Genome size, growth rate, and elemental stoichiometry of cultured *Calanus finmarchicus*

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A female *Calanus finmarchicus* awaiting preservation for further analysis. Photo by Lotte Thommesen and Kristina Kvile, September 2019.

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Lotte Thommesen

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ABSTRACT

The copepod *Calanus finmarchicus* seasonally dominates the zooplankton biomass in the North Atlantic. In this study, I compare body size, genome size, growth rate, and elemental stoichiometry between cultured *C. finmarchicus* and conspecifics from Norwegian coastal waters. At NTNU SeaLab in Trondheim, Norway, *C. finmarchicus* has been held continuously in culture for more than 65 generations. I collected developmental stages C4 to adults from the culture and wild C5 and females for comparison from Oslofjorden, Norway. Furthermore, I included data from an experiment conducted with the cultured copepods as a third group for comparison. To assess growth rate and genome size, I quantified nucleic acid content of individual copepods before using the resulting DNA content (µg and %) and the RNA:DNA ratio as proxies for genome size and growth rate respectively. Additionally, I measured carbon, nitrogen, and phosphorus content in the copepods to calculate elemental ratios as they can also be proxies of growth rate as given by the growth rate hypothesis.

Results from the study did not consistently follow the postulations of the growth rate hypothesis. The cultured copepods had a significantly higher RNA:DNA ratio than corresponding stages from the field, though they did not consistently have lower C:P or N:P ratios. The study indicated that differences between the environments of the cultured copepods and the experimental copepods were sufficient to produce a significantly higher growth rate, larger body size, and higher relative carbon content (%) in the experimental copepods. The similarities in total DNA content (µg) between C5 copepodites from the three origins suggested they had a similar genome size if copepods have cell number consistency. However, no conclusions could be made regarding genome size as this would require data on cell-specific genome size and stage-specific cell numbers.

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1. INTRODUCTION

Ecological stoichiometry, the study of the balance between chemical substances in ecological processes, establishes a tight coupling between growth rate and the elemental content of an organism through the growth rate hypothesis (Sterner & Elser, 2002). The growth rate hypothesis states that differences in organismal stoichiometry are caused by differential allocations to ribonucleic acid (RNA) necessary to meet the protein synthesis demands for biomass growth and development (Sterner & Elser, 2002). Carbon (C), nitrogen (N), and phosphorus (P) are of particular importance as they are the main components of macromolecules (Sterner & Elser, 2002; Weider et al., 2004). Typically, close to 50 % of organismal dry weight is C, while N and P usually constitute 5 - 10 % and <1 %, respectively (Sterner & Elser, 2002). When considering the major components contributing to cellular mass, nucleic acids have the highest P content, at nearly 9%. Furthermore, nucleic acids are also amongst the most N-demanding biomolecule at approximately 39% N, but the main pool of cellular N is bound in proteins (Elser et al., 1996). The growth rate hypothesis proposes that rapidly growing organisms should have higher P content (i.e., low C:P and N:P ratios) due to the high P content in RNA (Elser et al., 2000; Elser et al., 2003). Ribosomal RNA constitutes as much as 85 % of the bulk RNA (Hessen et al., 2009). Studies on the crustacean zooplankton *Daphnia spp.* indicate selection for high growth rate under P-limitation to be a driver towards reduced genome size due to reallocation of P from deoxyribonucleic acid (DNA) to RNA (Hessen et al., 2008; Hessen et al., 2009).

Genome size and cell size covary, with one being a potential determinate for the other, though the causality is not clear (Bennett, 1987; Cavalier-Smith, 1978; Hessen et al., 2013; McLaren & Marcogliese, 1983). The correlation between the two indicates that differences in body size at least partly reflect changes in cell size (Gregory et al., 2000; Hessen et al., 2013). Body size and genome size show a positive correlation among invertebrate taxa, including copepods (Ferrari & Rai, 1989; Hessen & Persson, 2009). Observations of corresponding cell numbers between similar stages of copepods (McLaren & Marcogliese, 1983) further support the possibility that body size differences in part are due to variations in cell volume. Adult body size in zooplankton is usually assumed to be closely related to fitness (Stearns, 1992), with one example being its direct effects on fecundity (Runge, 1984). Genome size shows no correlation to organismal complexity (Gregory, 2005; Mirsky & Ris, 1951). Differences in genome size between phylogenetic lineages are due to significant variations in amounts of

non-coding DNA and not in the number of genes (Gregory, 2001; Gregory, 2005). The decoupling of organismal complexity and genome size, known as the "C-value paradox" (Thomas, 1971), in combination with the influence of genome size on phenotypic traits related to fitness (Ferrari & Rai, 1989), has led to speculations of whether genome size in itself is an adaptive trait (Bennett, 1987; Cavalier-Smith, 1978; Ferrari & Rai, 1989).

The Copepoda form the second-largest Crustacean taxa and are considered the most numerous multicellular organisms on earth (Humes, 1994; Mauchline, 1998). Copepods represent a diverse and abundant group (Mauchline, 1998) and show a large diversity in genome size (Gregory, 2000). Furthermore, copepods play a vital role in marine food webs by transferring the energy of primary producers to organisms at higher trophic levels (Bron et al. 2011; Mauchline, 1998). Calanoid copepods of the genus *Calanus* are often considered keystone species due to their abundance and high lipid content (Jonasdottir et al., 2015). They represent an energy-rich food source for pelagic fish stocks (Beaugrand & Kirby, 2010; Dommasnes et al., 2004), seabirds (Kwasniewski et al., 2012) and marine mammals (Banas et al., 2021). *Calanus spp*. show consecutive replacement along a thermal gradient (Choquet et al., 2017), with species showing larger body size, larger genome size, and longer life cycle with increasing latitude (Leinaas et al., 2016). *Calanus finmarchicus* (Gunnerus, 1770) has a main distribution in the North Atlantic, partly extending into arctic waters (Choquet et al., 2017). In response to rising seawater temperatures, modeling predicts a northwards shift in distribution (Reygondeau & Beaugrand, 2011). *C. finmarchicus* seasonally dominates the zooplankton biomass in the northern North Sea and the North Atlantic (Melle et al., 2014; Planque & Batten, 2000).

Like all copepods, *Calanus spp*. undergo anamorphic development with 12 distinct post-embryotic developmental stages, with the animal progressing through 6 naupliar stages (N1-N6) followed by 5 copepodite stages (C1-C5) before molting into adult males or females (Fig. 1) (Miller & Tande, 1993). Copepod life-history stages are generally classified into eggs, nauplii, copepodites, and mature males and females. *Calanus spp.* perform a seasonal vertical migration in which late juvenile stages descend to deep waters and lower their metabolic rates to save energy and avoid predators during food scarcity (Edvardsen et al., 2006). Mid-winter, they ascend, molt to adults and start spawning in time for the spring bloom. *C. finmarchicus* mainly has a 1-year life cycle, though the life span varies greatly with the environment, resulting in up to three generations per year in its southern range while

possibly having a multi-year life cycle in its most northern distribution (reviewed in Melle et al., 2014)

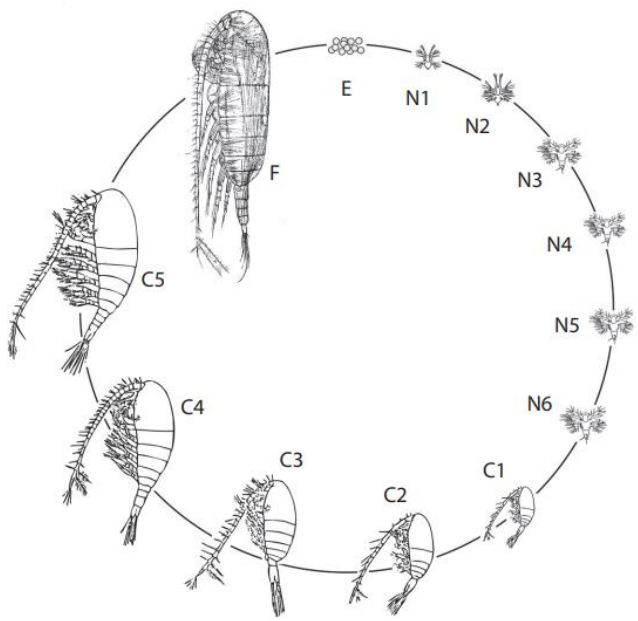

Figure 1. The life cycle of a calanoid copepod from egg, followed by 6 naupliar (N1-N6) and 5 copepodite stages (C1-C5) before molting into adult males or females. Figure from Baumgartner and Tarrant 2017.

Temperature may directly or indirectly affect multiple levels of biological organization, all the way from genome size through cell size and body size to community structure (Fig. 2) (Hessen et al., 2013). Biochemical reaction rates, metabolic rates, and other rates of biological activity, such as growth rate and developmental rate, increase exponentially with temperature (Brown et al., 2004). Studies report an inverse relationship between body size and temperature in a wide range of taxa, including copepods (Atkinson, 1994; Campbell et al., 2001). The processes of growth, defined as the increase in biomass and development, the transitions between life stages, are part of an organism's way to maturity. Both are affected by and may respond independently to environmental conditions (Forster et al., 2011; Horne et al., 2019; Kvile et al., 2020) The critical factors controlling stage development and growth rate in copepods are temperature, food quality, food quantity and predation (Campbell et al., 2001; Cook et al., 2007; Kvile et al., 2020). Typically, development under limited resources results in slow growth, with animals reaching maturity later and more petite than animals reared under optimal resource conditions (Campbell et al., 2001). There is a negative relationship between genome size and growth rate (Bennet, 1987; Gregory 2005; White and McLaren, 2000) and cell division rate (Gregory, 2005; Hessen et al., 2009). Simultaneously, the

relationship between RNA content and growth rate is positive (Bujellos et al., 2014a; Sterner & Elser, 2002). The growth rate is influenced by ambient temperature, food quantity and quality (Becker et al., 2005; Wagner et al., 1998; Wagner et al., 2001) and predation (Kvile et al., 2020). Furthermore, there may be significant interacting effects on copepod growth, such as between food quantity and temperature (Malzahn et al., 2016), food quantity and temperature (Campbell et al., 2001), or food quantity and predation (Kvile et al., 2020).

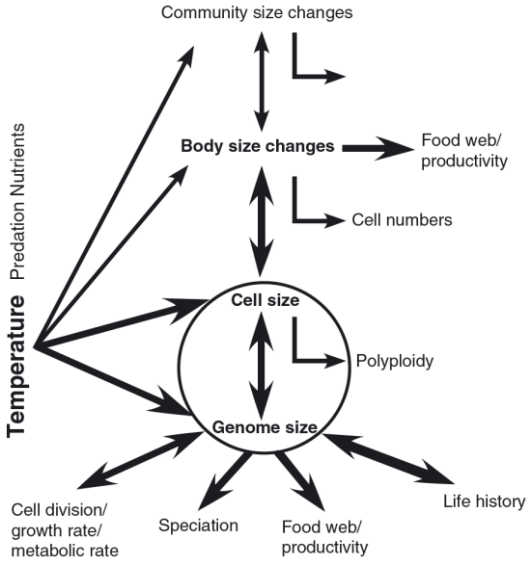

Figure 2. A conceptual flow-chart for evolutionary drivers and effects related to temperature-size responses. Bold lines represent the core drivers and responses. From Hessen et al., 2013.

Growth rate is often defined as the rate of biomass increase per unit of time (Hessen at al., 2009), though in this thesis, growth rate will be defined as the rate of protein synthesis using RNA:DNA ratio as a proxy. The RNA:DNA ratio can be used to assess ontogenetic, intraspecific, and relative interspecific variations in growth rate and has been shown for a range of organisms, including copepods (Bullejos et al., 2014b; Speekman et al., 2007; Wagner et al., 2001). E.g., the difference in RNA:DNA between cladocerans and copepods, 24.8 and 1.6 respectively, reflecting their different life-history strategies with cladocerans having a small genome and growing and maturing much faster than copepods (Hessen et al., 2008). Furthermore, the RNA:DNA ratio can be used as an indicator of nutritional status, as shown for *C. finmarchicus* (Wagner et al., 1998).

At the Norwegian university of science and technology (NTNU) SeaLab in Trondheim, Norway, *C. finmarchicus* has been continuously held in culture since 2004 (Hansen et al., 2007). As a result, this population has been cultured through more than 65 generations. The culture is fed *ad libitum* at 10 °C and has an average generation time of 11-12 weeks (Kvile et al., 2020). Continuously cultured copepods are subject to altered selection pressure compared to that experienced under natural conditions, often towards high reproductive rates and short generation times (Mauchline, 1998). With this culture comes an exciting opportunity to assess the effects of multiple years raised at higher temperatures and whether there are any observable changes in body size, genome size, growth rate or elemental stoichiometry. Comparing cultured *C. finmarchicus* to conspecifics from natural habitats is of interest due to the ecological importance of the studied species and its genus. Increased knowledge about basal physiology and plasticity in relation to changing temperatures and nutrient regimes will potentially shed light on the effects of rising seawater temperatures on *C. finmarchicus* and other *Calanus spp*.

2. AIM OF THE STUDY

The primary aim of my study was to investigate whether cultured *C. finmarchicus* at NTNU SeaLab showed different genome size and life history traits, such as growth rates and adult body size compared to conspecifics from natural conditions. The objective was to quantify nucleic acid content in individual copepods to calculate RNA:DNA ratio and relative DNA and RNA content (as % of dry weight). DNA content (µg and %) and RNA:DNA ratio was then to be used as proxies for genome size and growth rate, respectively. A secondary aim was to investigate C, N, and P stoichiometry since the relationship between these key elements also can be used as indicators of growth as given by the growth rate hypothesis (Elser et al., 2000; Elser et al., 2003; Sterner & Elser, 2002).

The cultured *C. finmarchicus* have spent more than 65 generations away from natural conditions and probably experience quite different selective pressures. Over the extended amount of time they have been in culture it is possible that they show quite different characteristics in the variables analyzed in this thesis compared to conspecifics from Norwegian coastal. Still, given the assumed long evolutionary time span required to change

genome size and configuration, I will assume a null hypothesis of there being no difference between them. That said, I predict the cultured copepods to have a smaller size than conspecifics from Norwegian coastal waters considering they develop at a constant temperature that is higher than bottom waters of the sampled fjord (Atkinson, 1994). I also predict the cultured animals to have a higher growth rate than the sampled conspecifics as they are fed continuously (Campbell et al., 2001; Cook et al., 2007). Furthermore, higher growth rates should also result in lower C:P and N:P ratios as given by the growth rate hypothesis (Elser et al., 2000; Elser et al., 2003; Sterner & Elser, 2002). Lastly, it was speculated that the cultured copepods might have evolved smaller genomes.

3. PRACTICAL CHALLENGES DUE TO THE PANDEMIC

Due to the pandemic, I had to shift my plans from using *C. finmarchicus* populations from Svalbard, Trondheimsfjorden, Oslofjorden, and the NTNU SeaLab culture to a more pandemic feasible solution with copepods from Oslofjorden and the culture. Furthermore, the sampling of the culture had to be down-scaled since live copepods were shipped to Oslo from Trondheim. The original plan was to collect copepods from one cohort of *C. finmarchicus* from the first nauplii stage to maturity. By sampling at regular intervals, interstage and intrastage variability could have been assessed for the measured variables. Instead, copepodite stages C4 to adults were sampled once each. While suboptimal, it still allowed me to examine my primary and secondary aim of whether the cultured copepods had modified their size, growth rate, C:N:P stoichiometry, or genome size compared to wild conspecifics.

4. MATERIALS AND METHODS

To achieve the aim of investigating genome size and growth rate between cultured and wild *C. finmarchicus* I quantified nucleic acid content using a high-range fluorometric assay. Total nucleic acid, DNA and RNA content (expressed as both µg per copepod and % of dry weight) and RNA:DNA ratio was calculated for individual copepods. The relative DNA content (%) in combination with total DNA content was used as proxies for genome size and the RNA:DNA ratio as a proxy for growth rate. The aim of investigating whether the copepods

also showed elemental ratios consistent with the growth rate hypothesis was evaluated by analyzing total elemental C, N, and P content, before calculating relative elemental contents (as % of dry weight) and elemental ratios. On two occasions, live copepods were shipped from the NTNU SeaLab to Oslo; the first shipment contained C4 and C5 copepodites, and the second shipment contained adults primarily. Field campaigns to collect wild *C. finmarchicus* were carried out in Oslofjorden between July and October 2020.

During sampling, the live copepods were held in a dark, temperature-controlled room. The stage was determined, and prosome length was measured before the copepods were preserved for further analysis. Nucleic acid quantification and P analysis were carried out on individual copepods from stage C4 to adults. For C:N analysis 3 C4 copepodites were grouped together, while C5 and adult copepods were analyzed individually. All analyses destroy the sample and sample weight is only acquired in C:N analysis, so a length-weight regression was used to calculate dry weight for the copepods used for nucleic acid quantification and P analysis. Weight was used to calculate relative content (% per DW) of the measured variables; total nucleic acid, DNA, RNA, P, C, and N. RNA:DNA, C:N, N:P and C:P ratios were calculated.

Throughout this thesis, I will refer to samples shipped from the culture as culture samples or of culture origin, while I refer to wild copepods as field samples or of field origin. However, I also include a third origin and refer to these as experimental samples or of experimental origin. This last group of copepods are also from the continuous *C. finmarchicus* culture at NTNU SeaLab but were collected during an experiment in 2019 conducted in collaboration with Kristina Kvile (Kvile et al., 2020).

4.1. Biological material

4.1.1. Cultured copepods from NTNU SeaLab

Both «culture» samples and «experimental» samples originate from the continuous *C. finmarchicus* culture at NTNU SeaLab in Trondheim, Norway (Hansen et al., 2007). The culture was established with *C. finmarchicus* collected by vertical net hauls in the Trondheimsfjord in 2004. Since then, the genetic identity of the culture has been confirmed using a molecular-based protocol (Choquet et al., 2017; Skottene et al., 2020). The culture is kept under a 16:8 h light:dark cycle at 10 °C in 250 L polystyrene tanks. The tanks are supplied with running natural seawater that is exchanged at a rate of 1x the tank volume per day. The seawater is filtered to 10 µm and is continuously collected from a depth of 70 m in

the adjacent fjord. The copepods are fed *ad libitum*, with a mixture of cultured *Dunaliella tertiolecta* and *Rhodomonas baltica.*

The samples referred to as "experimental" samples are those from the control group in the experiment described in detail in Kvile et al. (2020). Only details pertinent to the control group are described below. In the experiment, a 2 x 2 factorial design, with two levels of food (high, low) in combination with the presence or absence of a predator cue, was used to examine the effects of predation risk and food on copepod life-history strategies. Each of the 4 treatments had 3 replicates resulting in 12 tanks that were sampled in random order but on specific days throughout the 24 day experiment. Before starting the experiment 300 C4 copepodites were added to each experimental tank by aliquoting batches of 50 individuals from the culture to the experimental tanks until all tanks had a total of 300 animals. The 12 tanks were 45-L white HD polyethylene and held under the same conditions as the culture, though with an exchange rate of 1.5 x the tank volume per day. *R. baltica* was used as food for the zooplankton in the experiment, though still added continuously. The control received no predator cue and the high food treatment, i.e., *R. baltica* at 200 µg C/L.

Collection of cultured copepods was made possible by shipping live copepods from Trondheim to Oslo by plane and direct transport to and from the airport. Under transportation, the copepods were kept in 250ml flasks with seawater placed in coolers with cooling elements. Shipment occurred on two occasions, the first on 01.07.2020 with C4 and C5 copepodites. The second shipment came on 29.07.2020 and contained adults. At the University of Oslo, the flasks were placed in a temperature-controlled room at 5 °C over a ~24 h period for sampling. Flasks were emptied into large beakers to make collection easier. Subsets of copepods were taken with a ladle and carefully poured over a 100um sieve to condense the zooplankton before being placed on ice in preparation for handling at room temperature. Copepods were picked out with a wide bode pipette, placed in a shallow plastic well in a drop of seawater, and anesthetized by adding a drop of tricaine methanesulfonate solution (MS222) (Finquel, 1.5 g/L seawater, Argent Laboratories, Redmond, Washington, USA). The edge of the pipette was cut to create an optimal tip size to avoid any harm to the animals during handling. Only live individuals were selected for further inspection. Under a Leica MZ8 stereomicroscope (Leica Microsystems, Wetzlar, Germany) developmental stage was determined, and prosome length was measured using a measuring ocular. Prosome length, representing body size, was measured from the tip of the cephalosome to the distal

lateral end of the thoracic segment. The scale on the measuring ocular was calibrated by using a stage micrometer at respective magnifications. Copepodite stages and sex of the adults were determined based on prosome segmentation and the number and shape of the urosome segments (Mauchline, 1998, s.25). Individuals were then picked up by their antennules using fine-pointed forceps to avoid any damage to the body before being preserved for further analyses. Copepods were placed in pre-weighed tin boats and dried for CN analysis, plastic containers for P analysis, or Eppendorf tubes with RNalater for RNA:DNA analysis.

Collection of copepods during the experiment in Trondheim was performed as described above for the culture samples, though length measurements were obtained from image analysis. The copepods were photographed laterally to determine size and lipid content, using a CCD camera (Nikon DS-Fil/U2, Tokyo, Japan) mounted on a Leica MZAPO stereomicroscope (Leica Microsystems, Wetzlar, Germany). A drawing tablet (Wacom Cintiq 12wx; Wacom, Saitama, Japan) was used to manually outline the prosome area in each image, and this two-dimensional projected area was quantified in ImageJ. The pixel-to-mm ratio was calibrated by imaging a stage micrometer at respective magnifications. All work in ImageJ was performed by Kristina Kvile.

4.1.2. Copepods from Oslofjorden

A dominant feature of Oslofjorden is the narrow and shallow sill at Drøbak, dividing the fjord into inner and outer Oslofjorden (Staalstrøm et al., 2020). The sill creates a separation of water masses, preventing fresh oxygenated water from entering the inner Oslofjorden, resulting in slow deep-water renewal. The area inside the sill has depths varying from 15-160 m (Fig. 3). At the innermost part of Oslofjorden we find Bunnefjorden, which is separated from the rest of the fjord by two sills. The sills are of 50 and 55 m resulting in bottom-water often becoming hypoxic (Kaartvedt et al., 2021). The sea surface temperature of the fjord varies considerably trough out the year, from around 0 °C during winter months up to above 20 °C during summer (Lundsør et al., 2020). The deep-water temperatures on the other hand, remain quite stable though out the year at around 7-8 °C (Bagøien et al., 2000). In Oslofjorden, *C. finmarchicus* is less abundant than its more southern relative *C. helgolandicus* (Claus, 1863) (Bagøien et al., 2000; Kaartvedt et al., 2021; Leinaas et al., 2016).

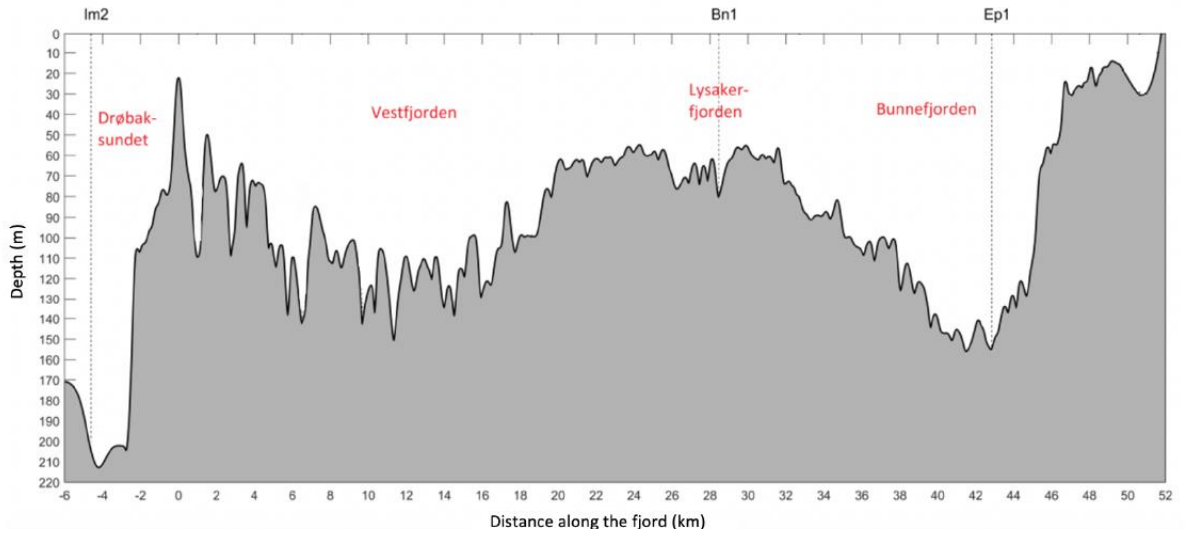

Figure 3. Depths and sills in inner Oslofjorden. A modified version of the figure from Staalstrøm et al. 2020.

Sampling campaigns to collect copepodites and adults of *C. finmarchicus* were carried out using the University of Oslo vessel Trygve Braarud. Vertical tows for zooplankton were collected using a WP2 net with 0.5 m diameter and 200 µm mesh size at three research stations in the Oslofjord the fall of 2020 (Table 1). All tows were from 10 m above the seafloor to 10 m below the sea surface. The collected zooplankton were transferred to 20 L coolers filled with seawater from the sampling station and transported to the laboratory within 2-4 h. At the laboratory, the coolers were placed in a dark temperature-controlled room (5 °C) and aerated gently (roughly one air bubble per second). Work in regard to sampling, length measurements, and preservation for further analysis was performed as described for the culture samples.

C5 copepodites were collected from all locations. C4 were collected from Drøbak and females from Bunnefjorden due to very few of these stages at the other sampling stations. No adult males were collected.

Table 1. Sampling dates and locations of field stations. Station codes correspond to codes in figure 3.

Station	**Date**	**Station code**	**Position (Latitude, Longitude)**
Drøbak	03.07.2020	Lm2	59.6220 , 10.6282
Bunnefjorden	24.08.2020	Ep1	59.7863 , 10.7238
Lysakerfjorden	21.10.2020	Bn1	59.8805 , 10.6467

Calanus copepods were attempted identified to species by checking the coxapodite curvature of the fifth pair of swimming legs under a dissecting microscope (Fleminger & Hulsemann, 1977). However, species identification based on morphological characteristics is unreliable (Choquet et al., 2018), so in this thesis, copepods from Oslofjorden will be considered as *Calanus sp.*

4.2. Analysis

2.2.1. Length-weight regression

Prosome length measurements combined with dry weight measurements were used to perform a regression analysis to establish the relationship between length and weight. The regression analysis was applied to calculate weight for samples from analysis where data on weight was not attained (nucleic acid quantification and P analysis). Multiple regressions were performed, all resulting in low R^2. For this reason, the regression equation presented in Kvile et al. (2020) was used, having an R^2 of 0.77.

$$log_e(W) = -4.57 + 3.51 \times log_e(L)$$

Where W is body weight (mg) and L is prosome length (mm).

This equation was later applied to convert prosome length to body mass of all individuals sampled for nucleic acid quantification and P analysis, in the interest of normalizing the total phosphate, RNA, and DNA content (µg) to dry weight, giving the relative content (%).

4.2.2. Nucleic acid quantification

Before the assay, length was measured and stage determined. The copepods were transferred to 0.5ml Eppendorf tubes with 0.3ml RNAlater (Thermo Fisher Scientific) for extraction and quantification of nucleic acids content. The Eppendorf tubes were kept at 4 °C for 24 h before storage at -18 °C until the day of analysis. Analysis was performed following the protocol by Bullejs et al. (2014a), which is a modification of the method given in Gorokhova and Kyle (2002) but for specific use on copepods. In short, nucleic acid content (µg) per sample was quantified using a microplate fluorometric high-range RiboGreen assay (Quant-iT Ribogreen RNA Assay Kit; Thermo Fisher Scientific) after nucleic acid extraction in 1% sarcosyl (prepared with N-Lauroylsarcosine and Tis-EDTA buffer; Merck Life Science, Darmstadt, Germany) and RNase digestion (RNase DNasefree, working solution 5µg/mL, Merck Life

Science). Fluorescence measurements were obtained using a BioTek Synergy Mx Microplate Reader. RNA and DNA content (µg) per sample was found by calibrating measured fluorescence against standard curves prepared with commercial standards of RNA and DNA (16S and 23S RNA from Escherichia coli, RiboGreen RNA Assay Kit, Thermo Fisher Scientific; DNA from calf thymus, Merck Life Science). Each sample was pseudo-replicated. RNA and DNA content were added to get the total nucleic acid (NA) content per sample.

Relative RNA, DNA, and NA content (%RNA, %DNA, and %NA) were calculated from the total RNA, DNA, and NA content (µg), respectively, divided by corresponding sample weight (calculated with the length-weight regression) before being multiplied by 100.

The protocol for quantifying nucleic acids allows for the preparation of samples the day before analysis. A methodological test was performed on 8 samples to see if the samples analyzed the day after preparation showed similar total nucleic acids content (µg) as those analyzed the same day.

4.2.3. Phosphate analysis

Total P content (µg) in samples was quantified using the Multitest MT19 (Method-No G-297-03, Seal Analytical), which is a modified molybdate blue method (Murphy & Riley, 1962) measuring orthophosphate in water using a SEAL AA3 HR AutoAnalyser (Seal Analytical, Norderstedt, Germany). Analysis was performed by Berit Kaasa at the University of Oslo. This analysis was performed on samples gathered specifically for this thesis, but not on samples gathered during the experiment. Relative P content (%) was calculated from the total P content (µg) divided by corresponding sample dry weight (calculated with the length-weight regression) before being multiplied by 100.

4.2.4. Carbon, nitrogen, and dry weight analysis

After length measurements as described above, copepods were transferred to pre-weighed tin capsules placed in a 96 well plate and dried at 60 °C for 24 h. Directly following the drying, samples were weighed again before being stored in sealed boxes until analysis. The difference between the first and second weighing of the tin capsules was used directly as individual dry weight measurements. Dry weight was determined by subtracting the weight of the empty tin capsules from the weight of the tin capsules containing the dried sample. Total C and N content (mg) per sample was measured using a Thermo Finnigan EA 1112 Series Flash

Elemental Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Analysis was performed by Berit Kaasa at the University of Oslo. For C4 copepodites, three individuals had to be grouped and analyzed together per replicate to reach the sensitivity threshold of the elemental analyzer. For C5 and adults, one individual per replicate was enough.

Relative C and N content (%) were calculated from the total elemental content (mg), divided by corresponding sample dry weight before being multiplied by 100. C:N ratio was calculated by dividing total C content (mg) by the corresponding total N content (mg) of each sample.

4.3. Statistical analysis

Statistical analyses and graphical representation of the data were performed in R statistics (R Core Team, 2017). All data were initially investigated through exploratory and descriptive statistical analyses. 4 samples were considered extreme outliers, all of which weighted >0.8 mg, and were removed.

A linear model was made for each variable with the grouping factors of stage and origin and their interaction. From the linear model, averages and standard deviations were predicted for all stages and origins. The predicted average with two standard deviations in each direction (representing 95% confidence intervals) was plotted for each stage of each origin to visualize the results. N:P and C:P ratios were calculated by dividing %N and %C of individual samples by the corresponding group average from the %P model. 1 sample was removed during modeling due to showing high residuals and high leverage when assessing the diagnostic plots. All copepods from the fjord were analyzed together when making models. Furthermore, no males and hardly any C4 were collected from Oslofjorden, resulting in model predictions of little relevance with large standard deviations. A one-way ANOVA was used on each linear model to assess if origin, stage, or their interaction were significant factors in explaining variance in the response variable. Significance was set at p-value < 0.05 for all tests.

Table 2. The number of samples per group (by origin and stage) used to produce each linear model.

Origin	**Field**				**Culture**				**Experiment**			
Stages	**C4**	**C5**	**C6F**	**C6M**	**C4**	**C5**	**C6F**	**C6M**	**C4**	**C5**	**C6F**	**C6M**
Length	12	159	50	0	79	148	94	96	68	198	132	61
Weight	6	39	18	0	18	45	28	26	19	58	43	25
DNA (%)	4	56	17	0	32	56	31	34	22	49	27	19
DNA (µg)	4	56	17	0	32	56	31	34	22	49	27	19
RNA (%)	4	56	17	0	32	56	31	34	22	49	27	19
RNA(µg)	4	56	17	0	32	56	31	34	22	49	27	19
NA (%)	4	56	17	0	32	56	31	34	22	49	27	19
NA (µg)	4	56	17	0	32	56	31	34	22	49	27	19
RNA:DNA	4	56	17	0	32	56	31	34	22	49	27	19
P (%)	1	12	6	0	6	6	6	6	0	0	0	0
P (µg)	1	12	6	0	6	6	6	6	0	0	0	0
N (%)	6	39	18	0	18	45	28	26	19	58	43	25
N (mg)	6	39	18	0	18	45	28	26	19	58	43	25
C (%)	6	39	18	0	18	45	28	26	19	58	43	25
C (mg)	6	39	18	0	18	45	28	26	19	58	43	25
C:N	6	39	18	0	18	45	28	26	19	58	43	25
N:P	6	39	18	0	18	45	28	26	19	58	43	25
C:P	6	39	18	0	18	45	28	26	19	58	43	25

5. RESULTS

Each linear model listed in Table 2 predicted averages and standard deviations for each stage within each origin. From the fjord, no males were collected and only a few C4 copepodites, resulting in unreliable averages with large standard deviations for these two groups. For this reason, they will not be referred to when making comparisons between origin or stages. Table 3 presents the model summaries for each model, all of which received significant p-values. The range of R^2 between models reflects how they, to a varying degree, explain the observed variation in the response variable. Results from the ANOVA for each model are presented in Table 4. Plots of model predictions for all groups are presented in four sections. First, length and weight (Fig. 4a,b), then nucleic acids (Fig. 5a-g), followed by individual elements (Fig. 6a-f), and finally the elemental ratios (Fig. 7a-c).

Table 3. Model summary of linear models. Lm is the linear model, Res.SE is the residual standard error, Df is the degrees of freedom.

Lm	Res. SE	Multiple R^2	Adjusted R^2	F-statistic	Df	p-value
Length	0.1309	0.8034	0.8016	443.8	10 and 1086	< 2.2e-16
Weight	0.08998	0.368	0.3479	18.29	10 and 314	< 2.2e-16
DNA (%)	0.4981	0.448	0.4315	27.26	10 and 336	< 2.2e-16
DNA (µg)	0.6278	0.371	0.3523	19.82	10 and 336	< 2.2e-16
RNA (%)	0.8044	0.7429	0.7353	97.11	10 and 336	< 2.2e-16
RNA (µg)	1.106	0.8631	0.8591	211.9	10 and 336	< 2.2e-16
NA (%)	1.094	0.6661	0.6562	67.04	10 and 336	< 2.2e-16
NA (µg)	1.406	0.8097	0.804	142.9	10 and 336	< 2.2e-16
RNA:DNA	0.635	0.817	0.8116	150	10 and 336	< 2.2e-16
P (%)	0.08973	0.412	0.314	4.204	6 and 36	0.002643
P (µg)	0.3018	0.6813	0.6281	12.82	6 and 36	1.068e-07
N (%)	3.057	0.2157	0.1907	8.634	10 and 314	1.749e-12
N (mg)	0.003352	0.6753	0.665	65.32	10 and 314	< 2.2e-16
C (%)	9.011	0.4814	0.4649	29.15	10 and 314	< 2.2e-16
C (mg)	0.02961	0.6044	0.5918	47.98	10 and 314	< 2.2e-16
C:N	1.271	0.6749	0.6645	65.17	10 and 314	< 2.2e-16
N:P	7.34	0.2898	0.2652	11.77	6 and 173	4.78e-11
C:P	20.12	0.2138	0.1866	7.843	6 and 173	1.762e-07

From Table 4 it is clear that stage, origin, and the interaction between them are significant factors in nearly all models. Origin was not significant in either P (%) or P (µg), and stage was not found to be significant for the C:P ratio.

Table 4. Results from ANOVA on linear models, assessing stage, origin, and their interaction. The table shows linear model (Lm), terms, degrees of freedom (Df), sum of squared (Sum Sq), mean sum of squares (Mean Sq), F value, and p-values. The p-values that are not significant are colored red.

Lm	Terms	Df	Sum Sq	Mean Sq	F value	p-value
Length	Stage	3	67.239007	22.4130023	1308.06059	0.0000000
	Origin	2	1.710180	0.8550899	49.90449	0.0000000
	Stage:Origin	5	7.086446	1.4172891	82.71538	0.0000000
	Residuals	1086	18.608099	0.0171345	NA	NA
Weight	Stage	3	0.8234185	0.2744728	33.897275	0.0000000
	Origin	2	0.2590837	0.1295418	15.998360	2e-07
	Stage:Origin	5	0.3980742	0.0796148	9.832398	0.0000000

	Residuals	314	2.5425193	0.0080972	NA	NA
DNA (%)	Stage	3	56.853837	18.9512791	76.386012	0.0000000
	Origin	2	4.803598	2.4017991	9.680817	0.0000817
	Stage:Origin	5	5.986562	1.1973124	4.825950	0.0002823
	Residuals	336	83.361202	0.2480988	NA	NA
DNA (µg)	Stage	3	56.819461	18.939820	48.055846	0.0000000
	Origin	2	3.281987	1.640993	4.163678	0.0163599
	Stage:Origin	5	18.005988	3.601198	9.137288	0.0000000
	Residuals	336	132.424671	0.394121	NA	NA
RNA (%)	Stage	3	203.2778	67.7592742	104.71765	0.0000000
	Origin	2	386.6260	193.3129839	298.75293	0.0000000
	Stage:Origin	5	38.4303	7.6860597	11.87832	0.0000000
	Residuals	336	217.4143	0.6470664	NA	NA
RNA (µg)	Stage	3	805.6507	268.550248	219.66678	0.0000000
	Origin	2	1187.3696	593.684801	485.61798	0.0000000
	Stage:Origin	5	597.7762	119.555239	97.79293	0.0000000
	Residuals	336	410.7716	1.222535	NA	NA
NA (%)	Stage	3	357.30006	119.100020	99.45841	0.0000000
	Origin	2	411.77320	205.886598	171.93242	0.0000000
	Stage:Origin	5	33.68162	6.736323	5.62539	5.38e-05
	Residuals	336	402.35517	1.197486	NA	NA
NA (µg)	STAGE	3	932.4234	310.807799	157.12312	0.0000000
	Origin	2	1308.0283	654.014164	330.62474	0.0000000
	Stage:Origin	5	587.0694	117.413887	59.35641	0.0000000
	Residuals	336	664.6471	1.978116	NA	NA
RNA:DNA	Stage	3	158.3348	52.7782639	130.87140	0.0000000
	Origin	2	312.8251	156.4125638	387.84776	0.0000000
	Stage:Origin	5	133.8798	26.7759582	66.39489	0.0000000
	Residuals	336	135.5032	0.4032834	NA	NA
%P	Stage	3	0.0888466	0.0296155	3.678125	0.0207760
	Origin	1	0.0231895	0.0231895	2.880033	0.0983151
	Stage:Origin	2	0.0910652	0.0455326	5.654955	0.0073166
	Residuals	36	0.2898649	0.0080518	NA	NA
P (µg)	Stage	3	3.586032	1.1953440	13.1225042	0.0000061
	Origin	1	0.023291	0.0232910	0.2556888	0.6161799
	Stage:Origin	2	3.399631	1.6998157	18.6606024	0.0000027
	Residuals	36	3.279281	0.0910911	NA	NA
N (%)	Stage	3	0.8234185	0.2744728	33.897275	0.0000000
	Origin	2	0.2590837	0.1295418	15.998360	2e-07

	Stage:Origin	5	0.3980742	0.0796148	9.832398	0.0000000
	Residuals	314	2.5425193	0.0080972	NA	NA
N (mg)	Stage	3	0.8234185	0.2744728	33.897275	0.0000000
	Origin	2	0.2590837	0.1295418	15.998360	2e-07
	Stage:Origin	5	0.3980742	0.0796148	9.832398	0.0000000
	Residuals	314	2.5425193	0.0080972	NA	NA
C (%)	Stage	3	1291.114	430.37120	5.300075	0.0014119
	Origin	2	15887.854	7943.92714	97.830461	0.0000000
	Stage:Origin	5	6487.687	1297.53744	15.979337	0.0000000
	Residuals	314	25497.101	81.20096	NA	NA
C (mg)	Stage	3	0.1501873	0.0500624	57.11914	0.0000000
	Origin	2	0.0954139	0.0477069	54.43163	0.0000000
	Stage:Origin	5	0.1748996	0.0349799	39.91063	0.0000000
	Residuals	314	0.2752072	0.0008765	NA	NA
C:N	Stage	3	41.21406	13.738020	8.497677	1.92e-05
	Origin	2	710.45674	355.228369	219.727162	0.0000000
	Stage:Origin	5	301.99407	60.398814	37.359798	0.0000000
	Residuals	314	507.63732	1.616679	NA	NA
N:P	Stage	3	1533.1635	511.05451	9.486284	7.80e-06
	Origin	1	626.1024	626.10238	11.621823	8.11e-04
	Stage:Origin	2	1644.3220	822.16100	15.261097	8.00e-07
	Residuals	173	9320.0279	53.87299	NA	NA
C:P	Stage	3	1837.616	612.5388	1.513054	0.2128685
	Origin	1	11823.214	11823.2139	29.204947	0.0000002
	Stage:Origin	2	5390.242	2695.1212	6.657316	0.0016392
	Residuals	173	70036.628	404.8360	NA	NA

5.1. Length and weight

Stage explained most of the variance in both length and weight (Table 4). The linear model for length received R^2 of 0.8034, while the linear model for weight received an R^2 of 0.368.

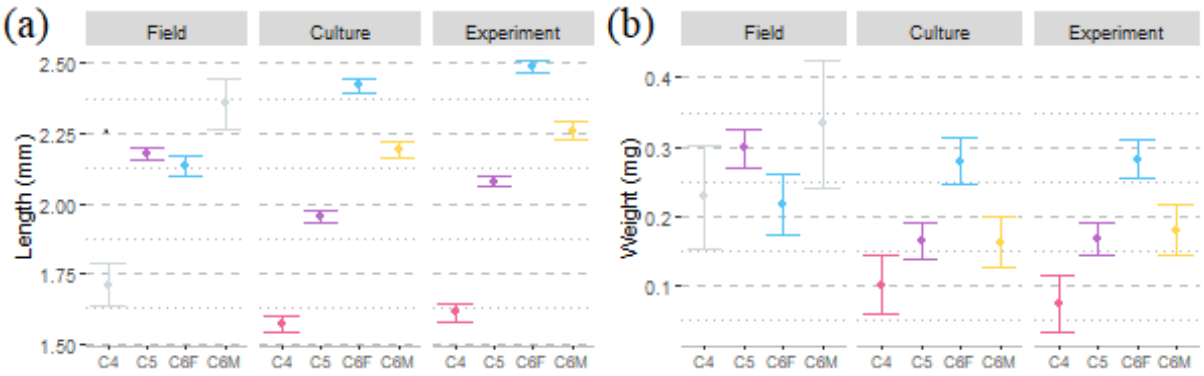

Figure 4. Model predicted average (dot) and 95% confidence interval for (a) prosome length (mm) and (b) dry weight (mg) per developmental stage and origin. Model predictions for stages C4 and males of origin field are unreliable due to lack of samples.

Copepods from culture and experiment show a substantial increase in body size with increasing stage (Fig. 4a), with females reaching a significantly larger size than males. All stages in culture were significantly smaller than corresponding stages from the experiment, though they show very similar body weights (Fig. 4b). Length is more distinctly different between stages than weight is. Females from the field weighed significantly less than C5 from the field, though they did not show significantly different body lengths (Fig. 4b). Copepodites of stage C5 from the field were significantly longer and heavier than C5 from the other origins (Fig. 4a,b). On the other hand, females from the field were significantly smaller than females from the culture and experiment, though their weight was not significantly different (Fig. 4a,b).

5.2. Nucleic acid quantification

The protocol for quantifying nucleic acids allows for the preparation of samples the day before analysis. The 9 samples used for the methodological test were C4 and C5 copepodites from the culture. Wilcoxon rank sum test confirmed there to be a significant difference between the samples analyzed one day apart (p-value of 0.0315). The ANOVA (Table 4) revealed the developmental stage to account for most of the variation observed in DNA content (Fig. 5a,b). In the remaining models (Fig. 5c-g) origin explained most of the variance.

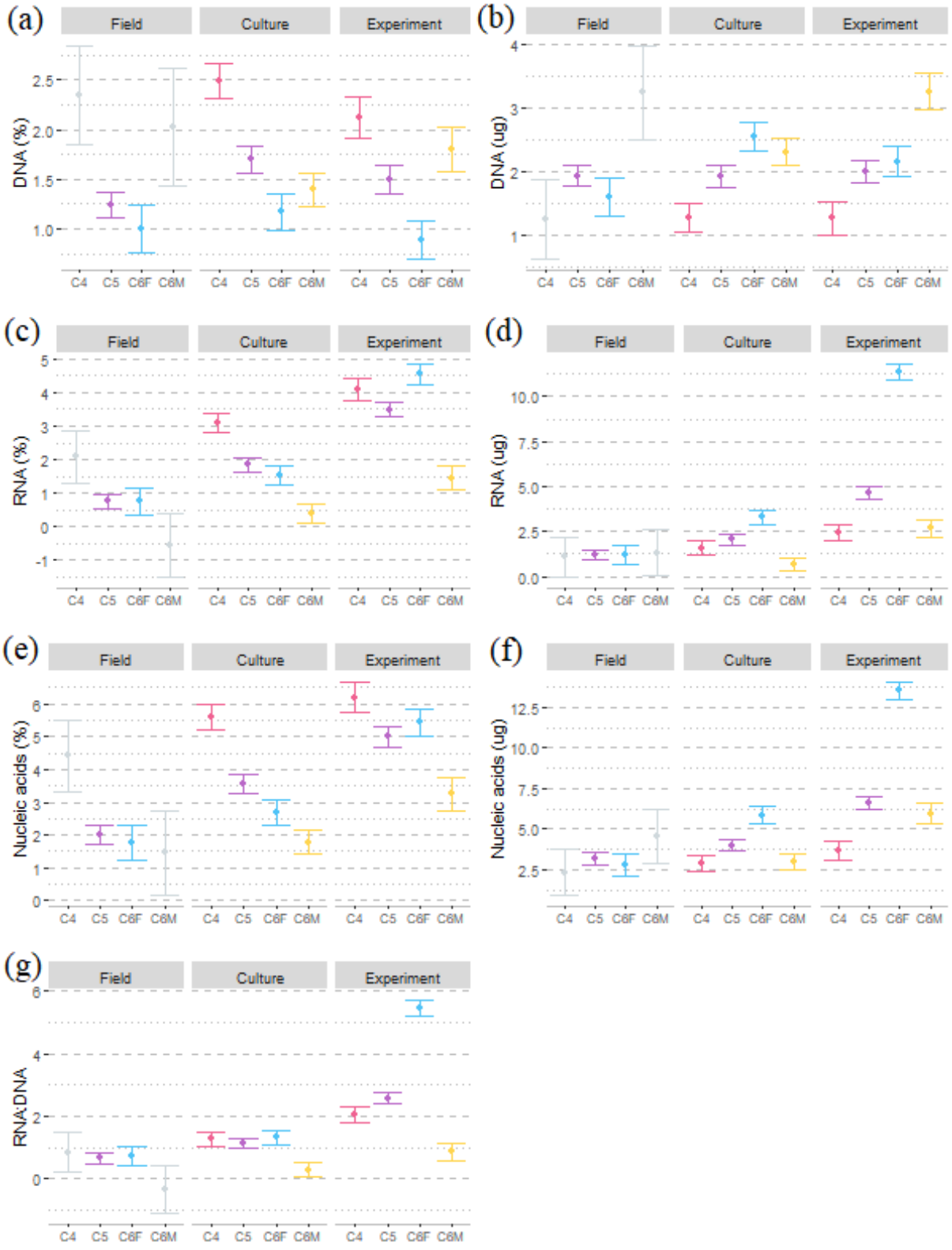

Figure 5. Model predicted average (dot) and 95% confidence interval for (a) relative DNA content (%), (b) total DNA content (µg), (c) relative RNA content (%), (d) total RNA content (µg), (e) relative nucleic acid content (%), (f) total nucleic acid content (µg), and (g) RNA:DNA ratio for each developmental stage and origin. Model predictions for stages C4 and males of origin field are unreliable due to lack of samples.

5.2.1. DNA content

Relative DNA content (%) was similar between females from different origins (Fig. 5a). C5 from the culture had a significantly higher relative DNA content (%) than C5 from the field but not significantly higher than C5 from the experiment, while C5 from the field and the experiment also had overlapping confidence intervals (Fig. 5a). There was a significant decrease in relative DNA content (%) with increasing stage in copepods of cultured and experimental origin (Fig. 5a). Field samples and males deviated from the trend, with males showing varied and significantly different relative DNA content (%) between origins and field stages showing overlapping model predictions (Fig. 5a).

Copepodite stage C5 showed similar total DNA content (µg) in the different origins (Fig. 5b). Females from the field show significantly lower total DNA content (µg) than females from the other origins (Fig. 5b). Total DNA content (µg) increased significantly with increasing stage in copepods from culture and experiment, though corresponding stages from the two origins were not significantly different (Fig. 5b). Again, males deviated and were significantly different between origins and showed varied total DNA content (µg) compared to the other stages (Fig. 5b). Males from the culture showed similar values as the females, while males from the experiment had a greater total DNA content (µg) than the females (Fig. 5b)

5.2.2. RNA content

All stages of experimental origin had significantly higher relative RNA contents (%) than corresponding stages from different origins (Fig. 5c). C5 and adult females from Oslofjorden had significantly lower relative RNA content (%) than corresponding stages from other origins (Fig. 5c). The relative RNA content (%) decreased significantly with increasing stage in copepods from the culture and the experiment, with only adult females deviating from this trend (Fig. 5c). In the experimental samples, adult females showed higher relative RNA content (%) than all other sampled stages, though not significantly higher than C4 from the experiment (Fig. 5c). The C5 copepodites and females from the field have similar relative RNA content (%), though the females had a higher standard deviation (Fig. 5c).

Copepods from the experiment showed higher total RNA content (µg) than copepods of the corresponding stage from the other origins (Fig. 5d). C5 and females from the field showed significantly lower total RNA content (µg) than copepods of the corresponding stage in the other origins (Fig. 5d).

5.2.3. Total nucleic acid content

Relative nucleic acid content (%) (Fig. 5e) and of total nucleic acid content (µg) (Fig. 5f) show similar patterns as those described for relative RNA content (%) (Fig. 5c) and total RNA content (µg) (Fig. 5d). The relative nucleic acid content (%) of C5 and females from Oslofjorden were significantly lower than for copepods corresponding stage in the other origins (Fig. 5e). Copepods from the culture and experiment showed significant interstage variability in relative nucleic acid content (%), while copepods from the field showed largely overlapping confidence intervals (Fig. 5e). Total nucleic acid content (µg) increased with increasing developmental stage (Fig. 5f), while relative nucleic acid content (%) decreased with increasing developmental stage in copepods from the culture and the experiment (Fig. 5e).

5.2.4. RNA:DNA ratio

Stages from the culture had significantly higher RNA:DNA ratios than corresponding stages from the field while at the same time being significantly lower than those of experimental origin (Fig. 5g). C5 and females from the field had very similar RNA:DNA ratios (Fig. 5g). This was also the case for C4, C5, and females from the culture (Fig. 5e). Males from culture and experiment showed significantly lower RNA:DNA ratios than all other stages from the same origin (Fig. 5e).

5.3. Elemental quantification

Interstage patterns within each origin are similar for total C, N and P content (Fig. 6b,d,f), the same pattern is seen for length and weight (Fig. 4a,b) implying that all three elements are closely linked to the copepods body size. Assessment of relative elemental content (%) (Fig. 6a,c,e) revealed some significant differences between origins, the most obvious being that relative C content (%) (Fig. 6e) was generally more variable than relative N and P content (%) (Fig. 6c and Fig. 6a, respectively).

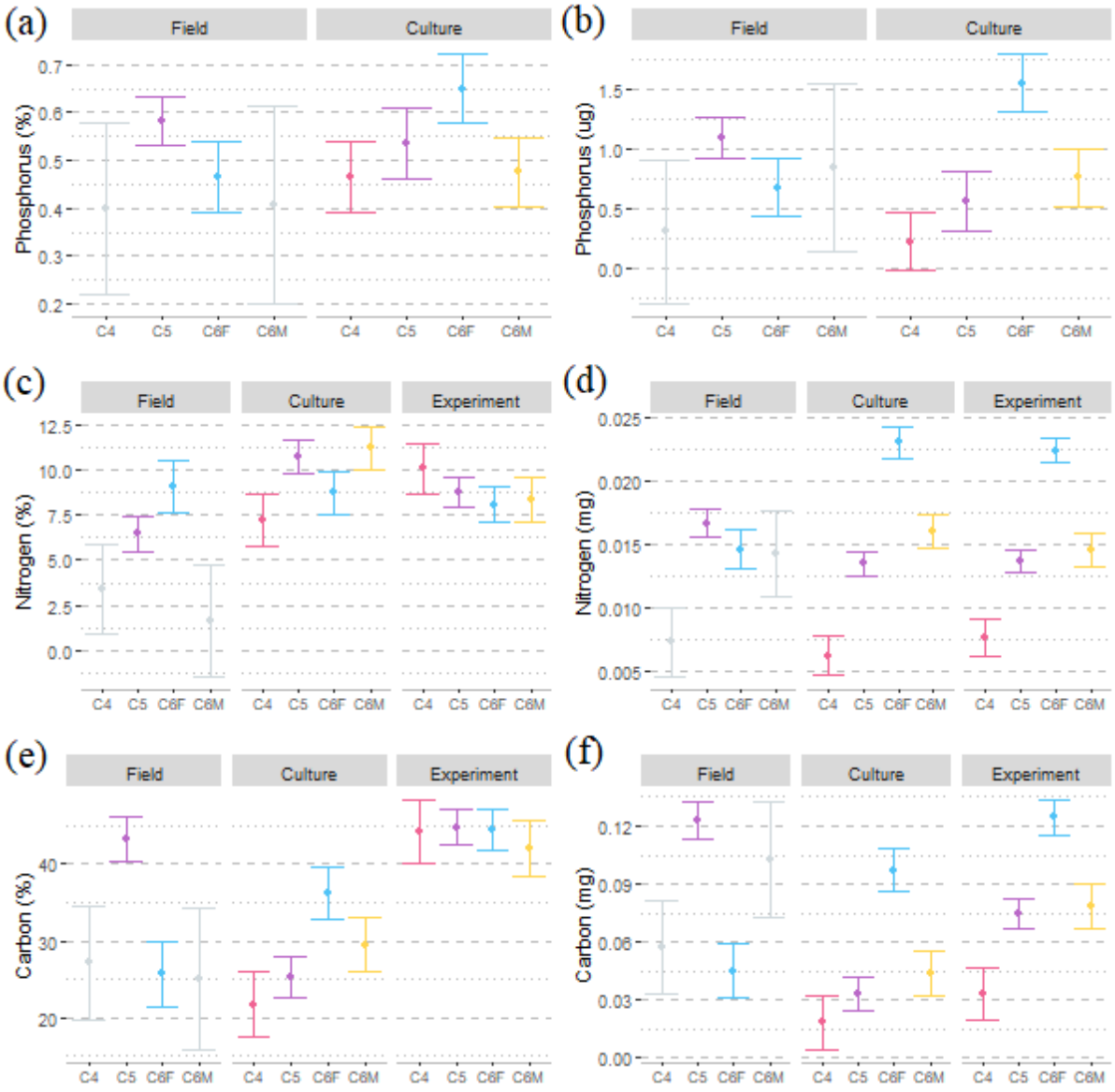

Figure 6. Model predicted average (dot) and 95% confidence interval for (a) relative phosphate content (%), (b) total phosphate content (µg), (c) relative nitrogen content (%), (d) total nitrogen content (mg), (e) relative carbon content (%) and (f) total carbon content (mg) for each developmental stage and origin. Model predictions for stages C4 and males from the origin field are unreliable due to lack of samples.

5.3.1. Phosphate content

ANOVA on the linear model (Table 4) found stage to be a significant factor regarding relative P content (%), while origin did not have a significant effect. When assessed their interacting effects, more of the variance in relative P content (%) was explained and is showed by the highest F-value. Females from culture had higher relative P content (%) than their field counterparts, while C5, on the other hand, had similar relative P content (%) between origins (Fig. 6a). Cultured females had significantly higher relative P content (%) than cultured males (Fig. 6a). Other than the above mentioned examples, no other groups were found to be

significantly different in relative P content (%) (Fig. 6a). Copepodite stage C5 from the field had significantly higher total P content (µg) than females from the field and C5 from the culture (Fig. 6b). Females from the culture had significantly higher total P content (µg) than all other stages in both origins (Fig. 6b)

5.3.2. Nitrogen content

All three origins show different interstage patterns for relative N content (%) (Fig. 6c). Females from all origins showed similar relative N content (%) (Fig. 6c). At the same time, all C5 were significantly different from each other, with C5 from culture having the highest relative N content (%) and C5 from the field having the lowest (Fig. 6c). C5 and males from the culture had significantly higher relative N content (%) than corresponding stages from the experiment (Fig. 6c). Total N content (mg) was similar between corresponding stages from the culture and experiment and increased with increasing developmental stage (Fig. 6d).

5.3.3. Carbon content

C5 from culture had significantly lower relative C content (%) than C5 from the other origins (Fig. 6e). Females from the three origins showed significant differences in relative C content (%), with copepods from the experiment having the highest relative C content (%), culture intermediate, and field lowest (Fig. 6e). The experimental samples had similar relative C content (%) between stages, all being higher than corresponding stages from the culture (Fig. 6e). In the field, C5 copepods had significantly higher relative C content (%) than females (Fig. 6e). Total C content (mg) showed similar patterns between corresponding stages from the different origins as for relative C content (%) (Fig. 6e,f).

5.3.4. Elemental ratios

ANOVA on the C:P model (Table 4) revealed origin and the interactions between stage and origin as significant factors in explaining the variance in the C:P ratio. However, stage alone was not a significant factor. For both C:N and N:P stage, origin and their interaction were significant factors in explaining the variance in elemental ratio. Origin explained most of the variance in C:N ratio, while the interaction between stage and origin explained most of the variance observed in the N:P ratio.

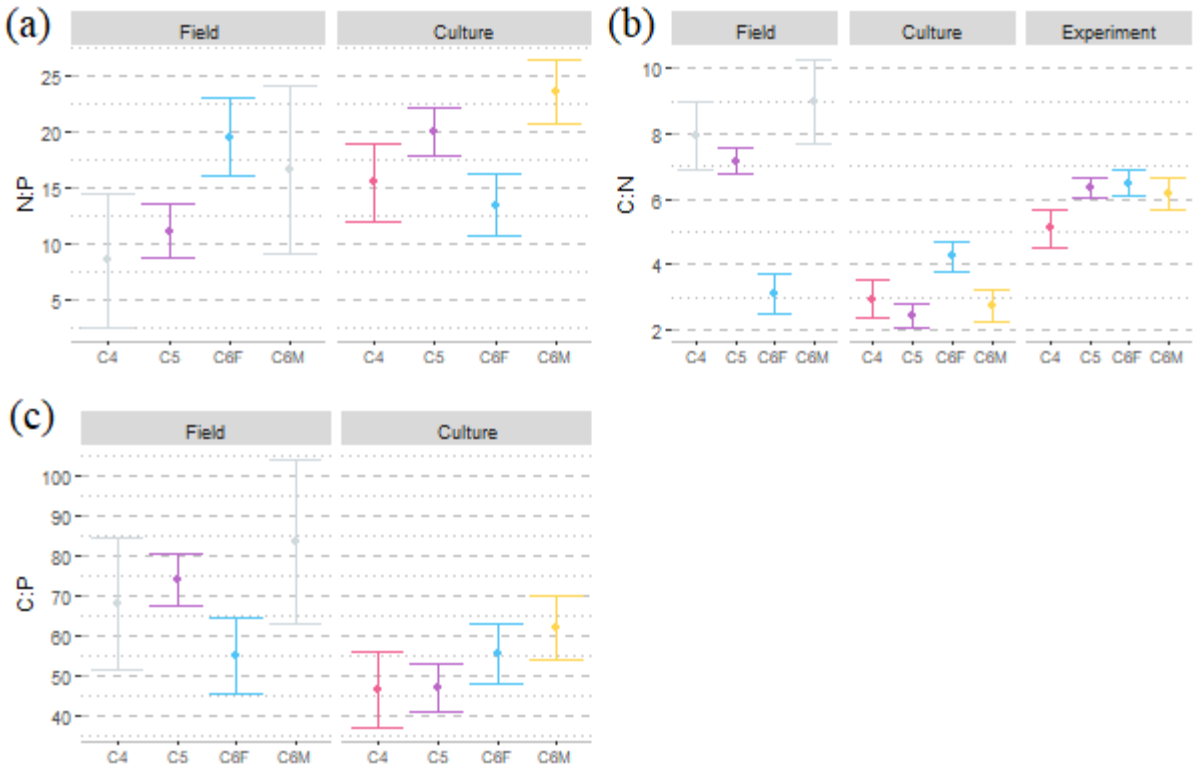

Figure 7. Model predicted average (dot) and 95% confidence interval for (a) N:P ratio, (b) C:N ratio, and (c) C:P ratio for each developmental stage and origin. Model predictions for stages C4 and males of origin field are unreliable due to lack of samples.

Regarding the N:P ratio the two origins showed different patterns between stages, with females having a significantly higher N:P ratio than C5 in field, but a significantly lower N:P ratio than C5 in the culture (Fig. 7a). C5 showed a significant difference in N:P ratio between origins, while females showed a slight overlap in confidence intervals and are therefore not considered to be significantly different (Fig. 7a). In culture, C4 and females had a significantly lower N:P ratio than males, while C5 showed overlapping confidence intervals with both males and C4 (Fig. 7a).

C5 copepodites from the field had a significantly higher C:P ratio than all other sampled stages, except for males from the culture who had a slightly overlapping confidence interval in the lower boundary (Fig. 7c). The predicted average for females was the same between origin, though females from the field show larger confidence intervals (Fig. 7c). There were no significant differences in C:P ratio between copepods of different stages from the culture (Fig. 7c).

All stages of experimental origin showed significantly higher C:N ratios than corresponding stages from the culture (Fig. 7b). There was significant interstage variability between C5 and females from the field, with C5 from the field also having a significantly higher C:N ratio than C5 from the other origins (Fig. 7b). Females from the field had a significantly lower C:N ratio than females from the other origins (Fig. 7b). In the culture, stages showed similar C:N ratios, except for females with a significantly higher C:N ratio than the other stages (Fig. 7b). Stages from the experiment showed similar averages, though C4 had a significantly lower C:N ratio than the rest (Fig. 7b).

6. DISCUSSION

It is challenging to disentangle the themes of this thesis, all of which, to a certain degree, are connected to and affected by the others. Still, I have chosen to structure the discussion into distinct sections, firstly in regards to body size, then growth rates, followed by genome size.

6.1. Body size

Due to the inverse relationship between body size and temperature (Atkinson, 1994; Campbell et al., 2001) the copepods from both the culture and the experiment were predicted to be smaller than conspecifics from Norwegian coastal waters. However, when comparing the length of the copepods from the culture and the experiment to the corresponding stages from the field, the continuously cultured copepods were not consistently smaller as expected (Fig. 4a). Females from the field were smaller than females from the two other origins, though the opposite was the case for C5 copepodites (Fig. 4a). All the wild females analyzed were collected from one location (Bunnefjorden) and had a median size larger than C5 sampled at the same location while smaller than C5 from other locations (Fig. 8a, Appendix). The females from the field were also smaller than the reported size of both *C. finmarchicus* and *C.hegolandicus* collected from the same fjord (Leinaas et al., 2016). The density plot of prosome length between C5 from different sampling stations in Oslofjorden (Fig. 8b, Appendix) supports the notion that the wild copepods sampled are of both species. However, the size difference cannot be explained by species alone, though it is possible that the fjord contains subpopulations (as possibly also indicated by the density plot of prosome length in Fig. 8b, Appendix). The presence of subpopulations is possible considering Bunnefjorden is

sheltered from the outer fjord (Fig. 3) with bottom water that often becomes hypoxic (Kaartvedt et al., 2021) and Leinaas et al. (2016) suggest that narrow sills may represent barriers to geneflow that could possibly result in local adaptions.

All stages from the culture and experiment were smaller than the reported lengths of *C. finmarchicus* reared at 10 °C (Campbell et al., 2001). Surprisingly, they are also smaller than the copepods raised at 12 °C (Campbell et al., 2001). One explanation may be that the changed selection pressure under culture conditions could favor a short generation time (Mauchline, 1998, s.297), promoting a smaller size to reach maturity as fast as possible. Furthermore, copepods show plasticity in size in response to food availability with low food concentration resulting in reduced size (Campbell et al., 2001). Hence, another possible explanation for the small size is that the copepods are nutrient-limited. However, this is unlikely since the cultured copepods are fed *ad libitum*. Still, different developmental stages may have specific nutritional requirements (Mauchline, 1998, s.169). It is a possibility that the two unicellular algae that are continuously supplied may represent suboptimal food at certain life stages, considering elemental ratios for optimal growth are stage-specific (Bujellos et al., 2014b). Notably, the nauplii with high growth rates may experience P-limitation (Bujellos et al., 2014b). If this were the case, growth limitation at certain stages could represent bottlenecks for adult size.

The copepods from the culture had lower relative C content (%) (Fig. 6e) than generally found in animal tissue (Sterner & Elser, 2002), which supports the notion that size may be limited by food availability. The cultured copepods, except for C4, were consistently smaller than corresponding stages from the experiment. Additionally, the developmental stages from the experiment had consistently higher relative C content (%) than the corresponding stage in the culture (Fig. 6e). Combined, this could imply that the copepods in the culture are experiencing limiting food conditions. However, the different external factors, such as food availability and temperature, have interacting effects on copepods growth (Malzahn et al., 2016), which could result in phenotypical changes even between copepods from fairly similar environments. Furthermore, life history strategies are typically state-dependent (McNamara & Houston, 1996), so the increased size and relative C content (%) observed in the experiment could be due to a changed strategy to maximize fitness in the experimental set-up.

Two differences between the culture and the experiment are that copepods in the experiment have more space and are fed only *R. baltica*. From personal observations, the copepods in the NTNU SeaLab culture would group right where the food was added, and distribution was sparse in the rest of the tank. Copepods in the experiment also crowded around the food source. However, the experimental tanks contained fewer copepods per m^3, and each sampling event resulted in even more space per copepod. Hence, the copepods in the experiment may be experiencing less competition for food. Becker et al. (2005) showed that increased copepod density resulted in decreased RNA:DNA ratios for *C. finmarchicus* due to the copepods grazing down all the large phytoplankton, hence becoming food limited. We may speculate that at high copepod densities, as found in the culture tanks, copepods are experiencing high competition for food, possibly promoting a smaller size, thus requiring less energy as energy requirements increase with size (Brown et al., 2004).

6.2. Growth rate

All copepods from the experiment had consistently higher relative RNA content (%), total RNA content (µg), and RNA:DNA ratio than corresponding stages from the culture (Fig. 5c,d,g). This means that the copepods in the experiment have a higher growth rate and implies that they are experiencing more optimal conditions, again indicating that cultured copepods may be developing under limiting conditions. However, copepods may show significant differences in RNA:DNA ratio within and between molts (Vrede et al., 2002), so it is possible that the observed differences are part of natural fluctuations within a molt cycle.

As expected, copepods from the culture and the experiment had significantly higher RNA:DNA ratios than corresponding stages from the field. However, both C5 copepodites and females from the culture showed a closer resemblance in the RNA:DNA ratio to corresponding stages from Oslofjorden than to corresponding stages from the experiment (Fig. 5g). In Oslofjorden *Calanus spp*. reportedly descends to deeper waters for overwintering in September (Bagøien et al., 2000), meaning that a large part of the wild copepods analyzed may be initiating or have already entered diapause. The lowered metabolic activity during diapause would expectedly result in low RNA:DNA ratios as they reflect the rate of protein synthesis. Furthermore, the copepods from the field inhabit different temperatures throughout the day and throughout the year as they perform both dial vertical migrations and seasonal

vertical migrations. The accumulated effect of these temperature changes on growth and development could not be assessed through work in this thesis.

In the culture and the experiment the RNA:DNA ratio increased with increasing developmental stage (Fig. 5g), similar to the reports in Wagner et al. (2001). Furthermore, Wagner et al. (2001) observed a large increase from C5 to adults, ascribing this to likely being due to female egg production. This same increase was found in females from the experiment, though not in females from culture. Ikeda et al. (2007) observed higher RNA:DNA in female *C. finmarchicus* and suggest it is due to gonad maturation or egg production. Hence, the large difference in the RNA:DNA ratio between females from the culture and the experiment (Fig. 5g) may be due to gonad maturation or active egg production in the females from the experiment.

According to the growth rate hypothesis, faster-growing organisms should show a higher RNA:DNA ratio and a lower C:P and N:P ratio (Elser et al. 2000; Elser et al., 2003). However, results from the different analyses showed contrasting results. The RNA:DNA ratio indicated that both C5 and females from culture had higher growth rates than corresponding stages from the field (Fig. 5g). This same significant difference is observed for C5 using the C:P ratio (Fig. 7c), hence implying that the C5 copepodites in culture are investing more in nucleic acids, promoting faster growth. On the other hand, the opposite was observed when assessing N:P ratio, in which C5 copepodites from the field had a significantly lower N:P ratio than C5 from the culture (Fig. 7a). Furthermore, females from the two origins were not significantly different in either C:P or N:P ratio even though they had a significantly different RNA:DNA ratio (Fig. 7a,c, and Fig. 5g, respectively). Weider et al. (2004) also observed results that did not correspond with the growth rate hypothesis and suggested that the three Daphnia species they examined had different strategies for allocating P among growth and biochemical pools to avoid stoichiometric bottlenecks at certain times in the life cycle.

Elser et al., (2003) suggest limitation by another nutrient than P to cause a decoupling of the relationships usually observed between P, RNA, and growth rate. Assessment of elemental content (Fig. 6) shows nutrient content around the expected range for animal tissue (Sterner & Elser, 2002) except for fairly low relative C content (%) in wild females and copepods from the culture, as well as a lower relative N content (%) than expected in C5 from the field. As already mentioned, each developmental stage may have specific and optimal nutritional

requirements (Bujellos et al., 2014b), opening the possibility that the deviations from the growth rate hypothesis are due to different limitations between the deviating groups. That said, P analysis was not performed on the experimental samples. Being the group with the highest growth rates it would have been interesting to see whether they followed the postulations of the growth rate hypothesis.

6.3. Genome size

As stated, in the absence of cell-specific analysis of genome size or cell-numbers per animal (neither of which are trivial to analyze in metazoans), relative DNA content (%) and total DNA content were used as proxies of genome size. Given the correlation between genome size and body size (Ferrari & Rai, 1989; Hessen & Persson, 2009), one could assume that the copepods from the continuous culture had a smaller genome as they were considerably smaller than reported for *C. finmarchicus* developing at the same temperature (Campbell et al., 2001). If genome size is an adaptive trait (Bennett, 1987; Cavalier-Smith, 1978; Ferrari & Rai, 1989) and culture conditions favor short generation times (Mauchline, 1998) it is possible that smaller genomes would increase fitness as it would reduce cell division time (Gregory 2005, Hessen et al., 2009) enabling the copepods to mature at a younger age.

C5 copepodites from the different origins had remarkably similar total DNA content (µg) even though they varied in size (Fig. 5b and Fig. 4a). As copepods grow with cell number consistency (McLaren and Marcogliese, 1983), one could assume that the copepods examined have the same amount of DNA per cell but that the size of the cells differs. However, the females from different origins did not show the same similarities in total DNA content (µg), though they showed similar relative DNA content (%). The *Calanus spp*. examined in Leinaas et al. (2016) showed that species with increasing northern distribution had larger genomes, with *C. finmarchicus* having the smallest genome and the most southern distribution. The more southern relative, *C. helglandicus*, of which most of the wild samples in this thesis probably are (Bagøien et al., 2000; Kaartved et al., 2021), was not examined in Leinaas et al., (2016). However, for the sake of discussion, I will assume that this species follows the same trend as the other species, hence having a smaller genome than *C. finmarchicus*. If this is the case, it is possible that the copepods in the NTNU SeaLab culture have evolved a smaller genome considering total DNA content (µg) of C5 copepodites was so similar across origins.

However, a conclusion on genome size variability between origins warrants data on stage-specific cell numbers and cell-specific genome size.

6.4. Considerations for further studies

A greater understanding of genome size would be gained using flow cytometry to find the cell-specific DNA content, and genome sequencing would provide the means to know which species one is comparing against. In future work, I would also recommend length measurements using image analysis as in Kvile et al. (2020), opening up the possibility of going back to assess individual copepods and to calculate the size of the lipid sac. Furthermore, the method is considerably less straining on the eyes. Additionally, it would have been interesting to compare the cultured *C. finmarchicus* to conspecifics from Trondheimsfjorden to quantify how the cultured copepods have changed during their years away from natural conditions. Lastly, with copepods readily available at NTNU SeaLab, sampling multiple times per developmental stage is very possible and may provide valuable data on the natural fluctuations in growth rate, and possibly C:N:P stoichiometry, within each developmental stage.

7. CONCLUDING REMARKS

In conclusion, the main aim of investigating genome size, growth rate, and body size of cultured copepods compared to wild conspecifics revealed how plastic some of these traits may be. In particular, it appears that the minor differences between the environment of the cultured copepods and the experimental copepods were sufficient to produce distinctly higher growth rates, larger body size, and higher relative carbon content (%) when copepods received more space. The differences mentioned might imply how the copepods from the culture are experiencing factors limiting growth, or just be evidence of how largely plastic copepods are to maximize fitness in a given environment. A trait that varied little between origins was genome size. The similarities in total DNA content (µg) between C5 copepodites from the different origins suggest a similar genome size if copepods have cell number consistency (McLaren & Marcogliese, 1983). However, no conclusions can be made in this regard without acquiring data on cell-specific genome size and stage-specific cell numbers. Lastly, the study did not provide results that consistently followed the postulations of the growth rate hypothesis.

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9. APPENDIX

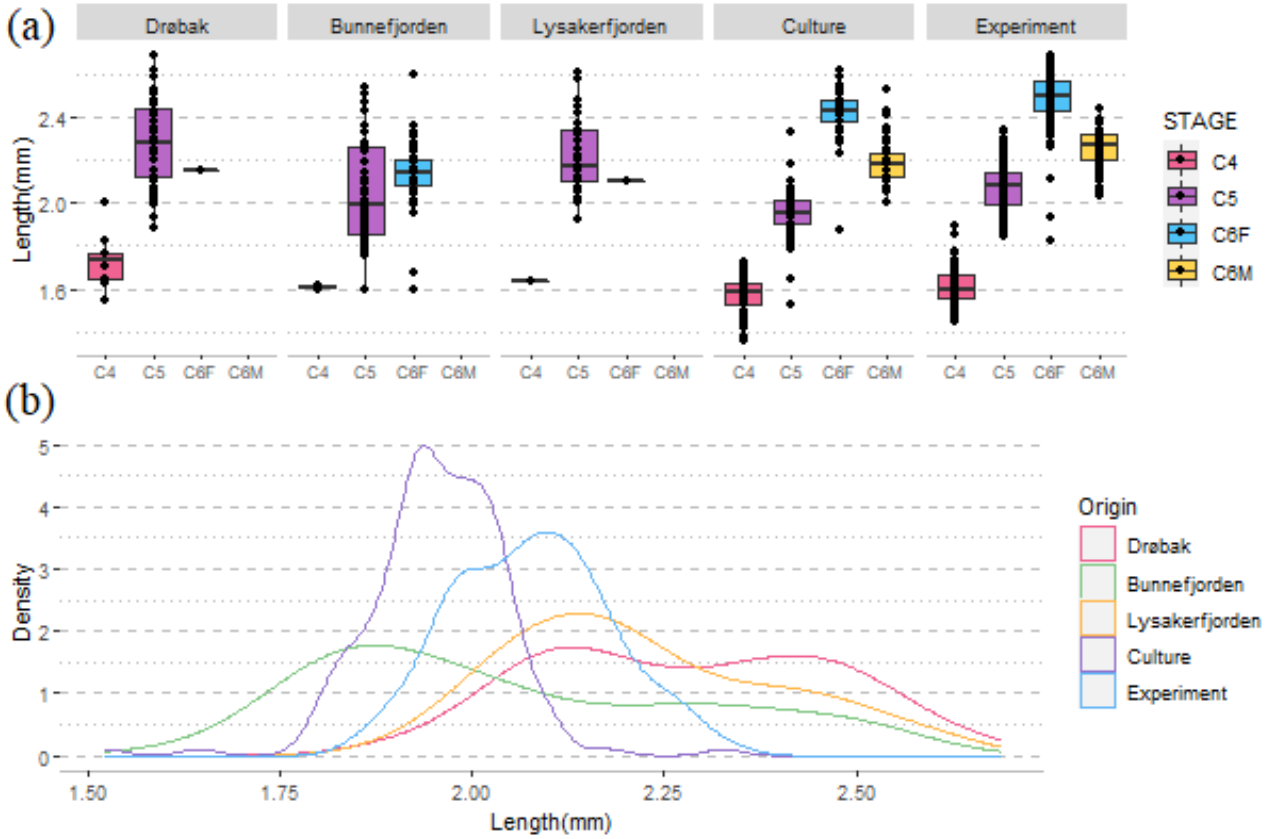

Figure 8. (a) boxplots of length (mm) for each stage and origin (dots). Boxplot with median (line), interquartile range (box), points outside the box are within 1.5x the interquartile range if pierced by the vertical line. Points not pierced are outliers. (b) density plot of copepodite C5 length (mm) from each origin, with the sampling locations from Oslofjorden held separate.