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Effects of produced water on growth and development of *Calanus finmarchicus* and *Calanus hyperboreus* early life stages at different temperatures

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Abstract

The temperature in the Arctic is increasing, which causes the sea ice to retreat. This opens for more offshore activity and longer shipping season for transportation vessels. The Arctic region is thought to contain large recoverable petroleum resources. Produced water (PW) is the main part of the waste water released from the platform during offshore activity. Polycyclic aromatic hydrocarbons (PAHs), present in produced water, has been shown to cause negative effects on growth, development and survival of aquatic organisms. The boreal species *Calanus finmarchicus* and the arctic species *C. glacialis* and *C. hyperboreus* are important constituents of the North Atlantic and Arctic food webs, and are essential prey species for fish larvae, in addition to juvenile and adults of smaller fish species. The early life stages of these species may be sensitive to produced water components. To examine this, the egg-stage of the two species *C. hyperboreus* and *C. finmarchicus* were exposed to the water-soluble fraction (WSF) of 11 selected PAHs at three different concentrations (100%: 1.80-13.23 µg L^{-1}, 50%: 1.08-6.33 µg L^{-1}, 10%: 0.38-1.22 µg L^{-1}) in addition to a control. They were reared at three different temperatures (*C. hyperboreus*: 3, 7.5, and 10°C, *C. finmarchicus*: 7.5, 10, and 12.5°C), to examine if the expected increase in temperature in the Arctic may cause additional effects. Hatching percentage was after PAH exposure of eggs at the different temperatures. From the egg-stage to the first feeding stage (NIII), biometry and dry weight) were analyzed. In addition, their ability to feed was measured at the NIII-stage, after 6 and 20 hours of algae exposure. Generally, the copepods developed faster with increasing temperature. Temperature seemed to have a larger impact on the development time and hatching of the eggs, than exposure. For dry weight the effects of both temperature and exposure remained inconclusive, with few significant differences within the different developmental stages, for both species. Considering the biometry, both temperature and exposure seemed to have some impact on the length of the nauplii. *C. hyperboreus* NII and NIII was longer at 7.5°C, compared with 3°C. However, NIII at 10°C was shorter than at 3°C. Therefore, higher temperature seems to be stimulating growth up to a point, however, beyond this point, the effect is negative. For *C. finmarchicus*, some effects were seen of both temperature and exposure, however, they did not seem to be very large. No significant effects were seen on *C. finmarchicus* with regard to temperature and exposure on their ability to feed. However, it seems that the combination of high temperature (10°C) and PAH-exposure may have a negative impact on *C. hyperboreus*' ability to feed, after 20 hours.

Sammendrag

Temperaturen i Arktis øker, noe som fører til at havisen minker. Dette åpner for mer oljeaktivitet og lengre shipping-sesong. Man forventer at store utvinnbare petroleumsressurser finnes i regionen rundt Arktis. Produsert vann utgjør hoveddelen av avfallsvannet som blir sluppet ut fra plattformen under offshoreaktivitet. Polysykliske aromatiske hydrokarboner (PAHer) finnes i produsert vann, og har vist seg å føre til negative effekter på vekst, utvikling og overlevelse på akvatiske organismer. Den boreale hoppekrepsarten *Calanus finmarchicus* og de arktiske artene *C. glacialis* og *C. hyperboreus* er en svært viktig del av de Nordatlantiske og Arktiske næringsnettene, og de er essensielle byttedyr for fiskelarver, i tillegg til juvenile og voksne av mindre fiskearter. De tidlige livsstadiene kan være sensitive for komponenter i produsert vann. For å teste dette, ble eggstadiene av de to artene *C. hyperboreus* og *C. finmarchicus* eksponert til den vannløselige fraksjonen av 11 utvalgte PAHer ved tre forskjellige konsentrasjoner (100%: 1.80-13.23 µg L^{-1}, 50%: 1.08-6.33 µg L^{-1}, 10%: 0.38-1.22 µg L^{-1}) i tillegg til en kontroll. De ble oppbevart i tre forskjellige temperaturer (*C. hyperboreus*: 3, 7,5 og 10°C, *C. finmarchicus*: 7,5, 10 og 12,5°C) for å undersøke om den forventede økningen av temperaturen i Arktis vil føre til ytterligere effekter. Klekkeprosent ble undersøkt både på eksponerte egg og ueksponerte egg ved de forskjellige temperaturene. Fra eggstadiet til første foringsstadiet (NIII), ble biometri og tørrvekt analysert. I tillegg ble evnen deres til å ta opp mat målt ved NIII-stadiet, etter 6 og 20 timer. Generelt, utviklet kopepodene seg raskere ved økende temperatur. Temperatur så ut til å ha en større innvirkning på utviklingstid og klekking av eggene enn eksponering. Når det gjelder tørrvekt, var effekten av både temperatur og eksponering uklar for begge artene. Med tanke på biometri, så det ut til at både temperatur og eksponering hadde noe innvirkning på lengden av naupliene. *C. hyperboreus* NII og NIII var lengre ved 7.5°C, sammenlignet med 3°C. Derimot var NIII kortere ved 10°C enn 3°C. Dermed ser det ut til at høyere temperatur er stimulerende for vekst opp til et punkt, men når dette punktet har passert, er effekten negativ. For *C. finmarchicus* ble det observert noe effekt for både temperatur og eksponering, men de virket ikke veldig store. Ingen signifikante effekter av eksponering og endringer i temperatur ble observert for *C. finmarchicus* sin beiteevne. For *C. hyperboreus* derimot, ser det ut til at kombinasjonen av høy temperatur (10°C) og PAH-eksponering gir en negativ innvirkning på *C. hyperboreus* beiteevne, etter 20 timer.

Table of contents

ACKNOWLEDGEMENTS I

ABSTRACT III

SAMMENDRAG V

ABBREVIATIONS 2

1. INTRODUCTION 4

1.1. PRODUCED WATER 4

1.1.1. Polycyclic aromatic hydrocarbons (PAHs) 5

1.2. CALANUS-SPECIES IN THE ARCTIC 5

1.2.1. Eggs to Nauplii-stage III development- Comparison between species 6

1.3. ACCUMULATION AND METABOLISM OF PAHS AND OTHER OIL COMPONENTS IN CALANOIDS 7

1.4. TOXIC EFFECTS OF PAHS AND OIL COMPONENTS IN CALANUS SPP. 8

1.4.1. Effects on the early life stages of copepods 9

1.5. PRODUCED WATER IN AN ARCTIC ENVIRONMENTAL PERSPECTIVE 10

1.6. AIM OF STUDY 11

2. MATERIALS AND METHODS 13

2.1. EXPERIMENTAL SETUP 13

2.1.1. Experimental animals 13

2.1.2. Experimental system 14

2.1.3. Exposure solution 14

2.2. EXPERIMENTAL PROCEDURE 17

2.2.1. Stage determination and development 18

2.2.2. Feeding experiment 19

2.2.3. Method development of the feeding experiment 21

2.2.4. Egg hatching 22

2.2.5. Biometric measurements 23

2.2.6. Dry weight analysis 23

2.2.7. Chemical analysis of water samples 24

2.3. STATISTICS 24

3. RESULTS 26

3.1. EXPERIMENTAL CONDITIONS 26

3.2. STAGE DURATION AND DEVELOPMENT 26

3.3. EXPOSURE CONCENTRATION 27

3.4. ALGAE CONSUMPTION 28

3.5. METHODOLOGICAL DEVELOPMENT OF THE FEEDING EXPERIMENT 30

3.6. GROWTH AND DEVELOPMENT 33

3.6.1. Egg hatching 33

3.6.2. Dry weight 37

3.6.3. Biometry 40

3.6.4. Relationship between growth and development 43
4. DISCUSSION 45
4.1. EXPOSURE TO PAHS 45
4.2. FEEDING 46
4.3. DEVELOPMENT 49
4.3.1. Egg hatching 49
4.3.2. Growth of nauplii 50
4.3.3. Dry weight 51
4.3.4. Biometry 52
4.4. ENVIRONMENTAL PERSPECTIVE 54
4.5. METHODOLOGY 55
4.5.1. Analysis of algae samples 55
4.5.2. Lugol fixation 56
4.5.3. Algae attaching to the walls 57
4.5.4. Suggestions for future feeding experiments 58
4.5.5. Nauplii mortality 58
4.5.6. Egg hatching 59
4.5.7. Dry weight 59
4.5.8. Biometry 59
5. CONCLUSION AND FUTURE PERSPECTIVES 61
REFERENCES 62
APPENDIX A 67
APPENDIX B 79
APPENDIX C 80
APPENDIX D 81
APPENDIX E 83
APPENDIX F 87
APPENDIX G 93
APPENDIX H 96
APPENDIX I 100
APPENDIX J 101
APPENDIX K 102
APPENDIX L 103
APPENDIX M 104
APPENDIX N 105
APPENDIX O 106

APPENDIX P 107
APPENDIX Q 109
APPENDIX R 111
APPENDIX S 112
APPENDIX T 114

Abbreviations

µg C L^{-1}	Microgram Carbon per Liter
ALCON	Algae Consumption
ANOVA	Analysis of Variance
BCF	Bioconcentration factor
CI-CV	Copepodite stages I-V
Ctrl	Control
CYP	Cytochrome P450
DW	Dry weight
FSW	Filtered seawater
GC-MS	Gas chromatography-mass spectrometry
GST	Glutathione S-transferase
N	Nitrogen
NI-NVI	Naupliar stages I-VI
NCS	Norwegian continental shelf
K_{OW}	Octanol/water partition coefficient
PAH	Polycyclic aromatic hydrocarbons
POPs	Persistent organic pollutants
PW	Produced water
rps	rounds per second
SD	Standard deviation
SPE	Solid-phase extraction
SPMD	Semi-permeable membrane devices
WAF	Water accommodated fraction
WSF	Water soluble fraction

1. Introduction

It is observed that the temperature on earth, including the oceans, has increased. The global mean surface temperature is expected to increase further. Relative to the period 1986-2005, models have predicted an increase up to 2°C in the period 2046-2065 and 3.7°C in the period 2081-2100 (IPCC 2015). However, in the Arctic, the temperature increase is higher. In fact, between 2011 and 2015, the Arctic was warmer than any time since around 1900, when instrumental records began. For the past 50 years, the Arctic has had twice the warming than the world as a whole (AMAP 2017). The models predict a further increase in the period 2081-2100 that could reach up to 11°C, relative to the 1986-2005 period (IPCC 2015)

Due to a warmer climate, the sea ice around the Arctic is retreating. In fact, the area is expected to become ice free in the summer already in the late 2030s (AMAP 2017). This will open for both more offshore activity, and a longer shipping season for transportation vessels. The Arctic region (north of the Arctic Circle) is thought to contain large recoverable petroleum resources (AMAP 2007), representing considerable economic values. Consequently, the exploration activity in the Barents Sea has increased in recent years. The latest example is the Goliat field, 50 km from the coast of the northernmost part of mainland Norway, which started production in 2015 (The Norwegian Oil and Gas Association 2015a). However, more fields are expected to open. In May 2016, the Norwegian Government offered 13 companies 10 production licenses in the Barents Sea during the 23rd licensing round. This is the first time since 1994 new exploration areas have become available for the industry in the southeastern Barents Sea (The Norwegian Oil and Gas Association 2016a).

Increased production of oil and related transport activities involve increasing operational emissions, including produced water (PW) and an increased risk of accidental oil spills.

1.1. Produced water

In the oil exploration and production process, PW constitutes the main part of the waste water that is released from the platform. It originates from water pumped up with the oil, and comprises water that is found naturally in the formation, in addition to water injected into the formation during drilling, to boost pressure and as a carrier for drilling mud and production chemicals. The PW is separated from the crude oil on the platform, before it generally is discharged to the sea. Because PW has been in contact with the sub-surface, it contains several naturally occurring substances including heavy metals, various inorganic salts, radioisotopes, organic acids and organic compounds, in addition to production chemicals, polycyclic aromatic

hydrocarbons (PAHs) and other hydrocarbons originating from oil. The composition of PW varies from field to field and is dependent on oil composition, sub-surface properties, and type and amount of production chemicals used. (Neff et al. 2011; The Norwegian Oil and Gas Association 2015b; The Norwegian Oil and Gas Association 2016b).

The discharges of PW in recent years, have been around 130 million standard cubic meters (scm) per year on the Norwegian continental shelf (NCS), however, discharges of 148 million scm was reported in 2015 (The Norwegian Oil and Gas Association 2016b). The legal limit of oil content in PW is 30 mg/L, while the oil content in PW in the NCS in 2015 was on average 12.3 mg/L, which is significantly below the legal threshold. On the NCS, PW represents the most important source of oil discharges (The Norwegian Oil and Gas Association 2016b).

1.1.1. Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) consists of two or more fused benzene rings, containing only hydrogen and carbon. They are formed by incomplete combustion of organic matter, both natural combustions such as volcanic eruptions and forest fires, or anthropogenic activity such as the burning of fossil fuels. They are also found naturally in oil. Anthropogenic activity is thought to be the dominant source of PAHs in the environment (Baek et al.). PAHs are semi-volatile organic compounds, and some of them are persistent in the environment. They are therefore considered to be part of the group persistent organic pollutants (POPs). PAHs have been detected in several environmental compartments, like air (Halsall et al. 1994), soil (Motelay-Massei et al. 2004), sediments (Laflamme and Hites 1978), and biological samples (Page et al. 2004).

The PAH congeners in PW are typically 2- and 3-ring PAHs, such as naphthalene and phenanthrene, as well as their alkyl homologues. The higher molecular weight 4- to 6-ring PAHs are rarely detected in PW. Primarily, they are associated with dispersed oil droplets, due to their low solubility. The concentration of PAHs in PW normally ranges from 0.040-3 mg/L (Neff et al. 2011)

1.2. *Calanus*-species in the Arctic

The boreal species *Calanus finmarchicus* (Gunnerus, 1765) and the arctic species *C. glacialis* (Jaschnov, 1955) and *C. hyperboreus* (Krøyer, 1838) are important constituents in the North Atlantic and Arctic food webs, as they represent a large part (50-80 %) of the total mesozooplankton biomass. (Blachowiak-Samolyk et al. 2008; Hirche and Mumm 1992).

Combined with krill (*Thysanoessa inermis* (Krøyer, 1846) and *T. raschii* (M. Sars, 1864)) and other zooplankton, the calanoids make up the basis for energy transfer from lower to higher trophic levels, as they are essential prey species. They are preyed upon by fish larvae as well as juvenile and adults of smaller fish species, like polar cod *(Boreogadus saida*), capelin (*Mallotus villosus*), and herring (*Cluppea harengus*). Atlantic cod (*Gadus morhua*), sea birds and marine mammals from higher trophic levels prey upon these smaller fish species (Sakshaug et al. 1994; Sakshaug et al. 2009). It has been shown that the food delivered to the little auk nestlings (*Alle alle*), primarily consists of the copepods *C. hyperboreus* and *C. glacialis* from the copepodite stages IV and V. *C. hyperboreus* seemed to be the most important species in the little auk chick diet in Northwest Greenland (Pedersen and Falk 2001).

1.2.1. Eggs to Nauplii-stage III development- Comparison between species

When the egg hatches, the nauplius (NI) is released. This is followed by 5 naupliar stages (NII-NVI) and 5 copepodite stages (CI-CV) before reaching the adult stages (Miller and Tande 1993). All Calanus species are considered to start feeding at the NIII stage.

C. finmarchicus starts to reproduce in late March, early April, and reproduction reaches a peak in late May, early June, when the spring bloom is at its highest. The eggs are transparent, and enclosed by a hard eggshell (Grenvald et al. 2013). They also sink in the water column (Melle and Skjoldal 1998). Time of development is temperature dependent, but even at low temperatures (4 °C), NIII is reached within 10 days. At low food availability, *C. finmarchicus* stays longer in the NIII stage, than with high food availability (Campbell et al. 2001).

In the Barents Sea, the spawning pattern of *C. glacialis* resembled that of *C. finmarchicus,* with low egg production during the pre- and early bloom periods and during the late bloom period. During the bloom period, the production is high, but variable. The eggs of *C. glacialis* can be recognized by a spiny membrane (Melle and Skjoldal 1998). At -1.2°C, *C. glacialis* reached NIII within 10 days. This species also stayed longer in the NIII stage without food available, and had lower survival than the controls who had food available (Daase et al. 2011).

C. hyperboreus eggs and the earliest naupliar stages (NI-NIII) are orange, which is assumed to be related to high fat reserves (Melle and Skjoldal 1998). In contrast to *C. finmarchicus,* they are not enclosed by a hard eggshell, but a thin membrane consisting primarily of fatty acids. The eggs are positively buoyant and therefore float towards the surface, after they have been spawned deep in the water column (Jung-Madsen et al. 2013). C. hyperboreus reproduce during winter, as early as November until March (Hirche and Niehoff 1996) before the spring bloom,

making egg production dependent on internally stored lipids only. The development time of the young stages is temperature dependent. Also for *C. hyperboreus*, development is faster at higher temperatures. At low temperatures (0°C), *C. hyperboreus* NIII can be reached in around 15 days (Jung-Madsen et al. 2013). The nauplii of the youngest stages (NI-NIII) have the same color as the eggs, indicating large fat reserves (Melle and Skjoldal 1998). Without food available, only a small proportion develops further from NIII to NIV (Jung-Madsen et al. 2013).

1.3. Accumulation and metabolism of PAHs and other oil components in calanoids

Copepods are filter-feeding organisms, i.e. they actively filter small particles. The smallest oil droplet fractions (<50 µm) may be ingested and thereby become bioavailable through the digestive system (Conover 1971; Hansen et al. 2009). However, the most important uptake of oil components for copepods is accumulation through non-dietary routes, called bioconcentration. The bioconcentration factor (BCF) is the proportion of the concentration of a chemical in an aquatic animal relative to the concentration in ambient water at steady state equilibrium. BCF quantifies the propensity of a chemical to accumulate in an aquatic animal. (Barron 1990). The lipophilicity of a compound is generally quantified as the octanol/water partition coefficient (K_{OW}) (often expressed by its log value), which is defined as the ratio of the concentration of the compound in the organic versus the aqueous phase of a two-phase system when in equilibrium (Klaassen 2013). Bioconcentration potential and the K_{OW} is generally found to correlate rather well (Müller and Nendza 2007). The log K_{OW} of PAHs ranges from 3.3 to around 6, making them potentially highly bioavailable for marine organisms (Hylland 2006).

C. finmarchicus copepodites and adults have been shown to accumulate oil compounds from both oil dispersions (with and without chemical dispersion), and solutions of dissolved oil components (water accommodated fraction (WAFs) and water-soluble fraction (WSF) (Hansen et al. 2011; Hansen et al. 2013; Nordtug et al. 2015; Nørregaard et al. 2015). Accumulation of the PAHs pyrene, phenanthrene, and benzo[a]pyrene has been shown in *C. hyperboreus* (Nørregaard et al. 2015).

Even if the concentration in the ambient water is low, the body burden of PAHs in marine biota may become high enough to cause toxic effects, due to bioconcentration (Hylland 2006; Müller and Nendza 2007). Processes to counteract and even reduce the accumulation of some

chemicals include enzymatic detoxification, excretion and maternal transfer to offspring. Dilution by growth may also be relevant in reducing concentrations in the body.

The enzymes assumed to be involved in the metabolism of PAHs in marine invertebrates are the cytochrome P450 (CYP) enzymes (Rewitz et al. 2006). In addition are glutathione S-transferase (GST) involved in processes regarding oxidative stress and lipid peroxidation (Barata et al. 2005b; Hayes et al. 2005). *C. finmarchicus* exposed to the WSF of oil showed expression of both CYP330A1 and GST, which may indicate some ability to metabolize PAHs (Hansen et al. 2009), and *C. glacialis* displayed higher expression of GST following exposure to WSF of marine diesel than *C. finmarchicus* (Hansen et al. 2013).

1.4. Toxic effects of PAHs and oil components in *Calanus* spp.

Copepods have been widely used in toxicological studies on effects of oil components and PAHs (Gardiner et al. 2013; Grenvald et al. 2013; Hansen et al. 2012; Hansen et al. 2011; Hansen et al. 2013; Jager et al. 2016; Olsen et al. 2013), making them an important model species group for environmental effects of oil components. The main focus has been on *C. finmarchicus* as it is a boreal species, and the Norwegian offshore oil industry has mainly been located in the North Sea. However, the Arctic species have gained more focus in recent years, as the Norwegian oil exploration is moving northwards.

In studies where *C. finmarchicus* and *C. glacialis* were exposed to pyrene, there was a time *(Jensen et al. 2008)* and temperature dependent (Hjorth and Nielsen 2011) reduction in egg production for C. finmarchicus while *C. glacialis* showed no significant reduction in neither of the studies. There was also a reduction in grazing in *C. finmarchicus* exposed to pyrene (Jensen et al. 2008) and lowered faecal pellet production with increasing temperatures combined with pyrene exposure in *C. finmarchicus* (Hjorth and Nielsen 2011). However, no significant effect was observed for *C. glacialis* in neither of the studies.

The effect on grazing was suggested to be due to narcosis and disturbed feeding pattern. Narcosis has in fact been suggested as a mode of action of PAHs in copepods (Barata et al. 2005a), and is described as a disturbance of membrane function, which results in decreased activity. This diminishes the ability to react to stimuli (Van Wezel and Opperhuizen 1995). Other studies have also suggested narcosis as an effect in *Calanus* spp. following exposure to pyrene or WSF of crude oil (Jensen and Carroll 2010; Nørregaard et al. 2014).

In a study where *C. glacialis* and *C. finmarchicus* were exposed to the WSF of crude oil, cumulative egg-production, hatching success and faecal pellet production were investigated inn *C. glacialis* and feeding efficiency was investigated in *C. finmarchicus. C. glacialis* showed no significant difference in egg- and faecal pellet production between the control and exposed, while hatching success was significantly reduced in the exposed group. *C. finmarchicus* showed reduced feeding efficiency in the high treatment group, compared to the control (Jensen and Carroll 2010).

There was a lowering of hatching percentage for *C. finmarchicus,* when females were exposed to pyrene, but not when the eggs were exposed directly (Jensen et al. 2008). For *C. glacialis*, there was no difference in hatching percentage between controls and exposed (Jensen et al. 2008). For *C. hyperboreus,* egg production and hatching success decreased with increasing concentration of pyrene (0.0202 – 20.2+ µg L^{-1}), however, there were no statistical difference between the exposed and the control, with the exception of hatching success at 20.2+ µg/L, which was significantly lower. (Nørregaard et al. 2014).

1.4.1. Effects on the early life stages of copepods

The effects of PAHs on early developmental stages of *C. hyperboreus* have, to the author's knowledge, not been examined previously. However, several studies have examined the effects of PAHs and other oil components on the early stages of *C. finmarchicus, C. glacialis* and other copepods (Bejarano et al. 2006; Calbet et al. 2007; Grenvald et al. 2013; Jager et al. 2016; Lotufo and Fleeger 1997; Saiz et al. 2009). *C. glacialis* experienced reduced growth, when exposed to pyrene. Both *C. finmarchicus* and *C. glacialis* experienced increased naupliar mortality with increased temperature and high pyrene exposure. It seemed that *C. finmarchicus* was more sensitive than *C. glacialis* (Grenvald et al. 2013). Jager et al. (2016) found that adult males *of C. finmarchicus* were the most sensitive to oil toxicity, followed by early copepodites, whereas nauplii were equally susceptible as late copepodites and adult females. The data reported by Bejarano et al. (2006), however indicated that the early stages of the harpacticoid copepod *Amphiascus tenuiremis* (Brady & Robertson D., 1880) were more sensitive than later stages when exposed to WAFs of crude oil during a full life cycle. Lotufo and Fleeger (1997) looked at stage-specific sensitivity to sediment-associated phenanthrene of two species of harpacticoid copepods *(Schizopera knabeni* (Lang, 1965) and *Nitocra lacustris* (Shmankevich, 1875)). *S. iknabeni* nauplii had a higher sensitivity than copepodites, while copepodites had a higher sensitivity than adults, and there was no sensitivity difference between males and females. The results were different for *N. lacustris,* where there were no significant differences

between nauplii, copepodites and males, while females were distinctly less sensitive. Saiz et al. (2009) found that the cycloid copepod *Oithona davisae* (Ferrari F.D. & Orsi, 1984) naupliar stages had lower tolerance than adults for the PAHs naphthalene and 1,2-dimethylnaphtalene, regarding narcotic and lethal effects. They also showed that feeding activity was sensitive to the presence of the two PAHs. Calbet et al. (2006) found no difference in mortality between nauplii and adults of the copepod *Paracartia grani* (Sars G.O., 1904) after 48 h exposure to naphthalene and 1,2-dimethylnaphtalene. However, in this experiment adults exposed for 48 h were compared to naupliar survival after eggs had been exposed for 48 h.

1.5. Produced water in an Arctic environmental perspective

The arctic and subarctic seas have high biodiversity and productivity. The southwestern Barents Sea is particularly productive, with several commercially important fish stocks, including capelin. This species can furnish about a fourth of the food requirements for many seabirds, marine mammals, and larger fish species, especially cod. Copepods including *Calanus* species, are the most important food source for capelin, as well as other fish species, such as polar cod and young Atlantic cod (Sakshaug et al. 2009).

When PW is discharged into the ocean, it is rapidly diluted, often by more than a 100-fold within 100 m of the discharge area. Because of this, in addition to biodegradation/transformation rates in receiving waters, the potential for acute toxicity beyond the immediate vicinity of the discharge area is likely to be limited. However, the continuing chronic exposure close to discharge areas may cause sub-lethal changes in populations and communities. This might affect their genetic diversity, reproduction success, growth, respiratory system, and development (Balk et al. 2011; Neff et al. 2011). In fact, biomarker response to PAHs in natural populations of haddock and Atlantic cod have been found in North Sea areas with widespread oil production (Balk et al. 2011).

In a field study performed in the Norwegian sector of the North Sea in 1997, Semi-permeable membrane devices (SPMDs) and blue mussels *(Mytilus edulis)* were used to obtain average concentrations of PAHs during a four-week period. Water samples were used to obtain water concentration of PAHs as well. These results showed that PAHs were present close to oil exploration sites in the Norwegian Sea. In blue mussel tissues, total PAHs ranged from 2540 to 18.1 ng/g, dry weight, while in the SPMDs total PAHs ranged from 9910 to 478 ng/g SPMD, between different sites. Highest concentrations were found in sampling sites closest to

platforms. (Røe utvik et al. 1999). With the expected increase in oil exploration in the Arctic, it is reasonable to believe that the emissions of PAHs to the sea water will increase as well.

During oil exploitation, there is a continuous emission of PW. Oil exploration in the arctic will therefore increase the potential of PAH accumulation in arctic *Calanus* species which will increase the risk of PAH transfer to higher trophic level organisms. In the marine food web, there has been reported a trophic dilution of PAHs (Baumard et al. 1998; Nfon et al. 2008; Wan et al. 2007). Zooplankton species have much higher concentrations, based on both dry- and lipid weight, than higher trophic level species, such as macro invertebrates, birds, and fish. This is likely because higher trophic level organisms have more efficient metabolic transformation, compared to lower trophic levels (Wan et al. 2007). Electrophilic PAH metabolites, formed by biotransformation, are known to form DNA adducts, in addition to attacking RNA and proteins (Balk et al. 2011), which can have serious consequences for organisms which metabolize PAHs efficiently. As Calanus spp. are capable of accumulating PAH, and are important prey for several higher trophic organisms, this may cause a risk for these organisms.

1.6. Aim of study

In this master thesis, effects of produced water components on growth and development of early stages of *C. finmarchicus* and *C. hyperboreus* at different temperatures are investigated.

Calanoid copepods have three big transition stages: Egg hatching, transition from non-feeding nauplii stage (NII) to feeding nauplii stage (NIII), and transition from the last nauplii stage (NVI) to the first copepodite stage (CI).

Hatching occurs because osmosis causes the egg-shell to burst and release the nauplius (NI). Proper development of the embryo is important for the nauplius to develop swimming capability. Studies have shown that when exposing *C. finmarchicus* eggs to PAHs (and dispersed oil droplets), effects have been minimal (Olsen et al. 2013), however, *C. hyperboreus* have thinner and more fragile egg shell characteristics, with only a lipid membrane, instead of a hard shell. Therefore, they might be more vulnerable to PW components.

The transition from non-feeding (NII) to feeding (NIII) is the transition between being dependent on maternally transferred energy to being able to gain energy through feeding, and may be sensitive to PW, as well. Adult *Calanus* spp. have shown to reduce feeding efficiency when exposed to pyrene and WSF of oil, and early life stages are thought to be more sensitive than adults. Survival, growth, and development will be dependent on their ability to initiate

feeding activity. Experiments have shown that this developmental phase is sensitive to food restriction and CO_2 exposure (Jung-Madsen et al. 2013; Pedersen et al. 2014).

The hypothesis is that these early developmental transition stages may be sensitive to produced water components, which may affect *C. hyperboreus* and *C. finmarchicus* ability feed.

The main aim of this study was to assess the possibility that PW components may impact development in *C. hyperboreus* and *C. finmarchicus* at different temperature regimes. A secondary aim was to assess whether feeding rate at the NIII-stage was effected by the presence of produced water PAHs, at different temperatures. A second secondary aim was to assess possible effects on growth and development from the egg-stage to the NIII-stage when exposed to produced water PAHs at the egg-stage, and exposed to different temperatures through the study period.

2. Materials and Methods

2.1. Experimental setup

Two of the experiments on *C. hyperboreus* (3 and 10 °C), as well as an investigation hatching success at different temperatures, were performed in a container at the Arctic Station, Disco Island, Greenland in February 2016. An additional experiment at 7 °C was started on the Arctic Station, but had to be terminated due to a mistake. Five experiments on *C. finmarchicus* (three temperature experiments, and two hatching success experiments) and two experiments on *C. hyperboreus* (7.5 °C, exposed hatching success of all temperatures) were performed at the laboratories of NTNU SeaLab in Trondheim, Norway November-December 2016 (*C. finmarchicus*) and March 2017 (*C. hyperboreus*).

2.1.1. Experimental animals

C. hyperboreus were collected (06.02.16, 07.02.16, and 08.02.16) through a hole in the ice, using a plankton net (300 µm mesh size), at 180 m depth, outside Disco Island, Greenland (69.13°N 53.25°W). The gathered zooplankton were transported to the Arctic Station in plastic containers (50L) by dog sledges. When returning to the laboratory, the ovulating *C. hyperboreus* were sorted out by gently capturing them into plastic scoops, and transferred to plastic buckets (10L) containing filtered seawater (FSW) at 3°C. *C. hyperboreus* is a large species, adult females have a length of 5.9-7.4 mm (Hirche 1997), and can therefore be distinguished without using a stereo microscope. As the eggs of *C. hyperboreus* are orange, ovulating females could be distinguished with orange marks in their otherwise transparent body (Figure 2.1.). If there was any uncertainty, a stereo microscope, was used. The plastic buckets were transferred to a container set at 3°C.

Figure 2.1. Ovulating females, A: *C. hyperboreus*, B: *C. finmarchimus*. *C. hyperboreus* eggs are orange, while C. finmarchicus eggs are transparent.

The *C. finmarchicus* used was collected from the continuous culture maintained at the research facility at NTNU SeaLab, as described by Hansen et al. 2007. The stock culture is maintained in 300 L tanks with flowing FSW, and are descendants from individuals collected from Trondheimsfjorden during autumn 2004. They are kept at 10 °C, and continuously fed a mixture of the microalgae *Rhodomonas baltica*, *Dunaliella tertiolecta*, and *Isochrysis galbana*.

2.1.2. Experimental system

Three experiments at three different temperatures were performed with each species. A series of three different exposure concentrations, plus control was included in the experiment. The selected temperatures in the experiments with *C. hyperboreus* were 3, 7.5, and 10 °C, and for *C. finmarchicus* 7.5, 10, and 12.5 °C. The animals were kept in VOC-vials from the egg stage to the decided developmental stage. To keep temperature variation at a minimum, the VOC-vials were placed in a pre-cooled water bath, in a temperature-controlled room/container set at the desired temperature. The temperature was monitored regularly through the experimental period, using a thermometer (Hach sension 156). Addition of *R. baltica* (200 µg C L^{-1}) was added already at the end of the NII-stage, to secure food availability for the nauplii immediately after hatching to the NIII stage.

At the Arctic Station, starting algae concentration was determined using a Bürker counting chamber. Cells from the stock solution were placed in the chamber, and number of cells/mL were estimated by counting three diagonally squares. An average of six counts was used to estimate concentration of stock solution (cells/mL). From this, it was calculated how much volume was needed from the stock solution, to get the wanted concentration in each VOC-vial (40 mL)

For the experiments performed at SeaLab, algae concentration in the production culture was calculated based on diluted samples (dilution 1:400), counted, using CoulterCounter® Multisizer 3^{TM} (BeckmanCoulter Inc.). Based on the production culture concentration, a stock solution was prepared (100mL, 18 500 µg C L^{-1}). 0.432 mL of the stock solution was then added to each VOC-vial (volume of 40mL), giving the desired algae concentration (200 µg C L^{-1}).

2.1.3. Exposure solution

An aqueous solution of 11 selected PAHs at sub-solubility concentration was used as exposure medium. This was generated from FSW and a PAH-spiked synthetic N-alkane oil (Table 1). These PAHs were chosen because they are typical components of PW, and represents a wide array of lipophilicities, which is shown here by the logarithm of their *n*-octanol-water partition

coefficient, K_{OW} (Table 1). Aqueous solubility and bioaccumulation characteristics are determined by the lipophilicity of the components. The Department of Chemistry and Biochemistry, Florida International University, USA, produced the PAH-spiked oil. The N-alkane oil itself was not expected to have any biological effect.

Table 2.1. List of PAHs used in the exposure experiments with *C. hyperboreus* and *C. finmarchicus*, with their corresponding log K_{OW} and molecular formula. Log K_{OW} values were obtained from SINTEF Materials and Chemistry (Environmental Technology department).

ID	Name	Log K_{OW}	Molecular formula
N	Naphtalene	3,30	$C_{10}H_8$
N1	2-Methylnaphtalene	3,79	$C_{11}H_{10}$
N2	2,6-Dimethylnaphtalene	4,24	$C_{12}H_{12}$
F	Fluorene	3,93	$C_{13}H_{10}$
P	Phenanthrene	4,58	$C_{14}H_{10}$
D	Dibenzothiophene	4,37	$C_{12}H_8S$
D1	4-Methyldibenzothiophene	4,86	$C_{13}H_{10}S$
D2	4,6-Dimethyldibenzothiophene	5,33	$C_{14}H_{12}S$
F1	Fluoranthene	5,19	$C_{16}H_{10}$
Py	Pyrene	5,13	$C_{16}H_{10}$
C	Chrysene	5,78	$C_{18}H_{12}$

A droplet generator system (Nordtug et al. 2011) was used to produce a dispersion of the N-alkane oil. This system consisted of a unit of four nozzles (diameter: 0.5 mm) in a series, connected by mixing chambers (chamber diameter: 8mm). A Q-SAN metering pump (Fluid Metering Inc., Syosset NY, USA) was used to control the input to the generator. The flowrate was 160 mL min^{-1}, which in the current system is enough to break the oil down to micro droplets by producing shear forces downstream the nozzle in each chamber. An Aladdin syringe pump (WPI, Sarasota FL, USA) was used to add the oil from a glass syringe (2.5 mL, SEG, Australia), through a Teflon capillary into the generator, just after the first nozzle. By using a flowrate of 0.889 µL m^{-1}, an oil dispersion with a concentration of 5 mg L^{-1} (5 ppm) was generated. To allow sufficient residence time to assume equilibrium between the PAHs dissolved in the water and in the droplets, the dispersion was led into an overflow chamber (4.5 L).

Sufficient dispersion for the experiment was supplied by a second outlet from the equilibrium chamber. To make the desired WSF solution the oil droplets were removed from the dispersion by filtration using a filter-unit consisting of fine glass wool (removing the biggest droplets),

with a Whatman Grade GF/C and a GF/F Class Microfiber filters under (Whatman Ltd., Madistone, UK; 1.2 and 0.7 µm, respectively) under. A Q-SAN metering pump was used to suck the dispersion through the filter unit. An overview of the system is shown in Figure 2.1.

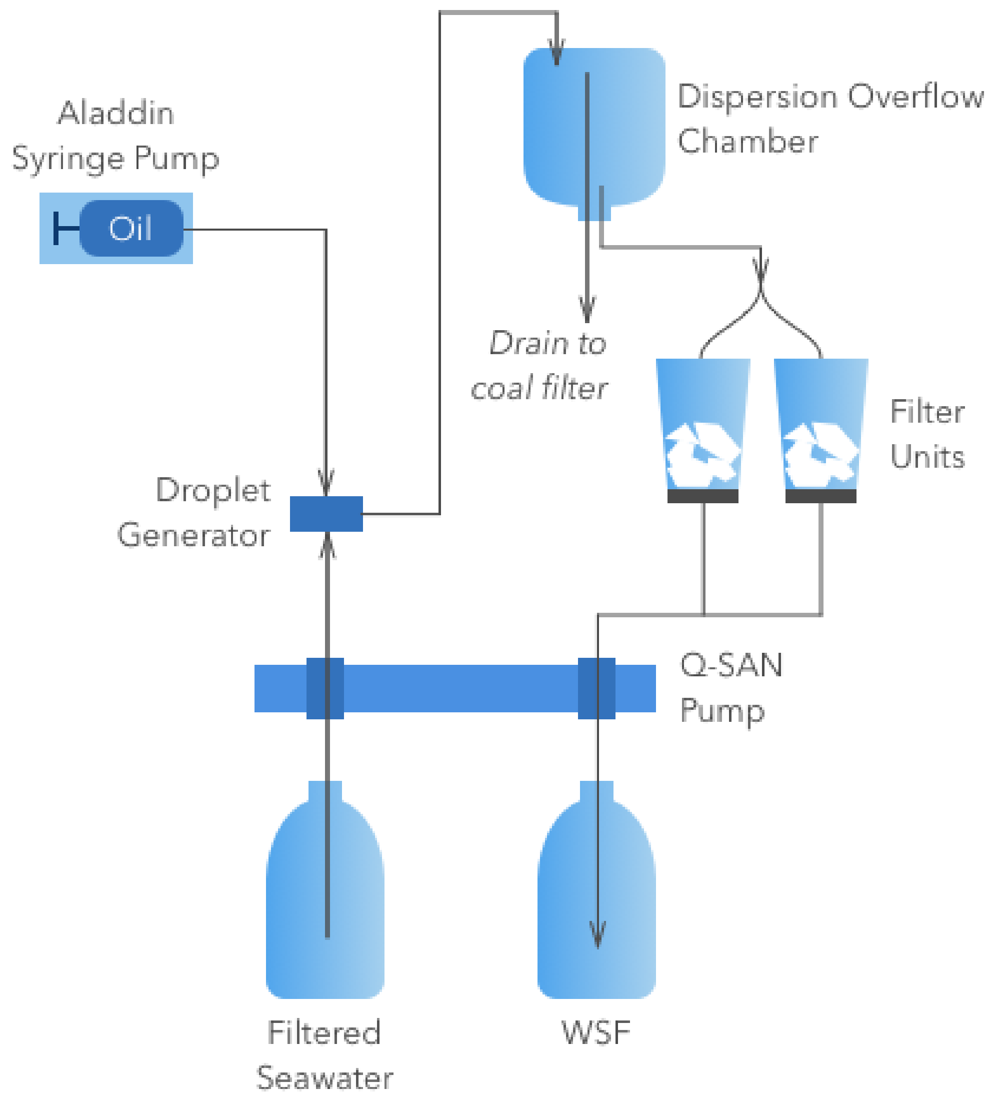

Figure 2.2. The system for generating the water-soluble fraction (WSF) of the oil (N-alkane oil, spiked with 11 different PAHs). Filtered seawater is pumped through the droplet generator at the rate of 160 ml m^{-1}. Oil with PAHs is added by a syringe pump to the filtered water passing the generator at 0.0889 µl m^{-1}. To allow sufficient time to assume equilibrium between the PAHs in the droplets and the PAHs dissolved in water, the dispersion is led to an overflow chamber defining a residence time. Dispersion for the experiment is taken from an additional outlet from the overflow chamber and filtered through a custom-made filtering unit to generate the desired WSF.

The WSF was produced in room temperature, using temperate FSW, to avoid crystal formation of the oil (which happens at low temperatures). The exposure solution (WSF) was collected in glass bottles (2 L). Further, some of the exposure solution was diluted, to three different final exposure solutions (10%, 50%, and 100%). Filtered SW was used as control.

Samples of the original exposure solutions, as well as the solutions used during the experiment, were preserved for analysis of PAH concentrations (at SINTEF Materials and Chemistry (Environmental Technology department).

2.2. Experimental procedure

Ovulating females were placed in plastic buckets (250 *C. finmarchicus* females per 20L buckets, 40 *C. hyperboreus* females per 10L buckets at the Arctic Station and 20 females per 5L buckets at SeaLab). Since *C. hyperboreus* eggs are floating, and in addition are orange of color, they could easily be seen in the water (Figure 2.3., A). They were collected by a siphon with a retaining chamber mounted inline. The eggs were retained in the chamber due to a sieve (40 µm mesh size), where the eggs were collected, while the water left through a tube. Eggs were collected with the Pasteur pipette, with an enlarged hole and rounded edges, to a Petri dish.

C. finmarchicus eggs sink to the bottom, and are more or less transparent (Figure 2.3., E). They were collected using a siphon with a sieve (300 µm mesh size) attached to the tip, to prevent accidently collecting adults. The eggs (< 12h old) were siphoned into a custom-made sieve cup (40 µm mesh size) placed inside a glass bowl containing a little FSW, to a level just enough to keep the sieve submerged. Eggs were collected from the sieve with the modified Pasteur pipette, referred above, to a Petri dish.

The eggs of both species were collected from the Petri dish, using micropipettes (eppendorf, 20-200 µL), and transferred to pre-marked VOC-vials. For each parallel (vials) 100 (*C. hyperboreus*) and 200 (*C. finmarchicus*) were collected. Due to the microscopic size of the eggs and nauplii, they were observed using a stereo microscope (Leica M80) during sampling. The collected eggs were then kept at the selected temperatures (*C. hyperboreus*: 3, 7.5, and 10 °C, *C. finmarchicus*: 7.5, 10, and 12.5 °C) and selected exposure regimes (0 (control), 10, 50, and 100% WSF solution) for 48h (*C. hyperboreus*) and 24h (*C. finmarchicus*). To prevent the eggs from drying out, there was a small amount of FSW in each VOC-vial, while sampling the eggs. A siphoning system (described below) was used to remove excess water, to secure that every sample had the same volume (5 mL) of FSW, before adding the WSF-solution. After exposure, the WSF-solution was removed using siphoning from inside a plexiglass tube inserted down to near bottom of the sample vial. To prevent the eggs and nauplii escaping with the siphoning water, a sieve (40 µm mesh size) was mounted at the distal (lower) end of the plexiglass tube. The exposure solution was then replaced with filtered seawater (FSW) using a procedure where

most of the volume was exchanged three times using the described siphoning system, and the eggs/nauplii then allowed to develop to the desired developmental stage (NI, NII, NIII) in clean seawater. During two of the experiments with *C. finmarchicus* (10 and 12.5°C) and one with *C. hyperboreus* (7.5°C) a pump (Watson Marlow 205U) replaced simple siphoning, during the water exchange procedure. Summary of Experimental setup, with exact number of eggs in each VOC-vial, and number of individuals used in the different experiments can be found in Appendix A.

2.2.1. Stage determination and development

The copepods were sampled when it appeared enough copepods had reached the desired developmental stage. Exact sampling dates and developmental times is described in Appendix B. All samples within the same temperature was sampled the same day. For *C. hyperboreus*, the developmental time was equal for the two highest temperatures, for both NI and NII, while NIII at 7.5°C developed one day faster than at 10°C. However, it should be noted that the experiment run at 7.5°C was performed at SeaLab, while the experiment at 10 and 3°C were performed at the Arctic Station. Developmental stage of the nauplii was determined by examining morphological characteristics (Figure 2.3., B-D, F-I), using a determination key developed by researcher Iurgi Imanol Salaverria-Zabalegui at the Department of Biology, NTNU. The determination key had close-up pictures, and a short description of the most prominent characteristics. Individual nauplii were collected from the VOC-vials using a rounded Pasteur pipette to a Petri dish, and used for further experiments and analyzes.

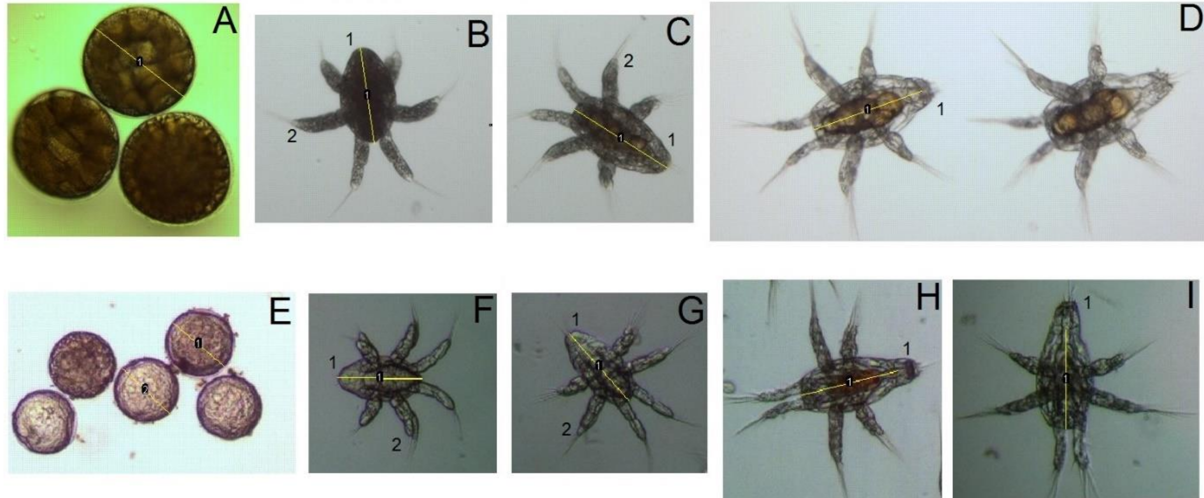

Figure 2.3. Developmental stages eggs-NIII of *C. hyperboreus* (A-D) and *C. finmarchicus* (E-I). Yellow line indicates the outline from the Image J software, which converts pixels to mm, and thereby derives the length. Diameter of the eggs, total body length of N1 and N2, and length of prosome of N3. Numbers indicate morphological characteristics on developmental stages. A and E: Eggs before hatching. B and F: NI; 2 relatively short and widely separately spines on the posterior end of the body (1), each antennule contains 3 thin setae (2). C and G: NII; more elongated body shape, 2 long and closely gathered spines on the posterior end of the body (1), structure of antennule is similar to NI (2). D, H, and I: NIII; 1 pair of long spines and 2 pair of short spines on the posterior end of body (1). *C. finmarchicus* is transparent, which makes it easy to see if an individual has eaten (H) or not (I), by distinguishing red matter inside the gut. *C. hyperboreus* has an orange lipid layer in the middle of its body, making it impossible to see if it has eaten, or not (D).

2.2.2. Feeding experiment

C. hyperboreus (Arctic Station) and *C. finmarchicus* (SeaLab) at the NIII-stage were exposed to a known concentration (200 µg C L^{-1}) of *Rodomonas baltica* for 6h and 20h. There were three parallels for each PAH-concentration, temperature and time period.

At the Arctic Station, starting algae cell concentration was determined using a Bürker counting chamber. Cells from the stock solution were placed in the chamber, and number of cells/mL were estimated by counting three diagonally placed squares. An average of nine counts was used. Based on the number of cells/mL, 500 mL of a solution with the concentration of 236 µg C L^{-1} corresponding to 200 µg C L^{-1}, was made (corrected for filtered seawater (2mL) added to each vial (13mL in total)) (Figure C.1., Appendix C). 12-20 *C. hyperboreus* nauplii (3 and 10°C) were added to each vial, depending on the availability of animals.

At Sealab, the initial algae concentration was determined using the same method as described earlier. The vials (13mL) were prefilled with FSW, and algae from the new solution (0,141 mL) was added to get the desired concentration (Figure C.2., Appendix C). 19-22 individual *C. finmarchicus* and 20 individual *C. hyperboreus* (Sealab) nauplii were added to each vial.

The vials (13mL) were filled with FSW with the correct concentration of algae, and placed on a rotating wheel (Stuart rotator SB3) to keep the cells from settling (2 rounds per second (rps) (Figure 2.2), and kept at the correct temperature. In the Arctic station, the rotating wheel with algae samples was kept in a fridge, and were therefore in darkness through the entire experimental period. For the samples at Sealab, the rotating wheel had to be kept in the room where all experiments were performed. Therefore, they were not kept in constant darkness throughout the experimental period. The exception was the *C. hyperboreus* 7.5°C, where a cardboard box containing holes, to keep the air flowing, was placed over the rotating wheel. When the feeding experiment was finished, the copepods were removed using a 40 µm sieve, and the algae solution was moved to a VOC-vial for later counting of algae density. At the Arctic station, the algae were fixated using Lugol solution (10% acetic acid), and sent to Sealab for further analysis. At Sealab, the algae were measured immediately after removing the animals. The algae concentration for both the *C. hyperboreus* and *C. finmarchicus* experiments was determined using a CoulterCounter® Multisizer 3™, assuming a sphear-normalized diameter range of *R. baltica*, of 7-8 µm (Figure D.1., Appendix D) (Berggreen et al. 1988; Båmstedt et al. 1999). For the last experiment (*C. hyperboreus* 7.5°C, 20h), 3 vials (13mL) were filled with algae solution in addition to the rest of the vials. The content in these vials was pored through the sieve (40 µm) and counted on the CoulterCounter. The result from this has been used as the assumed start concentration when determining number of algae cells eaten/*Calanus*/h (Equation 1) for all samples, as the start concentration in the other samples is unknown.

Cells eaten/*Calanus*/h = (Assumed star concentration (cells/mL)*13mL (1)
– concentration counted (cells/mL)*5(dilution) *13mL)
/number of individual *Calanus*/number of hours

At Sealab (*C. finmarchicus* and *C. hyperboreus* 7.5°C), after the algae solution was removed from the vials, the vials were properly rinsed with FSW, to determine if any algae were left on the walls. The rinsed water was transferred to new VOC-vials, and fixated with Lugol solution, for later counting. The vials were weighed before counting, and after the solution was removed, and they had become dry, to determine how much solution was present. Since the CoulterCounter gives number of cells/mL, number of cells could be calculated (Appendix E).

Figure 2.2. The rotating wheel with the vials wit algae solution was spinning at 2 rps to keep the cells from settling, and kept at the selected temperature.

2.2.3. Method development of the feeding experiment

As mentioned above, the algae concentrations in the feeding experiments were determined using the CoulterCounter. However, initially fluorescence of the chlorophyll in algae (*R. baltica*) was planned to be used as a measure of algae density. To examine if this method was accurate enough for this purpose, three solutions of known concentrations of algae (160, 180, and 200 µg C L^{-1}) was prepared as described in 2.1.2. Of these solutions, 10 sub-samples of each concentration were counted on the CoulterCounter. 10 new sub-samples of each concentration were then made, for extraction of chlorophyll for fluorescence measurements, using the following method: The algae were vacuum-filtrated through a 0.7 µm glass fiber filter (Whatman GF/F) and the chlorophyll was extracted from the filter, using pre-heated ethanol (10mL, 70°C) for 5 minutes. The ethanol with the chlorophyll was then sampled with a syringe through a syringe filter (0.7 µm glass fiber filter (Whatman GF/F)) connected to the tip, to prevent dispersed algae to enter with the extract. The fluorescence of the chlorophyll was then measured using a fluorometer (TD-700).

Because the algae samples from the Arctic station had to be fixated with Lugol solution, an experiment was performed as well to check for possible effects of fixation. 3 bottles (2L) of known concentration (160, 180, and 200 µg C L^{-1}) of *R. baltica* were prepared, as above. To know the exact start concentration, 10 samples were taken directly from each bottle and counted, using the CoulterCounter. The remaining algae solution was added directly to VOC-

vials (40mL). 5 samples of each concentration were counted without fixation. 3 samples were counted after being fixated for 5 hours. Further 5 samples of each concentration were counted after 21 hours, 46 hours, one week, and 25 days (called 4 weeks for simplicity). After counting the 4 week samples, remaining material in the vials were diluted 5x, and counted again, to see how dilution affects the algae concentration.

To test if the pipetting errors/variations could have an effect on the standard deviation of algae counts in the feeding experiment, a pipetting experiment was performed. Deionized water was pipetted using either a Shorty Phenix One-Piece Serological Pipette (20 mL) or eppendorf pipettes (0.141-5 mL). Each volume pas pipetted and weighed 10 times, to validate the accuracy of the pipetted amount.

2.2.4. Egg hatching

Two hatching success experiments were performed for each species. In the first experiment, 20 eggs of each species) were added in vials (12 mL), with 2mL FSW, and filled with the applied exposure solutions (Control, 10%, 50%, and 100% WSF), and fixated with Lugol solution after specified times (*C. finmarchicus*: 24h, 36h, 48h, and 72h, *C. hyperboreus*: 48h, 96h, and 144h). The different time scales for the two species is due to the development time differences between the species.

For *C. finmarchicus* starting point (zero time) was defined as when the ovigerous females were incubated in the egg-laying containers. For *C. hyperboreus* the females had been left undisturbed in the containers up to 48h when the eggs were collected, and the first fixation was made 12h after collection. Therefore, the eggs could be up to 60h of age, when the 48h fixation was performed.

In the second experiment, 30 eggs were added to vials (20 mL) filled with FSW. They were fixated with Lugol solution a given number of hours after being added to the vials (24h, 48h, 72h, and 96h for *C. finmarchicus* and 48h, 96h, 144h, and 192h for *C. hyperboreus*).

For both experiments, after each period, eggs/animals were transferred from the vials to a glass dish using a Pasteur pipette with an enlarged aperture and burned edges (described above). The number of eggs and the number of animals were counted manually by observing them through a stereo microscope (Leica M80) (Appendix F and G).

2.2.5. Biometric measurements

C. hyperboreus and *C. finmarchicus* were photographed, diameter of the eggs, body length (NI-NII) or prosome length (NIII) were obtained from the photographs. Copepods used for measuring respiration rate (another master thesis, 12-30 individuals, depending on stage and how many animals were found after the respiration experiment) were placed on concave microscope slides, and as much water as possible was removed, to prevent the copepods from moving around. Digital images were taken through a microscope (bright field, Leitz Biomed (Arctic Station), Nikon eclipse C*i* (SeaLab)) using a Unibrain firewire camera (Fire-i 785c color: 4605) operated by Fire-i software (Unibrain S.A., Athens, Greece), calibrated at magnification 4.0x0.5. The eggs of *C. hyperboreus* (3 and 10 °C) were taken at magnification 10x0.5. A unique ID-name was given to every photo, and the photos were stored on a personal computer.

Cross section (eggs), body length (NI-NII), and prosome length (NIII) was outlined manually, in the ImageJ software (National Institute of Health, Bethesda, MD, USA). A line was drawn of the diameter of the eggs, from the anterior dome of the head to the rearmost part of the body (NII-NII), and from the anterior dome of the head to the end of the prosome (Figure 2.3.). ImageJ was calibrated by measuring the length of an image of a ruler (0.01 mm) taken at magnification 4.0x0.5, giving the count 537 pixels mm^{-1} (*C. hyperboreus* 3 and 10 °C), 539.3337 pixels/mm^{-1} (*C. hyperboreus*, 7.5 °C and 538.5002 pixels/mm^{-1} (*C. finmarchicus*). For the eggs taken at magnification 10, a new calibration was made, giving the count 539 pixels/0.40 mm. The program converted pixels into mm, and thereby derived the marked length.

2.2.6. Dry weight analysis

In addition to biometric measurements, the same copepods were also subjected to dry weight (DW) analysis. The nauplii were collected with a micropipette (eppendorf, 10-20 µl), and rinsed in isotonic ammonium formate (CH_5NO_2, 0.5M), to avoid formation of salt crystals during the drying process. Crystals would add to the sample weight, and could thus corrupt the DW measurements. The rinsed copepods were placed in pre-weighed tin capsules (5x9 mm, empty weight: 30-32 mg) arranged in a 96 well plate. Number of individuals in each sample can be seen in Appendix A. The samples were dried in a heating cabinet (60°C) for a minimum of 24 hours. DWs of the nauplii were determined by subtracting the weight of the empty tin capsules from the weight of tin capsules containing copepods, using a microscale weight (Mettler

Toledo). Average weight of individuals was determined by dividing each sample weight with the number of individuals added to the tin capsule.

2.2.7. Chemical analysis of water samples

Solid-phase extraction (SPE), was used to extract the PAHs from the preserved experimental solution, using an Agilent Bond Elut PPL SPE column. The columns with the retained components were then kept at -20°C before analysis at SINTEF Material and Chemistry (Environmental Technology department) in Trondheim, GC-MS was used for the analyses, using a method based on the US EPA 8270D method to quantify PAH components (US EPA 2014). For each individual experiment, the different exposure solutions in the VOC-vials were combined, and analyzed

2.3. Statistics

Microsoft Excel (2013) was used for calculations and sorting of data, while the Sigmaplot 13.0 (Systat Software) was used for generation of graphs and statistical analysis. Two-way Analysis of Variance (ANOVA) was used for statistical comparisons of the differences between the samples. The procedure was selected to examine the influence of both temperature and exposure concentration. The significance level (α) was set to 0.05 for all tests. In a two-way ANOVA, there are three null hypotheses; the first factor (temperature) has no effect on the population mean, the second factor (exposure) has no effect on the population mean, and there is no interaction between the two factors (temperature x exposure), where all the null hypotheses are tested together. If any of these hypotheses are rejected further comparisons need to be carried out, to test how the factors have an effect or interact (Towned 2002)

If any of the null hypotheses were rejected ($p<0.05$), a Holm-Sidak post-hoc test (multiple comparison) was performed to identify the groups with a different mean. For the exposed hatching success experiment, the effects of temperature and exposure were tested for, within each time-interval. For the un-exposed hatching success experiment, a one-way ANOVA was used to test for the different temperatures within each time-interval.

Both two-way and one-way ANOVA assumes approximately normally distributed data and that the variance is equal (homoscedasticity), meaning that the standard deviations in the different groups are within the same range (Towned 2002). A Shapiro-Wilk test was used for testing normality, and a Brown-Forsythe test was used for testing for equal variance. In the cases where two-way ANOVA was used, and data was not normally distributed or homoscedastic, simple

transformations were made. First the data set was log transformed ($\log_{10}$), and if these data were not normally distributed or homoscedastic, a square root- or a square-transformation was performed. In some cases, the data was not normally distributed or homoscedastic after any transformation. In some of these samples, the normal distribution was assumed based on the histograms, and an ANOVA and Holm-Sidak post-hoc test was performed if the null hypothesis' were rejected, while in some of the hatching success experiments (*C. finmarchicus* 24h and 36h, *C. hyperboreus* 48h and 144h), normal distribution was impossible to achieve because almost all the samples were equal. A two-way ANOVA was still performed, followed by a Holm-Sidak post-hoc test if statistical significance was detected, however, one should be careful when interpreting these results.

One-way ANOVA was performed on all the experiments on eggs (biometry, dry weight) as temperature was the only variable factor, in addition to examining differences in dry weight and biometry between the nauplii stages. If the normality- or equal variance tests failed, a Kruskal-Wallis One-way ANOVA on ranks was performed. If he null hypothesis was rejected, i.e. there was a difference in mean between the samples, a Pairwise comparison using Dunn's Method was performed. If the samples were normally distributed, and had equal variance, and the null hypothesis was rejected, a Holm-Sidak post-hoc test was performed.

3. Results

3.1. Experimental conditions

The temperature in the water bath was measured regularly throughout the experiment (Table 3.1). Measured temperatures are used when describing stage development times. Nominal temperatures are used consistently to describe the rest of the results.

Table 3.2. Measured temperatures (mean±SD) in the water bath for each temperature. The temperature in the water bath was measured regularly throughout the developmental period. VOC-vials containing the copepods were incubated in the water bath.

Species	Nominal temperature (°C)	Measured temperature (°C)
C. hyperboreus	3.0	3.43±0.12
	7.5	7.68±0.30
	10	9.34±0.26
C. finmarchicus	7.5	7.56±0.29
	10	9.80±0.22
	12.5	12.35±0.34

3.2. Stage duration and development

The time (days) from incubation to sampling of the selected stage was plotted for each stage (eggs – N3) of *C. hyperboreus* and *C. finmarchicus* (Figure 3.1). *C. hyperboreus* developed slower than *C. finmarchicus*, and developmental time generally decreased with increasing temperature. However, *C. hyperboreus* (7.7°C), which was sampled at SeaLab, was an exception, and developed faster than *C. hyperboreus* (9.3°C), sampled at the Arctic Station. Time of development from egg to NIII was 14 days at 3.4°C, 8 days at 7.7°C and 9 days at 9.3°C for *C. hyperboreus*. There was no experiment performed for *C. hyperboreus* NII at 9.3 °C, due to high mortality, therefore, the day that is plotted in figure 3.1, is the day the NII experiment was planned to be performed. For *C. finmarchicus* time of development from egg to NIII decreased from 5 days at 7.6°C to 3 days at 12.3°C. Summary of temperatures, sampling dates, and development times can be found in Table B.1., Appendix B.

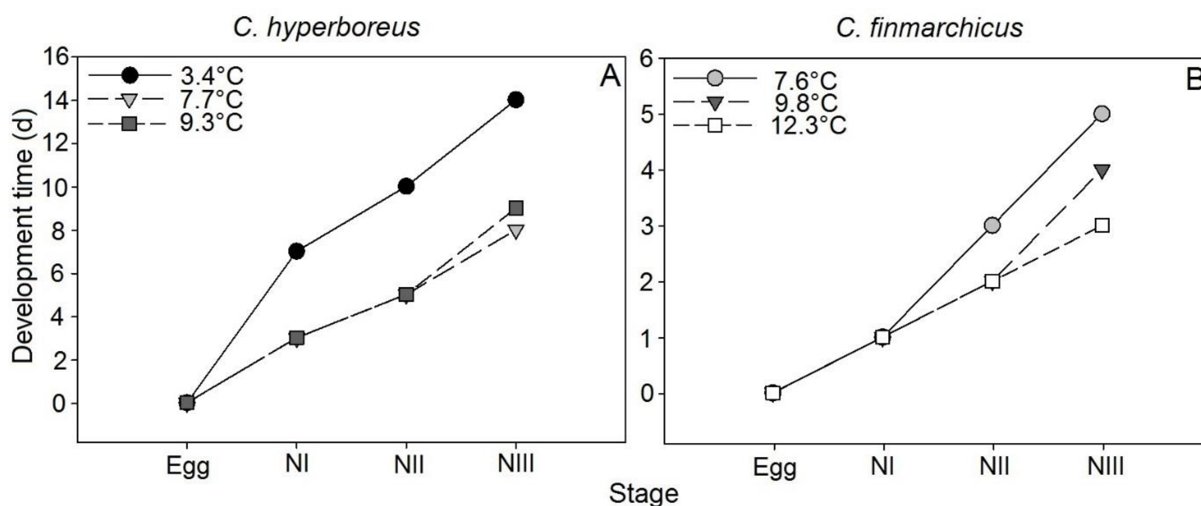

Figure 3.3. Development times of *C. hyperboreus* (A) and *C. finmarchicus* (B) nauplii at three different temperatures. NI, NII, and NIII represents each of the naupliar stages. Development time is the period from the eggs were incubated to the day the nauplii-stage was sampled. The nauplii were sampled as soon as there were enough copepods of the desired stage. All samples within a temperature group were sampled the same day; therefore, the data does not provide a basis for SD. For *C. hyperboreus* 7.5 and 10°C were sampled after the same number of days. For *C. finmarchicus* NI was samples after 1 day for all temperatures. NII was sampled the same day for the two highest temperatures. Number of treatments and samples in each treatment for each stage is summarized in Appendix A, while sampling dates and developmental time is summarized in Appendix B.

3.3. Exposure concentration

Results from the chemical analysis of the WSF-solutions are summarized in Appendix H, while the sum of all PAHs can be found in Table 3.2. For most samples, the concentration was measured both in the bottles containing the diluted samples, which was the remains, after the solution had been added to the VOC-vials, and in the water removed from the VOC-vials after exposure of the eggs. The VOC-vials generally had a lower concentration than the diluted WSF-samples. The vapor pressure of naphthalene is relatively high (Neff et al. 2011), and the lighter components could have evaporated from the bottles and VOC-vials while the caps were off. Evaporation and spreading via the air may explain why naphthalene could be detected in the controls.

Table 3.3. The measured concentration of ΣPAHs (μg L^{-1}) in water samples from the stock solution, WSF from bottle, which is the remains of the water that was added to the VOC-vials, and the WSF from VOC-vials after the exposure. The stock solution for *C. hyperboreus* at 3°C were measured 3 times (mean±SD shown), and at 7.5°C there were samples from two bottles (mean shown). The individual PAHs can be found in Appendix H. For hatching success, the same solution was used for all temperatures.

			WSF from bottle				WSF from VOC-vials			
		Concentration ΣPAHs (μg L^{-1})								
Species	Temperature (°C)	Stock solution	Ctrl	10%	50%	100%	Ctrl	10%	50%	100%
C. hyperboreus	3	13.34±1.27					0.14	0.38	1.08	1.80
	7.5	19.36	0.02	1.82	9.19	18.37	0.05	1.20	5.94	11.82
	10			1.34	5.99	11.46	0.18	0.62	3.41	7.31
	Hatcing success		0.10	1.82	9.55	18.74				
C. finmarchicus	7.5		0.03	1.82	9.60	19.12	0.07	1.22	6.33	13.23
	10		0.03	1.53	7.93	16.34	0.07	1.07	5.31	11.30
	12.5		0.10	1.35	8.13	12.76	0.05	0.94	5.54	9.75
	Hatching success	23.26	0.05	2.04	10.65	21.47				

3.4. Algae consumption

For the experiments performed at the Arctic Station (*C. hyperboreus* 3 and 10°C), algal densities based on measured samples fixated with Lugol solution, apparently had much lower concentrations of algae than the experiments where the algae were counted directly after finishing the feeding experiment (*C. hyperboreus* 7.5°C and *C. finmarchicus* 7.5, 10, and 12.5°C) (Figure I.1, Appendix I). Because of this, the algae consumption (ALCON) of *Calanus* appeared to be significantly higher for *C. hyperboreus* tested at 3 and 10°C (Greenland) than at 7.5°C (Trondheim).

For the 6 hour feeding period, there was a significant effect of the combination of temperature and exposure (P=0.013) (Table J.1., Appendix J). ALCON at 3°C was significantly lower than at 10°C in the Ctrl group (P = 0.008) and significantly higher than at 10°C in the 50% group (P = 0.006) (Figure 3.2., A). When mean ALCON of all exposures within the temperatures were analyzed, consumption at 7.5°C came out significantly different from the 3 and 10°C experiments, as well. (Table J.2, Appendix J). At 3°C, ALCON in the Ctrl group was significantly lower than in the 50% exposure group (P = 0.005) (Figure 3.2., A).

For the experiment that was ended after 20 hours feeding period, there was also a significant effect on the combination of temperature and exposure (P=0.002) (Table J.1., Appendix J). ALCON at 3°C was significantly higher than at 10°C within the 10% exposure group (P <0.001) (Figure 3.2, C). If mean consumption of all exposures within the temperatures were analyzed,

all temperatures yielded significantly different consumption from each other (Table J.2, Appendix J). Because there were only enough copepods for two parallels at 3°C/50% exposure, and one of these parallels had evaporated when arriving at SeaLab, after being transferred from the Arctic Station, there was only one parallel left, preventing further statistical treatment. Within 10°C, the ALCON in Ctrl was significantly higher than in all the other exposure groups (Figure 3.2, C).

For the 6 hours feeding period with *C. finmarchicus*, there was no effect on ALCON of the combination of temperature and exposure (P=0.575) (Table J.1., Appendix J), and there were no significant differences related neither to exposures nor temperatures (Figure 3.2, B). When ALCON mean of all temperatures were analyzed, the 10% exposure group was significantly different from the Ctrl group (P = 0.046) and the 100% group (P = 0.032) (Table J.3, Appendix J). Some values apparently were below, or close to 0. The number of consumed algae was obtained by subtracting the number of algae cells at a given exposure time from the assumed starting number of algae cells (Table I.1., Appendix I), and in some cases, the number of algae counted, was higher than the assumed number of algae in the beginning.

For 20 hours feeding period with *C. finmarchicus*, the combination of temperature and exposure had no effect (P=0.928) on ALCON (Table J.1., Appendix J). Consumption at 7.5°C was significantly lower than at 12.5°C within Ctrl (Figure 3.2, D). When analyzing mean of all exposures, ALCON at 7.5°C was significantly different from both 10°C (P = 0.006) and 12.5°C (P <0.001) (Table I.1, Appendix I).

The feeding experiment for *C. finmarchicus* should be interpreted carefully, as there were many of the curves given by the CoulterCounter that seemed to have a lot of background noise, making the integration of the number frequency peak quite difficult (Figure D.2., Appendix D).

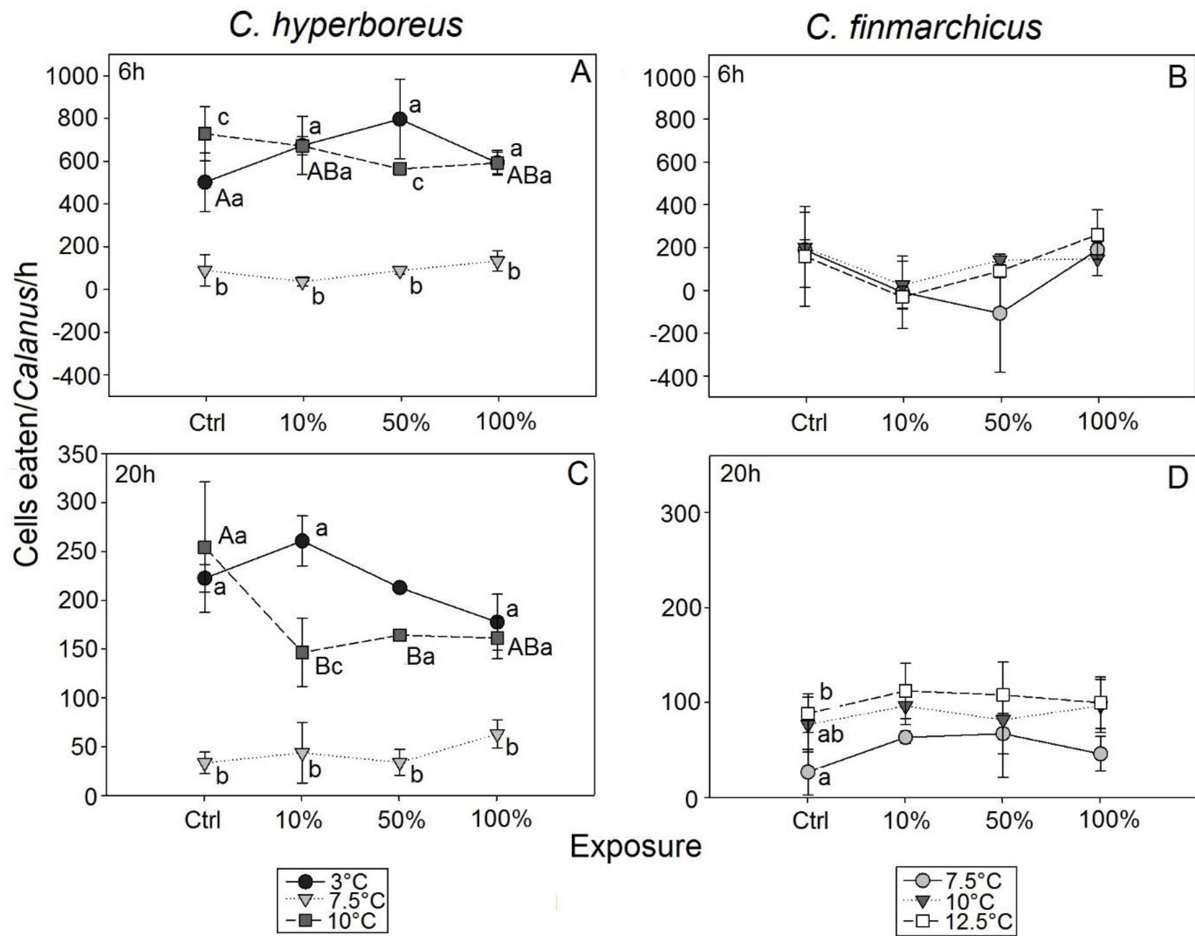

Figure 3.4. Number of algae cells eaten/*Calanus*/hour of A and C: *C. hyperboreus* and B and D: *C. finmarchicus* at different temperatures and treatments (means ± SD, n=3 in all groups except in the *C. hyperboreus* 50% group in the 20 hours time period, where there was only one parallel). A and B: 6 hours exposure. C and D: 20 hours exposure. Number of individuals in each parallel is given in table B.1 in Appendix B. Significant difference between the temperature groups within each exposure group is indicated with lower case letter, while significant difference between exposure groups within the same temperature group is indicated with upper case letter (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

3.5. Methodological development of the feeding experiment

Comparing fluorescence as a measure of algae concentration with counting on the CoulterCounter, revealed that the fluorescence method had much higher uncertainty, compared with particle counting (Figure 3.3, A and 3.4). As opposed to CoulterCounter measurements (Figure 3.3., B), the measurements of the fluorescence overlapped between the chosen algae concentrations (Figure 3.3., A).

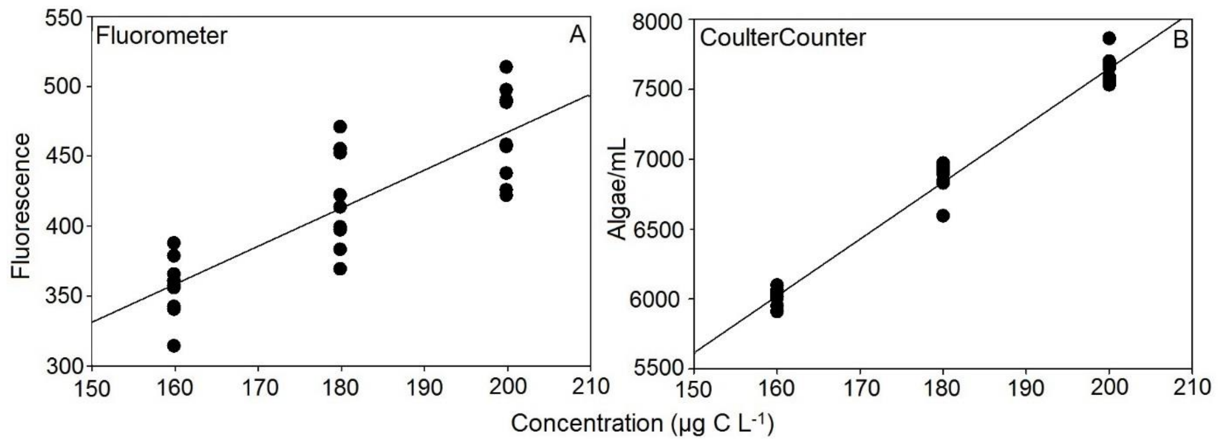

Figure 3.3. 10 samples of known algae concentration (160, 180, and 200 µg C L^{-1}) were filtrated, and chlorophyll extracted, to measure fluorescence on a Fluorometer (A), and counted on a CoulterCounter (B).

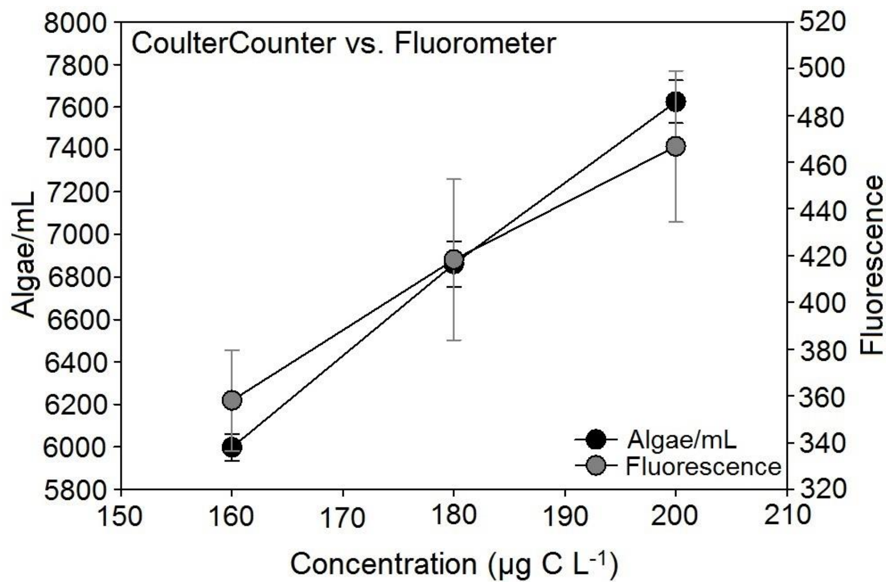

Figure 3.4. Data from Figure 3.3. plotted together (mean±SD). Error bars for Fluorescence is in grey, to better visualize the difference.

In the experiment where algae were fixated with Lugol solution, there was a clear decrease, compared to the unfixated samples, and it seemed that the difference increased with increasing concentration (Figure 3.5). However, the fixated samples remained fairly stable with time. On the other hand, when the 4 week old samples had been diluted 5x, and counted again, the standard deviation between the parallels had increased, when multiplying with 5, to calculate the original concentration.

After the feeding experiment was finished, the vials were emptied and rinsed with a volume of FSW. The main water content with suspended algae was then put forward to analyses. The algae in the rinsed water, assumed to be a fraction of the algae stuck to the bottom and inner walls of the vials, were then counted on the CoulterCounter. The number of algal cells in the

rinsed water varied much between the samples (3000-24000); however, algae were definitely present in most samples (Appendix D).

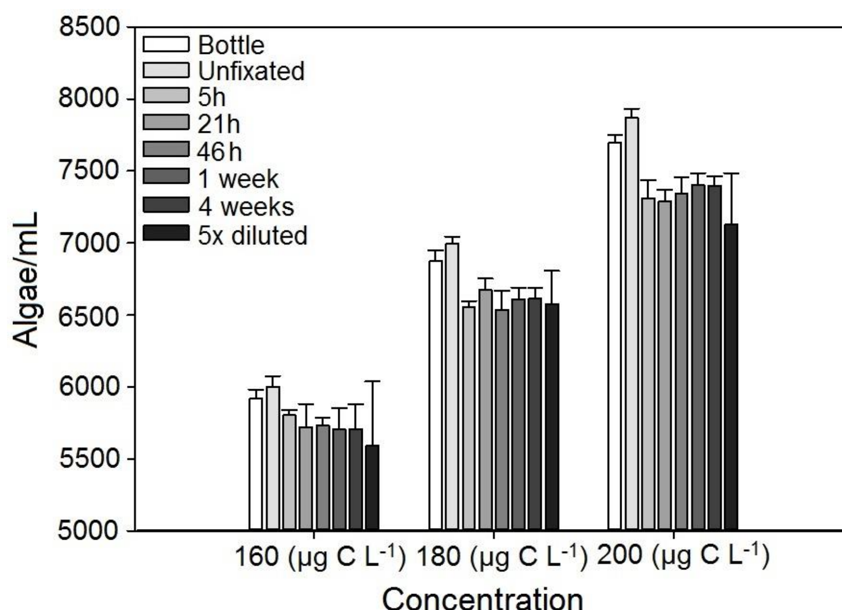

Figure 3.5. Fixation experiment. 3 concentrations (160, 180, and 200 (µg C L^{-1}) of *R. baltica* were prepared in bottles (2L). First, 10 samples of each concentration taken directly from each bottle was counted (Bottle). The solution was further added to VOC-vials, where 5 of each concentration was counted without being fixated (Unfixated). 3 fixated samples were counted after 5 hours. For the rest of the samples, there were 5 of each concentration. After the 4 weeks (25 days) samples had been counted, rest algae solution in the samples were diluted 5 times, and counted again (5x diluted). Before plotting these samples, they were multiplied with 5, to compare with the undiluted samples.

An experiment to examine if the pipetting could affect the results of the feeding experiment was performed (Table 3.3). Generally, the weight was a little lower than the expected volume, however, the standard deviation is very small. Thereby, it is unlikely that the pipetting caused the large standard deviation of algae density measurements seen in the feeding experiment.

Table 3.3. Pipetting experiment. To test if the pipetting was the reason for the high standard deviation of algal density measurements in the feeding experiment, ion free water was pipetted and weighed. For the 20 mL volume a Shorty Phenix One-Piece Serological Pipette was used, while for the remaining volumes, eppendorf-pipettes (0.200 mL-5mL) were used. Each pipetting was repeated 10 times, the results (mean±SD) showed in the table. The 5 mL volume test was repeated three times, with parenthesis indicating the different times.

Volume (mL)	Mean weight (g)	SD
20 mL	19.972	0.0228
5 (1)	4.996	0.0232
5 (2)	4.960	0.0369
5 (3)	4.969	0.0090
3	2.989	0.0361
1	0.991	0.0020
0.432	0.424	0.0009
0.200	0.199	0.0004
0.141	0.139	0.0005

3.6. Growth and development

3.6.1. Egg hatching

Exposure of PAHs did not seem to have much effect on developmental time and hatching of the eggs in neither *C. hyperboreus* nor *C. finmarchicus*. There were no significant differences between any of the exposed groups within any of the temperatures applied for *C. hyperboreus*, and it seemed as exposure of PAHs had no effect on final hatching success after 144 hours (Figure 3.6, A, C, E). For *C. finmarchicus*, after 24h, the 100% exposure group had significantly higher hatching percentage than the other exposure groups. Further, in the 100% group, significantly more eggs had hatched after 48 hours than the Ctrl group, at 12.5°C. However, for the 100% group at 12.5°C, more eggs had hatched after 48 hours, than after 72 hours. Within any of the other temperatures, there were no significant difference between the exposures. Regarding final hatching success after 72 hours, the mean of the 100% exposure group was lower than the other exposure groups at 7.5°C, although not significantly (Figure 3.6, B, D, F). For *C. hyperboreus* there did not seem to be any combined effect of temperature and exposure on egg hatching within any of the time periods. However, for *C. finmarchicus*, the combined effect of temperature and exposure on egg hatching was significant after 24h (P=0.026) and 48h (P=0.010), but not for the other time periods (Table L.1., Appendix L).

Temperature seemed to have a much larger effect on developmental time and hatching of the eggs for both *C. hyperboreus* and *C. finmarchicus*. For *C. hyperboreus*, within the different exposures, after 48 hours, all the different exposures showed almost zero hatching. It is clear that the eggs of *C. hyperboreus* develop slower at 3°C than 7.5 and 10°C. After 96 hours, the 3°C exposure group showed significantly lower hatching (around 50%) than both 7.5°C and 10°C, which had reached a hatching percentage close to 100% (Figure 3.7, A, C, E, G). When analyzing mean of all exposures, the hatching in the 3°C group was significantly lower than in the 7.5 and 10°C groups (Table L.2, Appendix L). After 144 hours, all the different exposures and temperatures had close to 100% hatching success. It should be noted that neither 48 hours nor 144 hours had normal distribution, therefore these results should be interpreted carefully. These numbers could not be normality transformed, as there were many samples with the same value. (Figure M.1., Appendix M).

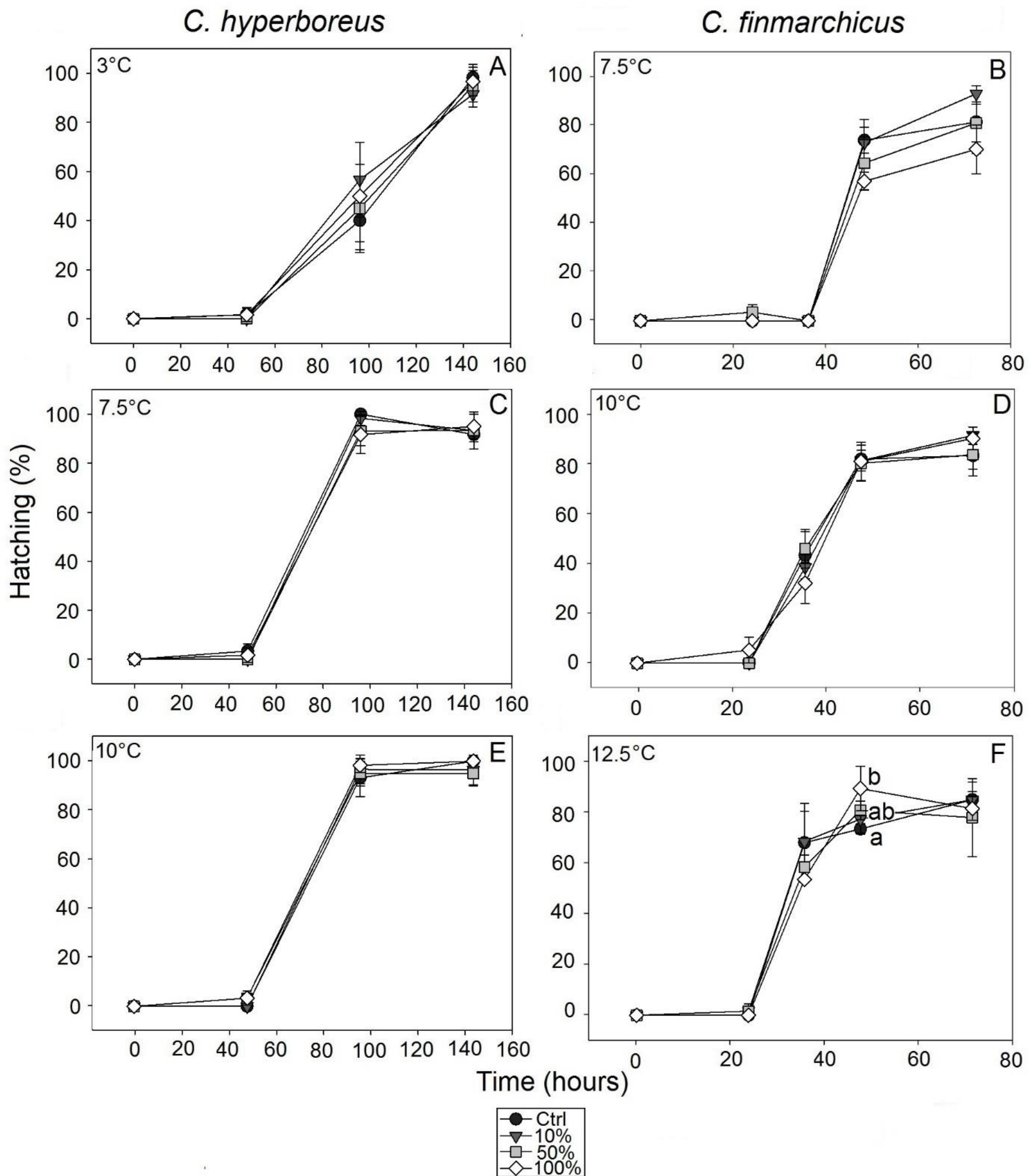

Figure 3.6. Hatching (%) of *C. hyperboreus* (A, C, E) and *C. finmarchicus* (B, D, F) at different temperatures (means ± SD). Number of eggs and nauplii is given in Appendix F. Significant difference between exposure groups is denoted with lower case letters (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Regarding *C. finmarchicus*, temperature seemed to have an effect on egg development, with an inverse relationship between temperature and egg hatching. After 36 hours, the 7.5°C group was still close to 0% hatching, the 10°C group had a significantly higher hatching percentage (around 40%), and 12.5°C had a significantly higher hatching percentage (around 60-70%) than the other two temperatures within all exposures (Figure 3.7, B, D, F, H). When mean of all exposures were analyzed, all temperatures were also significantly different from each other with

regard to hatching percentage (Table L.2, Appendix L). However, neither at 24 hours nor at 36 hours the percentage hatching showed normal distribution, due to many samples having zero hatching, making normal distribution transition impossible (Figure M.2., Appendix M). After 48 hours, hatching percentage in the 7.5°C group had reached almost 80% in the Ctrl and 10% exposure groups, while in the 50% and 100% exposure groups, the hatching percentage mean was only around 60%. Likewise, within the Ctrl and 10% exposure groups, there were no significant differences in hatching between the temperatures. Within the 50% exposure group, the 7.5°C group had significantly lower hatching percentage than both the 10 and 12.5°C groups (about 80%). Within the 100% exposure group, the 7.5°C group had significantly lower hatching success than the 10°C group (around 80%), while the 12.5°C groups showed significantly higher hatching percentage (about 90%) than at the other two temperatures (Figure 3.4, B, D, F, H). When mean of all exposures were analyzed, the 7.5°C group appeared significantly different from the other two temperatures with regard to hatching percentage (Table L.2., Appendix L). After 72 hours, it is assumed that full hatching potential is reached. Here, there were no significant difference in hatching success between the temperatures, and almost all groups had reached a hatching percentage around 80. The only exception was the 7.5°C group within the 100% exposure group, which had a mean hatching percentage around 60%. However, due to large standard deviation, there was no significant difference from the other groups (Figure 3.7, B, D, F, H).

In the second hatching success experiment, where only the effect of temperature was examined, the time periods referred to the time when the eggs were sampled. After 48 hours, the *C. hyperboreus* 3°C group exhibited significantly lower hatching success than the 7.5°C group, which was again significantly lower than in the 10°C. After 96 hours, the 3°C group had significantly lower hatching than both the 7 and 10 °C groups. After 144 hours, there were no significant differences between any temperatures. However, after 192 hours, the 3°C group had significantly higher hatching success than the10°C group, while no data could be obtained for the 7°C group. For *C. finmarchicus*, there were no significant differences between any of the temperatures (Figure G.1, Appendix G).

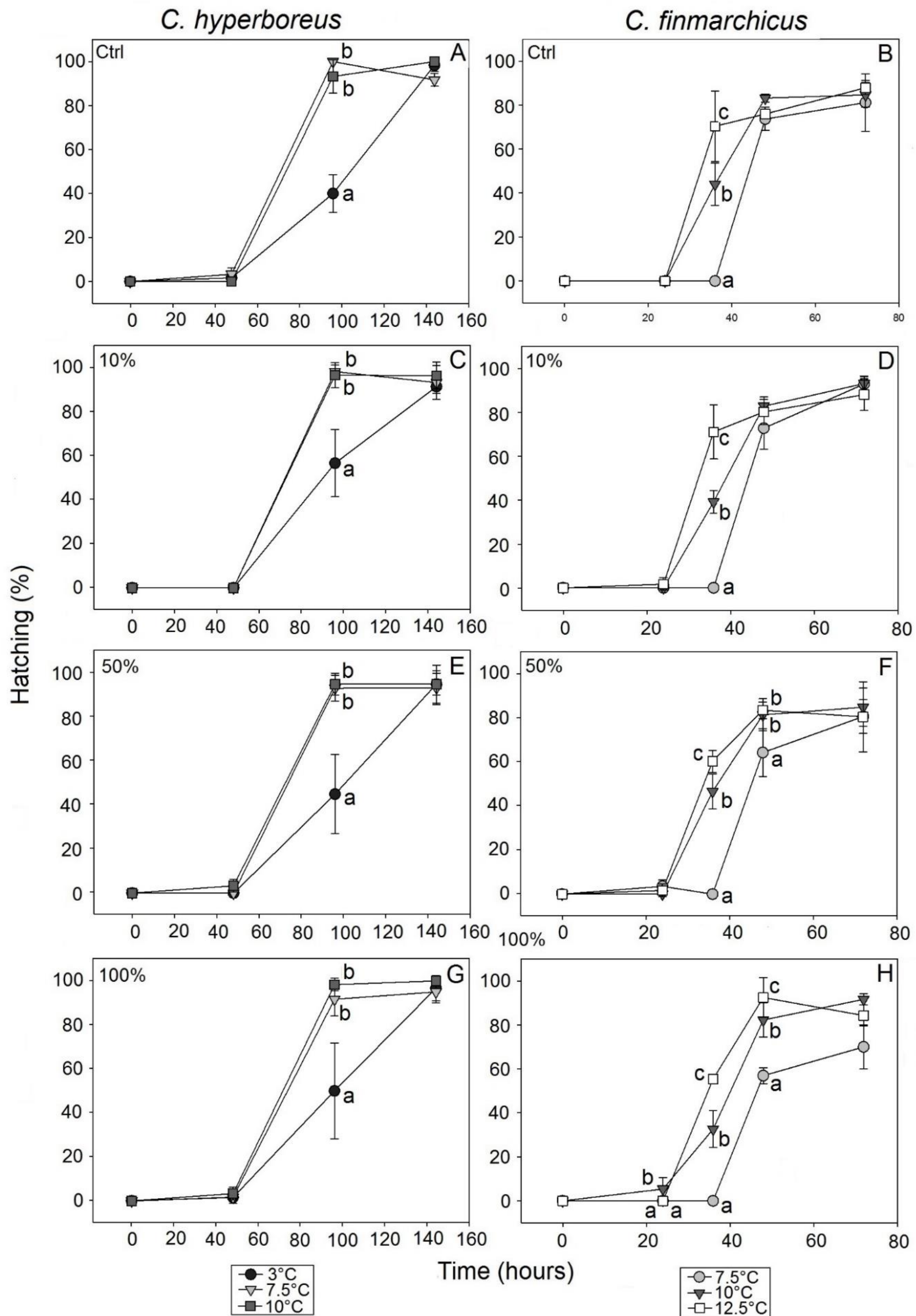

Figure 3.7. Hatching success (%) of *C. hyperboreus* (A, C, E, G) and *C. finmarchicus* (B, D, F, H) at different exposures (means ± SD). Number of eggs and nauplii is given in Appendix F. Significant difference between the temperatures within each exposure group, after a given number of hours, is indicated with lower case letters letter (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

3.6.2. Dry weight

There were no significant differences between the dry weight of the eggs of neither groups of *C. hyperboreus* nor *C. finmarchicus* (Figure 3.8)

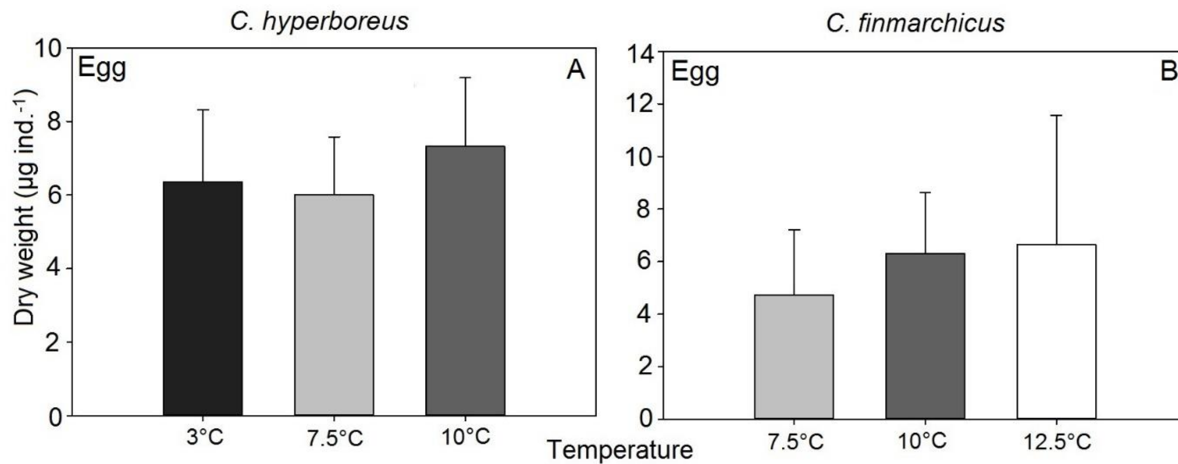

Figure 3.8. Dry weight (µg ind.$^{-1}$) of the eggs of *C. hyperboreus* (A) and *C. finmarchicus* (B) at different temperatures (means ± SD). Number of individuals is given in Appendix A. There were no significant differences between the different temperature groups (*C. hyperboreus* – Kruskal-Eallis One-way ANOVA on ranks, *C. finmarchicus* – One-way ANOVA, $p < 0.05$).

There were no significant differences in dry weight (µg ind^{-1}) within the different exposures nor the different temperatures for the developmental stages NI and NII of both *C. hyperboreus* and *C. finmarchicus* (Figure 3.9, A – D). However, for *C. hyperboreus* NI, there was a significant dry weight difference between the Ctrl and 50% exposure groups (P = 0.007), and the 50% and 100% exposure groups (P = 0.023), when mean of all temperatures within the different exposures were considered (Table N.1, Appendix N). The combination of temperature and exposure, had no significant impact on NI (P=0.952) nor NII (P=0.390) for *C. hyperboreus* (Table N2, Appendix N). For *C. hyperboreus* NII, there was a significant difference in dry weight between the 3°C and 7.5°C groups (P = 0.013), when mean of all exposure groups within each temperature were considered (Table N.3., Appendix N). At developmental stage NIII of *C. hyperboreus*, the Ctrl had a significantly higher dry weight than the 10% exposure group (P = 0.015) and the 100% group (P = 0.018), within 7.5°C. Within the other temperatures and all the exposure groups, no significant differences were found (Figure 3.9, E). In addition, there was no significant effect on dry weight of the combination of temperature and exposure (P=0.262).

For *C. finmarchicus* NII there was a significant difference between the 7.5°C and 12.5°C groups (P = 0.024) when mean of all exposures within the temperatures were considered (Table N.3, Appendix N). The combination of temperature and exposure had no significant impact on *C.*

finmarchicus NI dry weight (P=0.276), nor the dry weight of NII (P=0.728) (Table N2, Appendix N). At developmental stage NIII of *C. finmarchicus*, there were no significant differences in dry weight between the exposures, within any of the temperatures. However, there seemed to be a significant effect of the combination of temperature and exposure (P=0.026) (Table N2, Appendix N). For the 12.5°C group, there was an increasing trend from the Ctrl group, via the 10% and 50%, and up to the 100% groups. The Ctrl and 100% exposure groups were almost significantly different (P = 0.052). Within the Ctrl, the 7.5°C group dry weight was significantly higher than in the 12.5°C group (P = 0.025), and the 10% exposure group had almost a significantly higher dry weight than the 12.5°C group as well (P = 0.076). Within the other exposures series, there were no significant difference between the temperatures (Figure 3.9, F).

Differences between the stages within each species and treatment were also examined (Appendix P). Differences between the eggs and the other stages, were only examined within Ctrl, as eggs had not been exposed to PAHs. Within *C. hyperboreus* in the Ctrl 3°C group, NIII had significantly higher dry weight than the eggs, however, there were no significant differences between any other stages. Within the Ctrl 7.5°C group, NIII had significantly higher dry weight than the other stages. Within the remaining of *C. hyperboreus* treatments, the dry weight of the stages was not significantly different.

Within the *C. finmarchicus* the Ctrl and the 10% exposure groups at 7.5°C and the Ctrl at 10°C, NIII had significantly higher dry weight than the other stages within these treatment groups. Within the remaining *C. finmarchicus* treatment groups, the stages were not significantly different.

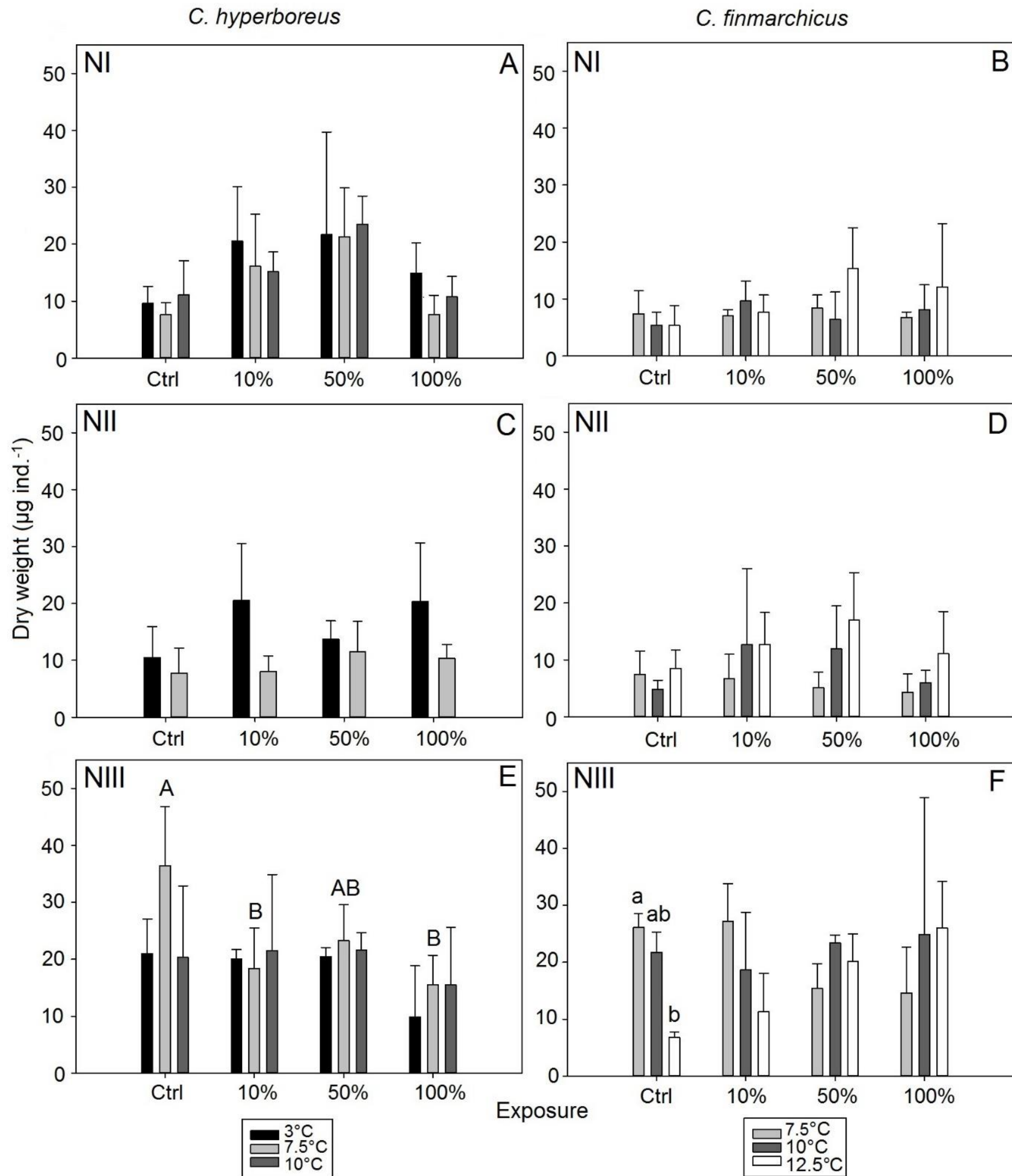

Figure 3.9. Dry weight (µg ind.$^{-1}$) of the different nauplii-stages of *C. hyperboreus* (NI – A, NII – C, NIII – E) and *C. finmarchicus* (NI- B, NII – D, NIII – F) at different temperatures and exposures (means ± SD). Number of individuals is given in in Appendix A. Significant difference between the temperature groups within each exposure group is indicated with lower case letter, while significant difference between exposure groups within the same temperature is indicated with upper case letter (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

3.6.3. Biometry

The egg diameters of *C. hyperboreus* showed significant variation between the different temperatures groups, where the 7.5°C group on the average had larger diameter than the 3°C group (P <0.001) and the 10°C group (P <0.001), and the 10°C group had larger diameter than the 3°C group (P <0.001). For *C. finmarchicus* the 7.5°C group had significantly larger diameter than the 10 (P <0.001) and 12.5°C (P = 0.002) groups. (Figure 3.12).

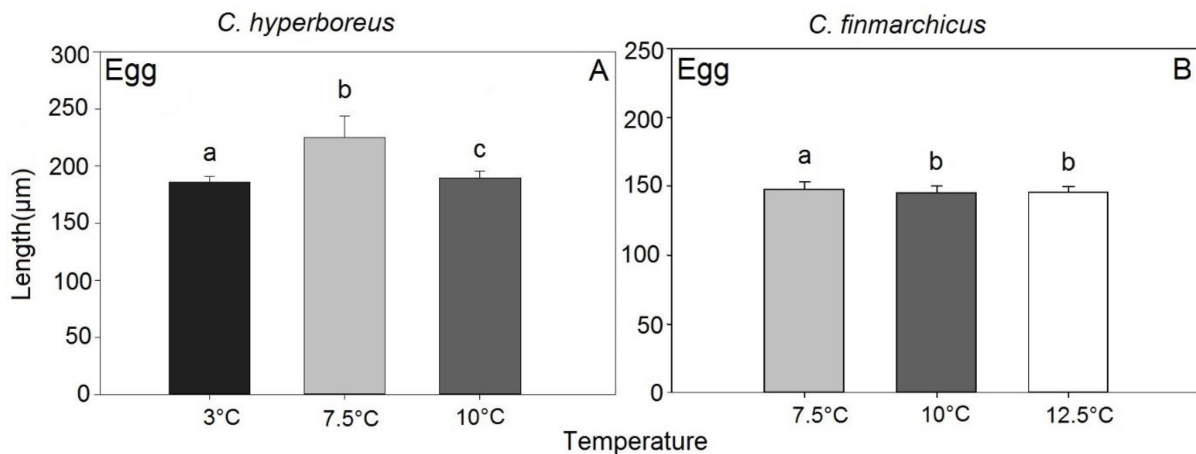

Figure 3.12. Average diameter (µm) of the eggs of *C. hyperboreus* (A) and *C. finmarchicus* (B) at different temperatures (means ± SD). *C. hyperboreus*: 3°C N=141, 7.5°C N=82, 10°C N=105. *C. finmarchicus*: 7.5°C N=121, 10°C N=112, 12.5°C N=73. There were cases where biometry could not be measured on the eggs, and cases where there were more biometry measurements than eggs present, because several pictures may have been taken of the same eggs, which causes the number of measurements to be different from number of individuals in the camera, as shown in Appendix A. Significant difference between the temperatures is indicated with lower case letter (*C. hyperboreus* – Kruskal-Eallis One-way ANOVA on ranks, Dunn's Method, *C. finmarchicus* - One-way ANOVA Holm-Sidak, $p < 0.05$).

Within *C. hyperboreus* developmental stage NI, there was a significant impact on length from the combination of temperature and PAH exposure (P=0.044) (Table Q.1, Appendix Q). The average lengths of the 7.5°C groups were significantly shorter compared to the 10°C groups in all exposure groups except the 10% group. Average length in the 3°C group was only significantly different from the other temperature groups at 50% exposure (Figure 3.13, A). However, when mean length of all exposures were considered, there was a significant difference between all temperature groups (Table Q.2, Appendix Q), and when mean of all temperatures were considered, the nauplii in the 100% exposure group was significantly longer than in all the other exposure groups. In addition, the 10% exposure group was significantly different in length from the 50% group (Table Q.3, Appendix Q).

Within developmental stage NII and NIII, the individuals in the 7.5°C groups were significantly longer than the corresponding individuals in the 3°C groups (Figure 3.13 – C and E) within all

exposures. However, for NII, there was no significant correlation between length and the combination of temperature and exposure (P=0.821), however, this was the case for NIII (P<0.001) (Table Q.1., Appendix Q). For developmental stage NII, there were no significant differences in length between any of the exposure groups (Figure 3.13 – C). Regarding developmental stage NIII, the individuals in the 10°C groups were significantly shorter than the individuals in the 3°C and 7.5°C groups in all exposures except 50%, where length was only significantly shorter than in the 7.5°C groups (Figure 3.13 – E). For the NIII stage, the length of Ctrl individuals was significantly longer than in the other exposure groups at 3°C. Within the 10°C groups, Ctrl individuals were significantly shorter than individuals from the 50% exposure groups (P < 0.001). When analyzing mean of all exposures within these temperatures the 3 and 7.5°C groups were significantly different (NII (P <0.001), NIII (P <0.001)) (Table Q.3., Appendix Q). In addition, for stage NIII, the length of the individuals in the 10°C group and the two other temperature groups were also significantly different (P <0.001) (Table Q.2, Appendix Q). It should be noted that these results should be interpreted carefully, as both the normality test, and the equal variance test failed. However, based on the histograms, the data looks normally distributed (Figure R.1., B-D, Appendix R).

Within *C. finmarchicus* developmental stage NI, there was a significant effect on length, when considering the combination of temperature and exposure (P=0.023). Within Ctrl, individuals of the 7.5°C group were significantly longer than nauplii in the 10 and 12.5°C groups, within the 10% exposure groups, nauplii exposed at 10°C is significantly longer than those exposed at 12.5°C, and within the 100% groups, individuals exposed at 12.5°C were significantly shorter than those exposed at 7.5 and 10°C (Figure 3.13, B). When mean of all exposures were considered, the length of individuals exposed at 12.5°C were significantly different from individuals exposed at both 7.5°C (P <0.001) and 10°C (P = 0.003) (Table Q.2, Appendix Q). Within the 7.5°C groups, Ctrl was significantly longer than corresponding individuals exposed at 50% (P <0.001) and 100% (P <0.001). When mean length of all temperatures within the different exposure groups were analysed, the lengths of the Ctrl groups and the 10% exposure groups were significantly different from the 50% and 100% exposure groups (Table Q.3, Appendix Q).

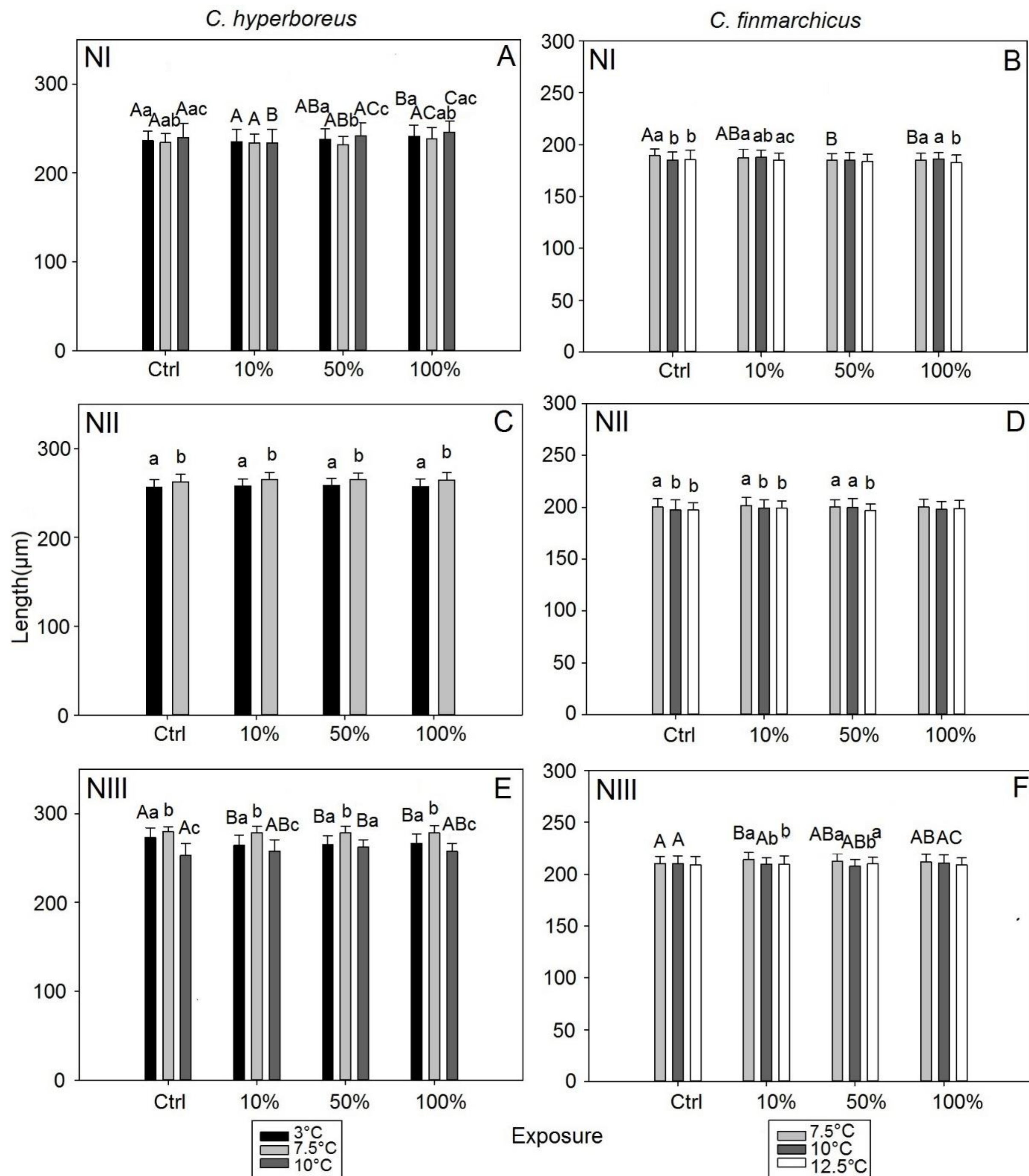

Figure 3.13. Average length (µm) of the individuals at different nauplii-stages of *C. hyperboreus* (N1 – A, N2 – C, N3 – E) and *C. finmarchicus* (N1- B, N2 – D, N3 – F) at different temperatures and exposures (means ± SD). Number of individuals analysed is given in Appendix A. Significant difference between the temperatures within each exposure group is indicated with lower case letter, while significant difference between exposures within the same temperature group is indicated with upper case letter (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Within developmental stage NII, there was no significant impact on length of the combination of temperature and exposure (P=0.136) (Table Q.1., Appendix Q). However, individuals exposed at 7.5°C was significantly longer than corresponding individuals exposed at 10 and 12.5°C within both the Ctrl and the 10% exposure regime. Within the 50% exposure groups,

individuals at 12.5°C were significantly shorter than individuals exposed at 7.5 and 10°C (Figure 3.13, D). When comparing mean of all exposures, individuals in the 7.5°C group was significantly longer than individuals in the 10 and 12.5°C groups (Table Q.2, Appendix Q). There were no significant differences between exposures found (Table Q.3., Appendix Q). It should be noted that neither NI nor NII had equal variance or were normally distributed, and should therefore be interpreted carefully. However, based on the histograms, the data looks normally distributed (Figure 2.R, B and C, Appendix R).

Within developmental stage NIII, the combination of temperature and exposure had a significant effect on length (P=0.011) (Table Q.1, Appendix Q). Individuals exposed at 7.5°C were significantly longer than individuals exposed at 10 and 12.5°C within the 10% exposure group. Individuals exposed at 7.5°C were also longer than individuals exposed at 10°C within the 50% exposure groups (Figure 3.13, F). When analyzing mean of all exposures, individuals exposed at 7.5°C were significantly longer than individuals from both the 10 and 12.5°C groups (Table Q.2, Appendix Q). Within the 7.5°C groups, Ctrl individuals were significantly shorter than nauplii in the 10% exposure groups. At 10°C, individuals in the 50% exposure groups were significantly shorter than corresponding individuals in the 100% exposure groups (Figure 3.13, F).

Differences between the stages within each species and treatment were also examined (Appendix S). Within both species, all stages were significantly different from each other within all treatments, except at 7.5°C.

3.6.4. Relationship between growth and development

Average length (µm) of the individuals transferred to the tin capsules, was plotted against the dry weight (µg) of the content of each respective tin capsule (Figure 3.14). *C. finmarchicus* is clearly divided into the different stages, based on the length, however, this is not as obvious, based on dry weight. Eggs and NI are gathered in their respective area. This could almost be seen for NII, as well, except for some high 10°C values, and many high 12.5°C. Within NIII, the dry weight is very spread, however, most of the "diamonds" seems to be gathered above 300 µg. *C. hyperboreus* cannot be divided into stages neither by length nor weight. When looking at the x-axis, the eggs kept at 3 and 10°C were clearly separated in its own group by diameter length, however, the length of eggs at 7.5°C overlaps with NI. The length of NI, with few exceptions, does not overlap with NII nor NIII, while NII and NIII clearly overlap with each other at all temperatures. The only exception is NIII of 7.5°C, which, with one

exception, is longer than any individuals of all other stages and temperatures. When considering dry weight, there were some very high outliers. If these were not considered, the eggs were generally lighter than all other stages. NI-NIII are very spread, when considering dry weight.

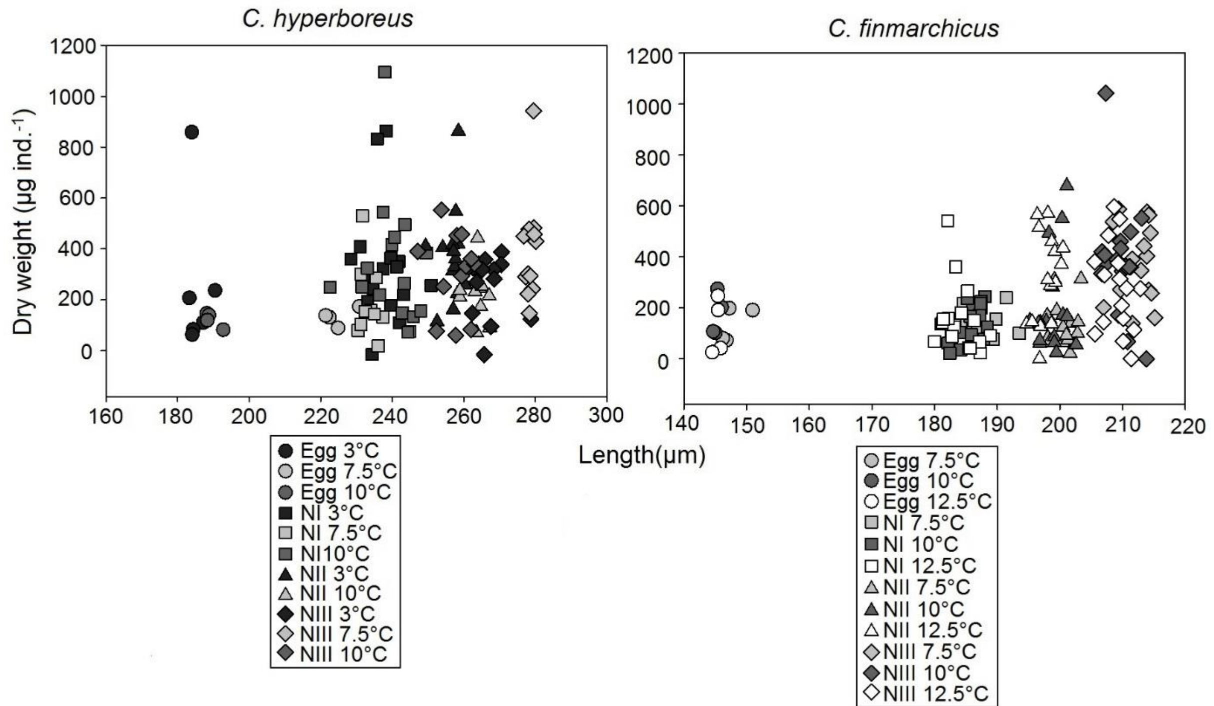

Figure 5.14. Relationship between average length (µm) of the individuals that were transferred to each tin capsule and dry weight (µg) of the content of each respective tin capsule of *C. hyperboreus* and *C. finmarchicus*. Number of individuals from both biometry and dry weight analysis can be found in Appendix A. It should be noted that number of individuals in each tin capsule varied.

4. Discussion

The results from the present study may indicate that PW components may significantly alter temperature-dependent processes in early stages of *C. hyperboreus* and *C. finmarchicus*. The combination of different temperatures and PAH-exposure seems to have altered the feeding efficiency of both *C. hyperboreus* and *C. finmarchicus*. This supports the findings of Hjorth and Nielsen (2011) who reported increased faecal pellet production, as a measure of grazing efficiency, with increasing temperatures in both adult *C. finmarchicus* and *C. glacialis*. When exposed to pyrene (0.202-20.2 µg L^{-1}), faecal pellet production was altered compared to the control, especially at the highest temperature (8°C). PAH-exposure seemed to have little impact on egg hatching, which is consistent with studies performed on *C. finmarchicus* (Grenvald et al. 2013; Hjorth and Nielsen 2011; Jensen et al. 2008). Regarding dry weight, the impact of different temperatures and PAH-exposure seemed to be small. However, there seemed to be some effects on the length of the nauplii, when they were exposed to PAHs at different temperatures.

4.1. Exposure to PAHs

The PAH compounds with lowest number of fused benzene rings and low log K_{OW} (Table 2.1) such as the naphtalenes (N, N1, N2) dissolve more easily in water than PAHs of higher log K_{OW} and were found in the highest concentrations in the exposure solutions. All PAHs had decreased concentrations in the solution from the VOC-vial than in the solution that was added to the VOC-vials (bottle) (Table 3.2). This was expected, as the VOC-vials contained a small amount of FSW (5mL) in them, to make sure the eggs were submerged in water, thereby diluting the solution. However, by using the dilution formula, it was found that the concentration from the VOC-vials was lower than expected. In the *C. hyperboreus* experiment at 7.5°C, for example, the 100% solution that was added to the VOC-vials had a measured concentration of 18.37 µg L^{-1}, based on this, the expected concentration in the VOC-vials was calculated to be 16.07 µg L^{-1}, while the measured concentration was 11.82 µg L^{-1}. A reason for this could be that naphtalenes have relatively high vapor pressure, making evaporation a possibility for the lower concentration in the VOC-vials. Another possibility is adsorption to the glass walls in the bottles and vials; however, this was not investigated further.

Total PAH concentrations in the VOC-vials ranged from 0.38-1.22 µg L^{-1} in the 10%-samples, 1.08-6.33 µg L^{-1} in the 50%-samples, and 1.80-13.23 µg L^{-1} in the 100%-samples. The *C.*

hyperboreus 3°C experiment had for unknown reasons, very low concentrations, which explains the large range of concentrations.

The concentration in the vials used for the egg hatching experiments were not measured, but it is expected to be lower than the concentration in the bottles, as 2 mL FSW was added to each vial to keep eggs submerged, before adding the exposure solution. However, the egg hatching experiment seemed to generally have higher concentrations than the other experiments.

The total concentrations of PAHs in PW emissions to the recipient ranges from 40-3000 µg L^{-1} (Neff et al. 2011), which are above the solutions used in this experiment. However, when PW is discharged to sea, it dilutes fast. Approximately 1 km outside the offshore installations in the North Sea, the total concentrations has been estimated to be in the range from 0.025 to 0.35 µg L^{-1} (Durell et al. 2006; Neff et al. 2011). This is in the same range as the lowest exposure solution in this study. Therefore, this study included concentrations which can be experienced by Arctic copepod populations in oil production areas.

Most studies investigating effects of PAHs on copepods have used sub-lethal concentrations, which is probably more realistic for oil spill accidents. Often, one or a few PAH are used as a proxy (proxies) for total oil content (Hansen et al. 2008; Hjorth and Nielsen 2011; Jensen et al. 2008). By using a mixture of several PAHs, potential additive toxic effects that may rise from PW emission, where a large number of PAHs is present, can be examined (Barata et al. 2005a).

4.2. Feeding

For the feeding experiments performed at the Arctic station, Greenland (*C. hyperboreus,* 3 and 10°C), the algae consumption appeared to be much lower than in the experiments performed at SeaLab (*C. hyperboreus* 7.5°C and *C. finmarchicus*). This may be due to methodological flaws regarding the treatment and analyses of the Greenland samples (see discussion later), and makes it difficult to compare the impacts on feeding within all the three different temperatures of *C. hyperboreus*. However, if only the temperatures 3 and 10°C (the Greenland samples) are considered (same treatment), some differences were observed.

After 6h, the number of algae cells eaten/*Calanus*/h was increasing from the Ctrl group via 10% to the 50% group, with a significant difference between the Ctrl and the 50% groups, while the 100% group was the same level as the Ctrl group. On the opposite, at 10°C there appeared to be a decrease from the Ctrl group, via the 10% group and down to the 50% and 100% groups, however, the decrease was not proved to be significant.

After 20 hours the number of algae cells eaten/*Calanus*/h at 3°C was higher in the 10% group than the Ctrl group, while the 50% group was at the same level as the Ctrl group, and the 50% group was higher than the 100% group, however, the differences were not significant. For 10°C there was a significant decrease from Ctrl, to 10% and 50%, the 100% was not significantly different from the Ctrl, probably due to large SD in both the Ctrl and 100% groups. Therefore, it seemed that exposure to PAHs affected the feeding ability of *C. hyperboreus* to some extent, but without proving a definite dose dependence. On the other hand, the response patterns seen at 3 and 10°C after 6 hours were largely retained after 20 hours. No significant effect of PAH exposure on ALCON in the 7.5°C *C. hyperboreus* experiment was observed. However, since the 3 and 10°C experiments seemed to have opposite trends in ALCON, with regard to exposure, it is a possibility that if the 7.5°C experiment had been performed under the same conditions as the two other temperature experiments, that the ALCON of the nauplii would have been somewhere between the 3 and 10°C experiments. However, this is only speculation.

Nørregård et al. 2014 found a reduction in cumulative faecal pellet production when adult *C. hyperboreus* was exposed to high concentrations of pyrene (20.2 µg L^{-1}), however, this experiment lasted for 14 days, and the difference in pellet production between exposed and control was not clear the first days of the experiment. Since cumulative faecal pellets were used as a measure of grazing, and the experiment was performed on adult females, it is difficult to compare the results with the present study. To the authors knowledge, no feeding experiments have been performed on *C. hyperboreus* nauplii.

Regarding *C. finmarchicus*, after 6 hours there was a non-significant decrease in ALCON from the Ctrl group to the 10% group at all temperatures, and consumption at 10 and 12.5°C 50% was at the same level as in the Ctrl group, while at 7.5°C the mean of the 50% group was lower than both the Ctrl and 10% groups. The ALCON at all three temperatures was in the same range in the 100% group.

After 20 hours, the ALCON in the 12.5°C group was significantly higher than the 7.5°C group within the Ctrl group, while the 10°C group was in the middle. Apparently, the same pattern is repeated also for the exposed groups, but the differences were not proved significant. Higher consumption at higher temperatures were also found in a previous study, where faecal pellet production was used as a measure of grazing (Hjorth and Nielsen 2011). For all temperatures, the consumption within the 10% group was higher than the Ctrl group, however, all temperatures were in the same range. They were also in the same range in the 50% and 100% groups. This contrasts with a study performed with *C. finmarchicus* in the CV stage, where the

authors found a concentration dependent increase in algae concentration (cells/mL) after *C. finmarchicus* of the CV-stage had been exposed to two treatments of the WSF of crude oil (High treatment: 7.0 µg L^{-1}, Low treatment 3.4 µg L^{-1}) and control, and incubated for 24 and 72 hours (Jensen and Carroll 2010). This indicates a concentration dependent decrease in feeding efficiency, which could also be seen by a concentration dependent reduction in faecal pellets. The high treatment and low treatment is in the same concentration range as the 10% and 50% treatments, respectively, in this study. As for *C. hyperboreus*, no feeding experiments performed on *C. finmarchicus* nauplii have been reported, and the studies performed on adults, usually use faecal pellets as a measure of grazing efficiency (Hjorth and Nielsen 2011; Jensen et al. 2008), making comparisons difficult.

Within the *C. finmarchicus* feeding experiments, there were some samples that apparently had a higher number of cells assumed in the start of the experiment, resulting in apparently negative feeding. These were also higher than in the Algae controls. The described pattern was especially pronounced in the case of the 6h samples. Such results were, however, probably artefacts, related to some methodological errors discussed below. Indeed, most animals observed had food in their stomachs, indicating active feeding.

It became clear that the feeding experiments were difficult to perform as unforeseen problems became evident. A number of possible errors were identified. The early-stage copepods only consume fractions of the algae present, as they are very small animals. Because of this, the algae density must be measured with high accuracy, to be able to properly calculate how much they have consumed. One source of error was the starting algae concentration in the VOC-vials (0h), which was only measured in one experiment (*C. hyperboreus* at 7.5°C). The start concentration in all the other experiments was assumed being similar to this measured concentration, with the dilution factor (5x) taken into consideration. Another source of error was that the applied algae (*R. baltica*) had a tendency and ability to attach to the walls and bottoms in the exposure vials. This was not recognized in the beginning of the experiments, however, later testing showed that a relatively large number of algae cells were left in the vials and therefore left out from counting (Appendix E). This could explain the peculiar results and the high SDs in many of the sample means. In addition, there were periods of light exposure during the *C. finmarchicus* experiments, which creates a possibility of algal growth. Further considerations regarding methodology, may be found in a separate section below.

4.3. Development

In general, developmental rate increased with increasing temperature (reduced stage duration) (Figure 3.1.), which was consistent with previous studies examining stage durations in *C. finmarchicus* and *C. helgolandicus* (Campbell et al. 2001; Cook et al. 2007). The exception was *C. hyperboreus* at 7.5°C in the experiment performed at SeaLab, which developed faster than *C. hyperboreus* at 10°C at the Arctic Station. No conclusion of this observation has been drawn. However, it may have a relation to the ovulating females being stressed during the transportation from Greenland to Trondheim, or that the females met different water condition in the laboratory (i.e. more/less food availability or different temperatures). An additional factor could relate to the descent of the females, since they were captured one year apart, they may belong to different sub-populations.

4.3.1. Egg hatching

The exposure to PAHs did not seem to have any effects on overall hatching success, for neither *C. finmarchicus* nor *C. hyperboreus*. This is consistent with other studies, where *C. finmarchicus* eggs were exposed to PAHs (Grenvald et al. 2013; Hjorth and Nielsen 2011; Jensen et al. 2008). However, the results in this study conflicts with the data from Nørregaard et al. 2014, who reported a concentration dependent-decline in hatching success of *C. hyperboreus*, when exposed to pyrene. They used a series of concentrations from a water control, and exposures ranging from 0.020 – 20.2+ µg L^{-1}, where hatching success at the highest concentration was significantly lower than the control. This study used a mixture of PAHs, however, the levels were similar (1.82-21.47 µg L^{-1}) and yet there were no significant differences between any of the exposure groups, including the control. In addition, the hatching percentage in the control at 2°C, 96 hours exposure in Nørregaard et al. (2014) was much higher than in the control in this study at 3°C, 96 hours. However, in Nørregaard et al. (2014), the females were exposed, and the eggs transferred to clean water, where they hatched, while in this study, the females laid the eggs in FSW, and the eggs were exposed separately. The different handling of the females and the eggs may explain some of the differences.

In this study, all *C. hyperboreus* temperatures and exposure groups reached close to 100% hatching success after 144 hours. However, 7.5 and 10°C reached close to 100% after 96 hours, indicating that the eggs develop slower at 3°C than at the higher temperatures (Figure 3.4., A, C, E, G). Since they reach full hatching eventually, this is not necessarily negative. Ideally, hatching percentage should have been examined after 72 hours as well, since the hatching

percentage at the two highest temperatures went from about 0% at 48 hours, to almost 100% after 96 hours. The reason this was not done, was because there were only enough eggs for three different time periods. The time periods were therefore chosen based on the hatching experiment performed at the Arctic Station, using only temperatures. Here it was shown that at 3°C, around 50% had hatched after 96 hours and almost 100% after 144 hours (Figure G.1., Appendix G).

It seems that full hatching potential for *C. finmarchicus* was reached after 72 hours for all exposures and temperatures, and is around 80%, except a few cases where they reached close to 90%, and one case (7.5°C, 100%) where they only reached around 70%. In this species, it is clear that the eggs developed faster at 12.5°C than 10°C, and that eggs at 10°C developed faster than eggs at 7.5°C (Figure 3.4, B, D, F, H).

It also seemed that *C. hyperboreus* reached a higher hatching percentage than *C. finmarchicus*, but used longer time to conclude hatching. This is interesting, as it was thought that *C. hyperboreus* would be more sensitive than *C. finmarchicus*, given their more fragile egg shell characteristics, with only a lipid membrane, and no hard shell (Jung-Madsen et al. 2013). However, it should be noted that the *C. finmarchicus* females were part of a continuous lab culture, while the *C. hyperboreus* females were caught in the wild.

4.3.2. Growth of nauplii

It has been assumed that the dry weight decreases from the egg to the NII-stage in *C. finmarchicus* and other *Calanus*-species (*C. marshallae* and *C. helgolandicus*), due to their inability to feed, while having a high growth rate at NIII, to recover the weight lost during the non-feeding stages (Krause and Radach 1993). This was not seen in this study. There were no significant differences between eggs, NI and NII in both species. However, when looking at the averages, there is in fact generally a small increase from eggs to NII, especially for *C. hyperboreus*. However, the large SDs indicate that mistakes could have been made for some of the measurements.

C. finmarchicus NIII had a significantly higher dry weight than the other stages within the Ctrl at 7.5 and 10°C, and within the 10% group at 7.5°C, but not in the other treatments (Table P.2., Appendix P). For *C. hyperboreus*, only the 7.5°C within Ctrl had a significantly higher dry weight than the other samples within NIII. At 3°C, within Ctrl, NIII was significantly larger than the eggs. For the rest of the samples, there were no significant differences. In 3°C 100%, NIII had in fact lower dry weight than the other stages, although not significantly so (Table

P.1., Appendix P). This should not be surprising, since a newly hatched NIII in fact is supposed to lose weight compared to NII (due e.g. to cuticular shedding). Then they start feeding and gain weight over the duration of the stage. Hence, depending on the exact sampling time, one may obtain NIII at different average weight.

If body length was considered, the NII stage was significantly longer than the NI stage, and the prosome of the NIII stage was significantly longer than the body length of the NII stage for both species and all treatment groups (Appendix S). It should be noted that the NII stage has a more elongated body than NI (Figure 2.3), therefore, an increase in length is not surprising.

4.3.3. Dry weight

There was no consistent trend between any of the exposures or temperatures regarding dry weight of the two species. Within *C. hyperboreus* NIII, at 7.5°C the Ctrl group had a significantly higher dry weight than the 10% and 100% groups exposure. At 3 and 10°C the Ctrl group seemed higher than the 100% group, however, not significantly so. The apparent trend that the Ctrl, 10% and 50% groups had higher weights than the 100% group was not seen in the younger stages. Within the NI stage, it could almost seem like the 10% and 50% exposure groups, had higher dry weight compared to the Ctrl and 100% groups, however, there were no significant differences. This trend could not be seen for neither the NII nor the NIII stages. Since only one of the samples (the NIII, at 7.5°C) had significant different weight between the different exposure groups, it seems that exposure had little impact on the dry weight of *C. hyperboreus*.

Within the NI stage, there were no differences between the temperatures regarding weight. Within the NII stage, the 3°C group was significantly heavier than the 7.5°C group, when comparing all samples. However, this trend could not be seen for the NIII stage, where there was no difference in weight between temperatures. The exception was in the Ctrl group, where the members of the 7.5°C group appeared to be heavier than those of the other temperatures groups, although this was not significant (Figure 3.9., A, C, E). It therefore seems that temperature had only a small, if any, impact on the dry weight of the tested *C. hyperboreus* eggs and nauplii.

Within NIII nauplii groups of *C. finmarchicus* the 12.5°C group had a significantly lower dry weight ind^{-1} than the 7.5°C group within the Ctrl group, while it was non-significantly lower in the 10% group. Within the NI stage, the nauplii in the 7.5 and 10°C groups were both similar to each other, and between the exposures, while in the 12.5°C group the nauplii were similar in

the Ctrl and 10% groups, with a non-significant increase appearing in the 50% and 100% groups. Within the NII stage, both in the 10 and 12.5°C temperature groups the weight of the nauplii were non-significantly higher than the 7.5°C group in all exposures except the Ctrl group. Therefore, it seemed that temperature *per se* had little impact on the dry weight on *C. finmarchicus*.

Vidal (1980) found that early copepodite stages of *Calanus pacificus* and *Pseudocalanus* sp. were relatively unaffected by temperature, while the dry weight of the later stages was inversely related to temperature. Unfortunately, the naupliar stages were not examined, however, since the early copepodite stages were unaffected by temperature, it can be speculated that the naupliar stages will be unaffected as well, which is in accordance with this study.

Regarding the possible exposure effects on *C. finmarchicus* nauplii in the 7.5°C group, there was a small, non-significant decrease from the Ctrl to the 100% group, while at both 10 and 12.5°C, the 10% and 50% groups were non-significantly higher than the Ctrl and 100% groups. At 12.5°C there was an increasing weight trend from the Ctrl group, via 10% and 50% to the 100% group, however, no significant differences were found. This trend also appears within the NI stage at 12.5°C, from the Ctrl group via 10% to the 50 and 100% groups, however, there were not any significant differences here either. The data therefore do not conclusively show that PAHs has any effect on the dry weight of early-stage *C. finmarchicus* (Figure 3.9., B, D, F).

4.3.4. Biometry

Some statistical significant differences between different temperatures and exposures were found, however, there was no consisting relationship between neither factor. Within *C. hyperboreus* NI, the body length in the 10°C groups were significantly longer than in the 7.5°C groups within all exposures except the 10% group. An inverse relationship was found for the NIII stage, where the body length in the 10°C group was significantly shorter than in the 7.5°C group in all exposure groups, and significantly shorter than the 3°C group in all exposure groups except the 50% group. The NII and NIII nauplii kept at 7.5°C had significantly longer bodies than in the 3°C group, while in the NI stage there were no significant differences between the two temperatures. It should be noted that when considering only the NIII stage, higher temperature seemed to have a stimulating effect on length up until 7.5°C, as the nauplii kept at 7.5°C were significantly longer than at 3°C for all treatments. However, at 10°C a possible stimulating effect seems to cease, as there is a decrease in length at this temperature. In fact, for

all exposure groups except the 50% group, at 10°C the length of the NIII stage of *C. hyperboreus* proved to be significantly smaller than in nauplii kept at 3°C. Temperature therefore seemed to have an effect on length, however, this varies between the stages. For the NII and NIII stages, 7.5°C seems to stimulate length growth. Unfortunately, there is no data for 10°C regarding the NII stage, but for the NIII stage, the length at 10°C is generally lower than at 3°C.

The impact of PAH exposure on *C. hyperboreus* length seemed to be difficult to assess. Within all temperatures within the NI stage, the Ctrl group was significantly shorter than the 100% group, in addition was the nauplii of the 10% group at 10°C significantly shorter than in the Ctrl and 100% groups. Within the NII stage there was no effect on length of exposure. While in the NIII stage, the Ctrl group at 3°C was significantly longer than all other exposure groups, and at 10°C the individuals of the Ctrl group were significantly shorter than the 50% group. Some effect of exposure was only present in the NI and NIII stages, and within these stages these effects seems very variable (Figure 3.13, A, C, E).

In the experiment with *C. finmarchicus*, the nauplii within the 7.5°C group seemed to be longer than in the other temperature groups, within all exposure groups. For nauplii of the Ctrl group in the NI stage, in the Ctrl and 10% groups in the NII stage and the 10% group in the NIII stage, this was significant. This could indicate an inverse relationship between length and temperature, however, mostly no difference between the two highest temperatures was observed. Therefore, if an inverse relationship between temperature and length exists, it seems at best to be non-linear. In fact, within the NI stage at 10% exposure, the nauplii kept at 12.5°C group were significantly shorter than in the 10°C group, and within the NI stage at 100% exposure and the NII stage at 50%, the nauplii of the 12.5°C group were significantly shorter than in the 7.5 and 10°C groups. Within the NIII stage, the nauplii in the 10°C groups were significantly shorter than in the 7.5 and 12.5°C groups experiencing 50% exposure, and within the 10% exposure group, the nauplii kept at 7.5°C were significantly longer than in the 10 and 12.5°C groups. Generally, there seemed to be only a small effect of temperature on length.

There have been reported an inverse relationship between temperature and body size in *C. helgolandicus* (Strickland 1970), however this study examined the CV-stage and adults, and not nauplii. It has also been shown an inverse between the length of *C. finmarchicus* and temperature. However, this was only for the later copepodite stages, and not for eggs and NIII, while NI and NII were not examined (Campbell et al. 2001), which makes comparison difficult.

There is to the authors knowledge, no studies examining the impact of temperature on body length of the first naupliar stages of *Calanus*.

Within the NI stage at 3°C the nauplii of the Ctrl group, was significantly longer than in the 50% and 100% groups, while at the remaining temperatures, within the NII stage all temperatures, and within the NIII stage at 12.5°C, there were no statistical differences. Within the NIII stage at 3°C, the nauplii of the Ctrl group was significantly shorter than those in the 10% group, and at 10°C the nauplii in the 100% group was significantly shorter than in the 50% group. As within the experiments with *C. hyperboreus* there were only a few significant differences between the exposure groups regarding length in NI and NIII, indicating the impact of PAHs on *C. finmarchicus* to be relatively small (Figure 3.13., B, D, F).

It should be noted that there were significant differences between the temperatures regarding the length of the eggs (Figure 3.12). This was strange, as the females of each species were kept at the same temperature for all experiments (3°C for *C. hyperboreus* and 10°C for *C. finmarchicus*). They were only exposed to the temperature treatment during experiments. However, this may be explained by age differences of the eggs. For *C. hyperboreus*, where there the differences are quite large, the eggs could be 0-3 days old, while for *C. finmarchicus*, the eggs age at sampling could be 0-24 hours. Another explanation for the large differences in size of the *C. hyperboreus* eggs, is the fact that the females used for the experiments at 3 and 10°C were collected a year before the females used for the 7.5°C experiments. Therefore, both food availability and temperature in the water could have been different during ontogenetic development of the females the two years. Another possibility is that the females collected the subsequent years originated from different populations, possessing different genetic characteristics. It could also be related to handling and other forms of stress experienced by the females during the transport from Greenland to Trondheim.

4.4. Environmental perspective

Considering that the temperature in the Arctic is expected to increase (IPCC 2015), and could reach temperatures higher than used in this experiment, this could pose a problem for *C. hyperboreus,* as the length of the nauplii seemed to be negatively affected by the highest temperature used (10°C). In addition, it seemed that the combination of high temperature (10°C) and PAH exposure decreased the ALCON of *C. hyperboreus*. Considering the oil exploration in the Arctic is expected to increase (The Norwegian Oil and Gas Association 2016a), together with the increasing temperature, this could potentially become a problem for populations of *C.*

hyperboreus living close to the platforms. However, even if the increase in temperature in the Arctic is higher than earlier expected (AMAP 2017), it is still happening gradually, meaning that the *C. hyperboreus* could possibly adapt to the increasing temperatures.

4.5. Methodology

During this study, there were some methodological difficulties, especially regarding the feeding experiment. First, some difficulties emerged regarding the analysis of the algae samples. It was decided to use the CoulterCounter to measure algae concentration, instead of using fluorescence of algae chlorophyll as a measure of density. But the vials (13mL) that were used, had a smaller volume, too low for direct counting on the CoulterCounter, and dilutions had to be performed causing uncertainties. In addition, it was shown that fixation with Lugol causes a small decrease in algae concentration. Another difficulty was the indications of algae attaching to the walls and bottoms of the vials, which could possibly explain some of the high SDs seen in the algae concentrations. Some suggestions to changes have been made, regarding the possibility of future feeding experiments. There were also some problems with nauplii mortality in the VOC-vials where the eggs had been collected, and a possible explanation for this have been suggested. At last methodologies of the other experiments performed have been evaluated.

4.5.1. Analysis of algae samples

Based on experience during the present work, glass VOC-vials (40 mL) appear as the superior container choice for small-scale experiment including free-living phytoplankton cells. They are easily cleaned, and contain enough volume for several analyses, including counting of algae in a particle counter. Plastic (polyethylene) vials (13 mL) may also be convenient as containers for small-scale experiments, they are cheap and have low weight, and accordingly low transport costs. But the smaller size gives low analyses volumes, and due to the affinity of PAHs to the plastic surface, they are not suited for exposure studies with lipophilic components.

13 mL polyethylene vials were used in the in the feeding experiments. They fit to the portable rotating wheel that was available, and was initially assumed to be the superior choice for these experiments. They were further assumed to contain enough algae material for algae density analysis, since fluorescence of algal chlorophyll was originally planned to be used as a proxy for density. Fluorescence measurements demand small volumes of sample, but after measuring the fluorescence of known/realistic algae concentrations, the signal showed such large

variations in subsample-to-subsample that small concentration differences could not be measured (Figure 3.3., A).

In a separate test, a comparison between density measurements using fluorescence and particle counting using the CoulterCounter was made (Figure 3.3., B). Particle counting gave a much smaller subsample-to-subsample variation, making this method more accurate (Figure 3.4). However, a CoulterCounter analysis needs 20 mL of sample to work properly. Since the plastic vials only had a volume of 13 mL, the samples had to be diluted before counting. In addition, material enough for a second analysis would be warranted, in case anything went wrong, and the samples were therefore diluted 5 times (5mL sample and 20mL FSW), giving a total of 25 mL sample. The CoulterCounter may have an intra-sample uncertainty of 50-100 cells/mL per count. When counting 6000-7000 cells, this is a fairly small number. However, because of the dilution, the number of cells/mL was much smaller. When taking the dilution into consideration, a difference of 50-100 cells/mL per count, may rise to a deviation of 250-500 cells/mL after back-calculation to the initial number. This may be more than the nauplii are expected to eat, making the results highly uncertain. If VOC-vials (40 mL) had been used instead, the dilution step could have been omitted, which would have made the results much more accurate. However, these problems were recognized after the analyses equipment, including all vials, were shipped to the Arctic Station at Greenland, and the equipment could not be replaced.

Unfortunately, this coincided with the discovery that fluorescence measurements were not appropriate for algal quantification in the present. But no CoulterCounter was available at the Arctic Station. Instead algal concentrations were measured using a Bürker counting chamber. The average of nine counts were used as a basis of quantification, however, because of rather high standard deviations between counts (Figure B.1, C, Appendix B), the calculated algal concentration was suspected not to have necessary accuracy.

After quantification of stock solutions using the Bürker chamber, the exposure algae solution was prepared by dilution. No check of algal density after dilution was made on fresh material, due to the difficulty in using the Bürker chamber with diluted samples. Instead all samples were fixated with Lugol, for later measurements on the CoulterCounter at Sealab.

4.5.2. Lugol fixation

A problem arose when it became clear that the amount of cells/mL of Lugol fixated algae proved to be much lower than expected, in both the algae control, and the in the feeding experiment. The measured densities were also much lower than in algae dilutions prepared at

SeaLab, where the initial density of the stock algae solution was in the same range as that at the Arctic Station. Also, a second count five months after the first counting, of the same fixated algae samples showed that the number had increased in most samples. Given the tendency of algae to sediment, an explanation could be that at the last occasion, more algae had loosened from the internal surfaces of the vials due to more extensive shaking.

To evaluate the impact of Lugol fixation on algae numbers, a small fixation experiment was performed. As can be inferred from Figure 3.5. there was a small density decrease in all samples after fixation, then the algae densities remained fairly stable for the rest of the period. This confirms that Lugol fixation causes some decrease in measured algae concentration, but not at the level seen in the samples from the Arctic Station (Figure 1.I., Appendix I). However, as expected, the diluted samples, when taking the dilution into account, had much higher variation than the undiluted samples, confirming the inaccuracy created by the dilution step (Figure 3.5).

4.5.3. Algae attaching to the walls

At the Arctic Station, the algae suspension in the plastic vials was transferred to VOC-vials prior to fixation. The possibility therefore remains that a significant fraction of the algae cells remained attached to the plastic walls of the vials, and thereby contributed to low and variable algae counts. To minimize the stress of the animals, the plastic vials were filled almost completely, only a circulating bubble was left during rotation. However, the bubble was probably insufficient for proper mixing, making it possible for the algae to attach to the walls. When the experiments on *C. finmarchicus* and the 7.5°C experiment on *C. hyperboreus* were performed (at SeaLab), all the plastic vials were properly rinsed with FSW, after the algae solution had been removed. The water used for rinsing, was fixated with Lugol, and counted within one week. The number of cells in the rinse water samples were highly variable, ranging from around 3000-24 000 cells, supporting the possibility of the algae attaching to the walls of the vials (Appendix E). However, the algae had attached to the walls in both the *C. hyperboreus* and *C. finmarchicus* experiments, hence this can therefore not explain the low and variable counts in the samples from the Arctic Station. However, it may explain some of the large SDs found in all samples, including the algae controls.

A particular problem in estimating algal consumption in the samples from Arctic Station, was the low and variable algae counts in the algae control samples (no copepods), which were in some cases lower than the samples containing copepods. Therefore, these samples could not be used to calculate algae consumption. A measure that partly could remedy the problem was

introduced within the *C. hyperboreus* experiment performed at SeaLab (7.5°C), where 3 vials were filled with algae solution of the start concentration, and immediately counted on the CoulterCounter. This gave a more accurate estimate of the start concentration, in this case 8190±106 cells/mL (mean±SD) (Table I.1., Appendix I). In retrospect, this procedure should have been included in all experiments done, preferably using even more parallels, to get a good indication of the spread in concentration.

4.5.4. Suggestions for future feeding experiments

Based on the experience from the present experiments, the use of the plastic 13 mL vials should have been omitted in favor of 40 mL VOC-vials. Compared to the plastic vials, they have less surface and more volume, which would hopefully decrease the number of algae attaching to the walls. However, more individuals would then be needed in each vial, because of the much larger volume. More individuals would probably give a clearer decrease in algae concentration, making it easier to investigate potential effects of temperature and exposure.

An alternative option to qualitatively decide if the nauplii had eaten or not is to study the gut content. In this case, the nauplii should have been collected after the feeding experiments were finished, and the fraction of nauplii with algae in the gut determined. With *C. finmarchic*us, this should be simple, as they are transparent, and the *R. baltica* is red and clearly visible using a standard microscope (Figure 2.3., H and I). Concerning *C. hyperboreus*, using standard microscopy is not as simple, since the nauplii have an orange lipid layer within their stomach area, obscuring any algae in the stomach (Figure 2.3., D). However, the algae are fluorescent and may be detected by fluorescence microscopy. This was tested within the last experiment on *C. hyperboreus* performed at SeaLab, where a couple of nauplii from two of the samples (Control and 100 %) were collected, and examined using a fluorescence microscope. It was, in fact, possible to detect if the nauplii had eaten or not.

4.5.5. Nauplii mortality

Due to the availability of eggs from *C. hyperboreus* at the Arctic Station, the number of eggs added to each vial was restricted to 100. This number is fairly low, considering that nauplii were needed for several experiments. Relatively high and variable mortality among the nauplii in the vials added to the uncertainty. Increasing the number was, however, not possible due to the resources and time available. A similar high mortality was not experienced within any of the experiments performed at SeaLab. A possible reason for the high mortality at the Arctic Station was water soluble components in the glue (Acrifix), used to adhere the sieve to the

plexiglass tube used for removing the WSF-solution and rinsing the eggs/newly hatched nauplii. During the stay in the Arctic Station the system was used without any previous treatment, while at later occasions the plexiglasses with sieves were kept in seawater one day and night before the experiments started. This was done to ensure that any toxic components left in the glue would dissolve. Given that the high mortality was not experienced at the later experiments at SeaLab, it is likely that the glue was the reason for the high mortality.

4.5.6. Egg hatching

The hatching percentage at the different time periods could have been confounded by when the eggs were laid. This is especially the case for the experiments only examining temperature, as time zero was when the eggs were collected, while age of the eggs were unknown. In the hatching experiment where the nauplii were exposed to PAHs, age of the eggs was taken into consideration. For *C. finmarchicus* the eggs were collected 12-15 hours after the females had been incubated in the egg-laying containers. However, for the *C. hyperboreus* females, the eggs were collected about 48 hours after the females had been incubated in the egg laying chamber, and first fixation was made 12 hours after collection. This was because there were some nauplii among the eggs collected, and eggs were deliberately chosen. Thereby, the eggs could have an age between 12-60 hours when the first fixation was made.

4.5.7. Dry weight

The eggs/nauplii were transferred to tin capsules, after rinsing in ammonium formate. However, the eggs and nauplii of both species are microscopic. Therefore, mistakes regarding the transfer to tin capsules cannot be excluded. In some samples, where the SD is very high, there is a possibility that one capsule has gotten two samples, while another is empty, which could have impacted the dry weight results. However, this is difficult to confirm, as no other analysis were performed on the nauplii in the tin capsules.

4.5.8. Biometry

There were some problems, both when taking pictures of the copepods, and when analyzing the diameter/length of the copepods. As much water as possible was removed, when taking the pictures of the *Calanus*, however, often there was enough water for them to swim in. They are also more or less identical in shape and size, making it very difficult to determine which individuals that were in the individual pictures, when they moved. When examining the pictures taken, it was impossible to determine which animals that had been analyzed. Therefore, as is

seen in Appendix A, analysis have often been made of more individuals than individuals present on the microslides. There were also some cases where for some reason, diameter/length could not be analyzed. In some cases, too much water had been removed, causing some of the eggs/nauplii to dry up. In addition, some nauplii were lying in angles were body length/prosome length could not be determined.

Another problem with the biometry, is that the quality of the pictures varied to some extent. The length was determined as accurately as possible, however the quality of the pictures caused some difficulties. In addition, when measuring prosome length in NIII, there were some nauplii, where the end of the prosome was difficult to determine, making it necessary just to assume where the prosome ended. This too may have caused inaccuracies in the results.

5. Conclusion and future perspectives

There is an indication that the combination of high temperature (10°C) and exposure may have an impact on *C. hyperboreus* feeding efficiency, while no effects were shown on *C. finmarchicus*. Therefore, more studies should be made on *C. hyperboreus* with regards to effect on temperature and PAH-exposure on feeding efficiency. Preferably, these potential studies should take in consideration the problems encountered with the methodology in this study.

Temperatures seem to have a larger impact than PAH-exposure on the length of *C. hyperboreus*, especially at NIII, where 7.5°C has a stimulating effect, compared to 3°C, while 10°C seem to negatively affect the nauplii. With regards to *C. finmarchicus*, neither temperature nor PAH-exposure seem to have any effect, with the exception of the control, where 12.5°C was significantly lower than 7.5°C. The effect on dry weight remains inconclusive, both with regards to temperature and PAH-exposure, in both species.

The limited knowledge of effects of PAHs and temperature on the early stages of *C. hyperboreus* have hopefully been expanded a little with this study. This study may thus contribute to more knowledge on the potential effects on *C. hyperboreus* with the combination of emissions from the increasing human activities in the Arctic and increasing temperature due to global warming.

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Appendix A

Table A.4. Summary of experimental setup for *C. hyperboreus* at 3°C, showing the number of individuals in the different analyses. There were cases where biometry could not be measured on the individuals, and cases where there were more biometry measurements than individuals present under the camera, because several pictures may have been taken of the same individuals, and they were so similar that it was impossible to distinguish them. The dead were sometimes added to the tin capsules, as they were hard to distinguish through the stereo microscope.

		Number of individuals						
Stage of development	Treatment	VOC-vials	In camera	Biometry measurements	Mark	DW	Feeding 6h	Feeding 20h
Egg			21	23		20		
			12	13		12		
			12	12		12		
			29	32		27		
			26	29		26		
			31	32		31		
						61		
						61		
						61		
						60		
NI	Control	100	22	21	1 dead	21		
		100	22	22	1 dead, 1 shell	22		
		100	20	23	3 shells	19		
		100	24	26	1shell	24		
	10%	101	22	21	1 dead	20		
		100	27	28	1 dead	26		
		102	21	22		19		
		101	26	29	1 dead, 1 shell	26		
	50%	101	25	25	1 dead	23		
		100	20	19	1 shell	16		
		100	21	21	2 shells	20		
		100	21	24		12		
	100%	100	21	24	1 dead	22		
		101	26	27		24		
		100	20	21		16		
		100	29	30		29		

Table A.1. Continued.

		Number of individuals						
Stage of development	Treatment	VOC-vials	In camera	Biometry measurements	Mark	DW	Feeding 6h	Feeding 20h
NII	Control	101	27	31	1 dead, 1 shell	25		
		100	27	29	1 shell	27		
		100	26	28	1 shell	25		
		103	27	24		26		
	10%	104	27	27		25		
		102	26	26		26		
		102	29	30		28		
		101	25	26		25		
	50%	100	29	33	1 dead	29		
		103	19	20	1 dead	19		
		101	27	27		28		
		101	26	26	1 dead	25		
	100%	101	25	24	1 shell	25		
		101	12	12		12		
		101	26	24		26		
		100	24	24		24		
NIII	Control	102	16	18		16	20	18
		102	15	18		15	20	19
		100	15	14		10	18	18
		100						
	10%	101	17	17		17	20	18
		100	18	20		18	14	17
		100	15	16	1 shell	15	18	14
		104						
	50%	100	17	21		17	20	20
		100	17	19		17	15	16
		100	15	20		15	12	-
	100%	101	17	17		14		
		100	14	17	1 shell	14	20	20
		100	15	15		14	19	20
		100	17	21		14	20	15

Table A.2. Summary of experimental setup for *C. hyperboreus* at 7.5°C, showing the number of individuals in the different analyses. There were cases where biometry could not be measured on the individuals, and cases where there were more biometry measurements than individuals present under the camera, because several pictures may have been taken of the same individuals, and they were so similar that it was impossible to distinguish them. The dead were sometimes added to the tin capsules, as they were hard to distinguish through the stereo microscope. For N1 there was problem with the animals disappearing in the pipette tips.

		Number of individuals						
Stage of development	Treatment	VOC-vials	In camera	Biometry measurements	Mark	DW	Feeding 6h	Feeding 20h
Egg			23	23	1 broken	23		
			20	22		20		
			20	19		20		
			19	18		19		
NI	Control	100	21	24	1 broken	18		
		100	20	18		18		
		100	21	21	1 shell	18		
	10%	100	18	17	2 dead	18		
		100	21	21		18		
		101	9	9		7		
	50%	100	22	25		21		
		100	18	28		15		
		100	18	17		12		
	100%	100	20	20	1 shell	18		
		100	11	16		7		
		100	18	16	1 shell	14		
NII	Control	100	29	29		28		
		100	28	30		28		
		101	30	29		30		
	10%	100	27	29		27		
		100	26	29		26		
		100	25	24	2 shells	22		
	50%	100	25	27		25		
		100	29	28		29		
		100	25	26	1 shell	26		
	100%	101	27	28	1 dead	27		
		100	28	28		28		
		100	26	28	1 dead	26		

Table A.2. Continued

		Number of individuals						
Stage of development	Treatment	VOC-vials	In camera	Biometry measurements	Mark	DW	Feeding 6h	Feeding 20h
NIII	Control	100	16	17		15	20	20
		100	19	19	1 dead	20	20	20
		100	17	17		16	20	20
	10%	100	19	19		19	20	20
		100	15	19		15	20	20
		100	20	19		20	20	20
	50%	100	19	20		19	20	20
		100	17	15		17	20	20
		100	19	19		18	20	20
	100%	100	18	20		18	20	20
		100	17	19		16	20	20
		100	17	20	1 shell	17	20	20

Table A.3. Summary of experimental setup for *C. hyperboreus* at 10°C, showing the number of individuals in the different analyses. There were cases where biometry could not be measured on the individuals, and cases where there were more biometry measurements than individuals present under the camera, because several pictures may have been taken of the same individuals, and they were so similar that it was impossible to distinguish them. The dead were sometimes added to the tin capsules, as they were hard to distinguish through the stereo microscope. There were many dead in the vials. The vials marked with N2 was instead used to get as many as possible in the N3-experiments.

		Number of individuals						
Stage of development	Treatment	VOC-vials	In Camera	Biometry measurements	Mark	DW	Feeding 6h	Feeding 20h
Egg			27	32		26		
			22	24		22		
			29	29		26		
			20	20		20		
NI	Control	100	26	29	1 dead	22		
		100	20	22	2 dead	20		
		100	27	30		22		
		100	26	25		26		
	10%	101	14	13	1 shell	14		
		100	19	20	2 shells	17		
		100	21	22	1 dead	22		
		100	20	24	3 shells	20		
	50%	100	24	28		20		
		100	27	27		22		
		100	23	23		21		
		104	23	23		22		
	100%	100	22	27	2 dead	19		
		100	17	18		16		
		100	21	23	2 shells	21		
NII	Control	102						
		100						
		100						
		100						
	10%	101						
		100						
		100						
		101						
	50%	100						
		100						
		102						
		101						
	100%	100						
		100						
		102						
		100						

Table A.3. Continued.

		Number of individuals						
Stage of development	Treatment	VOC-vials	In Camera	Biometry measurements	Mark	DW	Feeding 6h	Feeding 20h
NIII	Control	100	16	16		16	20	12
		101					16	12
		101	15	14	1 shell	14	20	16
		101	12	13	3 dead	12		
	10%	100	17	18		16	20	20
		100					21	20
		104	21	21		21	20	20
		103	15	18		15		
	50%	101	14	15	2 shells	14	20	20
		100					20	20
		101	17	19	1 shell	17	20	20
		100	20	18	1 shell	250		
	100%	101	19	21		19	20	20
		100					20	20
		100	16	15		16	20	20
		100	16	13		16		

Table A.4. Summary of experimental setup for *C. finmarchicus* at 7.5°C, showing the number of individuals in the different analyses. There were cases where biometry could not be measured on the individuals, and cases where there were more biometry measurements than individuals present under the camera, because several pictures may have been taken of the same individuals, and they were so similar that it was impossible to distinguish them. The dead were sometimes added to the tin capsules, as they were hard to distinguish through the stereo microscope.

		Number of individuals						
Stage of development	Treatment	VOC-vials	In Camera	Biometry measurements	Mark	DW	Feeding 6h	Feeding 20h
Egg			37	38		37		
			29	27		29		
			34	29		27		
			27	27	1 shell	25		
NI	Control	200	25	26		25		
		200	20	28	2 dead	19		
		200	26	22		25		
		201	25	27	1 shell, 1 egg	24		
	10%	201	21	23	2 dead	21		
		200	20	21		19		
		201	16	14		16		
		201	21	24		23		
	50%	200	23	23		24		
		200	18	21		18		
		201	23	22		23		
		200	24	27		24		
	100%	200	20	21	1 egg	20		
		200	26	28		24		
		200	19	20		19		
		200	18	15		18		
NII	Control	201	24	30	1 dead	23		
		210	22	25	1 dead	23		
		201	23	33		24		
		204	23	23		24		
	10%	200	24	33		24		
		203	20	26	1 dead	21		
		200	22	24		22		
		200	24	32	2 dead	25		
	50%	201	33	35	1 dead	33		
		200	25	27	1 dead	23		
		202	21	22	1 dead	19		
		200	17	27		17		
	100%	200	22	31		22		
		200	22	23		22		
		200	24	28	1 dead	23		
		200	24	25		23		

Table A.4.Continued.

		Number of individuals						
Stage of development	Treatment	VOC-vials	In Camera	Biometry measurements	Mark	DW	Feeding 6h	Feeding 20h
NIII	Control	203	18	11		18	20	21
		200	14	12	1 dead	14	20	20
		201	23	19		22	20	22
		200	21	20		20		
	10%	204	18	16		18	20	20
		202	19	18		19	20	20
		200	17	18		17	20	20
		201	20	20		20		
	50%	202	16	19		15	20	20
		201	19	17		19	20	20
		200	18	21		17	20	20
		202	19	23		19		
	100%	200	19	20		18	20	20
		201	19	19		19	20	20
		200	20	20		17	20	20
		200	19	17		19		

Table A.5. Summary of experimental setup for *C. finmarchicus* at 10°C, showing the number of individuals in the different analyses. There were cases where biometry could not be measured on the individuals, and cases where there were more biometry measurements than individuals present under the camera, because several pictures may have been taken of the same individuals, and they were so similar that it was impossible to distinguish them. The dead were sometimes added to the tin capsules, as they were hard to distinguish through the stereo microscope.

		Number of individuals						
Stage of development	Treatment	VOC-vials	In Camera	Biometry measurements	Mark	DW	Feeding 6h	Feeding 20h
Egg			30	33		30		
			25	23	1 shell	23		
			29	30		29		
			25	26		25		
NI	Control	200	24	26		21		
		200	20	13		16		
		200	17	14	2 dead	16		
		200	22	15		18		
	10%	202	26	25	2 dead	25		
		200	20	23	1 dead	20		
		200	19	14		18		
		205	26	36	1 dead	28		
	50%	200	23	27		22		
		200	18	14		20		
		200	19	15	1 dead	18		
		200	13	13	2 dead	12		
	100%	200	17	20	1 dead	17		
		201	18	16		16		
		200	22	24	3 dead	23		
		200	26	27	1 dead	17		
NII	Control	200	15	25		15		
		200	24	30		24		
		200	21	34	1 dead	21		
		200	14	21		16		
	10%	200	22	20	2 dead	22		
		200	21	21		21		
		200	23	18	3 dead	17		
		200	25	16		25		
	50%	200	25	34		25		
		200	33	33		33		
		200	22	24	1 dead	6		
		200	26	27	2 dead	27		
	100%	200	22	20		21		
		209	21	30		19		
		200	24	21		22		
		200	20	29	1 dead	20		

Table A.5. Continued.

		Number of individuals						
Stage of development	Treatment	VOC-vials	In Camera	Biometry measurements	Mark	DW	Feeding 6h	Feeding 20h
NIII	Control	200	19	16		19	20	20
		200	19	20		19	20	20
		200	14	13		14	20	20
		200	20	17		20		
	10%	200	19	21		18	20	20
		200	19	22		18	20	20
		200	15	18		16	20	20
		200	19	20		19		
	50%	200	18	15		14	20	20
		200	20	20	1 dead	20	20	20
		200	18	20		17	20	20
		200	18	23		18		
	100%	200	15	15		15	20	20
		200	20	20		20	20	20
		200	20	17		20	20	20
		200	17	21		17		

Table A.6. Summary of experimental setup for *C. finmarchicus* at 12.5°C, showing the number of individuals in the different analyses. There were cases where biometry could not be measured on the individuals, and cases where there were more biometry measurements than individuals present under the camera, because several pictures may have been taken of the same individuals, and they were so similar that it was impossible to distinguish them. The dead were sometimes added to the tin capsules, as they were hard to distinguish through the stereo microscope. For N1 there was problem with the animals disappearing in the pipette tips.

		Number of individuals						
Stage of development	Treatment	VOC-vials	In Camera	Biometry measurements	Mark	DW	Feeding 6h	Feeding 20h
Egg			23	22		22		
			11	9	2 broken	10		
			17	16	2 broken	17		
			25	26		24		
NI	Control	200	17	18		14		
		200	18	18		14		
		200	15	15	1 dead	14		
		200	17	21	1 dead	15		
	10%	200	20	20	2 dead	18		
		200	18	15	1 dead	15		
		200	21	20	4 dead	24		
		200	21	18	1 dead	13		
	50%	200	24	25	1 dead	15		
		200	25	28	1 dead	21		
		200	14	15	1 dead	14		
		200	17	22		13		
	100%	200	20	23	1 dead	11		
		200	21	24		14		
		200	15	17	2 dead	13		
		200	19	23	1 dead	16		
NII	Control	200	25	23	1 dead	26		
		200	26	31		26		
		200	21	30		21		
		200	25	29		25		
	10%	200	27	30		26		
		200	30	30		30		
		200	23	27		23		
		200	26	29		26		
	50%	200	31	32		31		
		200	25	29		25		
		200	24	31		24		
		200	29	30	1 dead	30		
	100%	200	24	27		24		
		200	31	30		30		
		200	21	24	1 dead	22		
		200	30	31		29		

Table A.6. Continued.

		Number of individuals						
Stage of development	Treatment	VOC-vials	In Camera	Biometry measurements	Mark	DW	Feeding 6h	Feeding 20h
NIII	Control	200	15	15		15	20	20
		200	19	18		19	20	20
		200	21	21			20	19
		200	20	17		20		
	10%	200	19	20		20	20	20
		200	19	22		20	20	20
		200	19	19		18	20	20
		200	14	15		14		
	50%	200	16	18		14	20	20
		200	20	20		18	20	20
		200	20	12		19	20	20
		200	23	16		23		
	100%	200	16	14		16	20	20
		200	18	19		18	20	20
		200	16	14		16	20	20
		200	20	19		20		

Appendix B

Table B.1. Summary temperatures, sampling dates and developmental times of the conducted nauplii experiments. *C. hyperboreus* NII at 9.3°C is in parenthesis because this experiment was not conducted due to high mortality. The date is the day the experiment was supposed to be performed.

Species	Development temperature (°C)	Sampling stage	Sampling date	Development time (days)
C. hyperboreus	3.4	Egg	09.02.2016	0
		N1	16.02.2016	7
		N2	19.02.2016	10
		N3	23.02.2016	14
	7.7	Egg	10.03.2017	0
		N1	13.03.2017	3
		N2	15.03.2017	5
		N3	18.03.2017	8
	9.3	Egg	12.02.2016	0
		N1	15.02.2016	3
		(N2)	(17.02.2016)	(5)
		N3	21.02.2016	9
C. finmarchicus	7.6	Egg	11.11.2017	0
		N1	12.11.2017	1
		N2	14.11.2017	3
		N3	16.11.2017	5
	9.8	Egg	23.11.2017	0
		N1	24.11.2017	1
		N2	25.11.2017	2
		N3	27.11.2017	4
	12.3	Egg	30.11.2017	0
		N1	01.12.2017	1
		N2	02.12.2017	2
		N3	03.12.2017	3

Appendix C

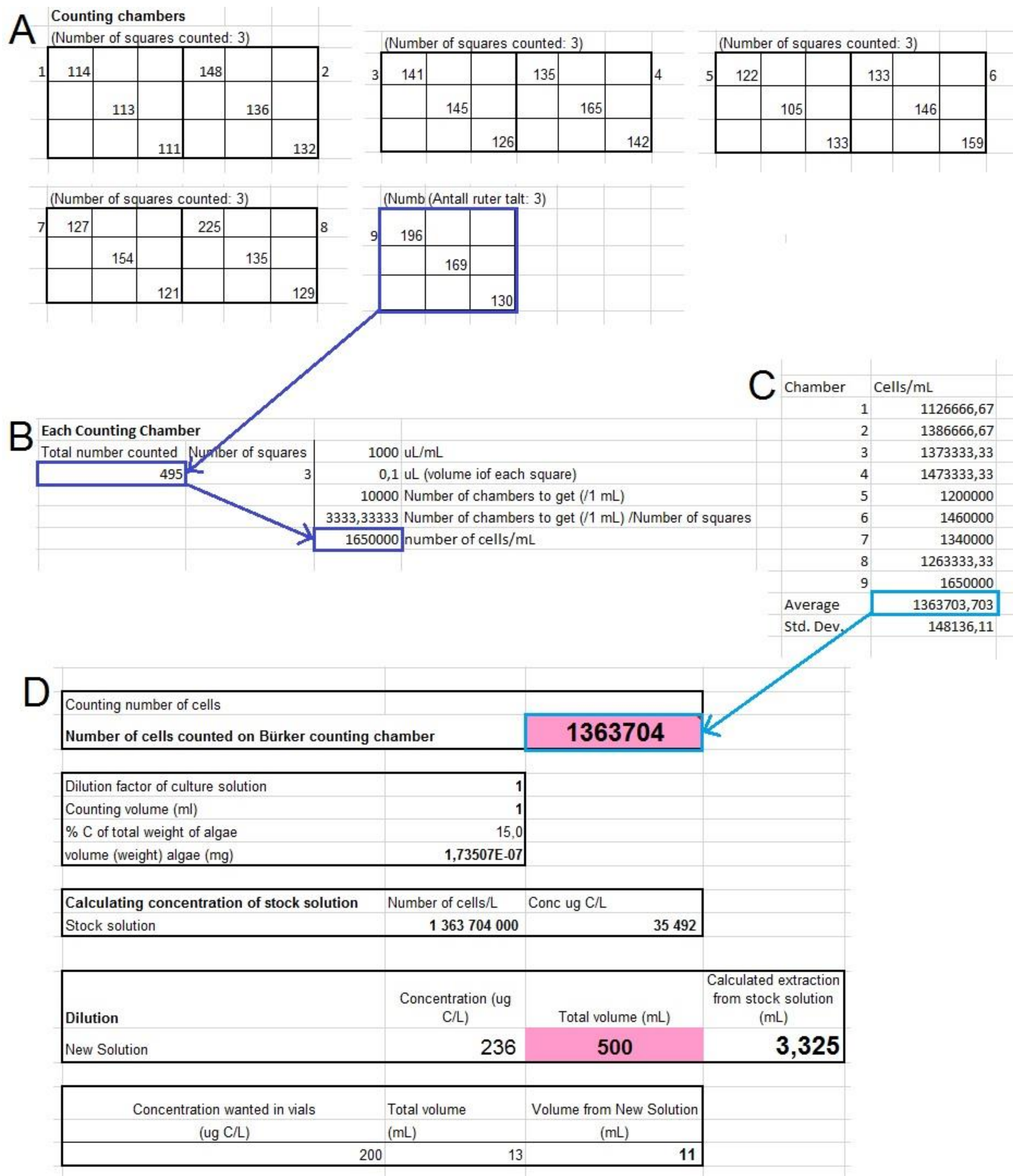

Figure C.6. Schematically drawing for determination of how much of the stock solution of *R. baltica* needed, to make the right concentration of algae for the feeding experiment. A: Large squares represent the chambers in a Bürker counting chamber, within each chamber, there are 9 smaller squares, where 3 diagonally squares were counted. The number of cells counted from each chamber were added together, and placed in B, which calculates cells/mL, based on the knowledge that each small square has a volume of 1 µL. The calculated cells/mL from each chamber were added to C, which calculates an average of all counts. This average is placed into D, which further calculates how much is needed from the stock solution, to 500 mL, with a concentration of 236 µg C L-1, to further get a concentration of 200 µg C L-1 in each vial. D: Acknowledgement: Ida Beathe Øverjordet, with some adjustments made to customize for Bürker chamber, as this was used for the Coulter Counter.

_1_	**Dilution of stock solution**			
	Take out 250 uL stock solution in a total volume of 100 mL			
_2_	Counting number in test solution			
	Counting number given by the coulter counter		**6740**	
	Dilution factor of culture solution	**400**		
	Counting volume (ml)	**1**		
	% C of total weight of algae	15,0		
	volume (weight) algae (mg)	**1,73507E-07**		
	Calculating concentration of stock so	Number of cells/L	Conc ug C/L	
	Stock solution	**2 696 000 000**	**70 166**	
_3_	**Dilution of Stock 1 - 18 500 ug C/L**	Concentration (ug C/mL)	Total volume (mL)	Calculated extraction from stock solution (mL)
	Stock 1	18 500	**100**	**26,4**
_4_	Total volume in vials		**13**	
	Concentration (ug C/L)	Total volume (mL)	Volume Stock 1 (mL)	
	200	13	**0,141**	

Figure C.7. Calculating how much of the stock solution of *R. baltica* is needed to make a Stock 1 (18 500 µg C L-1), and further, how much of this is needed to ad in each vial, to get the correct concentration (200 µg C L-1), based on a 400x dilution of the stock concentration. Acknowledgement: Ida Beathe Øverjordet, with some adjustments to adapt to the vials (13 mL).

Appendix D

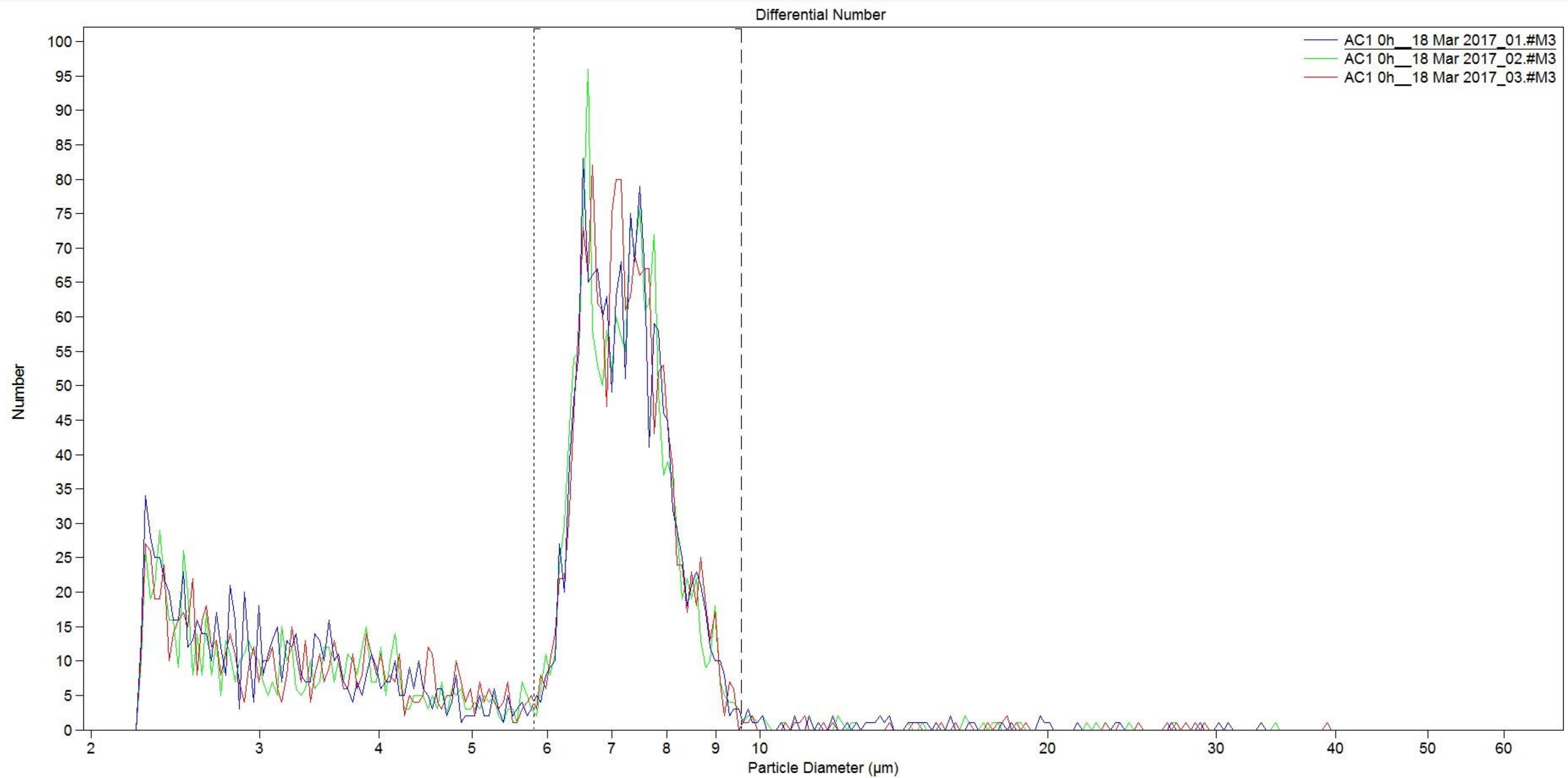

Figure D.1. Output given by CoulterCounter, the clear peak was integrated at the expected size of *R. baltica*, around 7-8 µm (between 6 and 10 µm, depending on the curves). The elution volume is 1 mL, giving a concentration of number of cells/mL, when integrating the average within the integrated area. example of a good curve, with a clear peak.

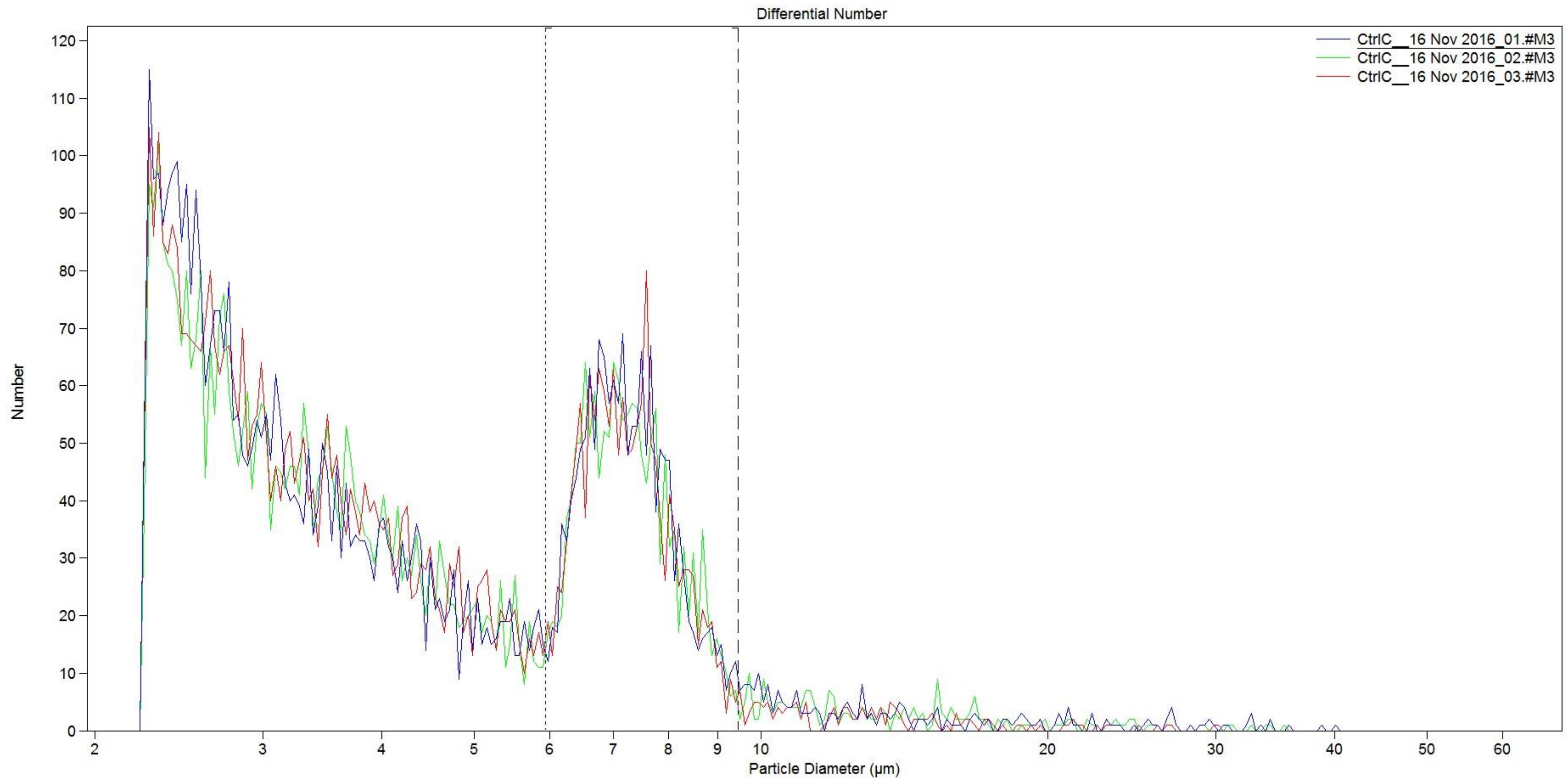

Figure D.2 Output given by CoulterCounter, the clear peak was integrated at the expected size of *R. baltica*, around 7-8 µm (between 6 and 10 µm, depending on the curves). The elution volume is 1 mL, giving a concentration of number of cells/mL, when integrating the average within the integrated area. Example of a bad curve, where there seems to be a lot of background noise, and should therefore be interpreted carefully. Many of the *C.* finmarchicus curves were like this.

Appendix E

Table E.1. Number of cells found in the FSW used to rinse the vials after the feeding experiment, to determine if the algae had been attached to the walls, for *C. hyperboreus* 7.5°C. Number of cells were calculated from cells/mL given from the CoulterCounter, and how many mL of FSW that was used to rinse the vials.

Time period	Treatment	Number of cells
6h	Ctrl	11748,65
		19159,71
		5289,02
	10%	5429,8
		4186,26
		7533,01
	50%	7823,4
		8817,9
		5735,34
	100%	34364,01
		13326,72
		10060,8
	Algae Ctrl	3776
		3664,24
		3072,72
20h	Ctrl	17525,76
		9129,6
		9794,02
	10%	5640,6
		8830,58
		22542,19
	50%	9525,86
		15838,08
		5735,79
	100%	6599,4
		14636,99
		9661,2
	Algae Ctrl	9462,15
		8867,43
		7926,1

Table E.2. Number of cells found in the FSW used to rinse the vials after the feeding experiment, to determine if the algae had been attached to the walls, for *C. finmarchicus* 7.5°C. Number of cells were calculated from cells/mL given from the CoulterCounter, and how many mL of FSW that was used to rinse the vials. If there were less than 3 parallels, this was because large particles caused the CoulterCounter to block so many times that there was not enough solution to count.

Time period	Treatment	Number of cells
6h	Ctrl	4153,54
		3268,8
		7317,24
	10%	6954,19
		5964,9
		7923,13
	50%	19051,27
		3066,8
		4214,4
	100%	4654,43
		3345,05
		3373,92
	Algae Ctrl	5155,77
		4036,45
		3796,26
20h	Ctrl	5825,28
		2818,02
		6230,7
	10%	12549,46
		13254,75
		19449,76
	50%	7844,54
	100%	9902,25
		15433,83
		6376,78
	Algae Ctrl	11874,24

Table E.3. Number of cells found in the FSW used to rinse the vials after the feeding experiment, to determine if the algae had been attached to the walls, for *C. finmarchicus* 10°C. Number of cells were calculated from cells/mL given from the CoulterCounter, and how many mL of FSW that was used to rinse the vials.

Time period	Treatment	Number of cells
6h	Ctrl	8371,59
		15749,3
		10095,44
	10%	6170,1
		11267,7
		4915,68
	50%	4262,2
		7107,36
		5236,42
	100%	6275,5
		5786
		4337,28
	Algae Ctrl	10421,86
		5044,52
		3787,68
20h	Ctrl	7594,7
		8283,75
		6860,3
	10%	6550,04
		14409,68
		11357,82
	50%	6858,6
		21725,08
		14059,8
	100%	18989,86
		9356,4
		17047,84
	Algae Ctrl	3376,59
		4205,47
		7566,96

Table E.4. Number of cells found in the FSW used to rinse the vials after the feeding experiment, to determine if the algae had been attached to the walls, for *C. finmarchicus* 12.5°C. Number of cells were calculated from cells/mL given from the CoulterCounter, and how many mL of FSW that was used to rinse the vials.

Time period	Treatment	Number of cells
6h	Ctrl	9196,3
		12490,32
		9434,94
	10%	7702,43
		10605,76
		11814,44
	50%	8817,55
		7584
		6485,76
	100%	19257,81
		11380
		12950,6
	Algae Ctrl	3391,4
		6758,78
		12611,3
20h	Ctrl	7770,75
		8106,12
		9229,92
	10%	13844,65
		12450,56
		18332,82
	50%	6886,88
		11065,68
		5962,07
	100%	12297,6
		15169,68
		18104,8
	Algae Ctrl	14250,9
		24361,22
		17247,27

Appendix F

Table F.1. Data for hatching percentage of exposed *C. hyperboreus* at 3°C. The females had been isolated for 48 hours, when the eggs were sampled. First fixation (48h) was performed 12 hours after sampling.

Hours	Treatment	Egg	Hatched	Total	Hatching (%)
48	Control	20	0	20	0
		20	0	20	0
		19	1	20	5
	10%	20	0	20	0
		20	0	20	0
		20	0	20	0
	50%	20	0	20	0
		20	0	20	0
		19	0	19	0
	100%	30	0	30	0
		20	0	20	0
		20	1	21	4,761905
96	Control	14	6	20	30
		11	9	20	45
		11	9	20	45
	10%	8	12	20	60
		6	14	20	70
		12	8	20	40
	50%	10	10	20	50
		8	12	20	60
		15	5	20	25
	100%	8	12	20	60
		15	5	20	25
		7	13	20	65
144	Control	0	20	20	100
		1	19	20	95
		0	19	19	100
	10%	1	19	20	95
		2	18	20	90
		2	17	19	89.4737
	50%	0	20	20	100
		0	20	20	100
		3	17	20	85
	100%	0	20	20	100
		2	18	20	90
		0	20	20	100

Table F.2. Data for hatching percentage of exposed *C. hyperboreus* at 7.5°C. The females had been isolated for 48 hours, when the eggs were sampled. First fixation (48h) was performed 12 hours after sampling.

Hours	Treatment	Egg	Hatched	Total	Hatching (%)
48	Control	19	1	20	5
		20	0	20	0
		19	1	20	5
	10%	19	0	19	0
		20	0	20	0
		20	0	20	0
	50%	17	0	17	0
		20	0	20	0
		19	0	19	0
	100%	20	0	20	0
		19	1	20	0
		20	0	20	5
96	Control	0	20	20	100
		0	18	18	100
		0	20	20	100
	10%	0	20	20	100
		0	19	19	100
		1	19	20	95
	50%	0	18	18	100
		2	18	20	90
		2	17	19	89.4737
	100%	0	20	20	100
		2	18	20	90
		3	17	20	85
144	Control	1	19	20	95
		2	18	20	90
		2	18	20	90
	10%	3	17	20	85
		0	20	20	100
		1	19	20	95
	50%	1	19	20	95
		3	17	20	85
		0	18	18	100
	100%	0	20	20	100
		2	18	20	90
		1	19	20	95

Table F.3. Data for hatching percentage of exposed *C. hyperboreus* at 10°C. The females had been isolated for 48 hours, when the eggs were sampled. First fixation (48h) was performed 12 hours after sampling.

Hours	Treatment	Egg	Hatched	Total	Hatching (%)
48	Control	20	0	20	0
		20	0	20	0
		20	0	20	0
	10%	18	0	18	0
		20	0	20	0
		20	0	20	0
	50%	19	1	20	5
		20	0	20	0
		19	1	20	5
	100%	19	1	20	5
		19	1	20	5
		20	0	20	0
96	Control	3	17	20	85
		1	18	19	94.7368
		0	17	17	100
	10%	0	18	18	100
		2	18	20	90
		0	19	19	10
	50%	0	20	20	100
		2	18	20	90
		1	19	20	95
	100%	1	19	20	95
		0	20	20	100
		0	19	19	100
144	Control	0	20	20	100
		0	20	20	100
		0	20	20	100
	10%	2	17	19	89.4737
		0	20	20	100
		0	20	20	100
	50%	2	18	20	90
		0	20	20	100
		1	19	20	95
	100%	0	20	20	100
		2	20	20	100
		1	20	20	100

Table F.4. Data for hatching percentage of exposed *C. finmarchicus* at 7.5°C. The first fixation (24h) was performed 24 hours after the females had been added to the egg laying containers.

Hours	Treatment	Egg	Hatched	Total	Hatching (%)
24	Control	20	0	20	0
		19	0	19	0
		19	0	19	0
	10%	18	0	18	0
		19	0	19	0
		19	0	19	0
	50%	19	1	20	5
		17	1	18	0
		19	0	19	0
	100%	18	0	18	0
		19	0	19	0
		20	0	20	0
36	Control	19	0	19	0
		20	0	20	0
		18	0	18	0
	10%	20	0	20	0
		20	0	20	0
		20	0	20	0
	50%	20	0	20	0
		18	0	18	0
		18	0	18	0
	100%	19	0	19	0
		18	0	18	0
		20	0	20	0
48	Control	6	13	19	68.4211
		4	15	19	78.9474
		5	14	19	73.6842
	10%	5	13	18	72.2222
		7	12	19	63.1579
		3	14	17	82.3529
	50%	7	8	15	53.3333
		3	9	12	75
		6	11	17	64.7059
	100%	8	9	17	52.9412
		8	11	19	57.8947
		6	9	15	60
72	Control	4	16	20	80
		1	18	19	94.7368
		5	11	16	68.75
	10%	1	16	17	94.1176
		1	19	20	95
		2	16	18	88.8889
	50%	5	15	20	75
		4	14	18	77.7778
		2	17	19	89.4737
	100%	4	16	20	80
		6	14	20	70
		8	12	20	60

Table F.5. Data for hatching percentage of exposed *C. finmarchicus* at 10°C. The first fixation (24h) was performed 24 hours after the females had been added to the egg laying containers

Hours	Treatment	Egg	Hatched	Total	Hatching (%)
24	Control	19	0	19	0
		20	0	20	0
		16	0	16	0
	10%	20	0	20	0
		20	0	20	0
		19	0	19	0
	50%	20	0	20	0
		20	0	20	0
		19	0	19	0
	100%	20	0	20	0
		17	1	18	0
		17	2	19	5.55556
36	Control	11	7	18	10.5263
		9	11	20	38.8889
		13	8	21	55
	10%	12	7	19	38.0952
		11	9	20	36.8421
		11	6	17	45
	50%	8	10	18	35.2941
		10	8	18	55.5556
		12	8	20	44.4444
	100%	12	5	17	40
		11	8	19	29.1053
		14	5	19	26.3158
48	Control	3	17	20	85
		3	13	16	81.25
		3	15	18	83.3333
	10%	3	17	20	85
		3	17	20	85
		4	14	18	77.7778
	50%	2	17	19	89.4737
		4	16	20	80
		5	15	20	75
	100%	3	16	19	84.2105
		2	16	18	88.8889
		5	14	19	73.6842
72	Control	2	18	20	90
		4	15	19	78.9474
		3	17	20	85
	10%	1	19	20	95
		2	16	18	88.8889
		1	18	19	94.7368
	50%	2	18	20	90
		2	18	20	90
		5	15	20	75
	100%	1	17	18	94.4444
		2	19	21	90.4762
		2	18	18	90

Table F.6. Data for hatching percentage of exposed *C. finmarchicus* at 12.5°C. The first fixation (24h) was performed 24 hours after the females had been added to the egg laying containers

Hours	Treatment	Egg	Hatched	Total	Hatching(%)
24	Control	20	0	20	0
		20	0	20	0
		19	0	19	0
	10%	20	0	20	0
		19	1	20	5
		19	0	19	0
	50%	20	0	20	0
		20	0	20	0
		19	1	20	5
	100%	20	0	20	0
		18	0	18	0
		20	0	20	0
36	Control	7	11	18	61.1111
		7	11	18	61.1111
		2	16	18	88.8889
	10%	3	16	19	84.2105
		8	12	20	60
		6	13	19	68.4211
	50%	9	11	20	55
		7	11	18	61.1111
		7	13	20	65
	100%	7	9	16	56.25
		9	11	20	55
		9	11	20	55
48	Control	4	13	17	76.4706
		5	14	19	73.6842
		4	14	18	77.7778
	10%	3	13	16	81.25
		5	14	19	73.6842
		3	17	20	85
	50%	2	14	16	87.5
		4	16	20	80
		3	15	18	83.3333
	100%	1	19	20	95
		3	14	17	82.3529
		0	20	20	100
72	Control	2	18	20	90
		2	17	19	89.4737
		3	16	19	.84.2105
	10%	1	16	17	94.1176
		2	17	19	89.4737
		4	16	20	80
	50%	1	17	18	94.4444
		7	12	19	63.1579
		3	16	19	84.2105
	100%	4	16	20	80
		3	15	18	83.3333
		2	17	19	89.4737

Appendix G

Table G.1. Hatching percentage of the experiment containing only temperatures, for *C. hyperboreus*. Hours were estimated from the eggs were sampled in the vials.

Temperature (°C)	Hours	Eggs	Hatched	Total	Hatching (%)
3	48	35	0	35	0
		34	0	34	0
		39	0	39	0
	96	24	14	38	36.84211
		12	18	30	60
		12	20	32	62.5
	144	2	39	41	95.12195
		1	39	40	97.5
		1	31	32	96.875
	192	0	30	30	100
		3	31	34	91.17647
		2	33	35	94.28571
7	48	28	4	32	12.5
		31	4	35	11.42857
		30	3	33	9.090909
	96	2	34	36	94.44444
		1	36	37	97.2973
		4	28	32	87.5
	144	2	29	31	93.54839
		3	28	31	90.32258
		3	30	33	90.90909
10	48	20	12	32	37.5
		19	11	30	36.66667
		15	15	30	50
	96	5	23	28	82.14286
		4	26	30	86.66667
		3	26	29	89.65517
	144	1	28	29	96.55172
		3	23	26	88.46154
		5	25	30	83.33333
	192	4	23	27	85.18519
		4	25	29	86.2069
		5	19	24	79.16667

Table G.2. Hatching percentage of the experiment containing only temperatures for *C. finmarchicus*. Hours were estimated from the eggs were sampled in the vials.

Temperature (°C)	Hours	Eggs	Hatched	Total	Hatching (%)
7.5	24	15	15	30	50
		21	6	27	22.2222
		13	14	27	51.85185
	48	6	24	30	80
		9	17	26	65.38462
		8	21	29	72.41379
	72	5	24	29	82.75862
		4	22	26	84.61538
		8	22	30	73.33333
	96	5	24	29	82.75862
		8	20	28	71.42857
		6	24	30	80
10	24	23	8	31	25.80645
		18	8	26	30.76923
	48	9	18	27	66.66667
		2	23	25	92
		4	22	26	84.61538
	72	6	22	28	78.57143
		4	18	22	81.81818
		5	20	25	80
	96	3	18	21	85.71429
		6	17	23	73.91304
		0	24	24	100
12.5	24	6	18	24	75
		13	14	27	51.85185
	48	2	21	23	91.30435
		4	21	25	84
		5	24	29	82.75862
	72	4	21	25	84
		1	26	27	96.2963
		4	22	26	84.61538
	96	1	29	30	96.66667
		6	23	29	79.31034
		6	26	32	81.25

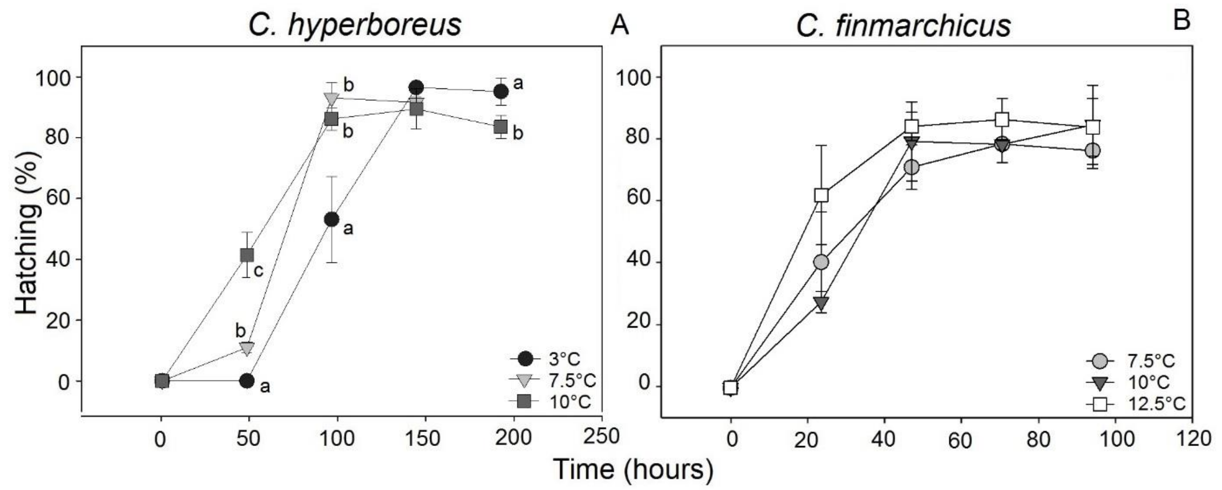

Figure G.1. Egg hatching (%) for the experiment were only temperatures were examined. A: *C. hyperboreus*, B: *C. finmarchicus*. Hours were estimated from when the eggs were sampled in the vials. Significant difference between temperatures, within a time period is indicated with lower case letters (One-way ANOVA, Holm-Sidak, $p < 0.05$).

Appendix H

Table H.1. Measured concentration (µg L^{-1}) of individual PAHs in the experiment *C. hyperboreus* 3°C. Refer to Table 1.1 for identification of PAHs. Dispersion refers to the raw dispersion (n=3), while Stock solution (n=3) is the filtered WSF, where droplets are removed (Figure 2.2), and is the remains of the solution added to the VOC-vials. WSF from VOC-vials refers to the water removed from the VOC-vials after exposure of the eggs. For stock solution, mean±SD is shown. There was no data for the WSF from bottles, which was the remains from the solution added to the VOC-vials.

		WSF from VOC-vials			
	Concentraton (µg L^{-1})				
PAH	Stock solution	Ctrl	10%	50%	100%
N	6.12±0.24	0.07	0.21	0.67	1.12
N1	0.06±0.00	0.01	0.04	0.04	0.03
N2	2.93±0.21	0.03	0.06	0.18	0.27
R	0.58±0.11	0.01	0.01	0.04	0.06
P	2.80±0.52	0.01	0.03	0.12	0.23
D	0.28±0.05	0.00	0.00	0.01	0.03
D1	0.30±0.06	0.00	0.01	0.01	0.02
D2	0.12±0.02	0.00	0.00	0.00	0.01
F1	0.02±0.01	0.00	0.01	0.00	0.00
Py	0.06±0.03	0.00	0.01	0.00	0.01
C	0.06±0.02	0.00	0.00	0.00	0.01
ΣPAH	13.34±1.27	0.14	0.38	1.08	1.80

TableH.2. Measured concentration (µg L^{-1}) of individual PAHs in the experiment *C. hyperboreus* 7.5°C. Refer to Table 1.1 for identification of PAHs. Stock solution (mean of two bottles) is the remains of the water that was used to make the dilutions. WSF from bottle is the samples that were added to the VOC-vials. WSF from VOC-vials refers to the water removed from the VOC-vials after exposure of the eggs.

		WSF from bottle				WSF from VOC-vials			
	Concentration (µg L^{-1})								
PAH	Stock solution	Ctrl	10%	50%	100%	Ctrl	10%	50%	100%
N	6.17	0.01	0.60	2.94	5.81	0.02	0.44	2.15	4.22
N1	6.27	0.01	0.58	2.98	5.99	0.01	0.38	1.93	3.85
N2	3.95	0.00	0.35	1.83	3.76	0.01	0.19	0.98	2.00
R	0.42	0.00	0.04	0.20	0.40	0.00	0.03	0.14	0.28
P	1.94	0.00	0.18	0.94	1.86	0.01	0.12	0.58	1.19
D	0.20	0.00	0.02	0.10	0.19	0.00	0.01	0.06	0.11
D1	0.19	0.00	0.02	0.09	0.17	0.00	0.01	0.04	0.08
D2	0.11	0.00	0.01	0.05	0.09	0.00	0.01	0.02	0.04
F1	0.02	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01
Py	0.05	0.00	0.01	0.03	0.04	0.00	0.01	0.02	0.03
C	0.06	0.00	0.00	0.03	0.04	0.00	0.00	0.01	0.02
ΣPAH	19.36±0.22	0.02	1.82	9.19	18.37	0.05	1.20	5.94	11.82

Table H.3. Measured concentration (µg L^{-1}) of individual PAHs in the experiment *C. hyperboreus* 10°C. Refer to Table 1.1 for identification of PAHs. There was no data for the stock solution and Ctrl of WSF from bottle. WSF from bottle is the samples that were added to the VOC-vials. WSF from VOC-vials refers to the water removed from the VOC-vials after exposure of the eggs.

	WSF from bottle			WSF from VOC-vials			
	Concentration (µg L^{-1})						
PAH	10%	50%	100%	Ctrl	10%	50%	100%
N	0.66	2.89	5.73	0.08	0.25	1.68	3.78
N1	0.03	0.03	0.05	0.01	0.01	0.02	0.04
N2	0.27	1.20	2.36	0.04	0.15	0.68	1.42
R	0.05	0.25	0.45	0.01	0.04	0.16	0.32
P	0.24	1.22	2.20	0.02	0.11	0.65	1.38
D	0.02	0.11	0.19	0.00	0.01	0.06	0.13
D1	0.02	0.12	0.21	0.00	0.01	0.06	0.12
D2	0.01	0.05	0.10	0.00	0.01	0.02	0.05
F1	0.01	0.02	0.03	0.01	0.00	0.01	0.01
Py	0.02	0.05	0.08	0.00	0.01	0.03	0.03
C	0.01	0.05	0.07	0.00	0.00	0.02	0.02
ΣPAH	1.34	5.99	11.46	0.18	0.62	3.41	7.31

Table H.4. Measured concentration (µg L^{-1}) of individual PAHs in the experiment *C. finmarchicus* 7.5°C. Refer to Table 1.1 for identification of PAHs. There was no data for the stock solution. WSF from bottle is the samples that were added to the VOC-vials. WSF from VOC-vials refers to the water removed from the VOC-vials after exposure of the eggs.

	WSF from bottle				WSF from VOC-vials			
	Concentration (µg L^{-1})							
PAH	Ctrl	10%	50%	100%	Ctrl	10%	50%	100%
N	0.01	0.63	3.29	6.43	0.03	0.44	2.25	4.70
N1	0.01	0.59	3.17	6.30	0.02	0.39	2.05	4.32
N2	0.00	0.33	1.79	3.66	0.01	0.20	1.05	2.23
R	0.00	0.05	0.22	0.45	0.00	0.03	0.18	0.36
P	0.00	0.18	0.90	1.82	0.01	0.13	0.66	1.33
D	0.00	0.02	0.09	0.18	0.00	0.01	0.07	0.13
D1	0.00	0.01	0.07	0.15	0.00	0.01	0.05	0.09
D2	0.00	0.01	0.04	0.07	0.00	0.00	0.02	0.03
F1	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01
Py	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.02
C	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.01
ΣPAH	0.03	1.82	9.60	19.12	0.07	1.22	6.33	13.23

Table H.5. Measured concentration (µg L^{-1}) of individual PAHs in the experiment *C. finmarchicus* 10°C. Refer to Table 1.1 for identification of PAHs. There was no data for the stock solution. WSF from bottle is the samples that were added to the VOC-vials. WSF from VOC-vials refers to the water removed from the VOC-vials after exposure of the eggs.

	WSF from bottle				WSF from VOC-vials			
	Concentration (µg L^{-1})							
PAH	Ctrl	10%	50%	100%	Ctrl	10%	50%	100%
N	0.01	0.54	2.71	5.57	0.02	0.40	1.95	4.06
N1	0.01	0.48	2.51	5.18	0.02	0.32	1.67	3.59
N2	0.00	0.28	1.46	3.09	0.01	0.17	0.85	1.90
R	0.00	0.03	0.18	0.36	0.00	0.03	0.13	0.29
P	0.00	0.14	0.76	1.53	0.01	0.11	0.51	1.08
D	0.00	0.02	0.08	0.15	0.00	0.01	0.05	0.11
D1	0.00	0.02	0.10	0.20	0.00	0.01	0.06	0.13
D2	0.00	0.02	0.08	0.17	0.00	0.01	0.04	0.08
F1	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.01
Py	0.00	0.00	0.02	0.04	0.00	0.01	0.01	0.02
C	0.00	0.00	0.03	0.05	0.00	0.00	0.01	0.03
ΣPAH	0.03	1.53	7.93	16.34	0.07	1.07	5.31	11.30

Table H.6. Measured concentration (µg L^{-1}) of individual PAHs in the experiment *C. finmarchicus* 12.5°C. Refer to Table 1.1 for identification of PAHs. There was no data for the stock solution. WSF from bottle is the samples that were added to the VOC-vials. WSF from VOC-vials refers to the water removed from the VOC-vials after exposure of the eggs.

	WSF from bottle				WSF from VOC-vials			
	Concentration (µg L^{-1})							
PAH	Ctrl	10%	50%	100%	Ctrl	10%	50%	100%
N	0.03	0.51	3.01	4.82	0.02	0.38	2.18	3.96
N1	0.03	0.39	2.51	3.72	0.01	0.27	1.70	2.86
N2	0.02	0.22	1.32	2.10	0.01	0.14	0.79	1.45
R	0.00	0.03	0.18	0.29	0.00	0.02	0.13	0.21
P	0.01	0.13	0.74	1.25	0.01	0.09	0.51	0.90
D	0.00	0.01	0.07	0.12	0.00	0.01	0.05	0.09
D1	0.00	0.02	0.10	0.19	0.00	0.01	0.06	0.12
D2	0.00	0.02	0.09	0.16	0.00	0.01	0.04	0.09
F1	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01
Py	0.00	0.01	0.03	0.03	0.00	0.01	0.03	0.03
C	0.00	0.01	0.05	0.06	0.00	0.00	0.03	0.03
ΣPAH	0.10	1.35	8.13	12.76	0.05	0.94	5.54	9.75

Table H.7. Measured concentration (μg L^{-1}) of individual PAHs in the exposed hatching success experiment for *C. hyperboreus*. Refer to Table 1.1 for identification of PAHs. Stock solution is the same as in table H.2. The WSF from bottle was added to all the vials.

	WSF from bottle			
	Concentration (μg L^{-1})			
PAH	Ctrl	10%	50%	100%
N	0.01	0.59	3.11	6.03
N1	0.00	0.54	3.07	6.05
N2	0.00	0.36	1.98	3.90
R	0.00	0.04	0.20	0.40
P	0.00	0.17	0.94	1.83
D	0.09	0.10	0.09	0.09
D1	0.00	0.02	0.09	0.19
D2	0.00	0.00	0.06	0.12
F1	0.00	0.00	0.00	0.02
Py	0.00	0.00	0.00	0.05
C	0.00	0.00	0.00	0.08
ΣPAH	0.10	1.82	9.55	18.74

Table H.8. Measured concentration (μg L^{-1}) of individual PAHs in the exposed hatching success experiment for *C. hyperboreus*. Refer to Table 1.1 for identification of PAHs.

		WSF from bottle			
	Concentration (μg L^{-1})				
PAH	Stock solution	Ctrl	10%	50%	100%
N	6.85	0.01	0.70	3.54	7.17
N1	7.80	0.01	0.64	3.36	6.83
N2	4.96	0.01	0.37	1.97	3.93
R	0.59	0.00	0.05	0.28	0.55
P	2.38	0.00	0.21	1.13	2.27
D	0.23	0.00	0.02	0.11	0.22
D1	0.24	0.00	0.02	0.11	0.22
D2	0.15	0.00	0.01	0.06	0.12
F1	0.01	0.00	0.00	0.01	0.02
Py	0.03	0.00	0.00	0.03	0.06
C	0.04	0.00	0.00	0.05	0.08
ΣPAH	23.26	0.05	2.04	10.65	21.47

Appendix I

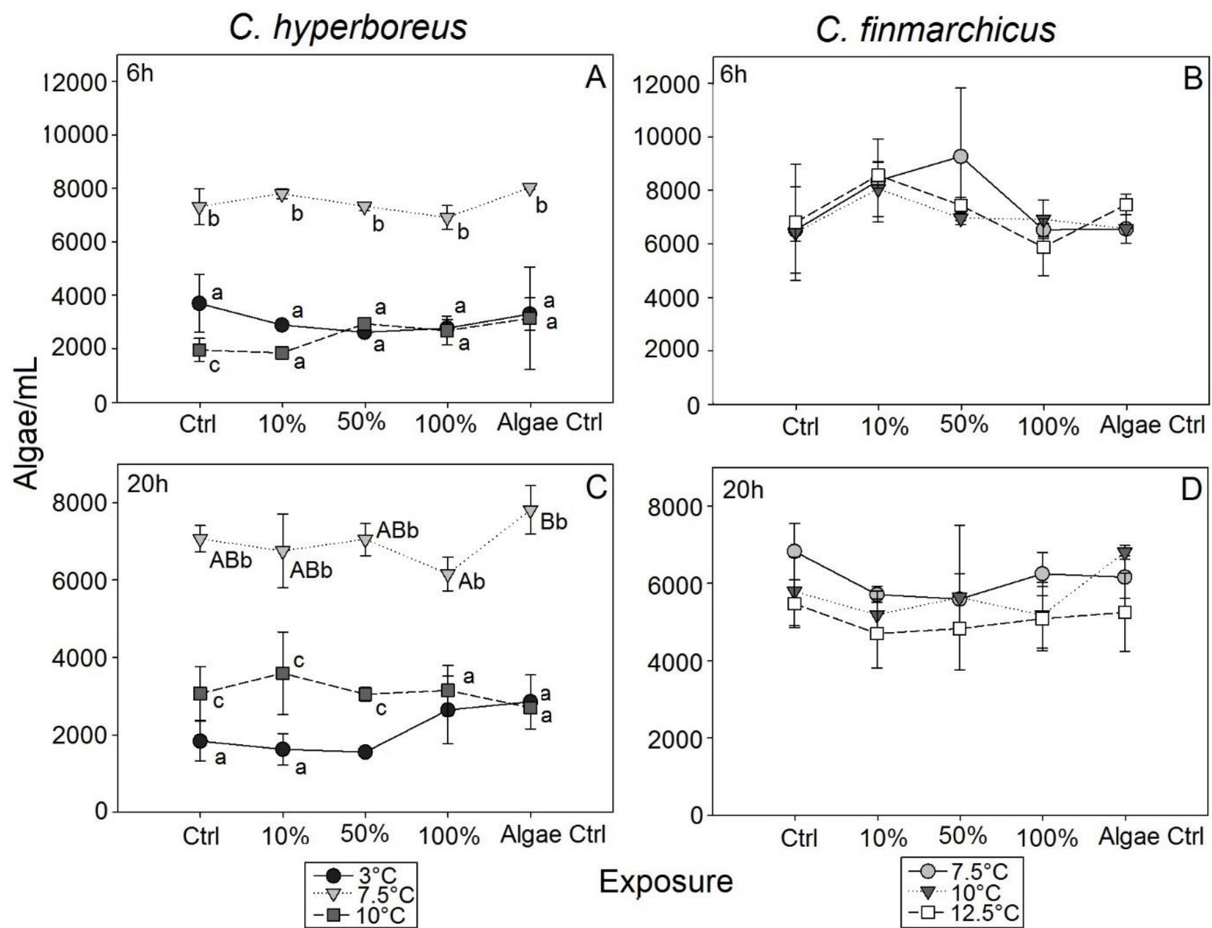

Figure I.1. Algae/mL counted on the CoulterCounter® Multisizer 3™, multiplied with 5, to make up for the dilution. A and C: *C. hyperboreus* and B and D: *C. finmarchicus* at different temperatures (means ± SD). Number of individuals is given in table B.1 in Appendix B. Significant difference between the temperatures within each exposure group is indicated with lower case letter, while significant difference between exposures within the same temperature is indicated with upper case letter (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Table I.1. Cells/mL of the algae samples counted after 0 hours. To take the 5x dilution into consideration, cells/mL was multiplied by 5. The average of this was used as assumed start concentration in all feeding experiments.

Sample	Cells/mL counted	Cells/mL *5
AC 1	1640	8200
AC2	1611	8055
AC3	1663	8315
Average	1638	8190

Appendix J

Table J.1. Statistics from the feeding experiments, comparing mean of all temperatures with mean of all exposure groups (Temperature x Exposure) within each time period for both *C. hyperboreus* and *C. finmarchicus*, statistical significance outlined in bold (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Species	Timeperiod	p-value
C. hyperboreus	6h	**0.013**
	20h	**0.002**
C. finmarchicus	6h	0.575
	20h	0.928

Table J.2. Statistics from the feeding experiments, with temperature as comparison factor, for each time period within *C. hyperboreus* and *C. finmarchicus*, mean of all exposures within each temperature, statistical significance outlined in bold (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Species	Time period	Comparison	p-value
C. hyperboreus	6h	3°C vs. 7.5°C	**<0.001**
		3°C vs. 10°C	0.957
		7.5°C vs. 10°C	**<0.001**
	20h	3°C vs. 7.5°C	**<0.001**
		3°C vs. 10°C	**0.029**
		7.5°C vs. 10°C	**<0.001**
C. finmarchicus	6h	All temperatures	0.516
	20h	7.5°C vs. 10°C	**0.006**
		7.5°C vs. 12.5°C	**<0.001**
		10°C vs. 12.5°C	0.216

Table J.3. Statistics from the feeding experiments, with exposure as comparison factor, for each time period within *C. hyperboreus* and *C. finmarchicus*, comparing mean of all temperatures within each exposure group, statistical significance outlined in bold (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Species	Timeperiod	Comparison	p-value
C. hyperboreus	6h	All exposures	0.742
	20h	All exposures	0.377
C. finmarchicus	6h	Ctrl vs. 10%	**0.046**
		Ctrl vs. 50%	0.149
		Ctrl vs. 100%	0.813
		10% vs. 50%	0.753
		10% vs. 100%	**0.032**
		50% vs. 100%	0.793
	20h	All exposures	0.219

Appendix K

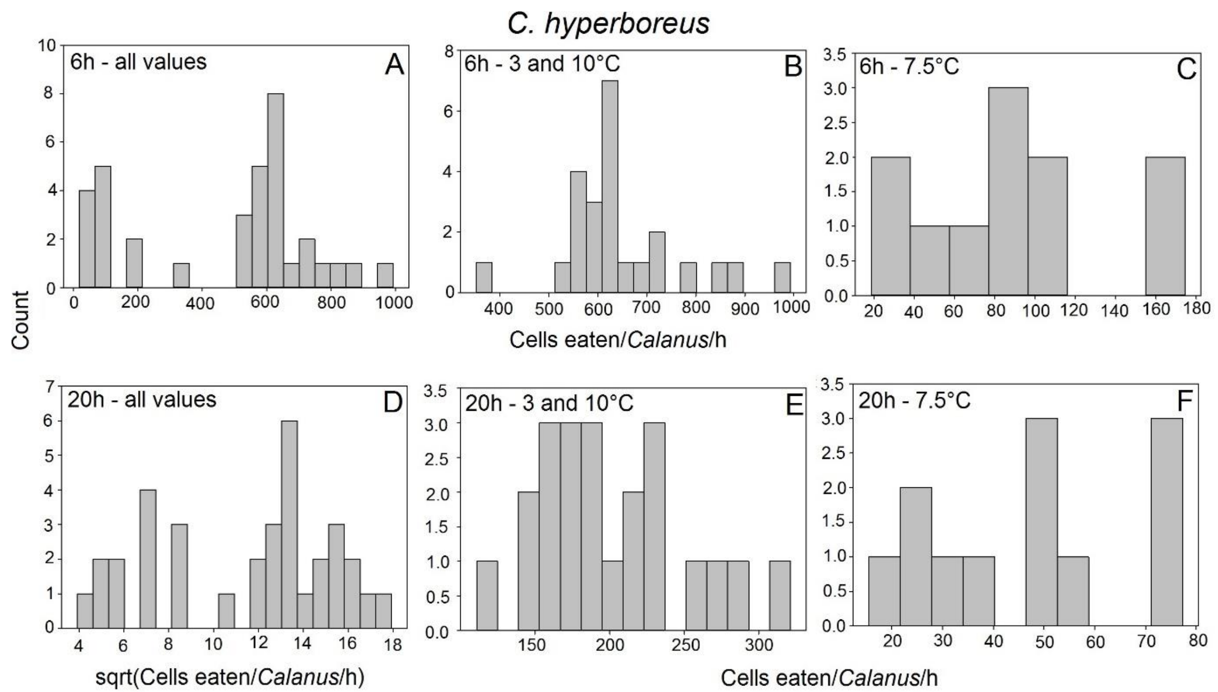

Figure K.1. Histograms showing the distribution of the Cells eaten/*Calanus*/h data of *C. hyperboreus*. The "count" at the y-axis represents number of copepods. Due to the large difference between 7.5°C and the two other temperatures, the distribution was divided in two areas (A and D), therefore the distribution of 3 and 10°C (B and E) and 7.5°C (C and F) is also shown separately. A-C: 6 hours of algae exposure. D-F: 20 hours of algae exposure.

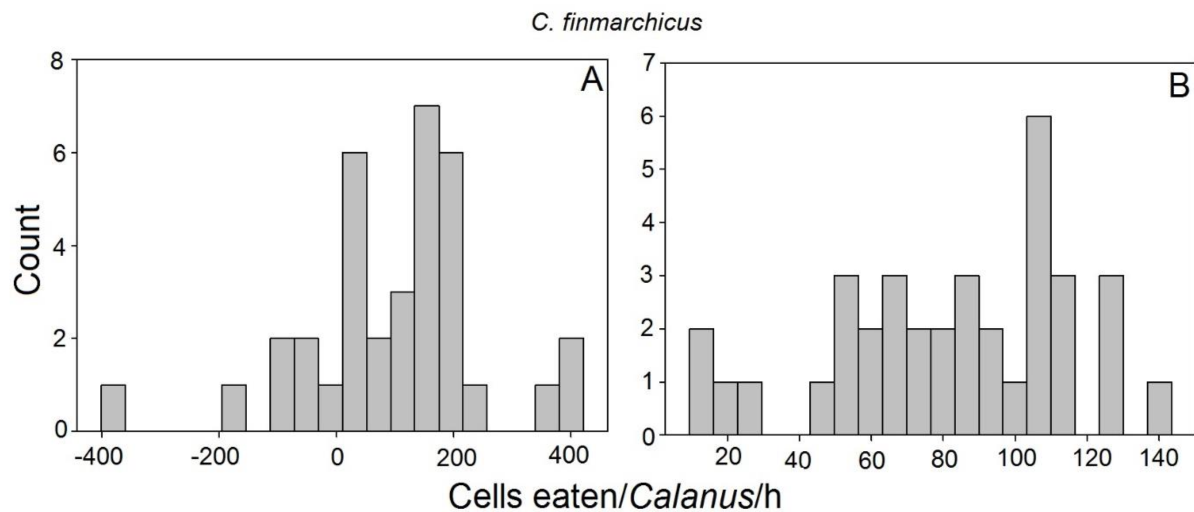

Figure K.2. Histograms showing the distribution of the Cells eaten/*Calanus*/h data of *C. finmarchicus*. The "count" at the y-axis represents number of copepods. A: 6 hours of algae exposure. B: 20 hours of algae exposure.

Appendix L

Table L.1. Statistics of Hatching Success, comparing mean of all temperatures with mean of all exposure groups (Temperature x Exposure), for each time period within *C. hyperboreus* and *C. finmarchicus*, statistical significance outlined in bold (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Species	Time (hours)	p-value
C. hyperboreus	48	0.180
	96	0.710
	144	0.840
C. finmarchicus	24	**0.026**
	36	0.275
	48	**0.010**
	72	0.282

Table L.2. Statistics of Hatching Success, with temperature as comparison factor, for each time period within *C. hyperboreus* and *C. finmarchicus*, mean of all exposures within each temperature, statistical significance outlined in bold (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Species	Time (hours)	Comparison	p-value
C. hyperboreus	48	All temperatures	0.594
	96	3°C vs. 7.5°C	**<0.001**
		3°C vs. 10°C	**<0.001**
		7.5°C vs. 10°C	0.996
	144	All temperatures	0.132
C. finmarchicus	24	All temperatures	0.812
	36	7.5°C vs. 10°C	**<0.001**
		7.5°C vs. 12.5°C	**<0.001**
		10°C vs. 12.5°C	**<0.001**
	48	7.5°C vs. 10°C	**<0.001**
		7.5°C vs. 12.5°C	**<0.001**
		10°C vs. 12.5°C	0.740
	144	All temperatures	0.115

Table L.3. Statistics of Hatching Success, with exposure as comparison factor, for each time period within *C. hyperboreus* and *C. finmarchicus*, comparing mean of all temperatures within each exposure group, statistical significance outlined in bold (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Species	Time (hours)	Comparison	p-value
C. hyperboreus	48	All exposures	0.150
	96	All exposures	0.572
	144	All exposures	0.457
C. finmarchicus	24	All exposures	0.222
	36	All exposures	0.097
	48	All exposures	0.943
	72	All exposures	0.075

Appendix M

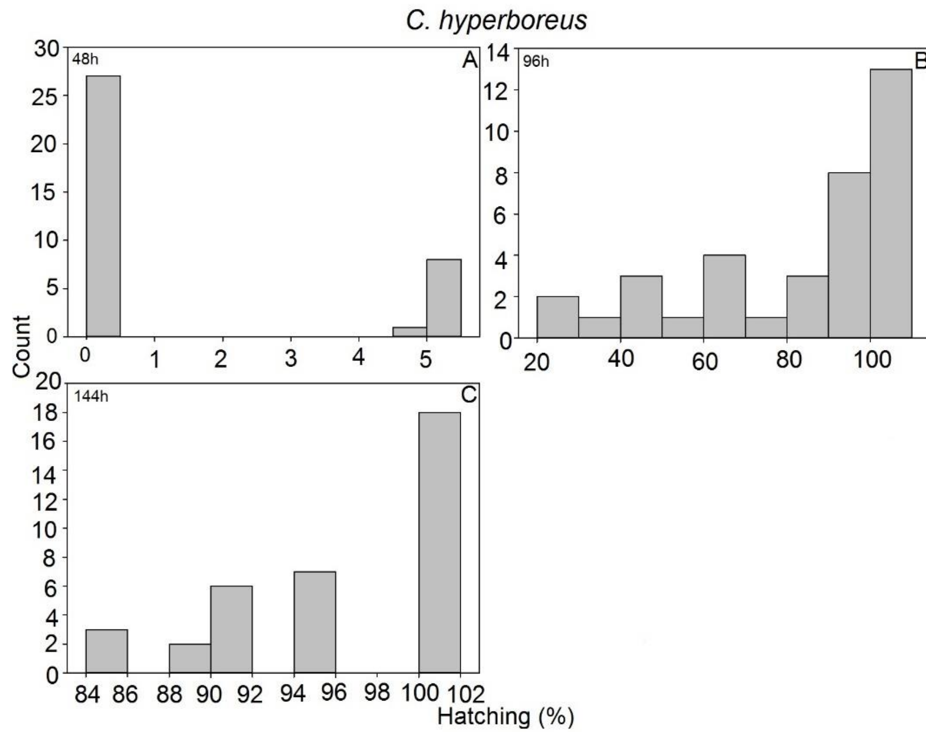

Figure M.1. Histograms showing the distribution of the Hatching (%) data of *C. hyperboreus*. The "count" at the y-axis represents number of copepods.

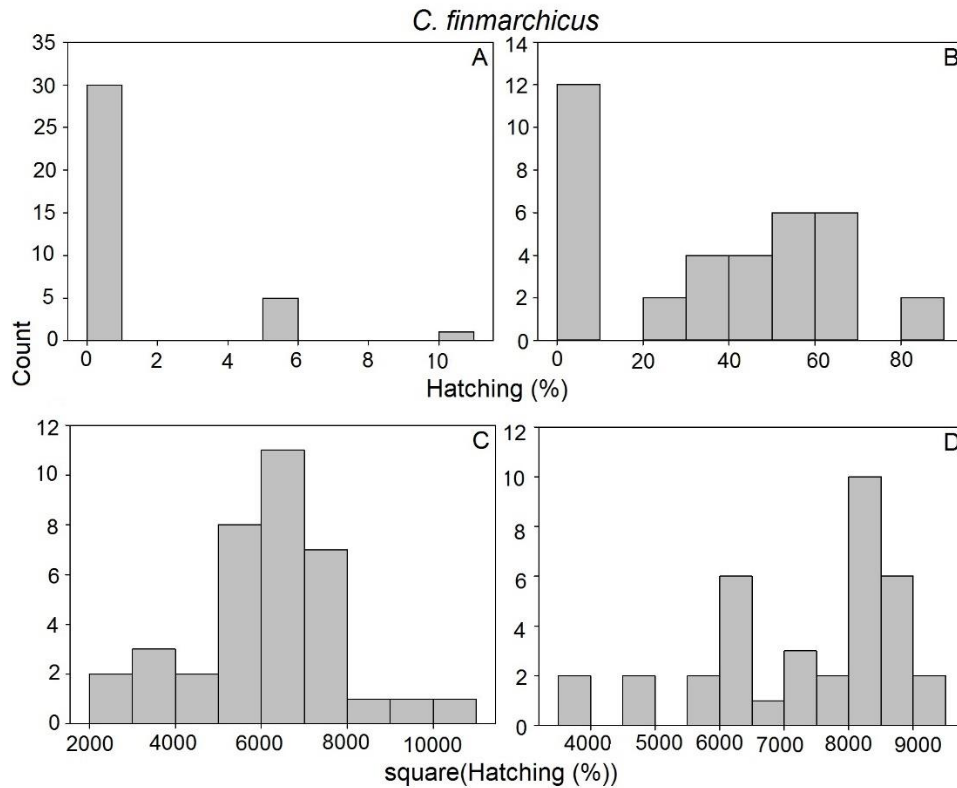

Figure M.2. Histograms showing the distribution of the Hatching success (%) data of *C. finmarchicus*. The "count" at the y-axis represents number of copepods C and D: data was square-transformed.

Appendix N

Table N.1. Statistics of dry weight, with exposure as comparison factor, for each developmental stage within *C. hyperboreus* and *C. finmarchicus*, comparing mean of all temperatures within each exposure group, statistical significance outlined in bold (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Species	Stage	Comparison	p-value
C. hyperboreus	NI	Ctrl vs. 10%	0.116
		Ctrl vs. 50%	**0.007**
		Ctrl vs. 100%	0.642
		10% vs. 50%	0.324
		10% vs. 100%	0.249
		50% vs. 100%	**0.023**
	NII	All exposures	0.352
	NIII	Ctrl vs. 10%	0.245
		Ctrl vs. 50%	0.496
		Ctrl vs. 100%	**0.016**
		10% vs. 50%	0.416
		10% vs. 100%	0.450
		50% vs. 100%	0.166
C. finmarchicus	NI	All exposures	0.202
	NII	All exposures	0.224
	NIII	All exposures	0.795

Table N.2. Statistics of dry weight, or each developmental stage, within *C. hyperboreus* and *C. finmarchicus*, comparing mean of all temperatures with mean of all exposure groups (Temperature x Exposure), statistical significance outlined in bold (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Species	Time (hours)	p-value
C. hyperboreus	NI	0.952
	NII	0.390
	NIII	0.262
C. finmarchicus	NI	0.276
	NII	0.728
	NIII	**0.026**

Table N.3. Statistics of dry weight, with temperature as comparison factor, for each developmental stage within *C. hyperboreus* and *C. finmarchicus*, mean of all exposures within each temperature, statistical significance outlined in bold (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Species	Stage	Comparison	p-value
C. hyperboreus	NI	All temperatures	0.528
	NII	3°C vs. 7.5°C	**0.013**
	NIII	All temperatures	0.371
C. finmarchicus	NI	All temperatures	0.548
	NII	7.5°C vs. 10°C	0.203
		7.5°C vs. 12.5°C	**0.024**
		10°C vs. 12.5°C	0.258
	NIII	All temperatures	0.163

Appendix O

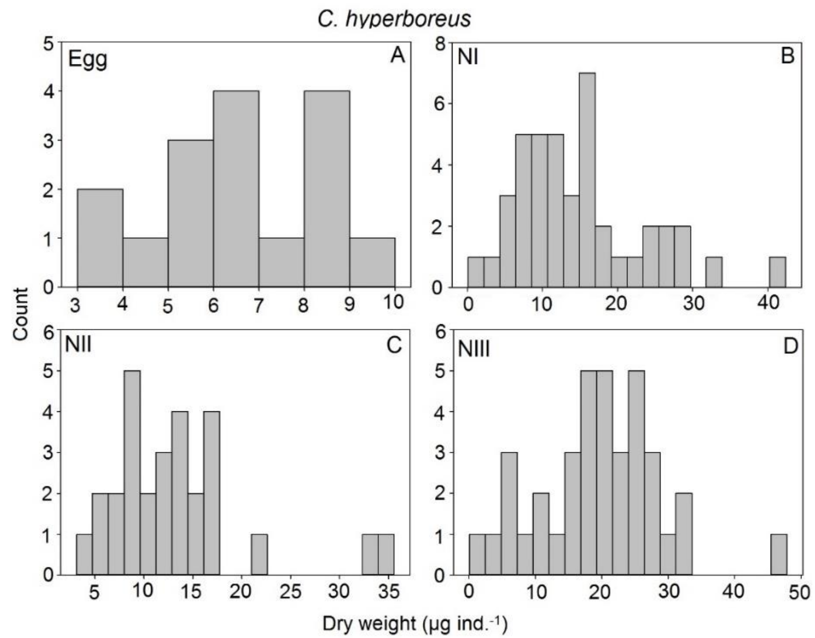

Figure O.1. Histograms showing the distribution of the Dry weight (µg ind.$^{-1}$) data of *C. hyperboreus*. The “count” at the y-axis represents number of copepods.

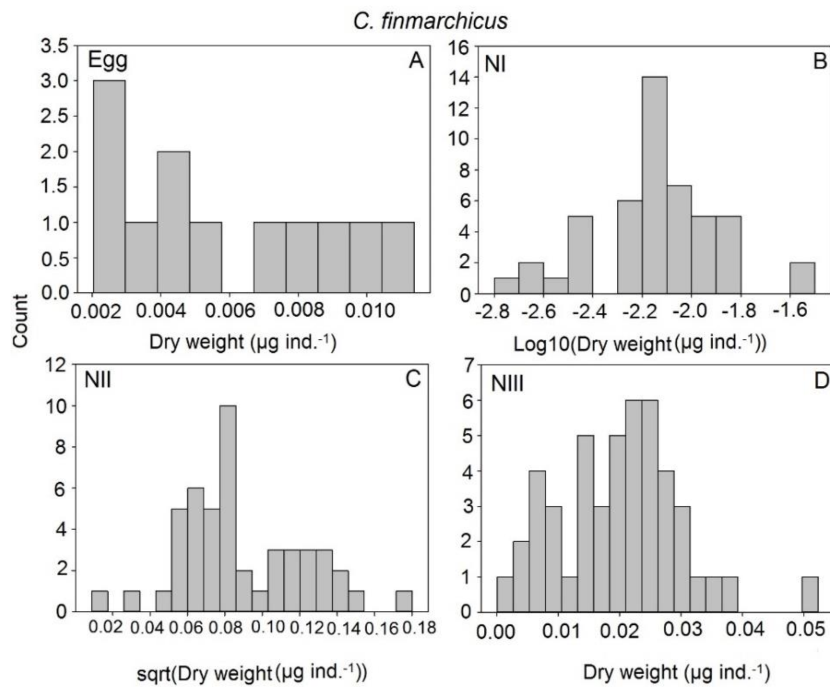

Figure O.2. Histograms showing the distribution of the Dry weight (µg) data of *C. finmarchicus*. The “count” at the y-axis represents number of copepods. B: was log10-transformed. C: was square-root-transformed.

Appendix P

Table P.1. Statistics of dry weight, comparison between developmental stages, within *C. hyperboreus*, statistical significance outlined in bold (One-way ANOVA, Holm-Sidak, Kruskal-Wallis One-way ANOVA on Ranks, Dunn's Method, $p < 0.05$). Kruskal-Wallis One-way ANOVA was used when Equal Variance Test (Brown-Forsythe) failed, and is denoted with *. Eggs are only compared within Ctrl, as they were not exposed.

Treatment	Temperature (°C)	Comparison	p-value
Ctrl	3*	Eggs vs. NI	1.000
		Eggs vs. NII	1.000
		Eggs vs. NIII	**0.016**
		NI vs. NII	1.000
		NI vs. NIII	0.725
		NII vs. NIII	0.645
	7.5	Eggs vs. NI	0.997
		Eggs vs. NII	1.000
		Eggs vs. NIII	**<0.001**
		NI vs. NII	0.981
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	10	All stages	0.092
10%	3	All stages	0.997
	7.5	All stages	0.231
	10	All stages	0.393
50%	3*	All stages	0.218 (0.071)
	7.5	All stages	0.161
	10	All stages	0.597
100	3	All stages	0.271 (0.486)
	7.5	All stages	0.486
	10	All stages	0.489

Table P.2. Statistics of dry weight, comparison between developmental stages, within *C. finmarchicus*, statistical significance outlined in bold (One-way ANOVA, Holm-Sidak, Kruskal-Wallis One-way ANOVA on Ranks, Dunn's Method, $p < 0.05$). Kruskal-Wallis One-way ANOVA was used when Equal Variance Test (Brown-Forsythe) failed, and is denoted with *. Eggs are only compared within Ctrl, as they were not exposed.

Treatment	Temperature (°C)	Comparison	p-value
Ctrl	7.5	Eggs vs. NI	0.482
		Eggs vs. NII	0.613
		Eggs vs. NIII	**<0.001**
		NI vs. NII	0.983
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	10	Eggs vs. NI	0.859
		Eggs vs. NII	0.822
		Eggs vs. NIII	**<0.001**
		NI vs. NII	0.768
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	12.5*	All stages	0.653
10%	7.5	NI vs. NII	0.903
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	10	All stages	0.446
	12.5	All stages	0.418
50%	7.5	NI vs. NII	0.185
		NI vs. NIII	**0.028**
		NI vs. NIII	**0.005**
100%	7.5	NI vs. NII	0.052
		NI vs. NIII	0.105
		NII vs. NIII	0.514
	10*	All stages	0.480
	12.5	All stages	0.081

Appendix Q

Table Q.1. Statistics of Dry Weight of each developmental stage, within *C. hyperboreus* and *C. finmarchicus*, comparing mean of all temperatures with mean of all exposure groups (Temperature x Exposure), statistical significance outlined in bold (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Species	Time (hours)	p-value
C. hyperboreus	NI	**0.044**
	NII	0.821
	NIII	**<0.001**
C. finmarchicus	NI	**0.023**
	NII	0.136
	NIII	**0.011**

Table Q.2. Statistics of biometry, with temperature as comparison factor, for each developmental stage within *C. hyperboreus* and *C. finmarchicus*, mean of all exposures within each temperature, statistical significance outlined in bold (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Species	Stage	Comparison	p-value
C. hyperboreus	NI	3°C vs. 7.5°C	**0.012**
		3°C vs. 10°C	**0.08**
		7.5°C vs. 10°C	**<0.001**
	NII	3°C vs. 7.5°C	**<0.001**
	NIII	3°C vs. 7.5°C	**<0.001**
		3°C vs. 10°C	**<0.001**
		7.5°C vs. 10°C	**<0.001**
C. finmarchicus	NI	7.5°C vs. 10°C	0.188
		7.5°C vs. 12.5°C	**<0.001**
		10°C vs. 12.5°C	**0.003**
	NII	7.5°C vs. 10°C	**<0.001**
		7.5°C vs. 12.5°C	**<0.001**
		10°C vs. 12.5°C	0.307
	NIII	7.5°C vs. 10°C	**<0.001**
		7.5°C vs. 12.5°C	**<0.001**
		10°C vs. 12.5°C	0.645

Table Q.3. Statistics of biometry, with exposure as comparison factor, for each developmental stage within *C. hyperboreus* and *C. finmarchicus*, comparing mean of all temperatures within each exposure group, statistical significance outlined in bold (Two-way ANOVA, Holm-Sidak, $p < 0.05$).

Species	Stage	Comparison	p-value
C. hyperboreus	NI	Ctrl vs. 10%	0.052
		Ctrl vs. 50%	0.772
		Ctrl vs. 100%	**<0.001**
		10% vs. 50%	**0.036**
		10% vs. 100%	**<0.001**
		50% vs. 100%	**<0.001**
	NII	All exposures	0.100
	NIII	All exposures	0.338
C. finmarchicus	NI	Ctrl vs. 10%	0.953
		Ctrl vs. 50%	**0.005**
		Ctrl vs. 100%	**0.008**
		10% vs. 50%	**0.007**
		10% vs. 100%	**0.011**
		50% vs. 100%	0.793
	NII	All exposures	0.084
	NIII	All exposures	0.457

Appendix R

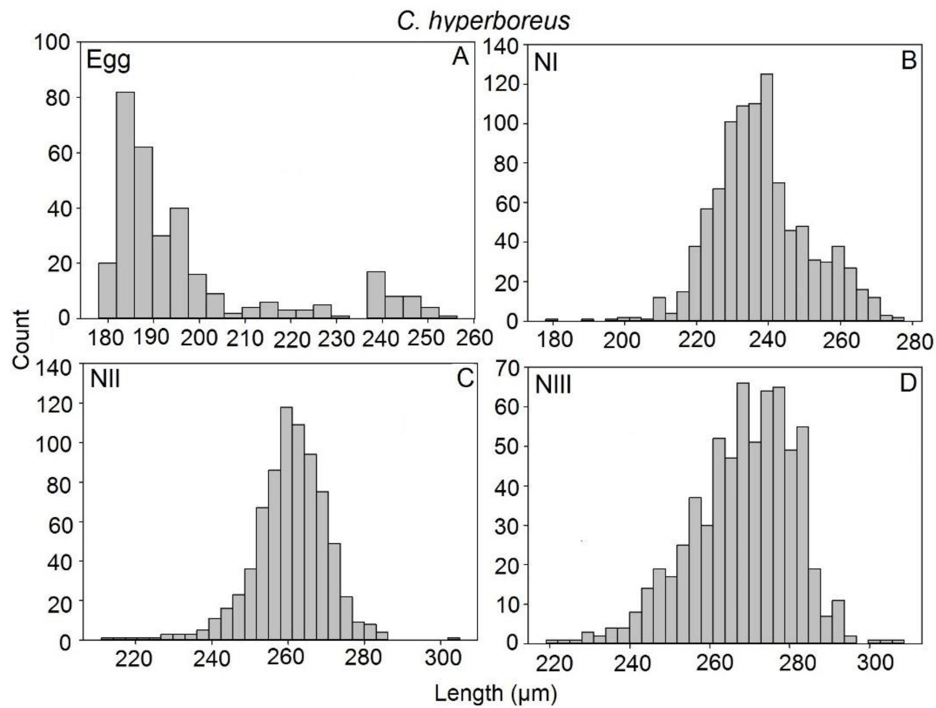

Figure R.1. Histograms showing the distribution of the Length (µm) from the biometry data of *C. hyperboreus*. The "count" at the y-axis represents number of copepods.

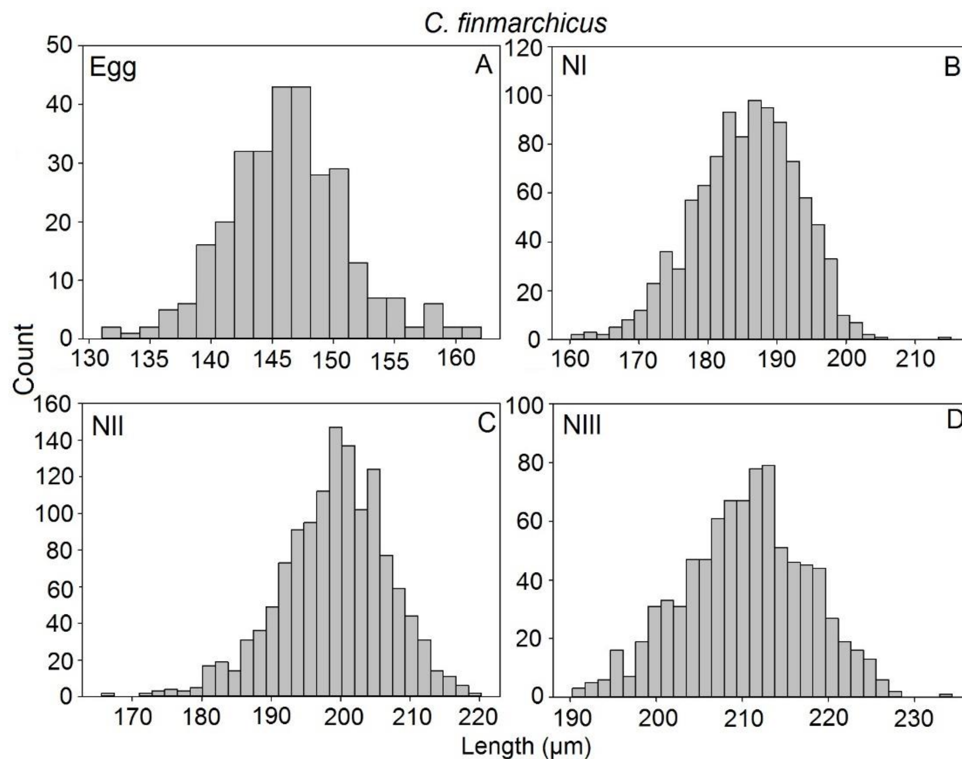

Figure R.2. Histograms showing the distribution of the Length (µm) from the biometry data of *C. finmarchicus*. The "count" at the y-axis represents number of copepods.

Appendix S

Table S.1. Statistics of biometry, comparison between developmental stages, within *C. hyperboreus*, statistical significance outlined in bold (Kruskal-Wallis One-way ANOVA on Ranks, Dunn's Method, $p < 0.05$). Kruskal-Wallis One-way ANOVA was used in all samples, as Equal Variance Test (Brown-Forsythe) and/or Normality Test (Shapiro-Wilk) failed. Eggs are only compared within Ctrl, as they were not exposed.

Treatment	Temperature (°C)	Comparison	p-value
Ctrl	3	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**0.003**
	7.5	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	10	NI vs. NIII	**<0.001**
10%	3	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**0.039**
	7.5	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	10	NI vs. NIII	**<0.001**
50%	3	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**0.005**
	7.5	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	10	NI vs. NIII	**<0.001**
100	3	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	7.5	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	10	NI vs. NIII	**<0.001**

Table S.2. Statistics of dry weight, comparison between developmental stages, within *C. finmarchicus*, statistical significance outlined in bold (One-way ANOVA, Holm-Sidak, Kruskal-Wallis One-way ANOVA on Ranks, Dunn's Method, $p < 0.05$). Kruskal-Wallis One-way ANOVA was used when Equal Variance Test (Brown-Forsythe) failed, and is denoted with *. Eggs are only compared within Ctrl, as they were not exposed.

Treatment	Temperature (°C)	Comparison	p-value
Ctrl	7.5*	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	10*	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	12.5*	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
10%	7.5	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	10*	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	12.5*	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
50%	7.5*	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	10	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	12.5*	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
100%	7.5	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	10	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**
	12.5	NI vs. NII	**<0.001**
		NI vs. NIII	**<0.001**
		NII vs. NIII	**<0.001**

Appendix T

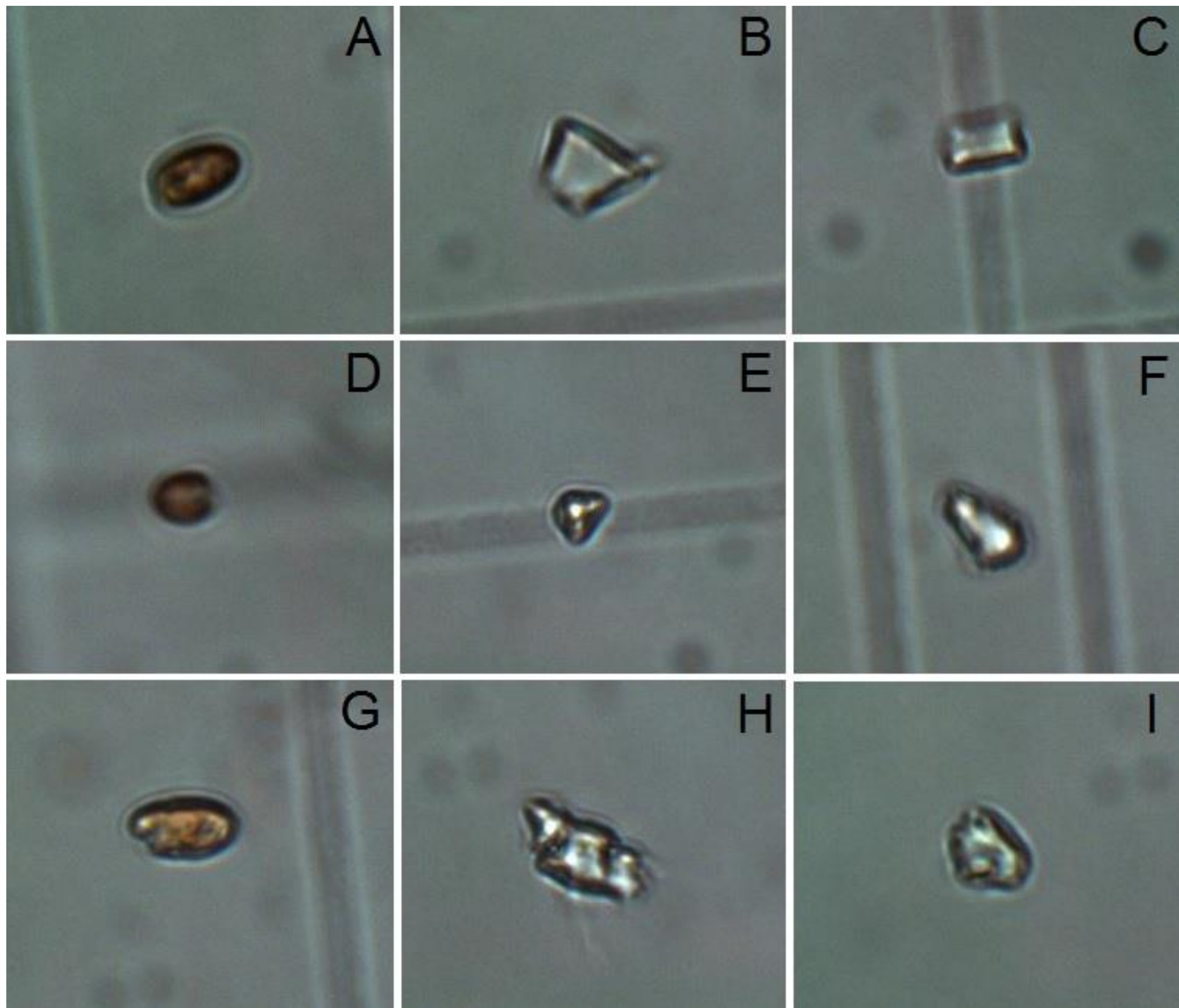

Figure T.1. Pictures taken of objects found in one of the *C. finmarchicus* algae samples, where there seemed to be a lot of background noise (shown in Figure D.2., B, Appendix D), All pictures were taken at magnification 40x0.5. A, D, and G