and Orr, 1972). Studies suggest that *C. finmarchicus* are still able to graze and survive on smaller microalgae at all life stages despite considerable growth from NI to adulthood (Meyer *et al.*, 2002). Phytoplankton are phototrophic and therefore depends on light energy for growth and development (Sebastiá, 2014). Since light is limited during winter months, the amount of bioproduction in the northern oceanic waters is low. Therefore, *C. finmarchicus* depend on building up energy stores to survive the winter. The lipid is stored in an internal fat sac, filling over 80% of the animal prosome in older animals (Lee and Hirota, 1973; Hagen and Auel, 2001; Vogedes *et al.*, 2010). Between 44-97% of the total lipid (TL) store is wax esters (WE) (Vogedes *et al.*, 2010), which consist mainly of long-chain fatty acids and fatty alcohols that are optimal for long term deposits (Sargent, Tocher and Bell, 2003; Lee, Hirota and Barnett, 1971). Long chained fatty acids contain more energy per unit mass, compared to short chained fatty acids (Falk-Petersen *et al.*, 2009). Additionally, the fat sac also contains triacylglycerols (TAG), and essential fatty acids which are important for the cell membrane, as well as tissue and gonad development (Lee, Hagen and Kattner, 2006). Phospholipids (mainly C16:0, C22:6n-3 (DHA) and C20:5n-3 (EPA)) and diacylglycerol ethers can be found in *C. finmarchicus* tissue and cells (Lee, Hagen and Kattner, 2006). Fatty alcohols, such as those we find in WE, are synthesised de novo by the *C. finmarchicus* from proteins and carbohydrates (Sargent, 1978). Fatty acids are mainly acquired dietary from phytoplankton (Bergvik *et al.*, 2012), but the fatty acids C20:1n-9 and C22:1n-11 (both found in WE) are also synthesised de novo by the animals (Sargent, 1978). The fatty acid composition can vary throughout the seasons (Kattner and Krause, 1989). According to Gatten *et al.* (1980), Hygum *et al.* (2000) and Miller, Crain and Morgan (2000), the level of lipid found in *C. finmarchicus* is mostly influenced by the size of the animal, and the food quality and quantity.

1.1.2 Growth and development

The size, growth rate and development time of the *C. finmarchicus* is depended on both temperature and food concentration (Campbell *et al.*, 2001). Low temperatures (high latitudes and deep-water layers) decrease the animal's heart- and metabolic rate (the ability to transform energy and material), hence slowing the growth rate and development time (Ikeda

et al., 2001; Maps, Record and Pershing, 2014; Marshall and Orr, 1972). On the other hand, the animals will grow larger than animals at higher temperatures because of a slower growth rate (Marshall and Orr, 1972). This function is also thought to be the reason for the vertical migration and diapause. As the food availability becomes short in winter months, the animal migrates down to colder waters. The reduced metabolism (caused by the cold water) makes it possible to survive the winter months only on the stored energy in the fat sac (Maps, Record and Pershing, 2014).

In temperate waters, the development time from egg to adult can be completed within one month, while in cold waters it can take up to one year, or more, to complete the cycle (Marshall and Orr, 1972). The time between development between different stages is mainly influenced by temperature (Marshall and Orr, 1972). Studies have also shown that the relative time period within each stage is constant at a constant temperature (Corkett, 1984).

1.1.3 Reproduction, egg production rate and hatching success

Sometime between December to January independent on latitude, the first moulting to adulthood occurs in overwintering animals, followed by a period of mating (Marshall and Orr, 1972). Males moult a little earlier than females, to prepare their sexual organs. Right after moulting, males starts producing spermatozoon (sperm), which is contained in a spermatophores (sperm ampulla) (Marshall and Orr, 1972). The male attaches the spermatophores close to the females genital opening, where it can retain for several days before it detaches. The eggs are presumably being fertilized on their way out of the female's genital opening by the attached spermatophore (Marshall and Orr, 1972). Since males moult earlier, they appear to have shorter developmental time than the females, and consequently shorter lifespan.

According to a study conducted by Pasternak *et al.* (2013), the egg production rate (EPR) is effected by temperature and food quality. The study showed that the EPR increases with temperature up to 10 °C under favourable feeding conditions. In another study (Pedersen and Hanssen, 2018), they found that the EPR decreased at 14 °C compared to the control group at 10 °C. This is probably due to less stored energy, caused by a higher metabolism.

As for the relationship between egg production and food quantity, Marshall and Orr (1972) stated that it is not a linear relationship and that the egg production stagnate a certain level of food. Marshall and Orr (1972) also stated that food taken up by mature is directly used to

produce eggs, and that stored lipids is not the only source of energy during this process. The laying of the eggs are linked to the amount of available food, as the female can delay the laying when the availability is short, until the conditions are favourable (Marshall and Orr, 1972). This may also be the explanation for the synchronization of *C. finmarchicus* spawning in algae blooms. In Marshall and Orr (1972) it is also stated that the species mainly lay their eggs during the night, though the reason for this is unclear.

The incubation time of the eggs after they have been laid is also depended on temperature (Marshall and Orr, 1972). At high water temperature (20 °C) it can take the eggs 19-22 hours to hatch, while at low temperatures (0 °C) it takes up to 120 hours. Preziosi and Runge (2014) found that hatching success (HS) was not significantly decrease by exposure to water temperature up to 19 °C.

1.2 Climate change and rising ocean temperatures

It is well accepted that the global temperature is rising due to high heat absorption from greenhouse gas emissions. Ever since the industrial revolution around 1850, the greenhouse gas emissions have increased due to burning of fossil fuels (IPCC, 2014). This increase is mainly driven by population and economic growth and is to this day higher than ever. The high emissions have led to an unprecedented concentration of carbon dioxide, methane, and nitrous oxide in the atmosphere. The effect of this concentration is most likely the dominant cause of the observed mean global warming since the mid-20th century (IPCC, 2014).

According to the Fifth Assessment Report (AR5) published by the Intergovernmental Panel on Climate Change (IPCC) in 2014, 93% of the excess heat from greenhouse gas emissions since the 1970s has been absorbed by the world's oceans, resulting in a rising ocean temperature. The IPCC's report of 2014 also predicts that the worlds mean ocean temperature will increase by 1-4 °C by the end of the 21st century, and the Arctic and Subarctic regions will warm at a higher rate than the global mean. Data collected from the National Oceanic and Atmospheric Administration (NOAA) in the United Stated of America shows that the anomalies of the mean sea surface temperature in the Northern Hemisphere already are close to +1°C (Figure 1.2.). According to Levitus *et al.* (2012), it is not just the surface level of the oceans that are warming. The data they provided shows that one third of the excess heat absorbed by the oceans are absorbed belove 700 m belove the sea surface.

1.2.1 Environmental impacts

In the report *Explaining ocean warming* published by the International Union for Conservation of Nature (IUCN), it is stated that a rise in global ocean temperatures will have a great effect on the ocean biodiversity, distribution and biomass (IUCN, 2016). In the past 50 years, there has been massive changes in many ecosystems (e.g., coral bleaching, change in plankton biodiversity and migration of species). Especially primary and secondary producers have been affected (Doney *et al.*, 2012), which have effected fishery industries (IUCN, 2016). The report estimates that if the temperature rises above the key threshold of 2 °C, up to 70% of the world oceans will experience large changes in the biodiversity.

The rise of ocean temperatures also creates other stressors, like ocean acidification due to a higher uptake of CO_2 (carbon dioxide) in the seawater, and a change in ocean stratification (both in salinity and temperature) due to the melting of polar ice caps (IPCC, 2014). While some of the effect of ocean warming is well documented, the total effects on the species found in the world's oceans are still uncertain (IUCN, 2016).

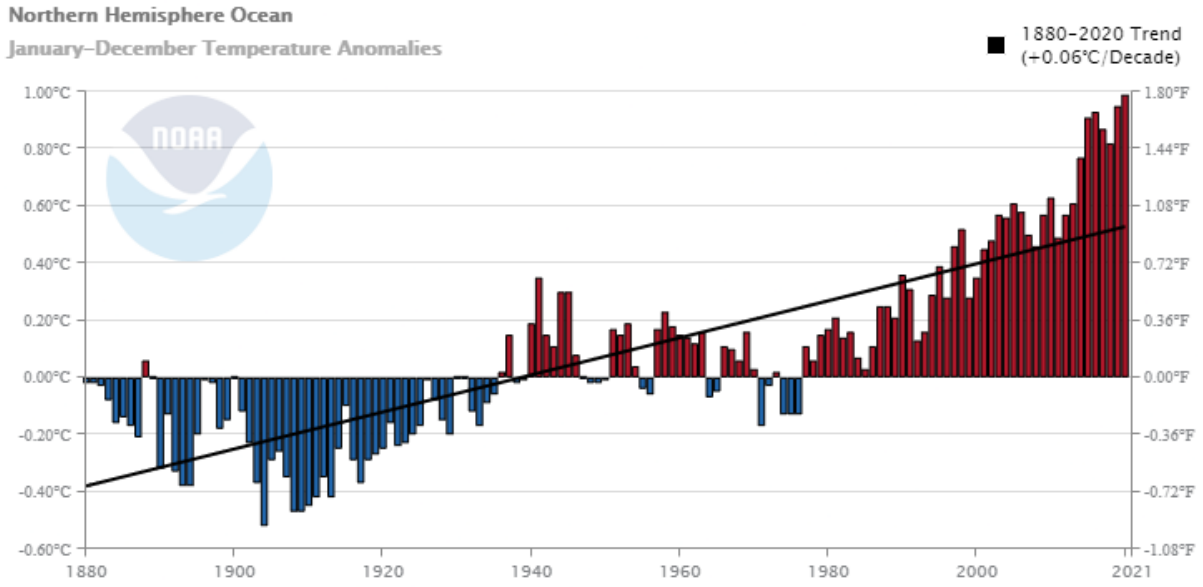

Figure 1.2. Annual sea surface temperature anomalies in the Northern Hemisphere from 1880 to 2015. Blue bars show negative anomalies (-) and red bars shows positive anomalies (+). The black line show the trend of the anomalies, with a mean increase of 0.06 °C per decade (NOAA, 2021).

1.2.2 Effects on *C. finmarchicus*

There have been several studies on the effects of increased water temperature on copepods (e.g. Cook *et al.* (2007), Preziosi and Runge (2014), Wilson *et al.* (2016), Grieve, Hare and Saba (2017)), and especially on the effect of ocean acidification (e.g. Pedersen and Hanssen (2018), Mayor *et al.* (2015)), due to higher carbon uptake caused by climate change.

Mayor *et al.* (2015) questions the viability of the copepod species if climate change also affects the microalgae productivity, phenology, and composition. If the phytoplankton communities are changed, then the copepods' ability to grow and store lipid may be at stake, influencing their ability to survive starvation in winter months. Some copepods (incl. *C. finmarchicus* in high latitudes) graze on ice algae that is totally depended on the sea ice to survive (Conover, 1988). Should the sea ice disappear, as predicted in some of the scenarios in the IPCC (2014) report, it would be devastating for the ice algae and the organisms depending on them.

Grieve, Hare and Saba (2017) estimates that the average density of *C. finmarchicus* may decrease by as much as 50% percent in a hight greenhouse gas emission scenario in the period spring and summer in the Gulf of Main and Georges Bank, by the end of the 21st century. Other studies predict that the species will shift its biogeographic range and migrate north, and totally disappear from the Gulf of Maine by 2050 (Reygondeau and Beaugrand, 2011). In the Atlantic Ocean, the species has contracted its distribution range, and Hinder *et al.* (2014) suggested that it is linked to rising ocean temperatures and that the species will further contract as the temperature rise in the near future.

On a more positive side, Møller *et al.* (2016) suggested that warmer temperatures and longer phytoplankton blooms caused by climate change might increase *C. finmarchicus* egg production in Arctic regions. Weydmann *et al.* (2015) found that *C. finmarchicus* has a faster egg development and therefore earlier hatching than other Arctic Calanus spp. This might give *C. finmarchicus* an advantage over the other Calanus spp. when it comes to hatching in time for the spring bloom.

1.3 Aim of study

The motivation for this study was to unravel some of the consequences of climate change, and *C. finmarchicus* was chosen as targeted species because of its key position in the North Atlantic Ocean (Runge, 1988). There is still much to learn about the climate effects on *C. finmarchicus* and other researchers in the field encourage more studies to be done to clarify the true effects of ocean warming. The aim of this thesis was to characterize the effect of elevated water temperatures, as a climate effect, on prosome size, fat sac development, egg production and hatching success in *C. finmarchicus*. Two microalgae species were chosen as diet source, to investigate additional effects of altered microalgal communities, caused by climate change.

The effects of elevated water temperatures and different microalgae diet were characterized on the following parameters:

Prosome size	Prosome length (mm). Prosome volume (mm^3).
Fat sac development	Fat sac size/Prosome volume ratio ($\mu g/mm^3$). Total lipid content (µg). Fatty acid and fatty alcohol composition (mol % of total lipid).
Egg production	Eggs laid per female (No.) (24-hour period).
Hatching success	Eggs hatched (%) (72-hour period).

The hypothesis (H) of the study were:

H1: Increased water temperature will negatively affect the prosome size of *C. finmarchicus.*
H2: Increased water temperature will decrease the size of the fat sac of *C. finmarchicus*.
H3: Increased water temperature will decrease the egg production per female.
H4: Increased water temperature will not affect the hatching success of *C. finmarchicus.*
H5: The animals fed *Dunaliella tertiolecta* (microalgae) will have poorer performance than animals fed *Rhodomonas baltica* (microalgae).

2. Materials and methods

2.1 Experimental setup

The experiments described in the present study were all conducted at the facilities of NTNU Centre of Fisheries and Aquaculture in Trondheim (SeaLab), in the period between November 2020 and April 2021. The rearing experiment was original planned to start in August 2020 but was delayed to November due to restrictions related to the global Covid-19 pandemic.

2.1.1 Laboratory cultures of *C. finmarchicus*

The animals used in this experiment was collected from the continuous stock culture of *C. finmarchicus* held at the research facility at NTNU SeaLab. The animals in the culture are descendants of individuals collected in Trondheimsfjorden in the autumn of 2004 (Hansen *et al.*, 2007) .There was conducted a DNA analysis on the animals which confirmed that the culture was in fact only consistent of *C. finmarchicus*, and not a mix between the species and the related species *Calanus glacialis*, which also are present in Trondheimsfjorden (Skottene *et al.*, 2020). The stock culture is maintained at 10 °C in 300 L tanks with filtered seawater and are continuously fed with algae mix containing the microalgae *Rhodomonas baltica* (Karsten 1898, clone NIVA 5/91) and *Dunaliella tertiolecta (*Butcher 1959, clone CCAP 19/27).

2.2 Experimental system

Three temperatures were used as the main treatment, at 10-, 12- and 14 °C nominal values (Table 2.1.). The 10-degree treatment was set to be a reference, as it was the same temperature as the stock culture was maintained at, from which the animals were collected. The 12- and 14-degree treatment groups were used to simulate two different scenarios of elevated water temperatures, caused by climate change. In addition, two microalgae were picked out as a diet treatment (*R. baltica* and *D. tertiolecta*) to simulate a change in diet caused by rising ocean temperatures. The main differences between the two microalgae are the biochemical composition and body size, *D. tertiolecta* (9-11 µm, 74.3 pg C/cell) being a generally smaller alga than *R. baltica* (18-30 µm, 54.6 pg C/cell) but having a greater carbon content per cell (Throndsen, 1997; Selnes, 2021). The nominal algae concentration in the

tanks was set to 200 µg C L^{-1}. The two forms of treatments were combined to six different treatment groups and all six combinations was replicated by three, making a total of 18 treatments and rearing tanks.

Table 2.1. There were six different treatment groups in the raring experiment of *C. finmarchicus*, each replicated by three (in total 18 rearing tanks). The groups were labelled with the first letter of their diet (R/D) followed by the temperature treatment value (10/12/14). The replicates were assigned with either the letter A, B or C after the treatment group label (e.g., R10A). In each tank there were 300 animals (*C. finmarchicus*). Animals fed with the same diet are referred to as either the *R. baltica* group or the *D. tertiolecta* group. Animals maintained at the same temperatures are referred to as the 10-degree, the 12-degree or the 14-degree groups.

TREATMENT GROUPS			
MICROALGAE DIET/ WATER TEMPERATURE TREATMENT	*R. baltica*	*D. tertiolecta*	**SUM**
10 °C	R10 (x3)	D10 (x3)	6 tanks
12 °C	R12 (x3)	D12 (x3)	6 tanks
14 °C	R14 (x3)	D14 (x3)	6 tanks
SUM	9 tanks	9 tanks	18 Total

A flow-through system was constructed in a temperature-regulating climate room for temperature control (Figure 2.1.). The system consisted of 18 50-L tanks (PE) based on the number of treatments. Both an inlet and an outlet were connected to the tanks. The inlet provided the tanks with filtrated seawater, as well as algae for the animals to feed on. The seawater was filtrated through two CUNO filters, first a 20 µm mesh, then a 1 µm mesh. The water flowed out of the filter through a 6 mm tube (PE, John Gust), and was distributed to the tanks through 2 mm tubes (PE, John Gust). The tubes hung a few centimetres above the bottom of the cultivation tanks. Algae was distributed with a pump (Watson Marlow, 205S) from six feeding tanks (10 L, HD PE), one for each treatment group. Aeration was added to the feeding tanks to prevent the algae from becoming sediment (High Blow, aquarium pump). The algae flowed from the feeding tanks through the pump in 3 mm silicon tubes (VWR) and from the pump through 1 mm silicon tubes (VWR), which was attached to the 2 mm inlet tubes (PE). The filtrated seawater flowed at a rate of 53 mL/min and the algae flowed at 2 mL/min, a total of 55 mL/min inflow to each tank. The outlet was located at the top of each tank, keeping the tanks at a constant level of 45 L and prevented the tanks from overflowing. A 100 µm bongo mesh was attached to the outlet to prevent any loss of animals and eggs.

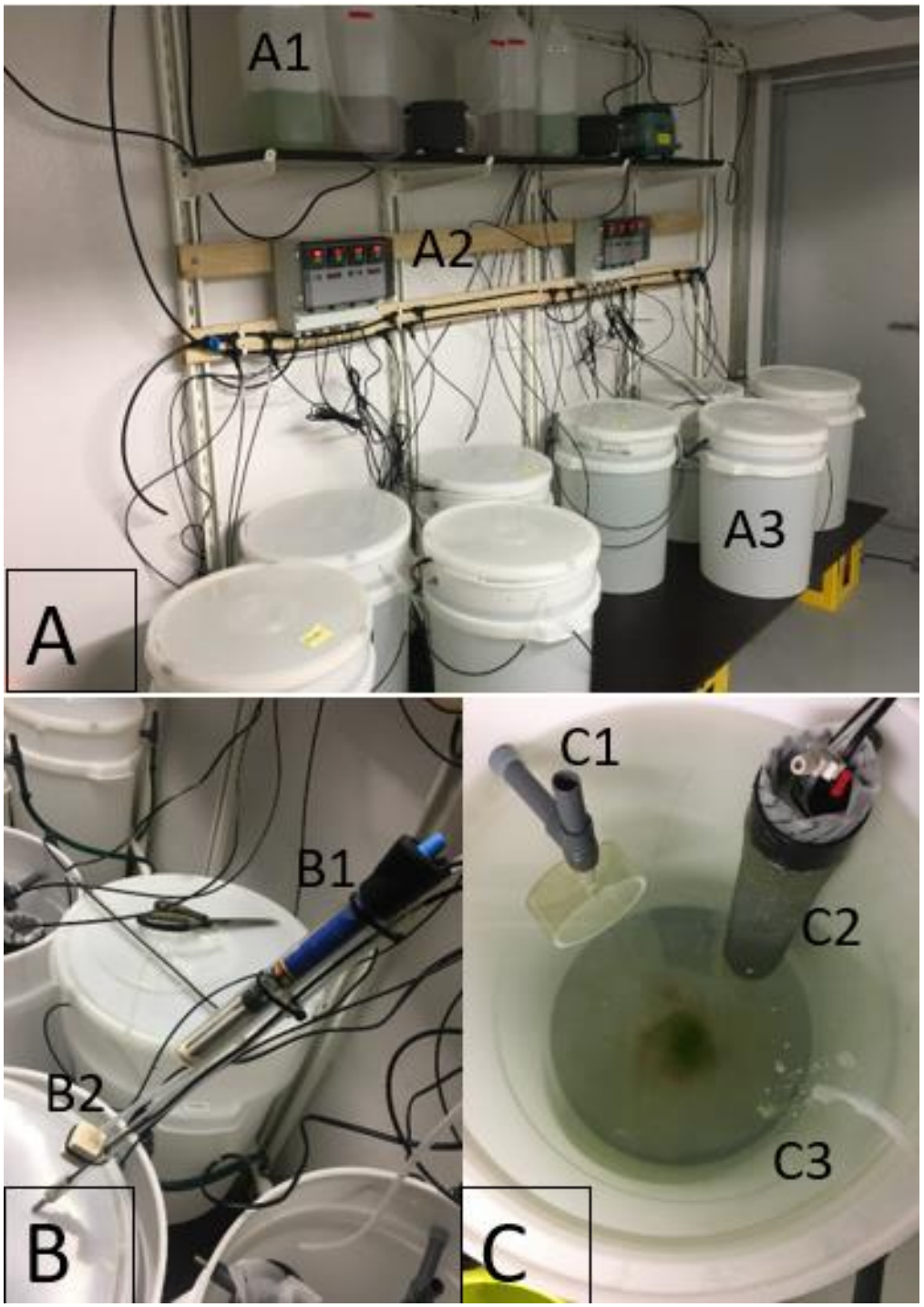

Figure 2.1. Collage of three images picturing several of the components in the flow-system used to cultivate the *C. finmarchicus* in the rearing experiment. Picture A shows the complete system on one side of the room, with the feeding tanks (A1), the thermostats (A2), the cultivation tanks (A3), as well as tubes and wires used for aeration, seawater and microalgae inlet, and electricity (for the thermostats and pumps). Picture B shows the heating element (B1) and the aeration stone (B2). Picture C shows the inside of the tanks, with the bongo mesh and the outlet (C1), the supporting mesh with the heating element (C2), and the inlet of filtrated seawater and algae with aeration (C3).

The climate room was set to 10 °C to provide the temperature for the six 10-degree group, while a heating element (Aquarium heater, 50W), connected to a thermostat (Auber, SYL-2372) and a sensor (PT100), were added in the remaining 12 tanks to provide the temperature for the 12- and 14-degree groups. Surrounding the elements were a supporting mesh (PE) covered in a 100 µm filter cloth to prevent animals from getting too close to the heating. Aeration was also added to the tanks through two plastic tubes (2 mm, PE, John Gust) using a pump (Watson Marlow, 205U) and compressed air from an oil-free compressor, to ensure circulation so that both temperature and algae was well mixed within the tanks. The first tube hung just belove the inlet of filtrated seawater and microalgae, and the second one hung just belove the heating element. The 10-degree group had just the aeration in the supporting mesh so that the tanks would have the same amount of aeration as the 12- and 14-degree groups. Aeration stones was attached at the end of the tubes to minimalize the air bubbles.

Temperature and algae concentration in the rearing tanks was measured and monitored every day during the experiment between 2:00 pm and 6:00 pm. Temperature was monitored using a calibrated thermometer (Digi-Sense, 91100-40) with a sensor (Omega, PL 052103). Any irregular temperatures above or belove 0.5 °C from the nominal temperature was corrected either by adjusting the thermostat or the aeration flow. Algae concentration was measured by running a 4 cl water sample from each rearing tank through a coulter counter (Beckman Coulter, Multisizer™ 3). The feeding tanks was refilled every 24 hours with algae concentrations based on the measured algae concentrations in the rearing tanks to keep the level close to the nominal value of 200 µg C L^{-1}. Since the treatment replicas shared feeding tanks, it was decided to base the daily algae refill of the feeding tank on the lowest measured algae concentration between the three replicas. The over-time measured temperature and algae concentrations are important for the use of interpreting and explaining other results.

2.2.1 Cultivation of microalgae

The two microalgae species *R. baltica* and *D. tertiolecta* was cultivated in a separate room from the animals. Two flat-bottomed 10-L flasks were used for each algae species. The algae were cultivated with a Conwy medium (Walne, 1966) (See Appendix A for nutrient content) at 20±2 °C and illuminated with three fluorescence light tubes (Philips, TLD 36w/965). The algae cultures were aerated with compressed air added 2350 ppm CO_2. A third of the algae culture was harvested every second day and refilled with filtrated seawater added Conway medium. 45 mL of each algae culture were sampled for lipid analysis three times during the

experiment (9th and 19th of November, and 7th of December). The sampled algae were stored in a minus 80 °C freezer (Panasonic, MDF-794-PE) before lipid analysis.

2.3 Experimental procedure

5400 CIII animals were sampled from the stock culture and counted using a magnifying glass (LUXO Wave) and divided into 18 tanks (300 animals in each tank). 50 animals from the stock culture were also sampled and frozen in a -80 °C freezer (Panasonic, MDF-794-PE) for lipid analysis. Algae was added directly to the tanks, to reach a concentration of 200 µg C L^{-1}. The animals in the 12- and 14-degree groups had to be acclimatised one degree a day so not to shock them from the temperature difference. The animals were raised and monitored until they reached the desired stages. At stage CV, 20 animals were sampled from each tank using a scoop, then photographed and frozen in a -80 °C freezer (Panasonic, MDF-794-PE) for biometrical and lipid analysis. After the sampling of eggs, the remaining animals in the tanks was sampled, and fixated with the use of Lugol's iodin (I_3K). The animals were then counted and divided by sex with the use of a microscope (Leica, M80 + Leica Base TL).

2.3.1 Stage determination, development time and sampling of CV animals

Since increasing water temperature speeds up growth rate and development time of *C. finmarchicus* (Campbell *et al.*, 2001; Cook *et al.*, 2007), it was not possible to sample from the different temperature groups at the same time. CV animals were therefore sampled from the different tanks after adults were observed in the tanks. This way, animals were sampled at approximately the same time of development. 20 animals were then collected and anesthetized (FINQUEL, Argent Chemicals) from each tank (14-degree group (Nov. 14th), 12-degree group (Nov. 16th) and 10-degree group (Nov. 17th)). The stage of the animals was determined based on morphological characteristics using a stereo microscope (Leica, M80 + Leica Base TL) and a short description of the most prominent characteristics by one of the supervisors (Researcher Dag Altin, Department of Biology, NTNU) (Morphological characteristics of *C. finmarchicus* in their copepodite stages are presented in Blaxter *et al.* (1998)). Before the animals were stored away for lipid analysis, a lateral-photograph was taken of each individual using a stereo microscope (Leica, MZ APO) with a camera (DS-FI1/DSU2) connected to a computer with an image software (NIS Elements F v/4.60).

After the sampling, it was noticed that there were only 18 animals from the D14B tank. It was by then too late to sample more CV, so the error had to be adjusted for during the statistical analysis of prosome length, prosome volume, and the ratio between fat sac size and prosome volume.

2.3.2 Egg sampling, incubation, and hatching

After observing that the majority of the animals had transitioned from CV to their adult stage and eggs were detected in the tanks, the sampling process of the eggs began (10th and 11th of December).

To ensure that the eggs in the tanks were laid in the period (24-hour), the tanks had to be emptied and cleaned from eggs that were already laid. The animals in the tanks were scooped out through a mesh (300 µm) and kept in a glass bowl. The tanks were cleaned using hot tap water and paper towels and refilled with filtered seawater together with the animals. 24 hours after the cleaning, the proses was repeated, only that the remaining seawater was filtrated through a 125 µm mesh to collect the eggs. The tank was also rinsed with a wash bottle filled with filtrated seawater to wash of any remaining eggs that might have stuck to the walls. The eggs were then transferred to a petri dish and photographed through a microscope (Nikon, Eclipse TS100) with a camera (Leica, MC170HD). 120 eggs from each tank were divided into six wells on a 24-well plate, 20 eggs in each well. The eggs were then incubated in incubation cabinets (Sanyo Incubator, MIR-253 and VWR, INCU-Line 150R) for 72 hours on the same temperature from which the eggs were laid in, well within the hatching-timeframe presented by Marshall and Orr (1972). After the incubation period, the unhatched eggs were counted and photographed using a microscope (Nikon, Eclipse 2000U) with a camera (DS-FI1/DSU2) connected to a computer with an image software (NIS Elements F v/4.60). Drops of Lugol's iodin (I_3K) were afterwards added to the wells to fixate the hatched nauplii. The next day, the nauplii was counted and photographed using the same equipment. Unhatched eggs and hatched nauplii is shown in Appendix B.

After the cleaning of the rearing tanks, algae were not directly added to the tanks with the filtrated seawater and the animals. It is therefore safe to assume that the algae concentration was low during the 24-hour egg laying period, as seen in Figure 3.2.

2.3.3 Lipid analysis

The frozen CIII (50 animals) and CV animals (20 from each tank. 10 were used, 10 were sampled as backup) as well as the microalgae samples were transferred to separate test tubes and mixed with a chloroform methanol solution with the use of a disperser (IKA T10 basic) with a dispersing element (4 mm). The lipid extractions were done according to the Folch method for lipid extraction (Folch, Lees and Stanley, 1957). The extracted fatty acids and fatty alcohols were analysed by a gas chromatograph (GC) (Agilent Technologies GC System 7890B, Agilent Technologies Autosampler 7693, Agilent Technologies 5977B MSD) according to Kattner and Fricke (1986). Fatty acids were identified using a fatty acid standard mixture (68D).

Notable equipment used during the lipid extraction are a centrifuge (Thermo Fisher Scientific, Heraeus Megafuge 16R), a heat block (Techne, Dri-Block DB 3D) and a nitrogen evaporator (Organomation, N-EVAP 111, OA-HEAT 5085).

Dry weight was also measured of the animal's and algae total lipid (TL) content using a micro weight (Mettler Toledo, UMX2). Glass vials were pre-weight.

2.3.4 Biometrical and morphometrical measuring and analysis

Length (L), height (H), width (W), and area of the CV animal prosome as well as the area of the fat sac was measured from the photographs taken, using a drawing board (Wacom Cintiq, DTK-1300) and the free image processing program ImageJ. The pixel-to-mm ratio was calibrated using a photo of a scale-bar (mm). The measurements made it possible to calculate the volume of the animal prosome (mm^3) as well as the size of the fat sac (mg).

The shape of the *Calanus* spp. prosome is strikingly similar to a horizontal ellipsoid, and the volume formula of an ellipsoid was therefore used to calculate the volume of the animal prosome. Equation 2.1. is the volume (V) of an ellipsoid, were (a) is length divided by two, (b) is height divided by two, and (c) is width divided by two (Aarnes, 2018).

$$V = \frac{3}{4}\pi abc \qquad [2.1.]$$

There was not taken any photographs of the animals from above after the sampling, making it impossible to measure the width of the animals. There were therefore conducted a side-study to see if there were any correlation between length/height and width of the animals. 50 adult individuals from the stock culture were photographed both from above and from a lateral view, and a ratio between prosome length and prosome width was found (0.331) (W/L). Length, height, and width of the prosome was measured at the largest respective distance. Specification about the location on the measurements conducted on the *C. finmarchicus'* prosome and fat sac is shown in Appendix C.

The fat sac size (mg) was estimated according to Vogedes *et al.* (2010) equation for total lipid (TL). In the study they found that the TL content of the fat sac can be estimated with a photographic method. They tested the results of this method against GC lipid measurements and found that the fat sac area had a high correlation with the TL content. The equations for TL are shown in [2.2.].

$$TL = 0.197A^{1.38}$$

[2.2.]

A ratio (r) was used to evaluate the fat sac size relative to the prosome volume of the *C. finmarchicus* (e.g., animals with no fat sac had a ratio at 0). The fat sac size was converted from mg to µg (x1000) to get a wider ratio. Based on wild sampled *C. finmarchicus* mentioned in Vogedes *et al.* (2010), with fat sacs covering over 80 % of the prosome volume, a maximum ratio was calculated and used as perspective (r = 260). The equation for this ratio is seen in [2.3.]

$$r = \frac{Fat\ Sac\ Size\ (\mu g)}{Prosome\ Volume\ (mm^3)}$$

[2.3.]

2.4 Statistics

The statistical analysis was performed with the software *IBN SPSS Statistics v.27*, while the graphs was generated with the software *SigmaPlot v.14.0*.

A Shapiro-Wilk-test was used to test for normality in the data, and a Levene's test was used to test for homogeneity of variance in the data (followed up with a Welch test if the data showed a significance in homogeneity). Bonferroni were used in the comparing of main effect to adjust for the multiple comparisons. One-Way ANOVA were used to test for significant effect between temperature treatments within the same diet group. Two-way ANOVA was used to statistically compare the different treatments.

A Bonferroni post hoc test were used for data that were normally distributed and had equal variance. A Gabriel post hoc test were used on data that were normally distributed and had equal variance but had a slight difference in sample sizes.

For data that were not normally distributed but had equal variance, the non-parametrical Kruskal-Wallis test were used (2-sided, asymptotic) to test for significance between treatment groups. For data that were normally distributed but were equal variance were not assumed, a One-Way ANOVA were used to test for statistically significance.

Level of significance was set to α (p) < 0.05 on all tests (i.e., when differences had a $p < 0.05$, it was considered significant), and test scores with alpha level between 0.05 and 0.1 was considered as trends.

2.5 Co-operation and assistance

A co-student helped with the daily measurements of water temperature- and algae concentration (as well as the refill of the feeding tanks) during the rearing experiment. For a few days, one of the supervisors did the daily chores, because both me and my co-student were in quarantine after suspecting infection of the Covid-19 virus.

The construction and design of the cultivation system was done with the assistance and guidance of two supervisors. The GC analysis as well as the fatty acid integration were conducted by two of the laboratory technicians at NTNU SeaLab.

3. Results

3.1 Culture parameters

Cultivation temperatures

Temperature measurements in each treatment tank is shown in Figure 3.1., while mean temperature with standard deviation (SD) in each tank is shown in Appendix D. The 14-degree group (except D14A) had a high temperature reading early in the experiment, some up to 15 °C. D14C had a longer period at around 15 °C and a peak at 16 °C at the end of the experiment. R14B had a peak in the middle of the experiment, at 15,5 °C. D14B was very turbulent during the experiment, having many high readings just below or above 15 °C.

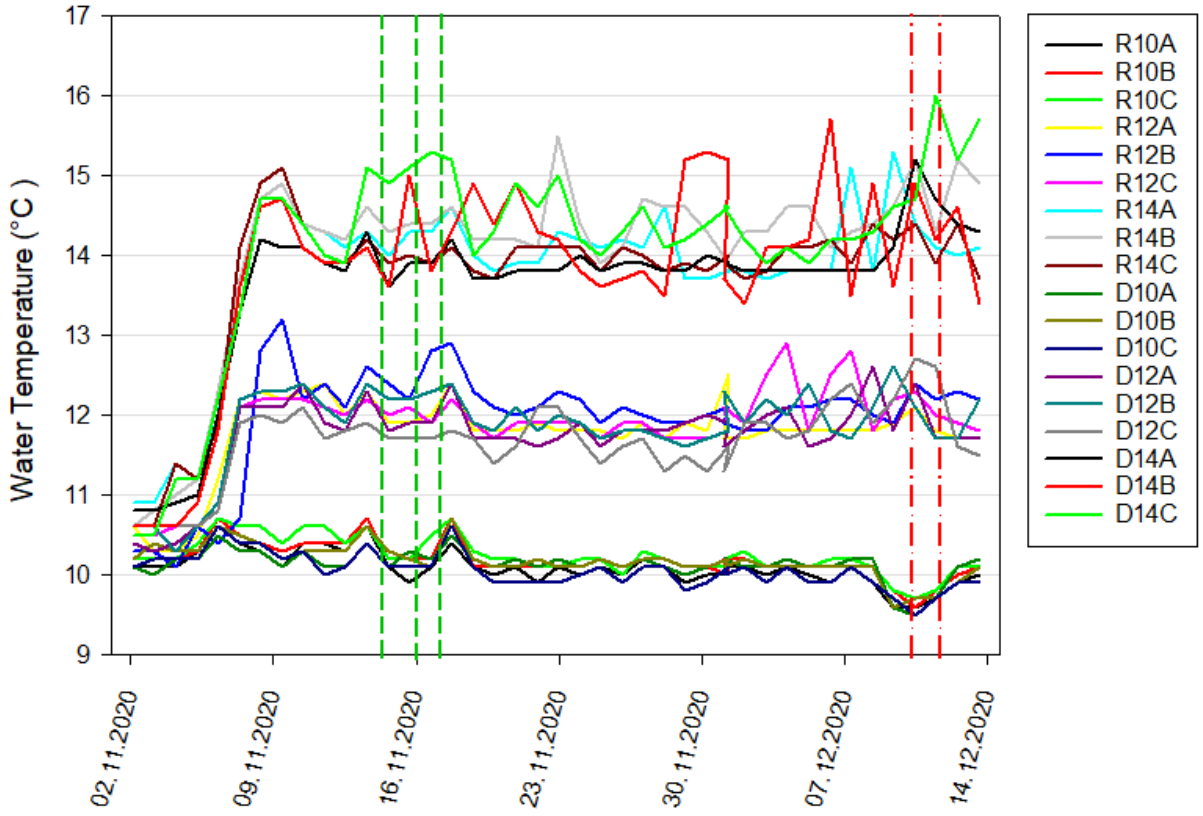

Figure 3.1. The water temperatures in the rearing tanks were measured every day during the cultivation experiment (02.11.2020-14.12.2020) (x-axis). The nominal value for the three temperature treatments were 10 °C, 12 °C and 14 °C. The green lines show the samplings of 20 *C. finmarchicus* (CV) individuals from each tank for lipid- and biometric analysis (14.11 (14 °C), 16.11 (12 °C), 17.11 (10 °C)), the red lines show the harvesting of eggs (10.12 (10 °C, and R12), 11.12 (14 °C, and D12).

R14A had some higher reading close to the end, at around 15 °C. The 12-degree group were more stable, although R12B (early) and R12C (late) measured over above 13 °C at a time. The 10-degree group was the most stabile with no major measurements (±1 °C).

Algae concentrations

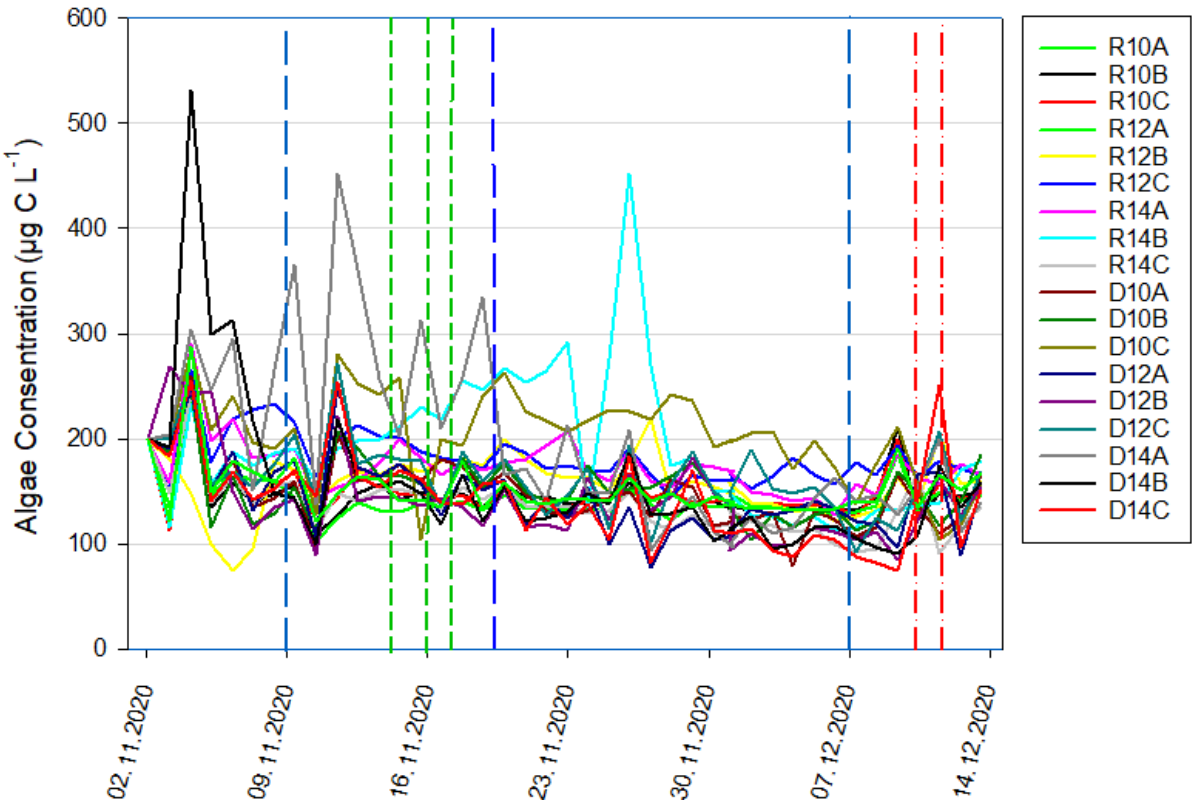

Figure 3.2. The algae concentration in the rearing tanks were measured every day during the cultivation experiment (x-axis). The nominal algae concentration was 200 µg C L^{-1}. The daily refills of the feeding tanks were based on the algae concentrations measured the same day. The blue lines show the sampling of algae (45 mL) for lipid analysis (9.11, 19.11, 7.12), the green lines show the samplings of 20 *C. finmarchicus* (CV) individuals from each tank for lipid- and biometric analysis (14.11 (14 °C), 16.11 (12 °C), 17.11 (10 °C)), the red lines show the harvesting of eggs (10.12 (10 °C, and R12), 11.12 (14 °C, and D12).

Algae concentration in each tank is shown in Figure 3.2., while mean concentrations with SD in each tank is shown in Appendix E. D14B had a high algae concentration reading early in the experiment, over 500 µg C L^{-1}. D14A had several high readings in the first half of the

experiment, peaking at 450 µg C L^{-1}. R14B had a high reading in the second half of the experiment, at 450 µg C L^{-1}. Several of the rearing tanks (D14 group, D12B, D14B, R10B, R12A and R12B) measured close to or below 100 µg C L^{-1} in the first half of the experiment, other tanks (D10A, D10B, D12 group, D14 group and R14C) measured close to or below 100 µg C L^{-1} in the second half of the experiment.

There were no critically low measurements of algae concentration (below 50 µg C L^{-1}), so it is fair to assume that there was no starvation in any of the tanks. This is confirmed later in Figure 3.6., as it's seems that all the animals had energy left to store.

3.2 Biometrical and morphometrical analysis

3.2.1 Prosome length

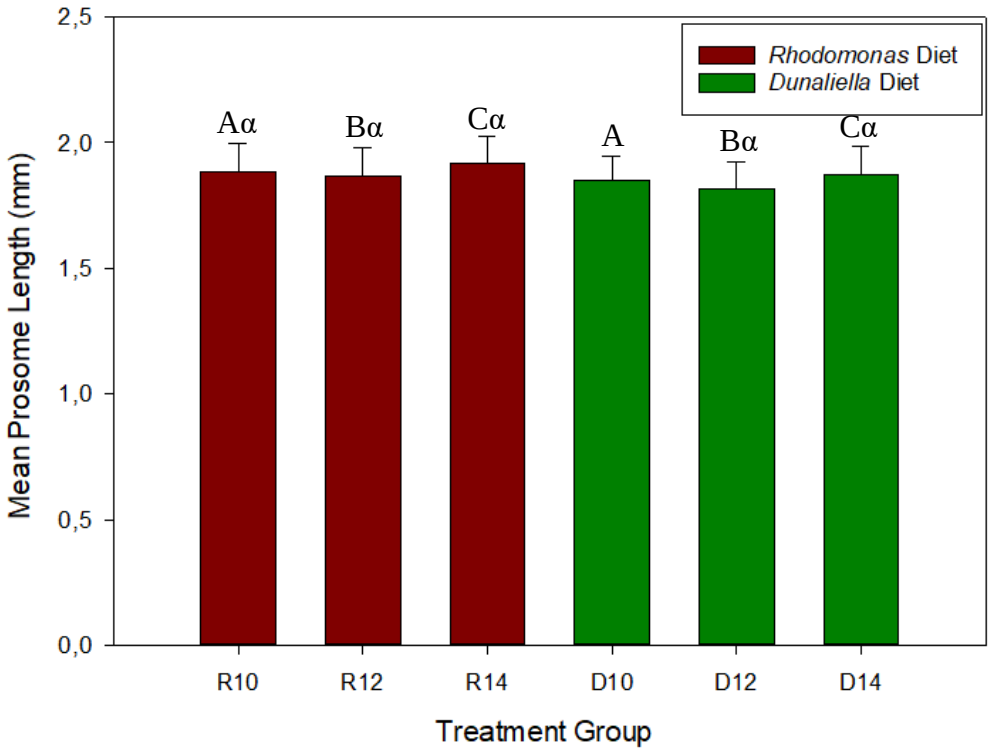

Figure 3.3. Mean prosome length measurement of 60 CV animals from each treatment group (20 animals from each tank) (means ±SD, n=60). Significant differences between algae diet treatments maintained at the same temperature is indicated with uppercase Latin letters, while significant differences between temperature treatments within each algae group is indicated with lowercase Greek letters.

A test between-subject effect in the Two-Way ANOVA shows that both temperature (p = 0.001) and diet (p = 0.000) had a significant effect on size (length), but with no interaction between the two (temperature*diet) (Figure 3.3.). The animals fed *R. baltica* were significantly longer than those fed with *D. tertiolecta* (p = 0.0, ANOVA). Animals maintained at 14 °C were significantly longer than those maintained at 12 °C (p = 0.0, Gabriel), and trended to be longer than the animals maintained at 10 °C (p = 0.064, Gabriel). Although animals in the 10-degree group were longer than the 12-degree group, the difference was not statistically significant. Within the *R. baltica* group, animals in the R14 group (1.918 mm) were significantly longer than animals in the R12 (1.868 mm, p = 0.018, Kruskal-Wallis) and R10 groups (1.882 mm, p = 0.038, Kruskal-Wallis). There was no significance between animals in the R12 and R10 groups. Within the *D. tertiolecta* group, animals in the D14 group (1.873 mm) were significantly longer than the animals in the D12 group (1.817 mm, p = 0.004, Kruskal-Wallis), but not significantly longer than the animals in the D10 group (1.846 mm). There was no significance between animals in the D12 and D10 groups.

3.2.2 Prosome volume

A test between-subject effects in the Two-Way ANOVA showed that both temperature (p = 0.001) and diet (p = 0.001) had significant effects on size (volume) (Figure 3.4.). There was no significant interaction (temperature*diet). Animals fed *R. baltica* were significantly larger than those fed *D. tertiolecta* (p = 0.0, ANOVA). Animals maintained at 14 °C were significantly larger than those maintained at 12 °C (p = 0.001, Gabriel) and 10 °C (p = 0.021, Gabriel). Although the animals in the 10-degree group were larger than those maintained at 12 °C, the differences were not statistically significant. Within the *R. baltica* group, animals in the R14 group (0.37 mm^3) were significantly larger than the animals in the R12 (0.34 mm^3, p = 0.046, Kruskal-Wallis) and R10 groups (0.345 mm^3, p = 0.041, Kruskal-Wallis). There were no differences between animals in the R12 and R10 groups. Whitin the *D. tertiolecta* group, animals in the D14 group (0.325 mm^3) were significantly larger than animals in the D12 group (0.29 mm^3, p = 0.004, Kruskal-Wallis), and trended to be larger than animals in the D10 group (0.3 mm^3 p = 0.087, Kruskal-Wallis). There were however not significant differences between animals in the D12 and D10 groups.

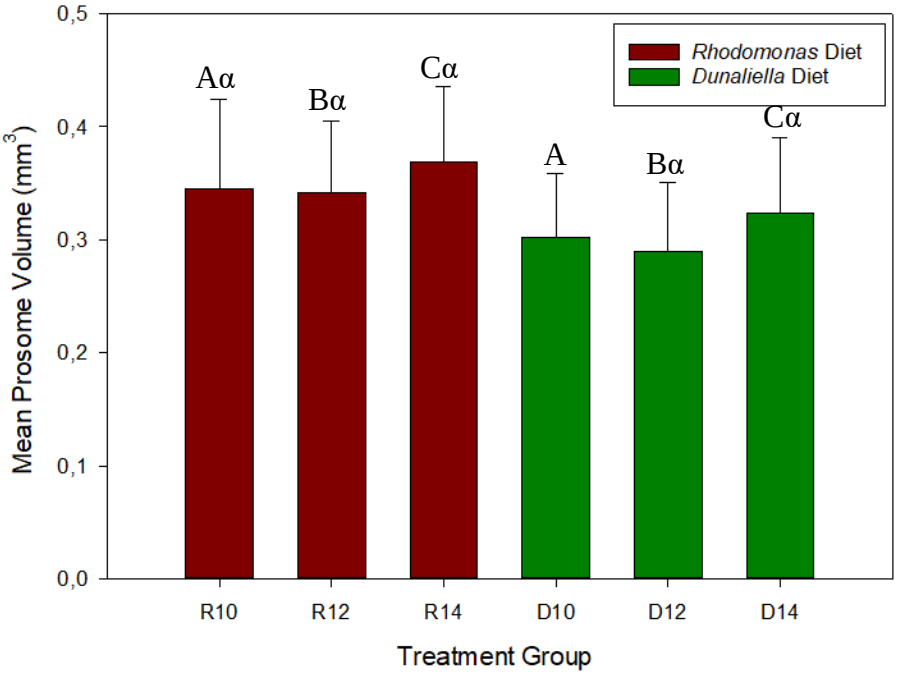

Figure 3.4. Mean prosome volume estimates of 60 CV animals from each treatment group (20 animals from each tank), calculated by using the volume equation of an ellipsoid (means ±SD, n=60). Significant differences between algae diet treatments maintained at the same temperature is indicated with uppercase Latin letters, while significant differences between temperature treatments within each algae group is indicated with lowercase Greek letters.

3.2.3 Ratio between fat sac size and prosome volume

The ratio between fat sac size and prosome length were not normally distributed, consequently, a non-parametric test was used for statistical comparisons. Both temperature ($p = 0.0$, Kruskal-Wallis) and diet ($p = 0.0$, Kruskal-Wallis) had significant influence on the data (Figure 3.5.). Animals fed *R. baltica* had a higher ratio than those fed *D. tertiolecta* ($p = 0.0$, Kruskal-Wallis). Animals maintained at 14 °C had higher ratio than those maintained at 12 °C ($p = 0.001$) and 10 °C ($p = 0.0$). There were no statistically significant differences between the 10- and 12-degree groups. Within the *R. baltica* group, animals in the R14 group ($r = 75.6$) had a significantly larger ratio than animals in the R12 ($r = 60$, $p = 0.042$, Kruskal-Wallis) and R10 groups ($r = 46.7$, $p = 0.0$, Kruskal-Wallis). In this case, animals in the R12 group were significantly larger than animals in the R10 group ($p = 0.045$, Kruskal-Wallis). Within the *D. tertiolecta* group, animals in the D14 group ($r = 45.3$) had significantly higher ratio than animals in the D12 group ($r = 27.7$, $p = 0.002$, Kruskal-Wallis), and trended to have a higher ratio than animals in the D10 group ($r = 35.2$, $p = 0.072$, Kruskal-Wallis). There was so statistically significance between animals in the D12 and D10 groups.

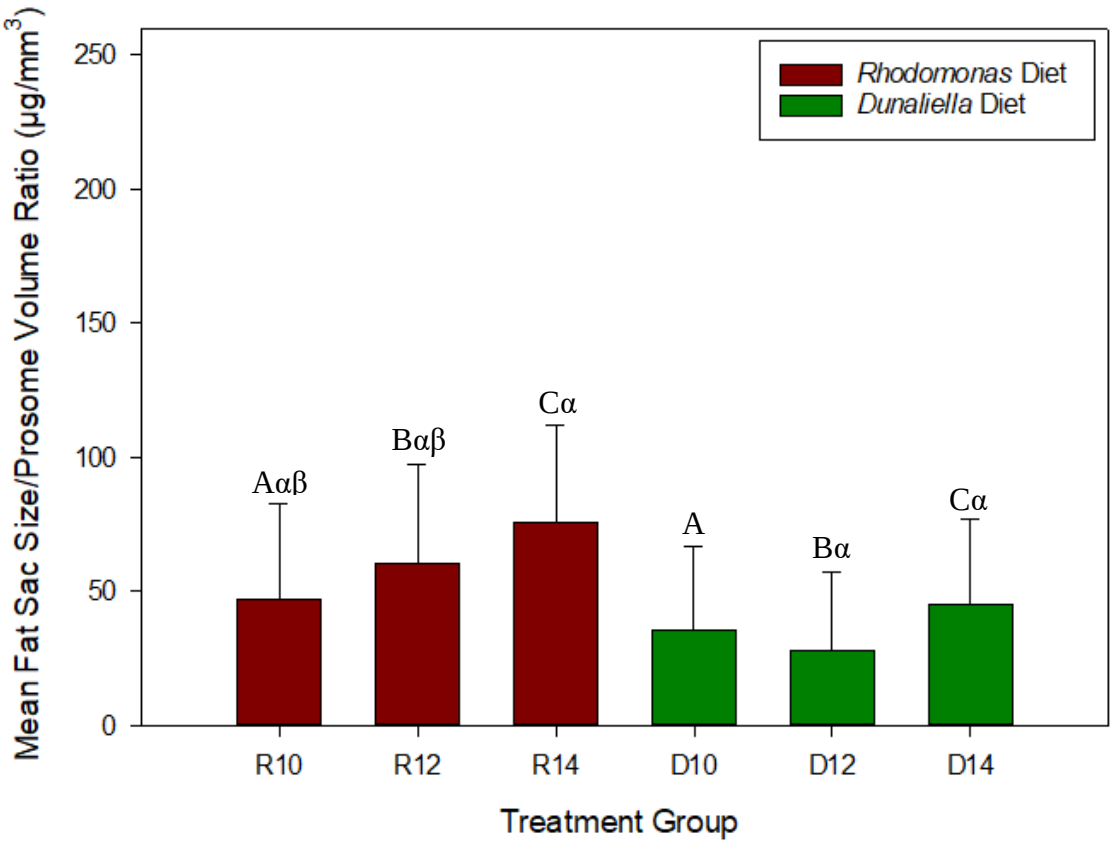

Figure 3.5. Mean ratio between the fat sac size and prosome volume of 60 CV animals from each treatment group (20 animals from each tank) (means ±SD, n=60). The ratio was calculated using the estimated TL content and dividing it on the estimated prosome volume. The Y-axis scaling ranged from animals with no fat sac (0) to animals with a fat sac covering 80% of the prosome (ratio: 260 mg/mm^3), based on the highest measurement fat sac size from wild animals (Vogedes *et al.*, 2010). Significant differences between algae diet treatments maintained at the same temperature is indicated with uppercase Latin letters, while significant differences between temperature treatments within each algae group is indicated with lowercase Greek letters.

3.3 Lipid and fatty acid

3.3.1 Fatty acid composition

Table 3.3. shows the most abundant fatty acids found in the *C. finmarchicus* (CV) after conducting a lipid analysis through a gas chromatograph (GC). The fatty acids are given as percentage (mol %) of total fatty acids (TL). Statistical tests and analysis were conducted on the data regarding level of saturated fatty acids (SAT), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) found in the sampled CV animals.

There was no interaction between temperature and diet on the level of SAT, MUFA and PUFA found in the *C. finmarchicus* ($p > 0.05$).

The data on the fatty acid composition from the second and third sampling of microalgae were added together and given as mean between the two, as it better describes the distribution of fatty acids over time between the sampling of CV animals and until the sampling of eggs.

Microalgae

The mean total lipid (µg) of the two microalgae can be seen in Appendix F.

R. baltica had a relatively high content of SAT amounting to more than 60% of the fatty acids in the first sampling but decreasing below 50% in the second two samplings (Table 3.1.). The main SAT were 14:0 (23.1/19.2%) and 16:0 (36.5/24.2%) in the first, second and third sampling respectively. The content of MUFA were around 13-14% in all samplings, and the main fatty acids were 16:1n-5 (5.3/4.3%), 18:1n-9 (2.3/2.0%) and 18:1n-7 (2.9/3.8%). The content of PUFA were around 20 % in the first sampling and over 30% in the second two samplings. The main PUFA were 18:3n-3 (5.8/9.0%) and 18:2n-6 (9.0/14.5%).

D. tertiolecta had around 30% SAT in the first sampling and a relatively high increase to over 80% in the second two samplings. The main SAT was 16:0 (29.6/73.8%) in the first, second and third sampling respectively. The content of MUFA were around 25% in the first sampling but decreased to 12% in the second two. The main fatty acids were 16:1n-14 (8.0/0.8%) and 18:1n-9 (9.3/4.4%). The content of PUFA were over 35% in the first sampling but decreased relatively low to under 3% in the second samplings. The main PUFA were 16:4n-3 (8.6/0.4%), 18:3n-3 (11.5/0.5%) and 18:2n-6 (6.0/0.8%).

The variability in the fatty acid content, especially seen in the *D. tertiolecta*, may be linked to the status of the microalgae culture. Though the culture had a stabile level of fatty acids (see Appendix F), the distribution changed from less MUFA and PUFA, to more SAT in the period between the algae sampling, suggesting that the culture of *D. tertiolecta* were about to crash.

Diet effect on *C. finmarchicus*

SAT, MUFA and PUFA in the animals were significantly affected by diet ($p = 0.032$, $p = 0.028$, $p = 0.004$, ANOVA). 14:0, 16:0 and 18:0 were the main SAT in all animals, but the level of each fatty acid was affected by the diet. For example, 14:0 was very low in animals fed *D. tertiolecta* which was devoid of this fatty acid while they were elevated in those fed *R. baltica*. In all cases, 16:0 was the main SAT, and the SAT content was around 40% of the fatty acids regardless of dietary origin.

The MUFA were rather low in all groups averaging (ca. 16% in animals fed *R. baltica,* and ca. 18% in animals fed *D. tertiolecta*), and the main fatty acids were 18:1n-9 and 22:1n-11. The animals also appeared not to incorporate 16:1n-7 despite it was found in both algal species in relatively high amounts.

The PUFA had a significant effect on *C. finmarchicus* fatty acid deposition. *D. tertiolecta* had high amounts of C_{16} PUFA, and these were also found in the *C. finmarchicus* fatty acids, but not in those fed *R. baltica* where these were absent. The animal fatty acids also showed clear signs of extensive elongation and desaturation of the major n-3 and n-6 PUFA. Animals fed *R. baltica* (9%) had relatively high levels of 18:2n-6 (around 6%), but also high amounts of the desaturation product 18:3n-6 (around 3%) and in particular 20:4n-6. Animals fed *D. tertiolecta,* which only had 6% of 18:2n-6, deposited some unchanged (ca 3.5%) and appeared to have little activity in elongation and desaturation to 18:3n-6 (0.5%) and 20:4n-6 (1%).

18:3n-3 was relatively low in *R. baltica* (5.8%), but the level tended to increase to around 9-10% in animals feeding on it. These animals also showed extensive elongation and desaturation to especially 18:4n-3 (ca 10%), and then 20:5n-3 (6%) and 22:6n-3 (ca 5%) despite being very low in the algae. *D. tertiolecta* did on the other hand contain 11.5% 18:3n-3, but this did not generate higher stores in *C. finmarchicus* (7.5%) and did also lower levels

of 18:4n-3 compared to animals fed *R. baltica*. The levels of 20:5n-3 and 22:6n-3 were also generally lower than those fed *R. baltica*. This was particularly true for 22:6n-3.

Temperature effect on *C. finmarchicus*

Temperature had a significant effect on the amount of SAT, MUFA and PUFA in the sampled CV animals ($p = 0.005$, $p = 0.025$, $p = 0.02$, ANOVA).

Animals maintained at 12 °C (over 40% with *R. baltica* diet, over 45% with *D. tertiolecta* diet) had a significantly higher level of SAT than the animals maintained at 14 °C (under 35% with *R. baltica* diet, over 35% with *D. tertiolecta diet*) ($p = 0.004$, Bonferroni). The main fatty acids were 16:0, which were around 21/16% with the *R. baltica* diet and around 30.0/23% with the *D. tertiolecta* diet. There was no significant difference between the animals in the 10-degree (over 35% with *R. baltica* diet, over 40 % with *D. tertiolecta* diet) and 12-degree groups, nor the animals in the 10-degree and the 14-degree groups.

Animals maintained at 14 °C (under 20 % with both diets) had a significantly higher percentage of MUFA than the animals maintained at 10 °C (under 15 % with the *R. baltica* diet, over 15 % with the *D. tertiolecta* diet) ($p = 0.027$, Bonferroni). The main fatty acid was 22:1n-11, which were around 8/4% with the *R. baltica* diet and 6/4.5% with the *D. tertiolecta* diet. There was no significant difference between the animals in the 10-degree and the 12-degree groups (around 16 % with both diets), nor the animals in the 12-degree and the 14-degree groups.

Animals maintained at 14 °C (over 45% with *R. baltica* diet, over 40% with *D. tertiolecta* diet) had a significantly higher percentage of PUFA than the animals maintained at 12 °C (over 40% with *R. baltica* diet, over 35% with *D. tertiolecta* diet) ($p = 0.027$, Bonferroni). The main fatty acid was 18:4n-3, which were around 10/1% with the *R. baltica* diet and 7/5% with the *D. tertiolecta* diet. Animals fed *D. tertiolecta* also had differences in 16:4n-3 between the 14- and 12-degree groups (around 7% and 5%). There was no significant difference between the animals in the 10-degree (over 45% with *R. baltica* diet, under 40% with *D. tertiolecta* diet) and 12-degree groups, nor the animals in the 10-degree and 14-degree groups. However, the animals in the 10-degree group showed trends to have a higher level than the animals in the 12-degree group ($p = 0.079$, Bonferroni).

Table 3.1. Fatty acid analysis of 30 CV animals from each treatment group (10 from each tank) (means mol % of TL ±SD, n=30). There were only conducted one sampling of algae (45 mL) before the sampling of CV animals. Two additional algae samples (45 mL) were done before the sampling of *C. finmarchicus* eggs (these samples are added together and given as a mean of the two). The fatty acid content within the animals can therefore only be seen relative to the fatty acid content in the first algae sample. The numbers displayed in the table show the percentage (mol %) of the total fatty acid content.

FATTY ACIDS	*R. BALTICA* BEF. CV SAMP.	*R. BALTICA* BEF. EGG SAMP.	R10	R12	R14	*D. TERTIOLECTA* BEF. CV SAMP.	*D. TERTIOLECTA* BEF. EGG SAMP.	D10	D12	D14
SAT										
C14:0	23.1	19.2±4.9	12.9±1.5	13.4±3.7	12.1±0.4	0.8	1.6±0.2	5.0±1.3	4.2±1.7	5.5±1.3
C16:0	36.5	24.2±1.4	17.7±1.9	20.9±2.7	16.5±1.1	29.6	73.8±0.7	27.2±3.9	30.7±5.2	23.5±2.5
C18:0	2.0	1.5±0.4	4.5±0.9	5.9±1.0	3.9±0.6	2.8	5.0±0.2	8.1±2.2	10.3±2.9	5.8±1.0
MUFA										
C16:1N-14	1.3	2.6±0.7	0.2±0.2	0.2±0.1	-	8.0	0.8±0.2	0.9±0.1	0.8±0.1	1.1±0.1
C16:1N-5	5.3	4.3±0.5	0.2+0.2	0.3±0.1	0.2±0.1	2.1	4.6±0.1	0.1±0.1	0.1	0.1±0.1
C18:1N-9	2.3	2.0±0.8	3.4±0.2	4.1±0.5	3.7±0.2	9.3	4.4±0.7	6.7±0.1	7.0±0.4	6.9±0.4
C18:1N-7	2.9	3.8±1.2	0.7±0.2	0.8±0.1	0.7±0.1	3.5	1.7±0.3	1.2±0.1	1.2±0.1	1.1±0.1
C22:1N-11	0.2	-	4.0±0.7	6.0±0.5	8.1±0.8	-	-	4.5±1.8	3.5±1.9	5.9±2.0
PUFA										
C16:4N-3	-	-	-	-	-	8.6	0.4	5.8±1.2	5.3±1.8	7.1±1.0
C18:3N-3	5.8	9.0±5.2	9.1±0.4	10.3±0.1	9.2±0.7	11.5	0.5±0.2	7.5±0.8	7.3±0.8	8.3±0.9
C18:4N-3	2.2	4.1±2.6	11.5±0.6	1.0±0.4	10.4±1.2	4.4	-	5.5±1.1	4.7±1.2	7.0±1.4
C20:5N-3 (EPA)	0.5	1.2±1.0	6.6±0.3	7.5±0.2	5.6±0.1	2.4	-	5.5±0.4	5.8±0.8	5.4±0.9
C22:6N-3 (DHA)	0.5	1.0±1.0	6.0±0.1	7.3±0-9	4.6±0.2	0.2	0.3±0.1	3.0±0.3	3.5±0.8	2.5±0.3
C16:3N-6	-	-	-	-	-	1.9	0.2±0.1	1.6±0.1	1.5±0.5	1.6±0.2
C18:2N-6	9.0	14.5±1.3	5.6±0.5	6.4±0.1	6.1±0.5	6.0	0.8±0.3	3.7±0.2	3.7±0.2	3.5±0.4
C18:3N-6	0.9	1.6±0.4	2.7±0.1	3.0±0.3	2.8±0.2	0.9	0.4±0.1	0.5±0.1	0.5±0.1	0.6±0.2
C20:4N-6	0.4	0.8±0.1	2.7±0.1	3.2±0.1	2.6±0.1	0.6	0.1±0.1	1.0±0.1	1.1	1.1±0.1
TOTAL SAT	63.1	46.0±6.7	36.7±1.0	42.1±0.4	34.3±1.8	33.7	82.2±2.1	42.1±5.0	47.0±6.8	36.9±3.3
TOTAL MUFA	13.3	14.5±1.8	13.0±0.8	16.5±1.6	18.1±1.0	24.6	12.6±1.4	18.0±2.6	16.6±2.8	19.8±1.9
TOTAL PUFA	20.8	32.4±7.9	46.0±0.7	40.1±1.8	45.5±1.5	36.7	2.8±0.8	38.9±3.3	35.7±4.7	41.4±4.6

3.3.2 Total lipid content in CV animals

A test between-subject effects in the Two-Way ANOVA showed that both temperature ($p = 0.03$) and diet ($p = 0.01$) had a significant effect on the amount of lipid found in each CV animal (Figure 3.6.). There was however no interaction between the two (temperature*diet). Animals fed *R. baltica* had significantly higher lipid levels than those fed *D. tertiolecta* ($p = 0.001$, ANOVA). Animals maintained at 14 °C had significantly higher lipid levels than those maintained at 12 °C ($p = 0.039$, Bonferroni), but not significantly higher than those maintained at 10 °C. It was no significant difference between the animals in the 10- and 12-degree groups.

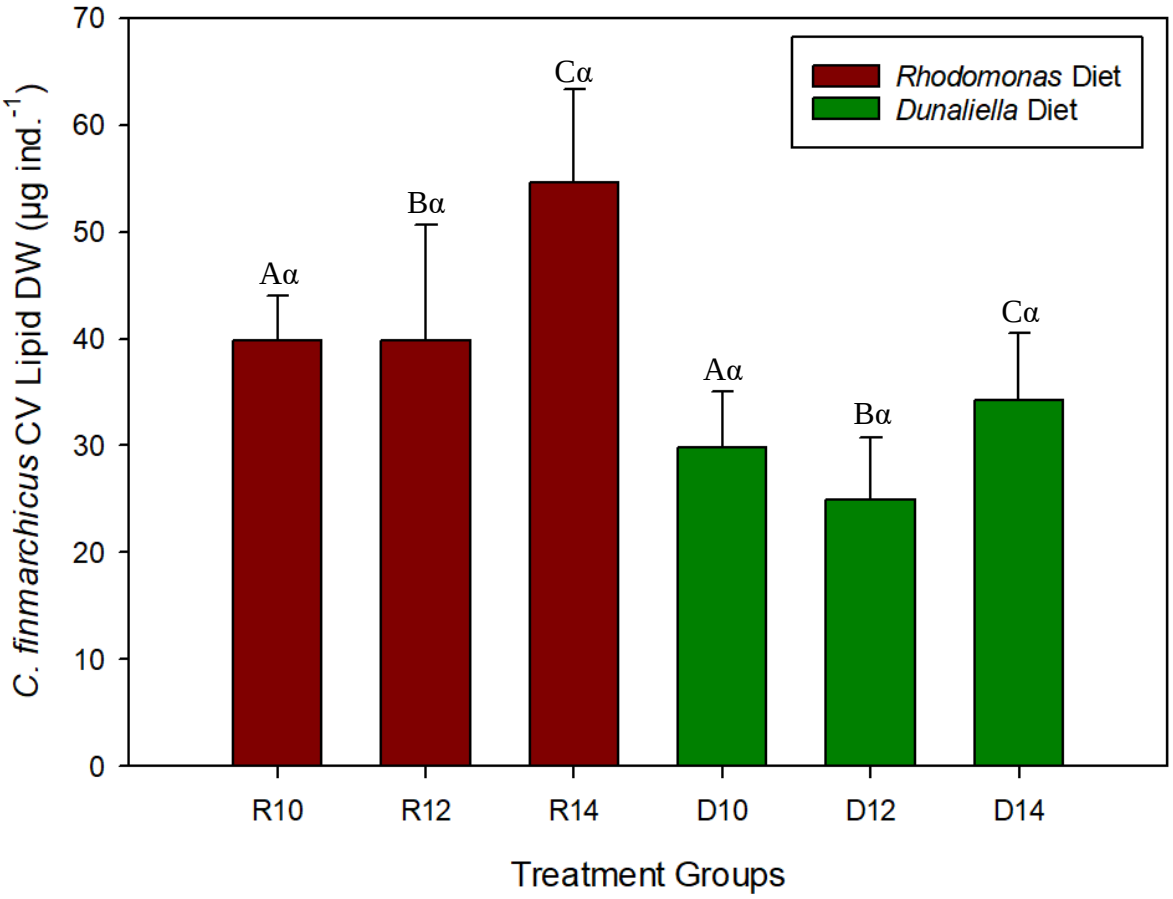

Figure 3.6. Mean lipid dry weight (DW) per individual, measured from 30 CV animals from each treatment group (10 animals from each tank) (means ±SD, n=30). Measurements were done before the lipid extractions, separation, and GC analysis. Significant differences between algae diet treatments maintained at the same temperature is indicated with uppercase Latin letters, while significant differences between temperature treatments within each algae group is indicated with lowercase Greek letters.

3.4 Survival rate

A test between-subject effects in the Two-Way ANOVA showed that diet had a significant effect on the survival in the tanks ($p = 0.001$), but that there was no effect from temperature nor the interaction between the two (temperature*diet) (Figure 3.7.). Animals fed *R. baltica* (around 60 % survival) had a significantly higher survival than of those fed *D. tertiolecta* (around 40 % survival) ($p = 0.001$). There were not significantly differences between the temperature treatments within either the *R. baltica* group or the *D. tertiolecta* group.

Both females and males were counted, and as predicted from Marshall and Orr (1972), the majority of *C. finmarchicus* where females, as the males moult earlier and have a shorter lifespan than the females (see Appendix G).

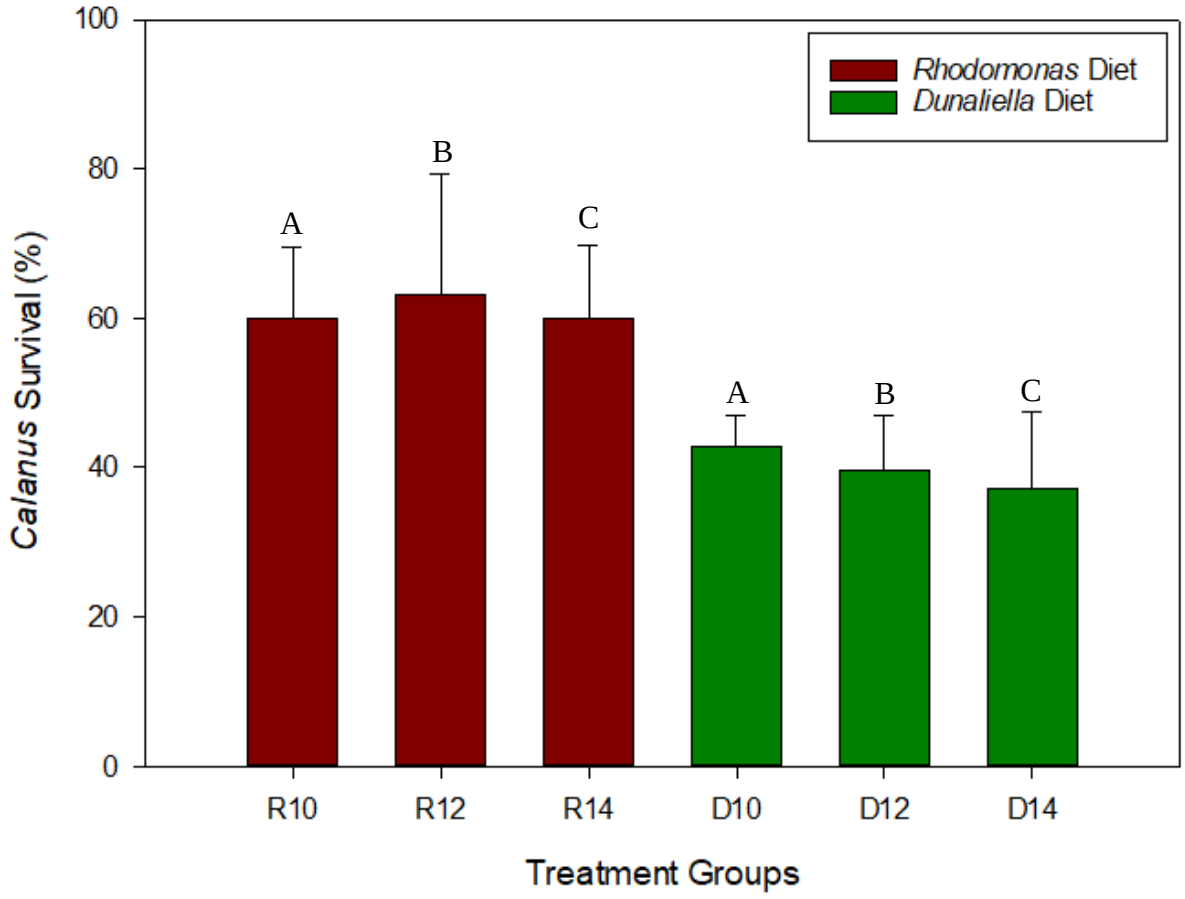

Figure 3.7. Mean survival rate in the treatment groups after the sampling of eggs (three replicas of each treatment group) (means ±SD, n=3). There were no significant differences between the temperature treatment groups. Significant differences between algae diet treatments maintained at the same temperature is indicated with uppercase Latin letters.

3.5 Egg production and hatching

3.5.1 Eggs laid per female

Egg production data were not normally distributed. Consequently, the data were analysed using a non-parametric test. Due to significant variation between data, it appeared that neither temperature (p = 0.459, Kruskal-Wallis) nor diet (p = 0.310, Kruskal-Wallis) had any effect on the egg production in the female *C. finmarchicus* (Figure 3.8.). The replicas for the different treatment groups also varied. For example, in the D14 group, were D14A laid 0.6 eggs per female, D14B laid 0.0 egg per female, and D14C laid 6.3 egg per female. A graph showing eggs per female in all rearing tanks can be seen in Appendix H.

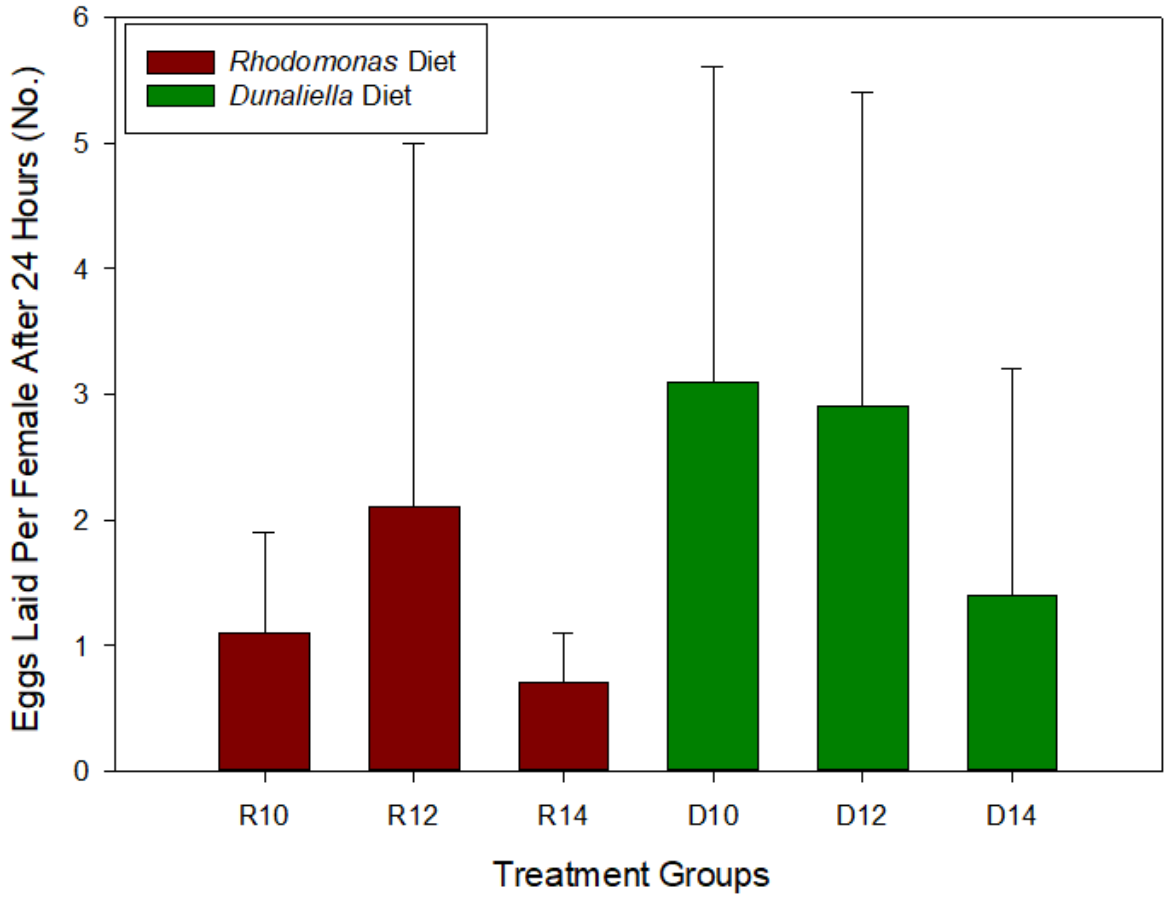

Figure 3.8. Mean number of eggs produced per female over a period of 24 hours by the *C. finmarchicus* in each treatment group (three replicas of each treatment group) (means ±SD, n=3). Data was not normally distributed and were analysed using a non-parametric test. There was no statistically significant effect from diet nor temperature on the produced eggs, due to significant variation between data

3.5.2 Hatching success

As for egg production, hatching data sorted by temperature groups were not normally distributed, and a non-parametric test was used to analyse the data. Neither temperature nor diet showed a statistically significant effect on the hatching success of the *C. finmarchicus* eggs (Figure 3.9.). However, the analysis showed that both temperature (0.081, Kruskal-Wallis) and diet (0.085, ANOVA) trended to affect the hatching.

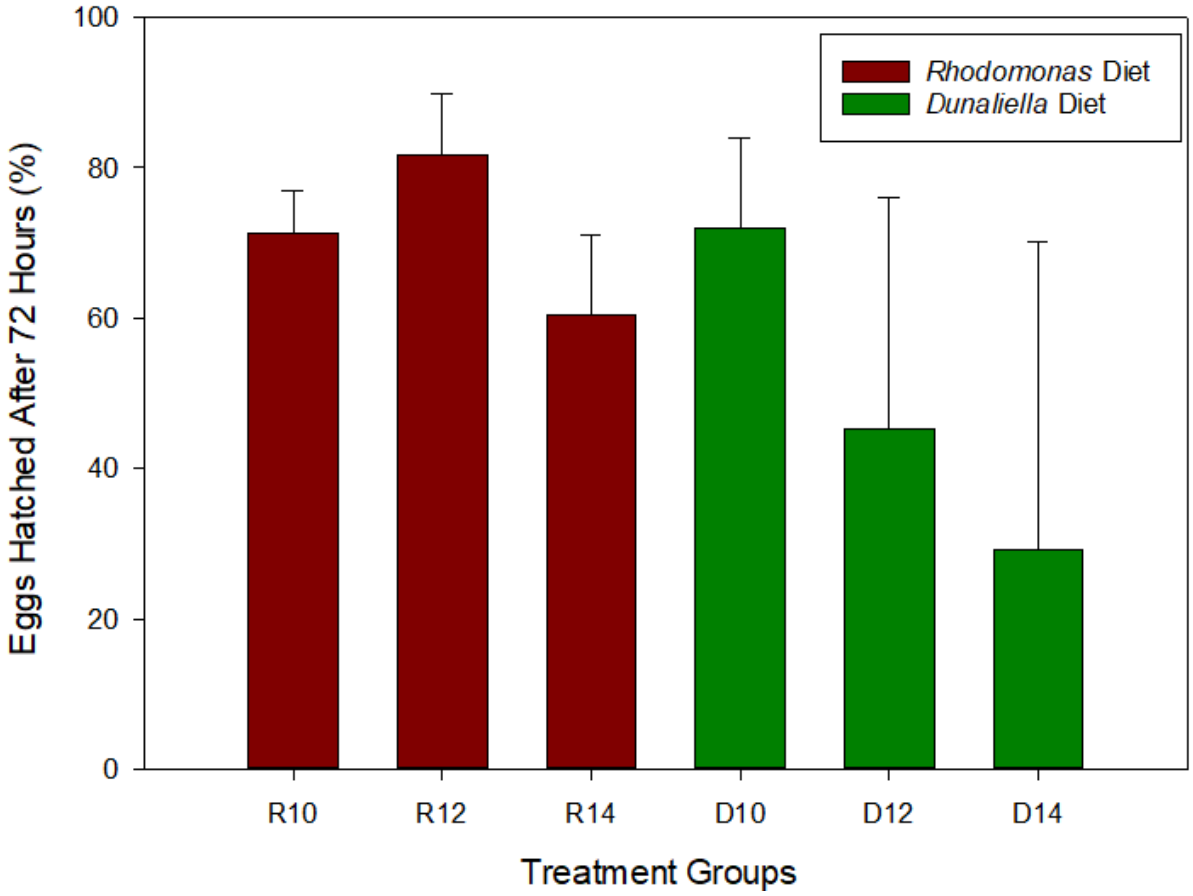

Figure 3.9. Percentage of hatched eggs laid by the *C. finmarchicus* in the different treatment groups (three replicas of each treatment group) (means ±SD, n=3). The incubation was done over a period of 72 hours in incubation cabinets holding the same degree (10/12/14 °C) as the tanks from which the eggs was laid in. There were no significant differences between diet treatment groups or temperature treatment groups. However, there were trends that suggested differences in both diet treatment groups and temperature treatment groups.

4. Discussion

The results are seen in context with the measured temperature and microalgae concentrations in the rearing tanks over time and demonstrate the effect of temperature and diet on prosome size, fat sac development, egg production and hatching success.

4.1 Treatment effects on *C. finmarchicus*

4.1.1 Effects from water temperature

It was expected that increasing water temperature would have a negative impact on the prosome size of the animal, causing animals maintained at higher temperatures to become smaller than animals maintained at lower temperatures. This is based on results from other studies (Marshall and Orr, 1972; Campbell *et al.*, 2001; Krause and Radach, 1993). It was also expected that the fat sac size would be smaller relative to the prosome at higher temperatures due to higher metabolism and energy acquirement (Ikeda *et al.*, 2001). A third expectation was that there would be less eggs per female from animals maintained at higher temperatures (Pasternak *et al.*, 2013; Campbell *et al.*, 2001; Pedersen and Hanssen, 2018), and that the hatching success would not be affected by the temperature (Preziosi and Runge, 2014; Weydmann *et al.*, 2015). Other expectations were that increasing water temperature would have a positive effect on the growth rate and development time (Campbell *et al.*, 2001), and that the animals maintained at higher temperatures would have to be earlier than those at maintained at lower temperatures, because of this rapid development.

It was therefore surprising that animals maintained at higher temperatures were larger (both in length and volume) and had a larger fat sac than of those maintained at lower temperatures. It was theorised that the animals maintained at higher temperatures grew closer to their maximum potential (Campbell *et al.*, 2001). However, the theory would not explain the high accumulation of lipids that should have been lower than the lipid levels of the lower temperature treatments, due to a higher metabolism (Figure 3.6.). Also, all animals were sampled at around the same time of development, thus all tanks should have grown to the same potential. Another possibility is that the surprising results may be linked to the fact that many of the 10- and 12-degree groups had several measurements of low algae concentration (under 50 µg C L^{-1} from the nominal value) in the period before the CV sampling. The 14-degree groups on the other hand either had relatively balanced numbers (R14 group) or high

concentration of algae (over 50 µg C L^{-1} from the nominal value) (D14 group). This means that the 14-degree groups would have more resources to grow than the animals in the 10- and 12-degree groups. Comparing the size of the animals with the animals measured in Campbell *et al.* (2001) maintained at the same temperature (12 °C), where the concentration of food was much higher (500 µg C L^{-1}), shows that the animals in the present study were smaller. Hygum *et al.* (2000) found that *C. finmarchicus* grew significantly larger with an enhanced food resource. This may confirm the theory of decreased size due to low food availability. The theory would also explain the higher accumulation of lipids by the 14-degree group, compared to the 10- and 12-degree groups (Figure 3.6). Mayor *et al.* (2015) found that animals maintained at high temperatures had an upregulated metabolism of lipids and proteins when the food availability were poor. This might also explain why the 10- and 12-degree groups were both smaller in size than the 14-degree groups and had lower levels of lipids stored. As for the fat sac size, Pedersen and Hanssen (2018) found that it is negatively affected by increasing temperature. Consequently, a clear effect from temperature cannot be seen in the present study, and it is therefore not possible to prove the hypothesis regarding prosome (H1) and fat sac size (H2).

It was expected to find a representative number of eggs in all treatment groups by sampling from the whole tanks. The low egg production, and the big variety between the replicas, made it impossible to test the data statistically. Appendix H gives a good illustration on this variability. It was theorized that the brief periods of high temperatures in some of the tanks might have made the males sterile. But the egg production was not directly related to these instances, and there is no literature data to support this as a cause. It is also possible that tanks with low number of eggs lacked males. However, comparing Appendix G and H contradicts this as a possibility. A third possibility could be a delayed of egg laying caused by some environmental factors such as low food availability after the cleaning of the tanks. Again, this does not explain the difference in egg production between the treatment groups and the replicas. Also, Marshall and Orr (1972) stated that females were observed laying their eggs within a day after handling, but had no explanation why. A fourth possibility is that the sampling was done in a period of egg production, as wild females wild tend to lay their egg in a series of bursts, with a couple of weeks between the bursts to produce new eggs (Marshall and Orr, 1972).

Since adult females were observed already at the CV sampling (and assuming that the majority of males moulted first), it is possible that the sperm from the males were less viable

than at an earlier period as suggested by (Marshall and Orr, 1972) and that the sampling of eggs were done too late. Egg production is relatively low in all the rearing tanks in the present study compared to egg production in other studies, like in Pasternak *et al.* (2013) when animals maintained at 10-degrees produced between 10-20 eggs per female, or in Campbell *et al.* (2001) were animals maintained at 8-degrees produced between 40-50 eggs per female. On the other hand, Campbell suggested that egg production rate (EPR) decreased with increasing temperature, and this suggestion is also supported by Pedersen and Hanssen (2018). Pasternak *et al.* (2013) on the other hand suggested that EPR increased with temperature under favourable feeding conditions, though this is not the case with the data in the present study. An explanation could therefore be that the level of food available was too low to support the relatively high temperatures, hence causing low production. Although animals fed *D. tertiolecta* seems to have produced more eggs with less food, compared to the animals fed *R. baltica*. Additionally, as seen in Figure 3.6., animals from all treatments were able to store lipids, hench they had energy and resources to both grow and produce eggs. Though Figure 3.8. may indicate a decrease in egg production as temperature increase, the data is not sufficient to draw any conclusions, hence making it impossible to conclude the hypothesis regarding egg production (H3).

As for the production of eggs, the hatching success (HS) was also difficult to test statistically. As expected, the non-parametric test did not show any statistical significance between the temperature groups, but the big variance in sample size due to low egg production makes the results less valid and therefore impossible to prove the hypothesis regarding hatching success (H4). On the other hand, the test did show trends of difference between the temperature groups that would have been interesting with a complete data set. As mentioned in the discussion about production of eggs, the time of sampling might have been late. Marshall and Orr (1972) suggested that the egg was less likely to hatch due to less healthy eggs and/or poorer viability of the sperm. Weydmann *et al.* (2015) found that there was no significant effect from temperature on HS of *C. finmarchicus* eggs. On the other hand, Pedersen and Hanssen (2018) found that increasing temperature affected the HS negatively (10-14 °C).

Mayor *et al.* (2015) stated that *C. finmarchicus* are adapted to survive within a “thermal window”. It is possible that the animals in the stock culture (maintained at 10 °C) over the years have adapted to higher temperatures, compared to wild *C. finmarchicus*. In a study mentioned by Marshall and Orr (1972) it was suggested that summer generations were more tolerant to higher temperatures than the winter generations, hence suggesting that the summer

generation adapted to a warmer environment. In that case, the temperature range in the present study might have been too small to make any significant effect. This could be a possible explanation for the surprising results on the effects of elevated temperatures.

4.1.2 Effects from microalgae diet

Hypothetically, in two tanks with exact 200 µg C L^{-1} with the algae (one with *R. baltica* and one with *D. tertiolecta*), it would be less *D. tertiolecta* than *R. baltica* due to the difference in carbon content per cell. Additionally, the *D. tertiolecta* is a smaller alga compared to *R. baltica*, and with these two factors added together, it is safe to assume that the density of algae in the tank with *D. tertiolecta* is lower than the tank with *R. baltica*, making it harder for the *C. finmarchicus* to graze on. This led to the expectation that the animals fed the *R. baltica* would perform better on all the analysis (prosome size, fat sac development, egg production and the hatching success) than those fed *D. tertiolecta*, due to less available food. Less food would possibly also lead to lower levels of lipids (most importantly fatty acids), both because it's less algae in the tank, and because that the animals would have to use more energy to find food. The content of fatty acids in the animals was expected to be affected by the content of fatty acids in the algae diet (Bergvik *et al.*, 2012).

As expected, the animals fed *D. tertiolecta* seem to have stored less lipids in general and in relation to body size, compared to the animals fed *R. baltica*. Additionally, the animals fed *R. baltica* seem to have had greater success in elongating the fatty acids. This difference in stored energy is also reflected on size, especially volume of the animal prosome (Figure 3.4.). This might be explained by less food availability for the animals fed *D. tertiolecta*. It is well known that more available food, lead to more energy to grow on and more energy to store as lipids (Hygum *et al.*, 2000; Skottene *et al.*, 2020; Campbell *et al.*, 2001). Mayor *et al.* (2009) found that *C. finmarchicus* catabolise protein in higher rates when food availability was low to meet their metabolic demands, to produce eggs. Niehoff (2004) found that the animals catabolise carbon (C) and nitrogen (N) to produce eggs when food availability is low. This would also explain the difference in size (both length and volume) between the two diet groups. In that case it would be likely that the animals in the present study fed *D. tertiolecta* utilized proteins as energy to survive, as well as C and N as resources to produce eggs. However, as already discussed, all sampled animals were able to store lipids, hence they had energy to spare and resources to produce egg, though the animals fed *R. baltica* stored more

than those fed *D. tertiolecta*, relative to the prosome size (Figure 3.5.). It is therefore unlikely that the theories from Mayor and Niehoff applies to the animals in the present study.

As already discussed, the big variety between the replicas, made it impossible to test the data on egg production statistically. The effect from diet on egg production is therefore inconclusive. Pasternak *et al.* (2013) found that *C. finmarchicus* produced more eggs when food availability was high, though Marshall and Orr (1972) stated that the relationship between food quantity and egg production is not a linear one. Nevertheless, it is not the case in the present study. It is also important to remember that the egg production from the wild *C. finmarchicus* (winter generation) is done by starved animals (Marshall and Orr, 1972), although in lesser numbers. However, Lee, Hagen and Kattner (2006) stated that *C. finmarchicus* must feed to produce large amounts of eggs and that the egg production is highly depended on phytoplankton blooms. They also found that a wild female with a total of 105 µg of lipid in her fat sac had transferred around 42 µg and divided it between 600 eggs (0.07 µg per egg), suggesting that around 7 % of the total lipid in the fat sac were used to produce 100 eggs. If that is the case, the animals in the present study should have been able to produce more eggs per female than what was registered based on the lipids measured of the CV animals.

The level of wax esters (WE) in herbivorous copepods are often characterised by the amount of the monounsaturated fatty acid 22:1n-11, which is not usually found in great levels in phytoplankton. As seen in Table 3.1., the animals fed *R. baltica* seem to have synthesised more WE than those fed *D. tertiolecta*. That means that the *R. baltica* group not only had a higher amount of lipids, but also a larger distribution of WE. This also suggest a higher production of eggs, which is not observed in the present study.

The animals fed *R. baltica* seem to have a higher survival than the animals fed *D. tertiolecta*. This may indicate that the concentration of algae at a point was too low to sustain that many animals, and/or that the nutritional value of the *D. tertiolecta* was too poor for some of the animals to survive on. On the other hand, relatively many animals survived within both diet groups, so the reason why some animals would perform worse is unclear. A possibility is that there were moulted a greater number of males in the tanks fed *D. tertiolecta*, and as mentioned by (Marshall and Orr, 1972), the males lives shorter lives than the females. The reason why this could/would happen can possibly be explained by the nutritional value of the diet, but no literature was found to support this theory.

Compared to the studies listed by Miller *et al.* (1998) on lipid content from sampled CV animals in the summer months, the lipid content from the animals in the present study are relatively low in both diet groups. Miller did several samples over the course of several months (46-341 µg ind.$^{-1}$ (April), 35-334 µg ind.$^{-1}$ (May), 48-332 µg ind.$^{-1}$ (June), 37-258 µg ind.$^{-1}$ (July)). The level of lipid from the animals in the present study only compares to the lowest samples measured by Miller.

Jonasdottir *et al.* (2002) suggested that low HS could be caused by low food quality. In the present study we might see tendencies of a mixed effect between increasing temperature and low food availability, as the HS of the animals fed *D. tertiolecta* seem to decrease with temperature. This tendence is not observed with the animals fed *R. baltica*. This is still only speculations, as the data is too incomplete to draw any conclusions.

Skottene *et al.* (2020) found that the animals in the stock culture compared to wild animals sampled in Trondheimsfjorden, had an upregulation of a protein (FABP) connected to fatty acid binding during periods of rapid lipid synthesis. Additionally, animals from the stock culture had an upregulation of lipid catabolism. This information also substantiates the fact that the stock culture might have adapted in other ways as vell, including tolerance of higher water temperatures, as discussed earlier. It is therefore important to point out that the results acquired from cultivated animals might not be representative for wild *C. finmarchicus*.

There were no major differences in the distribution of the major phospholipids 16:0, 22:6n-3 (DHA) and 20:5n-2 (EPA) in the animals, though a statistical analysis on each fatty acid was not conducted. As suggested by Lee, Hagen and Kattner (2006), the distribution of these lipids is high independent of the diet (Table 3.1.). These phospholipids are especially important for the structure and function in the cell membrane, thus important for the growth and development of the animal, as well as for development of tissue and gonads. As suggested in other studies, the significantly larger animals (*R. baltica* group) had higher levels of lipids than the smaller animals (*D. tertiolecta* group) (Gatten *et al.*, 1980; Hygum *et al.*, 2000; Miller, Crain and Morgan, 2000).

4.2 Methodological consideration

4.2.1 Rearing of *C. finmarchicus*

It proved to be difficult to maintain a constant temperature regime and algae concentration in several of the tanks. The aeration and the thermostat had to be adjusted frequently to lower or raise the temperature in one or several tanks that had gone above or belove 0.5 °C of the nominal temperature value (10 °C, 12 °C or 14 °C). This problem was mainly observed in the 12- and 14 -degree tanks, and what separated these tanks from the 10-degree tanks was the source of heating. It is possible that the heating elements did not provide a strong enough heating effect in a 50 L tank, and that the room temperature had a greater influence on the 12- and 14-degree tanks because of it. Another possibility is that the aeration in each tank was connected to the same source, thus effecting all tank when meaning to adjust only one (e.g., one tank had its aeration elevated because of hight temperatures, the other tanks would have their aeration lowered, causing temperature in those tanks to increase). A suggestion to others is to use "heating bandages" around the tank, instead of aquarium heaters, to prevent temperature disturbance from the room and to better distribute the temperature.

It was also noticed that animals in the start of the experiment were sucked into the supporting mesh due to the flow of water caused by the aeration from within the supporting mesh. No casualties were observed because of this, but it is unknown if this had any effect on any of the results from the rearing experiment. The problem was solved by lowering the aeration in the tanks.

4.2.2 Sampling of eggs

Originally it was planned to construct a micro flow system to hold individual females and sample eggs per individual. The system was constructed and tested on a few animals, but no eggs were observed in the following days. Since eggs were observed in the rearing tanks, it was therefore decided to collect eggs from the whole tanks, using the method describes in section 2.3.2. It was believed that by extracting eggs this way, it would be enough data on eggs to give viable results on both egg production and hatching. As seen in Appendix H, some of the tanks provided few or even zero eggs. A suggestion for other is to extend the sampling interval from one to at least three samplings (3x 24h), to possibly get more data on egg production, and more samples to hatch.

4.2.3 Extraction of lipids and analysis on gas chromatograph

The original plan was to analyse both fatty acids and fatty alcohol from the lipid extracted from the CV animals. The extraction of the lipids (as well as the separation of fatty acids, fatty alcohol, and cholesterol) went according to plan. But the level of fatty alcohols that was analysed proved to be too low to give any valid results. It was decided to not redo the extraction and the separation, since the main data of interest was in the extracted fatty acids.

4.2.4 The Covid-19 pandemic

Originally it was planned to start the experimental part of the study in August 2020, with good time to redo the experiment before Christmas break, should anything go wrong or if more data was needed. After the experimental part of the present study, a co-student was to start his experimental part with the same flow-through system and technical equipment. National restrictions regarding the Covid-19 pandemic postponed the original start date until November the same year, preventing any chances of redoing the experiment. This was also the reason why more eggs were not sampled after realising the differences between replicas.

5. Concluding remarks

Elevated water temperature affected the targeted species *C. finmarchicus* at a various degree. Prosome size as well as the fat sac size (both in general and relative to the prosome size) seem to increase significantly with temperature, though these results are contradicted by other studies. The explanation of these surprising results may lie in the concentration of food available to the animals in the time before sampling and measuring. Animals fed *R. baltica* performed better than animals fed *D. tertiolecta* on several of the analysis, being both significantly longer and larger, as well as having significantly more lipids (both in general and relative to the prosome size). The data on egg production and hatching did not show any statistical differences between the treatment groups (both diet and temperature), most likely because of high variety in data. However, animals fed *R. baltica* as well as animals maintained at lower temperatures showed trends of having a higher hatching success than those fed *D. tertiolecta* and those maintained at higher temperatures. Nevertheless, further research is needed to clarify the long-term effect of temperature and dietary change, as climate effects, on the species *C. finmarchicus*.

5.1 Future perspectives and suggestions

It's encouraged to conduct similar experiments in bigger scale, with a wider temperature range, several more measurements over time, and a cultivation of *C. finmarchicus* over several generations. Additionally, it would be interesting to sample wild animals to compare effect from temperature and diet. That may provide important new information regarding the effect of climate change and rising ocean temperatures on *C. finmarchicus'* different physiological processes and ability to survive.

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Appendix A – Conwy medium nutrient content

Table A.1. The nutrient content of the Conwy medium used to feed the algae *R. baltica* and *D. tertiolecta* is listed below. The algae were used as food for the *C. finmarchicus* during the rearing experiment. The medium was added to the cultivation flasks after daily harvesting and refilling of filtrated seawater. 1.55 ml Conwy medium was used per litre seawater.

Algal nutrient stock solution – content per 1000 ml:

$FeCl_3 \cdot 6H_2O$	1.30 g
$MnCl_2 \cdot 4H_2O$	0.36 g
H_3BO_3	11.2 g
Na-EDTA	30.0 g
$NaH_2PO_4 \cdot H_2O$	20.0 g
$NaNO_3$	100.0 g
$ZnCl_2$	0.0211 g
$CoCl_2 \cdot 6H_2O$	0.0200 g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.0900 g
$CuSO_4 \cdot 5H_2O$	0.0200 g
Thiamin HCL	0.10 g
Cyanocobalamin (B_{12})	0.005 g
Deionised water to 1000 ml	-

Appendix B – Photograph of nauplii and egg (*C. finmarchicus*)

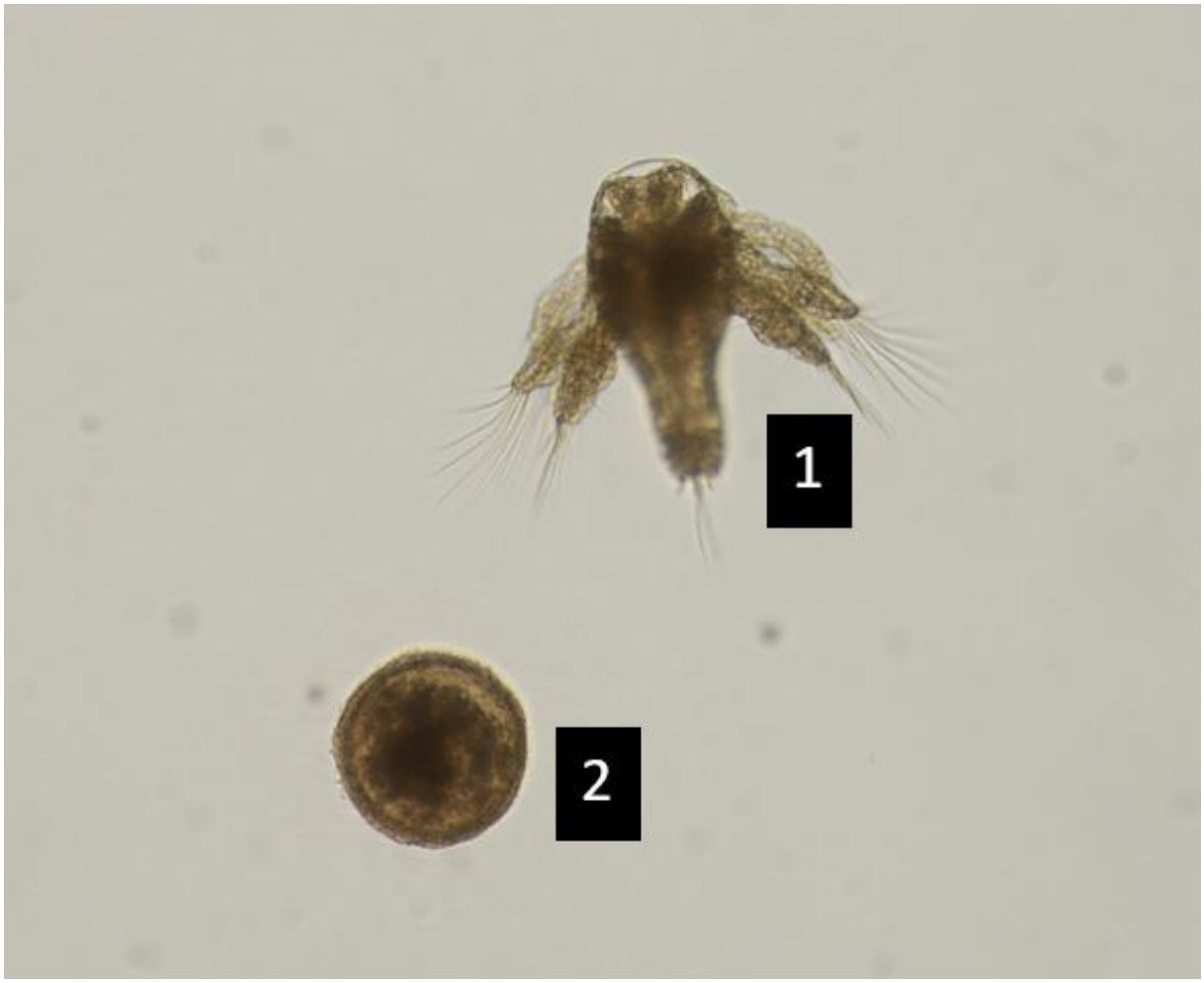


Figure A.1. The picture shows a *C. finmarchicus* hatched nauplii (NI) (1) and an unhatched egg (2). The dark yellow colour on the nauplii and egg is due to the collar of the fixation chemical Lugol's iodin (I_3K).

Appendix C – Size measurement specifications

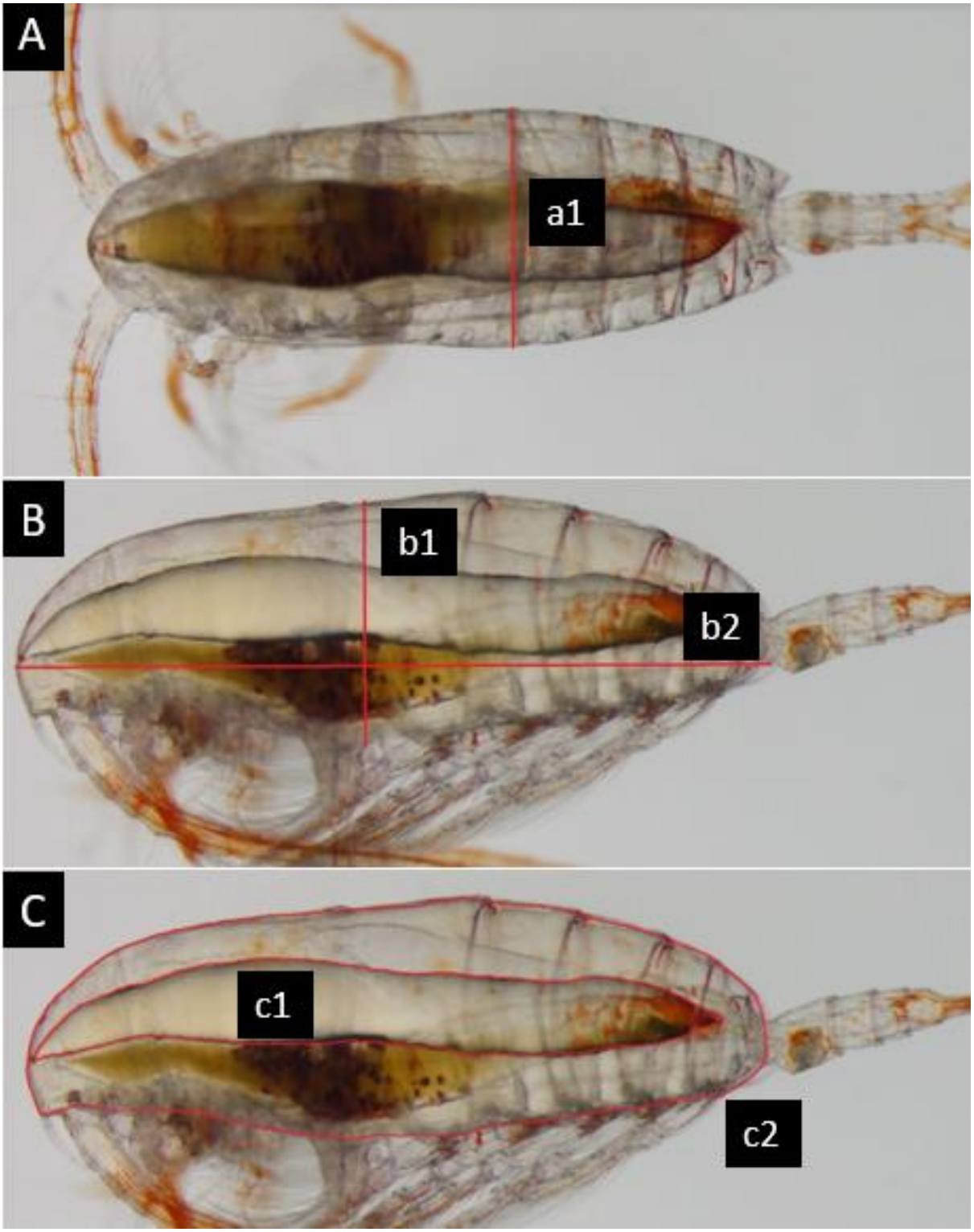


Figure A.2. Picture A shows with a red mark how the prosome width (a1) was measured on the animals. Picture B shows how the prosome height (b1), and length (b2) was measured on the animals. Picture C shows how the fat sac area (c1), and the prosome area (c2) was measured on the animals. Length, height, and width of the prosome was measured at the largest respective distance.

Appendix D – Water temperature measurements

Table A.2. Measured mean water temperatures (°C) with SD in each treatment tank during the rearing experiment of *C. finmarchicus*. The temperature water temperature was measured every day during the experiment.

TANK	NOMINAL TEMPERATURES (°C)	MEASURED TEMPERATURE (°C)
R10A	10	10.1±0.2
R10B	10	10.1±0.2
R10C	10	10.2±0.2
R12A	12	11.9±0.2
R12B	12	12.2±0.3
R12C	12	12.0±0.3
R14A	14	12.2±0.4
R14B	14	14.5±0.4
R14C	14	14.1±0.3
D10A	10	10.1±0.2
D10B	10	10.1±0.2
D10C	10	10.0±0.2
D12A	12	11.9±0.3
D12B	12	12.0±0.3
D12C	12	11.8±0.3
D14A	14	14.0±0.3
D14B	14	14.3±0.6
D14C	14	14.6±0.5

Appendix E – Algae concentration measurements

Table A.3. Measured mean algae concentration (C L^{-1}) with SD in each treatment tank during the rearing experiment of *C. finmarchicus*. The algae concentration was measured every day during the experiment.

TANK	NOMINAL ALGAE CONSENTRATION (C L^{-1})	MEASURED ALGAE CONSENTRATION (C L^{-1})
R10A	200	147±16
R10B	200	143±15
R10C	200	145±15
R12A	200	137±13
R12B	200	161±22
R12C	200	178±19
R14A	200	167±18
R14B	200	192±70
R14C	200	130±20
D10A	200	141±26
D10B	200	149±24
D10C	200	199±44
D12A	200	138±33
D12B	200	131±28
D12C	200	159±35
D14A	200	186±87
D14B	200	133±28
D14C	200	135±42

Appendix F – Total lipid in microalgae

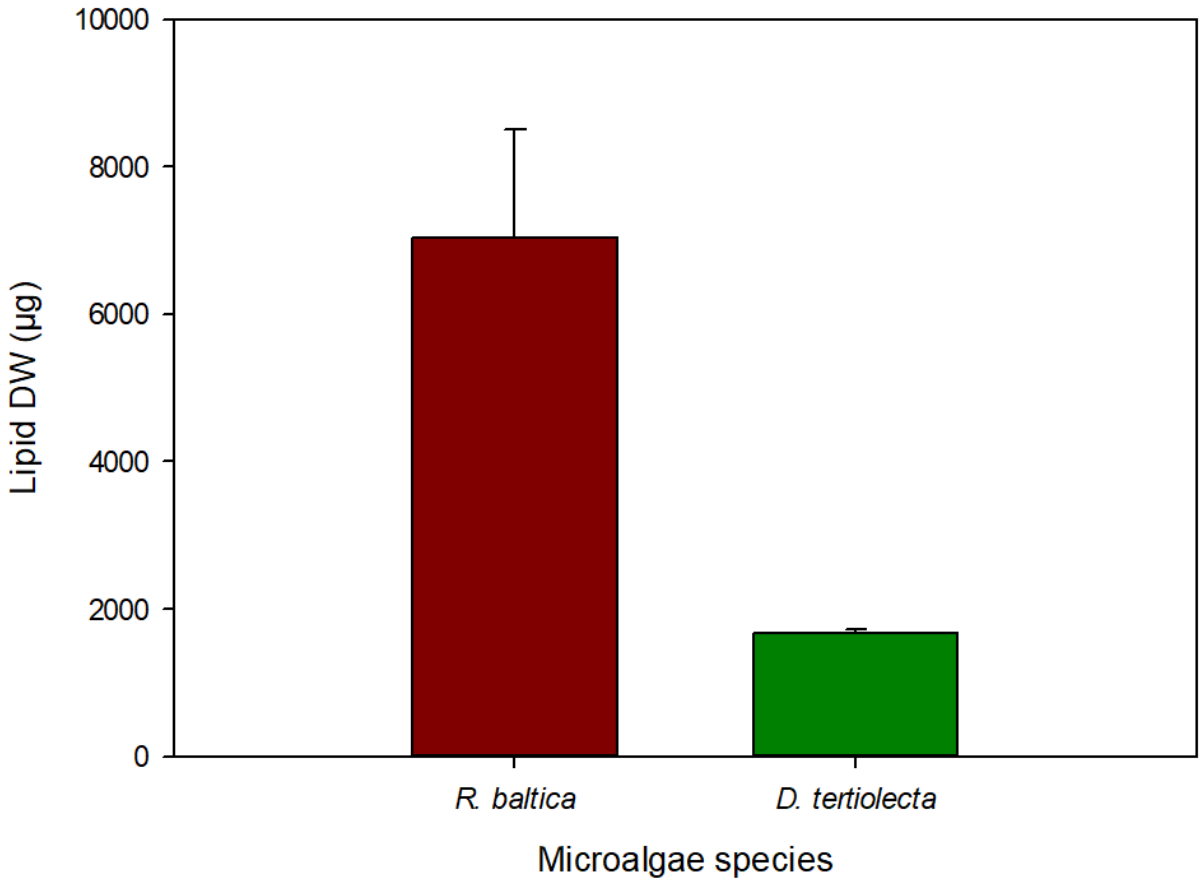


Figure A.3. Mean total lipid (±SD, n=3) in 45 mL samples of the microalgae *R. baltica* and *D. tertiolecta* used as a diet for *C. finmarchicus* during the rearing experiment.

Appendix G – Survival rate

Table A.4. Registered survival of *C. finmarchicu*s in each rearing tank. After the sampling of eggs, the surviving *C. finmarchicus* was scooped out, fixated, and counted.

TANK	SURVIVAL (NR.)	SURVIVAL (%)	MALES (%)	FEMALES (%)
R10A	150	50.0	3.3	96.7
R10B	183	61.0	4.9	95.1
R10C	207	69.0	3.8	96.2
R12A	225	75.0	1.3	98.7
R12B	209	69.6	4.8	95.2
R12C	134	44.6	0.7	99.3
R14A	199	66.3	1.5	98.5
R14B	146	48.6	3.4	96.6
R14C	195	65.0	3.1	96.9
D10A	134	44.6	13.4	86.6
D10B	137	45.6	16.1	83.9
D10C	113	37.6	8.0	92.0
D12A	97	32.3	3.1	96.9
D12B	118	39.3	6.7	93.3
D12C	141	47.0	9.2	90.8
D14A	99	33.0	3.0	97.0
D14B	147	49	5.4	94.6
D14C	89	29.6	3.4	96.6

Appendix H – Egg production in *C. finmarchicus*

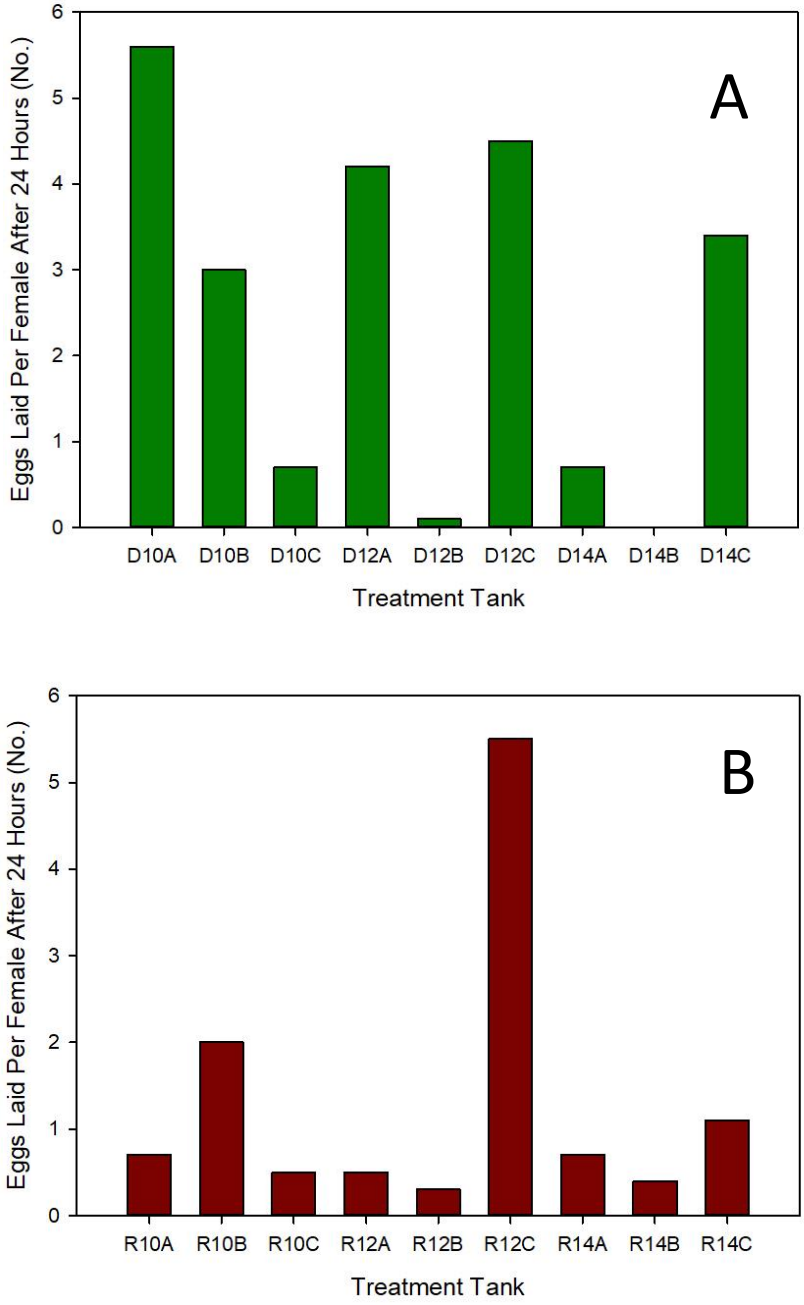
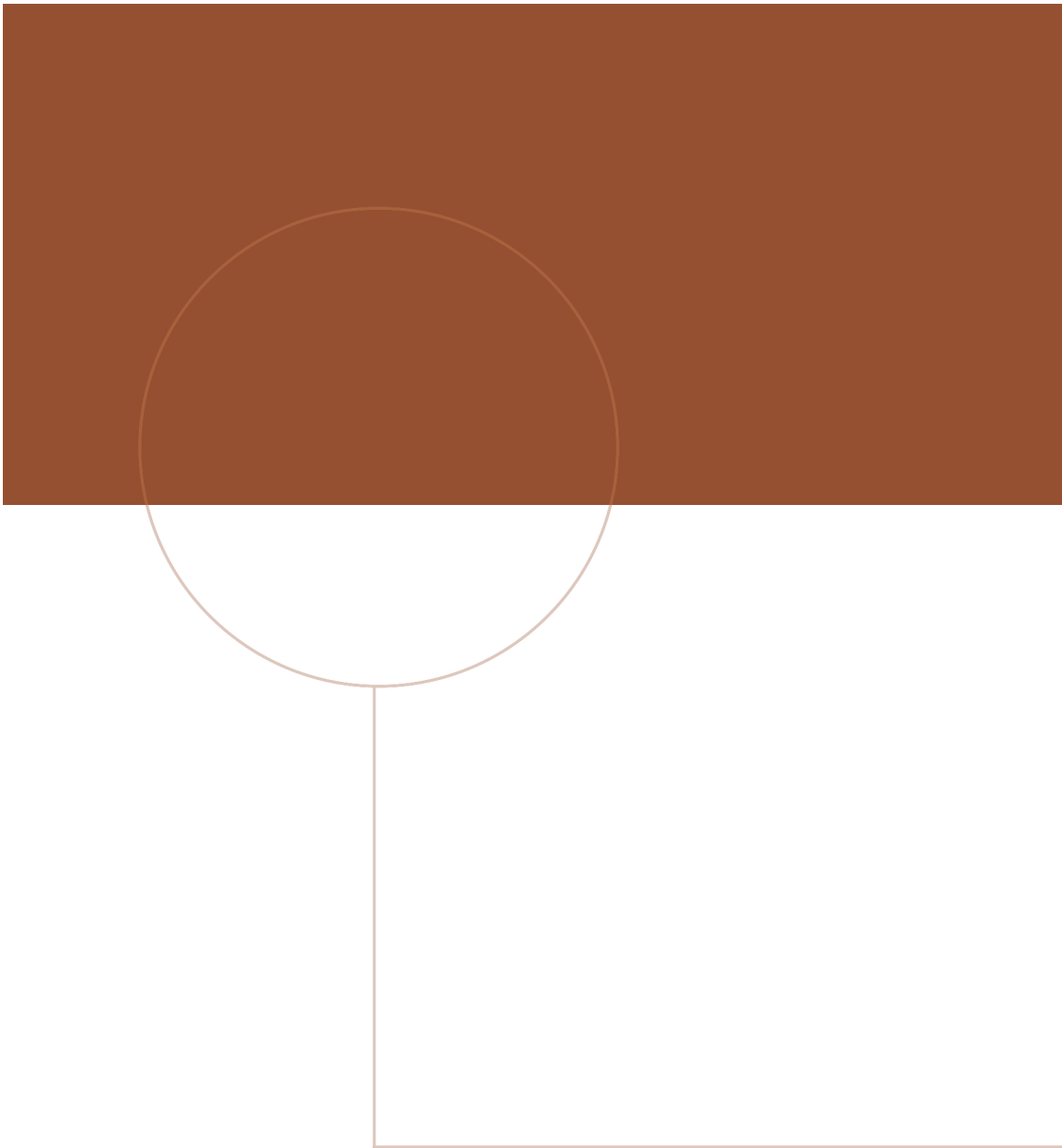


Figure A.4. Mean number of eggs laid per female *C. finmarchicus* in the rearing experiment. (A) shows the animals fed *D. tertiolecta*, (B) shows the animals fed *R. baltica*.



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