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Dynamics of astaxanthin accumulation in *Calanus* spp., from Norwegian coastal waters

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Abstract

The copepod *Calanus finmarchicus* contains astaxanthin, a red carotenoid, synthesized from phytoplankton precursor pigments. Due to this red pigment, large scale surface swarms of *C. finmarchicus* along the Norwegian coast can be detected by ocean color satellite remote sensing. The objective of this thesis was to explore the dynamics of astaxanthin accumulation in *Calanus* spp. from coastal habitats during the productive season. *Calanus* spp. and phytoplankton were collected during two cruises outside the North Norwegian coast in spring and summer, and from two fjords throughout the productive season. Phytoplankton and copepod pigments were analyzed with high performance liquid chromatography (HPLC). Large variations in astaxanthin concentration in the copepods were found, with particularly high values (up to 4.1 μg ind.$^{-1}$) at Tromsøflaket during summer 2018. Such high values have not been reported elsewhere for marine copepods. Astaxanthin content in the copepods were likely related to feeding history, with increasing values with progression of the phytoplankton bloom. In the fjords, phytoplankton biomass, including possible precursor pigments reached high values, nevertheless pigmentation in the copepods were low compared to *C. finmarchicus* in the swarms off the Norwegian coast. Swarm formation might promote astaxanthin accumulation in copepods, as costs associated with increased conspicuousness are reduced. Potential factors regulating astaxanthin synthesis, including ultraviolet radiation, are discussed.

Keywords: Calanus finmarchicus, phytoplankton, pigments, North Atlantic, zooplankton swarms.

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Bodø, May 2020

Mathilde Servan

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Abbreviations

AF Adult female

Allo Alloxanthin

Asta Astaxanthin

ββ-Car β,β-Carotene

βε-Car β,ε-Carotene

But-fuco 19'-Butanoyloxyfucoxanthin

Chl Chlorophyll

CIV Copepodite stage 4

CTD Conductivity, temperature and depth profiler

CV Copepodite stage 5

Diadino Diadinoxanthin

Diato Diatoxanthin

DVM Diel vertical migration

Fuco Fucoxanthin

Hex-fuco 19'-Hexanoyloxyfucoxanthin

HPLC High Performance Liquid Chromatography

Lut Lutein

c-Neo 9′-*cis*-Neoxanthin

PCA Principal Component Analysis

Peri Peridinin

Pras Prasinoxanthin

UVR Ultraviolet radiation

Viola Violaxanthin

Zea Zeaxanthin

1.0 Introduction

Species in the *Calanus* genus, mainly *C. finmarchicus* (Gunnerus, 1770), are among the most numerous copepods in the North Atlantic, and dominate in terms of biomass (Broms et al., 2009; Jaschnov, 1970; Melle et al., 2014). *C. finmarchicus* are commonly named "red feed", referring to its distinctive red color (fig. 1). This color is caused by astaxanthin, a ketocarotenoid pigment (Matsuno, 2001). Copepods, small planktonic crustaceans such as *C. finmarchicus*, are one of few organisms able to synthesize astaxanthin from precursor carotenoids through their algal diet (Rhodes, 2006; Ringelberg, 1980).

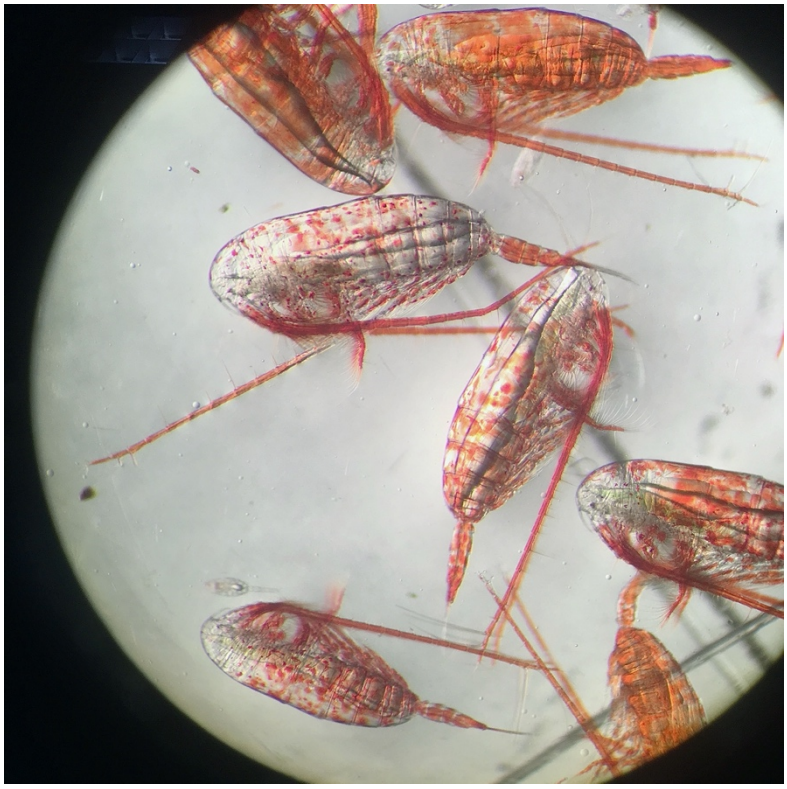

Figure 1. Pigmented individuals of *C. finmarchicus* photographed through the lens of a microscope at Tromsøflaket, summer 2018.

1.1 Dynamics of astaxanthin accumulation in copepods

Carotenoids are originally derived from photosynthesizing organisms, where they function as accessory light-harvesting pigments, or protect against oxidative damage of cellular components (Lohr, 2011, p. 135). Astaxanthin is synthesized by some microalgae under stressful environmental conditions (Henley et al., 2004; Watanabe & Fujii, 2016), such

as the freshwater chlorophyte *Haematococcus pluvialis* (Boussiba & Vonshak, 1991). Marine phytoplankton rarely contain astaxanthin, although some microalgae have been found to produce astaxanthin when grown in culture (Antia & Cheng, 1982; Foss et al., 1985; Lubián et al., 2000).

While the proximate cause for astaxanthin synthesis may be straightforward, the ultimate causes have been much discussed. Astaxanthin is a powerful antioxidant due to its many conjugated double bonds, protecting cells against oxidative stress by removal of free radicals and reactive oxygen species such as singlet oxygen (Kobayashi & Sakamoto, 1999; Shimidzu et al., 1996). Oxidative stress can cause destructive effects to macromolecules, such as polyunsaturated fatty acids (Caramujo et al., 2012). Oxidative stress can be induced by external factors, such as xenobiotics and ultraviolet radiation (UVR; UV-A wavelength 320-400 nm and UV-B 280-320 nm) (Lesser, 2006). It is well known that *H. pluvialis* up-regulates genes responsible for astaxanthin synthesis when faced with various stressors, such as high light intensity (Boussiba & Vonshak, 1991). Copepods could possibly also up-regulate genes responsible for astaxanthin synthesis in stressful environments, although no studies have confirmed this. Some studies show a positive correlation between astaxanthin concentration and survival when exposed to UVR (Byron, 1982; Caramujo et al., 2012; Hairston, 1976; Karanas et al., 1979), while other studies show no link between these variables (Speekmann et al., 2000). On the other hand, a study by Schneider et al. (2016) suggest that astaxanthin accumulation is related to lipid metabolism and reproduction, rather than UVR protection, as the concentration of astaxanthin was highest when lipid concentration was at its highest, which was during wintertime.

Astaxanthin accumulation has been proposed to be a trade-off between threats from UVR exposure (Hansson, 2000, 2004; Hylander et al., 2009), or growth benefits (Gorokhova et al., 2013), and predation threats. Being a red copepod residing in the illuminated surface layer does present a challenge when visual predators are nearby, as demonstrated by Brüsin et al. (2016), Byron (1982) and Hairston (1979) with predation experiments. The freshwater copepods *Daphnia* spp. has been found to respond to fish kairomones by reducing pigmentation (Tollrian & Heibl, 2004), indicating that pigmentation is a plastic trait. In addition, it has been shown that copepods from lakes without predators are generally more pigmented than individuals of the same species in lakes with predators (Hairston, 1976).

One way to avoid visual predators is by diel vertical migration (DVM); descending to deeper depths during the day when the surface layer is illuminated. Another tactic for predator avoidance (among other physical and biological drivers) could be swarming behavior (Folt & Burns, 1999), as demonstrated by *C. finmarchicus* in the North Atlantic Ocean (Cushing & Tungate, 1963; Wishner et al., 1988). In a swarm, the probability of being eaten is reduced compared to solitary individuals (Hamilton, 1971; Milinski, 1977; Olson et al., 2013). Thus, if all individuals in the swarm are pigmented, the benefits of having a high concentration of astaxanthin could outweigh the costs.

Astaxanthin concentration in *C. pacificus* and *C. helgolandicus* has been shown to be highly dynamic and related to recent feeding history, as animals rapidly loose or gain pigmentation after a few hours of starvation or feeding (Juhl et al., 1996; Sommer et al., 2006). When *C. pacificus* was fed a high concentration of the cryptophyte *Rhodomonas* sp., pigmentation increased with 50 % from initial values (Juhl et al., 1996). The amount of astaxanthin per copepod has been found to be related to the composition and biomass of phytoplankton in the surrounding water, favoring a diverse community dominated by chlorophytes, dinoflagellates and diatoms with thin silica frustules (heavily silicified diatoms are harder for the copepods to digest) (Andersson et al., 2003). This indicates that the surrounding phytoplankton community could be responsible for the variations in copepod pigmentation. However, a study by Van Nieuwerburgh et al. (2005) showed that astaxanthin concentration per copepod was not affected by phytoplankton community composition, and decreased with phytoplankton biomass. They suggested that the dense phytoplankton bloom functioned as a "shield" against UVR, which decreased the copepods demand for photoprotection by astaxanthin. Holeton et al. (2009) found that astaxanthin concentration in the copepod *Acartia bifilosa* only increased with food availability towards a certain threshold, when above this threshold, astaxanthin concentrations in the copepods were low. They hypothesized that as feeding activity increased, gut evacuation rates increased, leading to decreased assimilation efficiency and uptake of precursor molecules.

Diurnal variations in astaxanthin concentration have been found for species with synchronized feeding activity, with peak concentrations at night or early morning when feeding activity is highest (Andersson et al., 2003; Kleppel et al., 1985; Ringelberg & Hallegraeff, 1976; Sommer et al., 2006). Sommer et al. (2006) found on average 60 % higher astaxanthin concentrations in the temporal species *C. helgolandicus* from the North Sea at night compared to day, while Hylander et al. (2015) found no variations in diel astaxanthin

concentration in arctic *Calanus* spp. from Disko bay in Greenland. DVM, and thus feeding activity, has been shown to be unsynchronized in arctic and subarctic *Calanus* spp. during the productive season (Basedow et al., 2010; Blachowiak-Samolyk et al., 2006), which could explain this lack of diel variations in astaxanthin concentrations.

Three forms of astaxanthin have been identified in copepods, depending on degree of esterification of the hydroxyl groups at each end of the molecule; free astaxanthin, astaxanthin monoesters and astaxanthin diesters (Foss et al., 1987) (fig. 2). The fraction of esterified astaxanthin has been shown to increase with developmental stage, demonstrating its important role in lipid metabolism and oxidation (Łotocka et al., 2004). The concentration of astaxanthin have been found to depend on the dynamics of the esterified fraction, which again depends on available food and illumination. The fraction of astaxanthin esters in *C. helgolandicus* has been shown to decrease in starved copepods in the dark, increase when food is available in the light, and remain at initial levels when food was available in the dark (Sommer et al., 2006). Illumination could thus possibly trigger astaxanthin esterification in copepods, which would enhance protection of lipids against oxidative stress caused by UVR.

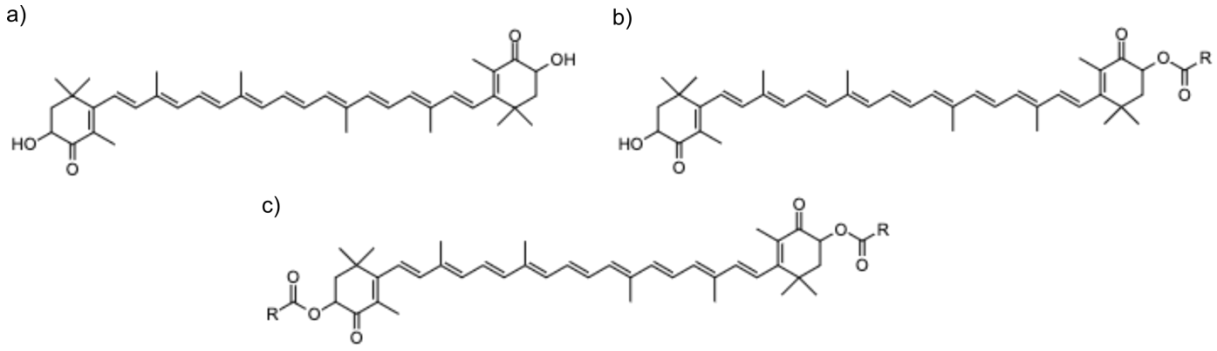

Figure 2. The structure of a) free astaxanthin, b) astaxanthin monoester, c) astaxanthin diester. R represents saturated or unsaturated alkyl chains, see Falk-Petersen et al. (2009) for composition of fatty acid chain lengths.

It has been shown that esterified astaxanthin is incorporated to a higher extent than free astaxanthin in the copepod *Amphiascoides atopus* (Caramujo et al., 2012). As astaxanthin is rare in marine phytoplankton (Jeffrey et al., 2011, pp. 45-55), pigmentation must originally

be derived from precursor molecules. It is not known if copepods could have different assimilation efficiencies for these precursor molecules.

1.2 Phytoplankton pigments and the synthesis of astaxanthin by copepods

All phytoplankton contain chlorophyll *a*, which absorbs light at blue and red wavelengths (max. absorption at 431 nm in acetone) (Egeland et al., 2011, p. 677). In addition to chlorophyll *a*, phytoplankton contains accessory pigments, which absorbs light at different wavelengths, e.g. chlorophyll *b* which has maximum absorption at 459 nm (in acetone) (Egeland et al., 2011, p. 683). Phytoplankton descended from the same ancestors usually have similar accessory pigments. It is therefore useful to divide the phytoplankton into three groups based on evolutionary history; the cyanobacteria, the red algal lineage and the green algal lineage (Jeffrey et al., 2011, p. 9). Some accessory pigments are rather common, e.g. chlorophyll c_1 and c_2 which are present in most phyla from the red algal lineage, while others are found in fewer phyla, e.g. alloxanthin which is present in the cryptophytes and the dinoflagellate *Dinophysis norvegica* which contain a cryptophyte endosymbiont (Meyer-Harms & Pollehne, 1998), and prasinoxanthin which is present in prasinophytes and dinoflagellates with prasinophyte endosymbionts (Jeffrey et al., 2011, p. 37). Because of the extensive research on, and identification of, signature pigments from phytoplankton groups, it is possible to identify many phyla from filtered water samples using high performance liquid chromatography (HPLC) (Jeffrey et al., 2011). Fucoxanthin has been shown to correlate with the presence of diatoms, although this pigment is present in many phyla from the red algal lineage (Wright et al., 1996). Peridinin is used as a marker for dinoflagellates, although many dinoflagellates do not contain peridinin (Jeffrey et al., 2011, p. 37). High concentrations of chlorophyll *b* indicates high concentrations of phytoplankton from the green algal lineage (Jeffrey et al., 2011, pp. 45-55).

Copepods synthesize astaxanthin from β,β-carotene by the addition of keto and hydroxy groups to the β-rings (Harker & Young, 1995; Matsuno, 2001). Two pathways have been proposed from β,β-carotene to astaxanthin; through β-cryptoxanthin, zeaxanthin and adonixanthin, or through echinenone, canthaxanthin and adonirubin (Misawa et al., 1995). A combination of these routes could also take place (Harker & Hirschberg, 1997). Of these possible precursors, β,β-carotene is the most common phytoplankton pigment, found in nearly all phyla, although high concentrations of β,β-carotene is usually associated with

phytoplankton from the green algal lineage (Jeffrey et al., 2011, pp. 45-55). Zeaxanthin is also a common phytoplankton pigment, found as major or minor accessory pigment in many phytoplankton phyla, with highest concentrations in cyanobacteria and some phyla of green algae. Cryptoxanthin, echinenone and canthaxanthin are mostly present in the cyanobacteria, while adonixanthin and adonirubin are rarely encountered in marine phytoplankton (Jeffrey et al., 2011, pp. 45-55). In addition, some aquatic animals can convert lutein to astaxanthin (Katayama et al., 1973), but it is not known if this pathway exists in copepods.

1.3 *Calanus finmarchicus*

The calanoid copepod *C. finmarchicus*, is of substantial scientific and commercial interest, due to its high abundance (Melle et al., 2014) and nutritional value (Bergvik et al., 2012). Their relatively large body size (2-3 mm) and lipid reserves makes them an attractive prey for higher trophic levels, such as herring, mackerel and Atlantic cod (Falk-Petersen et al., 2009; Gislason & Astthorsson, 2002; Kaartvedt, 2000; Sundby, 2000). *C. finmarchicus* is also trending as a nutritional supplement for human consumption, valued for its high amount of astaxanthin and omega-3 polyunsaturated fatty acids (Cook et al., 2016; Eysteinsson et al., 2018; Tande et al., 2016).

The distribution of *C. finmarchicus* is mainly associated with the North Atlantic water masses (Choquet et al., 2017; Conover, 1988; Melle et al., 2014), including the Norwegian continental shelf and fjords (Espinasse et al., 2016; Hopkins et al., 1984; Skreslet et al., 2015; Wiborg, 1954). The large lipid stores, mainly consisting of wax esters, serve as energy reserves for the hibernating copepods during winter (Falk-Petersen et al., 2009; Lee et al., 2006). The life cycle for *C. finmarchicus* consists of six naupliar stages followed by five copepodite stages (CI-CV) and a final molt into either adult male or female (Mauchline, 1998, p. 23). Developmental time from egg to CV usually takes between three to eight weeks, largely determined by food concentration and temperature (Campbell et al., 2001; Møller et al., 2012). The older stages, mainly CVs, descends to great depths during summer and enters diapause, a dormant state where energy consumption is minimized during the low productive winters (Hirche, 1996a). In some areas, CVs have been found to molt directly into adults during summer and produce a second generation, evident by a second, although smaller, abundance peak (Gislason & Astthorsson, 1996; Lie, 1965). The individuals who enters diapause stay dormant for many months prior to molting into adult forms during late winter.

The sex ratio is usually skewed towards females, except for a short period as males develop first (Hirche, 1996b; Tande & Hopkins, 1981). Egg laying occurs before, during and after the spring bloom, although highest survival rate probably occurs when the feeding nauplii can utilize the phytoplankton bloom for growth (Kaartvedt, 2000; Melle & Skjoldal, 1998; Stenevik et al., 2007). In addition, recruitment of the new generation largely depends on phytoplankton biomass due to the cannibalistic behavior of females when phytoplankton are in short supply (Basedow & Tande, 2006; Plourde et al., 2008). The seasonal development of *Calanus* spp. stages usually varies between water masses, with earlier development in coastal waters, due to the later progression of the phytoplankton bloom and colder temperatures in Atlantic water (Broms & Melle, 2007). In the coastal waters off Northern Norway, the bloom usually forms around April-May when the pycnocline shallows due to freshwater runoff and warming of the surface layer (Head et al., 2013).

C. finmarchicus are primarily herbivorous, selectively feeding on larger phytoplankton cells such as diatoms and dinoflagellates (Meyer-Harms et al., 1999; Mullin, 1963). Diatoms and dinoflagellates contain the precursor molecule β,β-carotene in minor amounts, while zeaxanthin can occur in some diatoms exposed to high light (Jeffrey et al., 2011, p. 44). *C. finmarchicus* have been shown to avoid smaller phytoplankton cells, such as cyanobacteria, pelagophytes, chlorophytes and prasinophytes, throughout the productive season (Meyer-Harms et al., 1999). Cyanobacteria and pelagophytes contain zeaxanthin as major pigment, while chlorophytes and prasinophytes contain β,β-carotene as a major pigment (Jeffrey et al., 2011, pp. 45-55). Selective grazing could thus complicate the association between phytoplankton community composition and astaxanthin accumulation in *C. finmarchicus*.

Although *C. finmarchicus* resides in surface layers during seasons with midnight sun, they seem to be little affected by UVR (Skreslet et al., 2005). It is tempting to attribute this to high concentrations of astaxanthin, but a study by Hylander et al. (2015) on *Calanus* spp. from arctic environments showed no relationship between UVR and astaxanthin. However, *Calanus* spp. accumulate other forms of "sunscreen" molecules, such as mycosporine-like amino acids (Hylander et al., 2015; Karentz et al., 1991), which could explain the lack of correlation between UVR and *Calanus* spp. pigmentation.

Marshall et al. (1934) observed that the brightest individuals of *C. finmarchicus* often were infected with parasites. This unusual coloration was also observed by Torgersen et al.

(2002) in parasite infected copepods from Oslofjorden. Intense pigmentation was thought to be induced by the parasites to enhance visibility and thus transmission to new hosts. This parasite induced coloration is often bright yellow-red and completely occupies the body cavity (see Torgersen et al. (2002) for photographs), contrary to normal red pigmentation which is usually found in the antennules, oil sac and carapace.

Since the species *C. finmarchicus* and *C. glacialis* cannot be distinguished based on morphological features, especially in Norwegian fjord communities (Choquet et al., 2017; 2018), they will be referred to as *Calanus* spp. throughout this thesis.

1.4 Aim

C. finmarchicus tend to aggregate in large surface swarms in the Norwegian sea which can extend for >1 000 km^2, with > 1 000 *Calanus* spp. m^{-3} (Basedow et al., 2019). Because of their red pigment, it is possible to detect these swarms by satellite, and this ocean color remote sensing could be a revolutionary approach to estimate the abundance and to track the movement of these swarms in space and time. If reliable abundance estimates are to be made from satellite observations, spatial and temporal variations in astaxanthin concentration in the copepods have to be accounted for. This thesis aims to explore the dynamics of astaxanthin accumulation in *Calanus* spp. during the productive season by answering these questions:

1. How does astaxanthin concentration and astaxanthin forms in *Calanus* spp. vary spatially and temporally?
2. Are there any relationships between astaxanthin concentration in *Calanus* spp. and phytoplankton biomass or community composition?

2.0 Methods

2.1 Sampling

2.1.1 Cruise, Summer 2018

17 stations off the northern Norwegian coast were sampled on board R/V "Helmer Hanssen" during a cruise as part of the "Sea Patches" project (hereafter referred to as summer 2018) from June 17-25, 2018 (fig. 3). The stations were mostly above the continental shelf were bottom depth ranged from 122-371 m (table 1). Two stations were located on the shelf break, and three were off shelf where bottom depths ranged from 1569-2755 m. Samplings were conducted at various times during the day and night.

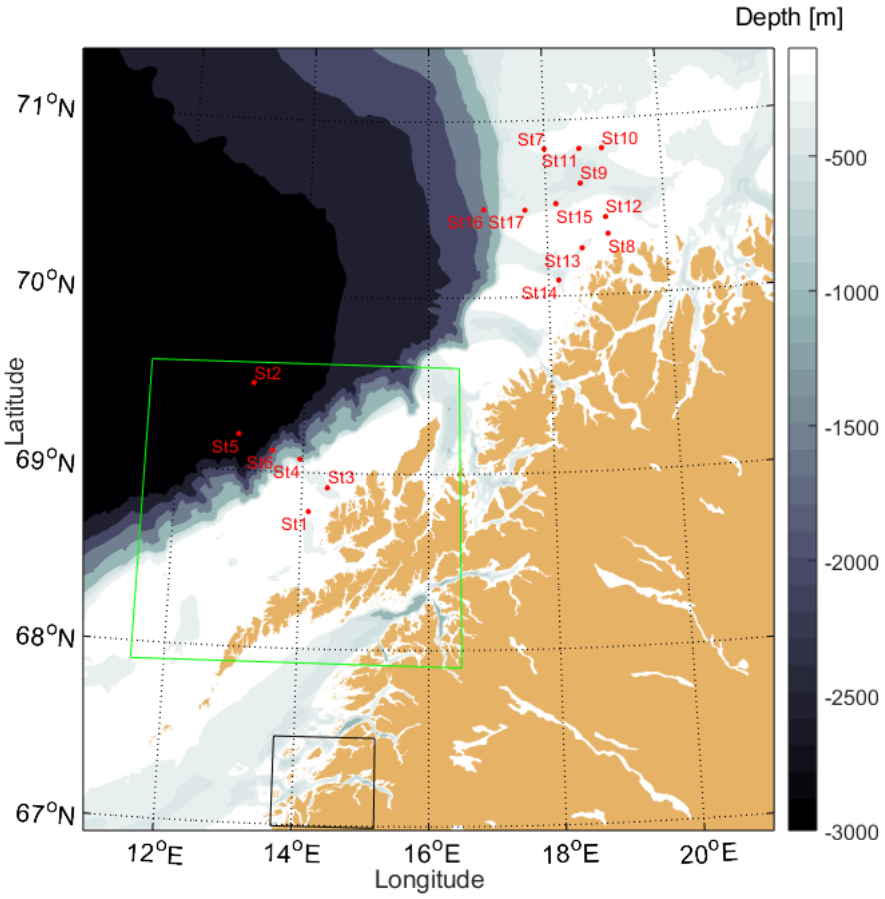

Figure 3. Map of sampling stations, summer 2018. Green square indicates sampling area spring 2019, and black square indicates fjord samplings.

Copepods for pigment analysis were sampled by vertical net hauls (0.5 m s^{-1}) using a WP2 net (mesh size 200 µm, diameter 57 cm, Hydro-bios) with a messenger-operated closing mechanism. Different depth layers were sampled to measure potential differences in pigmentation between *Calanus* spp. from the surface and deeper depths. Copepods were collected from 10-0 m and 40-20 m, with exceptions of station 1 where they were collected from 30-0 m, and station 2 and 3 where they were collected from 40-10 m and 10-0 m (table 1). Additional samples were taken at deeper depths on station 9 (350-100 m), station 10 (210-100 m), station 12 (265-100 m) and station 14 (340-100 m), to compare astaxanthin concentration in copepods from productive and non-productive layers. After net sampling, the zooplankton were collected in 10 l buckets and stored in a cold room at 4°C until processing. Processing began immediately after sampling. For each depth, three replicates of 30 *Calanus* spp. CVs were gently picked out using forceps and placed on a tissue paper to dry. The CVs were identified by having four segments on their urosome (Lebour, 1916), using a stereo microscope. The sample replicates of dried animals were wrapped in aluminum foil, put in zip lock bags, and placed in a -80°C freezer on board.

Table 1. *Calanus* spp. stage (CV = copepodite stage V), sampling depths, number of replicates x individuals in each replicate collected for pigment analysis, bottom depth and local time at each station from summer 2018.

Station	Depth (m)	Stage	N	Bottom depth (m)	Time (local)
1	30-0	CV	3 x 30	122	13:30
2	10-0	CV	3 x 30	2755	11:30
	40-10	CV	3 x 30		
3	10-0	CV	3 x 30	268	01:00
	40-10	CV	3 x 30		
4	10-0	CV	3 x 30	518	11:00
	40-20	CV	3 x 30		
5	10-0	CV	3 x 30	2750	22:00
	40-20	CV	3 x 30		
6	10-0	CV	3 x 30	1569	10:15
	40-20	CV	3 x 30		
7	10-0	CV	3 x 30	170	03:40
	40-20	CV	3 x 30		

Table 1. (*continued*)

Station	Depth (m)	Stage	N	Bottom depth (m)	Time (local)
8	10-0	CV	3 x 30	172	06:30
	40-20	CV	3 x 30		
9	10-0	CV	3 x 30	371	18:50
	40-20	CV	3 x 30		
	350-100	CV	3 x 30		
10	10-0	CV	3 x 30	224	00:15
	40-20	CV	3 x 30		
	210-100	CV	3 x 30		
11	10-0	CV	3 x 30	216	10:00
	40-20	CV	3 x 30		
12	10-0	CV	3 x 30	280	13:00
	40-20	CV	3 x 30		
	265-100	CV	3 x 30		
13	10-0	CV	3 x 30	236	01:15
	40-20	CV	3 x 30		
14	10-0	CV	3 x 30	357	02:30
	40-20	CV	3 x 30		
	340-100	CV	3 x 30		
15	10-0	CV	3 x 30	303	17:50
	40-20	CV	3 x 30		
16	10-0	CV	3 x 30	1109	11:15
	40-20	CV	3 x 30		
17	10-0	CV	3 x 30	278	18:30
	40-20	CV	3 x 30		

Seawater was collected for phytoplankton pigment analysis from 0 m and 5 m at each station, and from 10, 20 and 50 m at stations 9, 8, 11 and 13. Seawater was sampled with 5 l Niskin bottles mounted on a rosette. 1 l from each depth was filtered through glass microfiber GF/F filters (diameter 25 mm, pore size 0.7 µm, Whatman®) using a vacuum pump. The filters were individually wrapped in aluminum foil, placed in zip lock bags, and stored in a -80°C freezer on board.

After the cruise, the filters and copepod samples were kept in a -80°C freezer at the University in Tromsø, before being transported to Bodø on dry ice. The filters were kept in a -80°C freezer, and the copepods in a -40°C freezer before pigment extraction. Filters from all depths at station 4, and from 5 and 50 m at station 9 were missing at arrival in Bodø.

2.1.2 Cruise, spring 2019

21 out of 28 stations were sampled off the coast of Lofoten and Vesterålen, on board R/V "Helmer Hanssen" during a cruise as part of the "Stressor" project (hereafter referred to as spring 2019) from April 27 to May 12, 2019 (fig. 4). Most of the stations were located on the shelf and shelf break area, with a few stations off shelf. Water depth ranged from 73-2772 m (table 2).

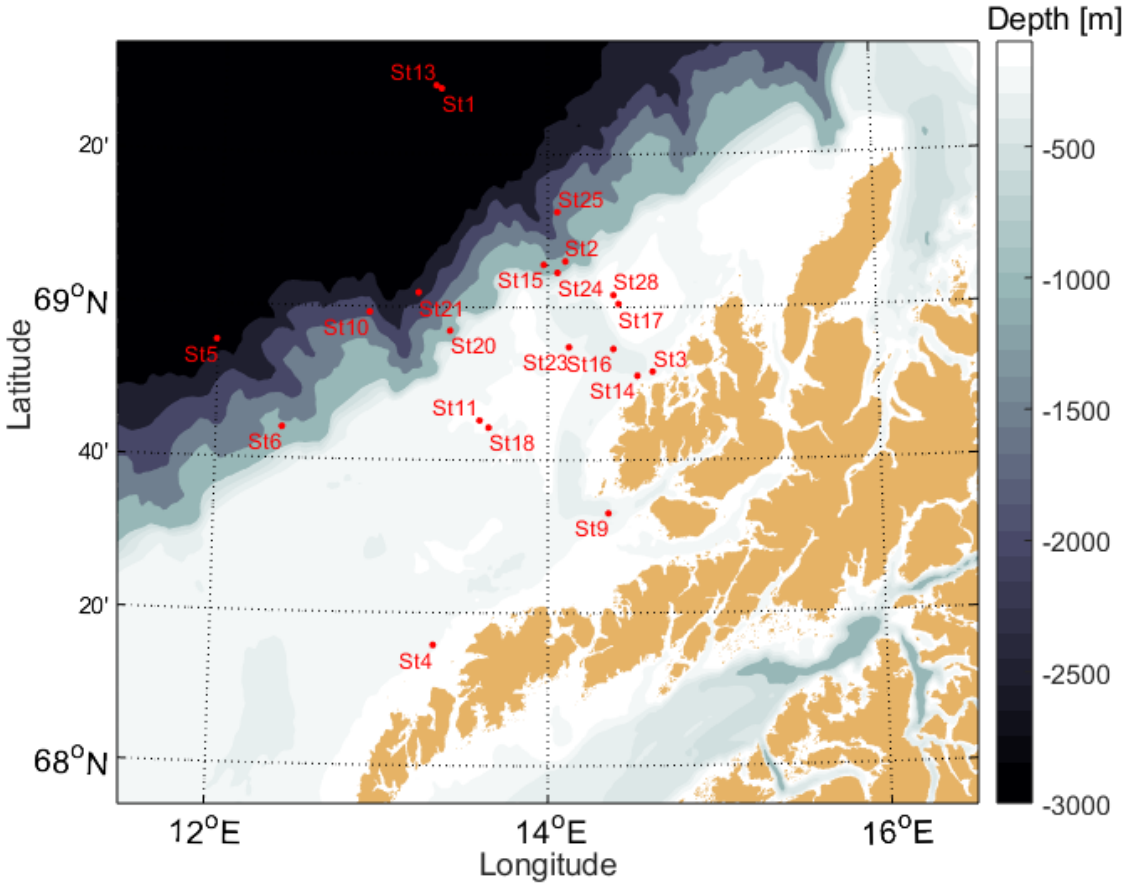

Figure 4. Map of sampling stations, spring 2019.

Copepods were sampled for pigment analysis using the same method and equipment as in summer 2018; two vertical hauls from 10-0 m and 40-20 m at each station (except

station 1 where sample depth was 100-40 m) using a WP2 net. Because this cruise took place at an earlier time in spring, CVs where often outnumbered by females and younger copepodite stages, or not present in the samples at all. CVs were collected at 11 stations, females at 6 stations, and CIVs at 9 stations. Females were identified by their genital somite, and CIVs by having three segments on their urosome (Lebour, 1916). Not all stations were sampled due to bad weather, or lack of sample preparation time between stations (these stations are not included in the map). At station 10 there were too few *Calanus* spp. in the sample to analyze pigments.

At each of the 21 stations, seawater was sampled from 5 and 30 m for pigment analysis. Unfortunately, the filters were thawed on the way from Tromsø to Bodø, which caused substantial degradation of chlorophylls. The number of peaks from degradation products in the chromatograms made estimation of pigment concentration unreliable, as the pigment peaks overlapped with each other. Consequently, this data is not reported.

To investigate how fast *Calanus* spp. lose pigmentation with starvation, 20 CVs were placed individually in small numbered plastic containers with filtered seawater, immediately after collection with the WP2. They were then left for 24 h in the cold room at 4°C. To check if light exposure could affect the pigment loss, the light in the cold room was turned on for 12 hours, then off for 12 hours (12 h light:dark) at stations 9, 11 and 14, kept off for 24 hours (24 h dark) at stations 16, 17 and 28, and kept on for 24 hours (24 h light) at stations 18, 23 and 24. The copepods were gently collected from the containers, dried, wrapped in aluminum foil and frozen at -80 °C. Two individuals were lost at retrieval from station 9 and one at station 24. Copepods collected immediately for pigment analysis from the same stations were considered control groups. One individual from station 14 was photographed before and after starvation for illustrative purposes.

Table 2. *Calanus* spp. stage (AF = adult female, CIV = copepodite stage 4, CV = copepodite stage 5), sampling depths, number of replicates x individuals in each replicate collected for pigment analysis, bottom depth and local time at each station from spring 2019.

Station	Depth (m)	Stage	N	Bottom depth (m)	Time (local)
1	100-40	AF	1 x 30	2762	11:36
2	100-40	AF	1 x 30	778	21:25
3	10-0	CIV	3 x 30	150	01:21
	40-20	CIV	3 x 30		

Table 2. (*continued*)

Station	Depth (m)	Stage	N	Bottom depth (m)	Time (local)
4	10-0	CIV	3 x 30	142	08:23
	40-20	CIV	3 x 30		
5	10-0	AF	3 x 30	2704	02:40
6	40-20	AF	1 x 30 + 1 x 22	739	08:02
9	10-0	CV	3 x 30	224	22:30
	40-20	CV	3 x 30		
10	-	-	-	1622	14:00
11	10-0	CV	3 x 30	93	21:00
	40-20	CV	3 x 30		
13	10-0	CIV	3 x 30	2772	17:23
14	10-0	CV	3 x 30	175	09:30
	40-20	CIV	3 x 30		
15	10-0	CIV	3 x 30	725	12:10
	40-20	CIV	1 x 26		
16	10-0	CV	3 x 30	266	20:19
	40-20	CV	3 x 30		
17	10-0	CV	3 x 30	86	01:24
	40-20	CV	3 x 30		
18	10-0	CV	3 x 30	85	13:44
	40-20	CV	3 x 30		
20	10-0	CIV	3 x 30	765	22:46
	40-20	CIV	3 x 30		
21	40-20	AF	1 x 30	2219	01:07
23	10-0	CIV	2 x 30	169	13:20
	40-20	CV	3 x 30		
24	10-0	CV	3 x 30	500	17:04
	40-20	CV	3 x 30		
25	10-0	CV	1 x 30	1750	01:08
	40-20	CV	2 x 30		
	40-20	AF	1 x 30		
28	10-0	CIV	3 x 30	73	13:12
	40-20	CV	3 x 30		

2.1.3 Seasonal fjord study

Two fjords in northern Norway were sampled for *Calanus* spp. and phytoplankton pigments from late winter to mid-summer 2019 on board R/V "Tanteyen". Sampling was conducted at a fixed station in each fjord, Saltfjorden (67.15°N, 14.38°E), and Nordfjorden (67.08°N, 14.18°E) (fig. 5). Both fjords were sampled on the same day between 9:00-15:00, except for one sampling in June where Nordfjorden was sampled on the 25 and Saltfjorden on the 26 (table 3).

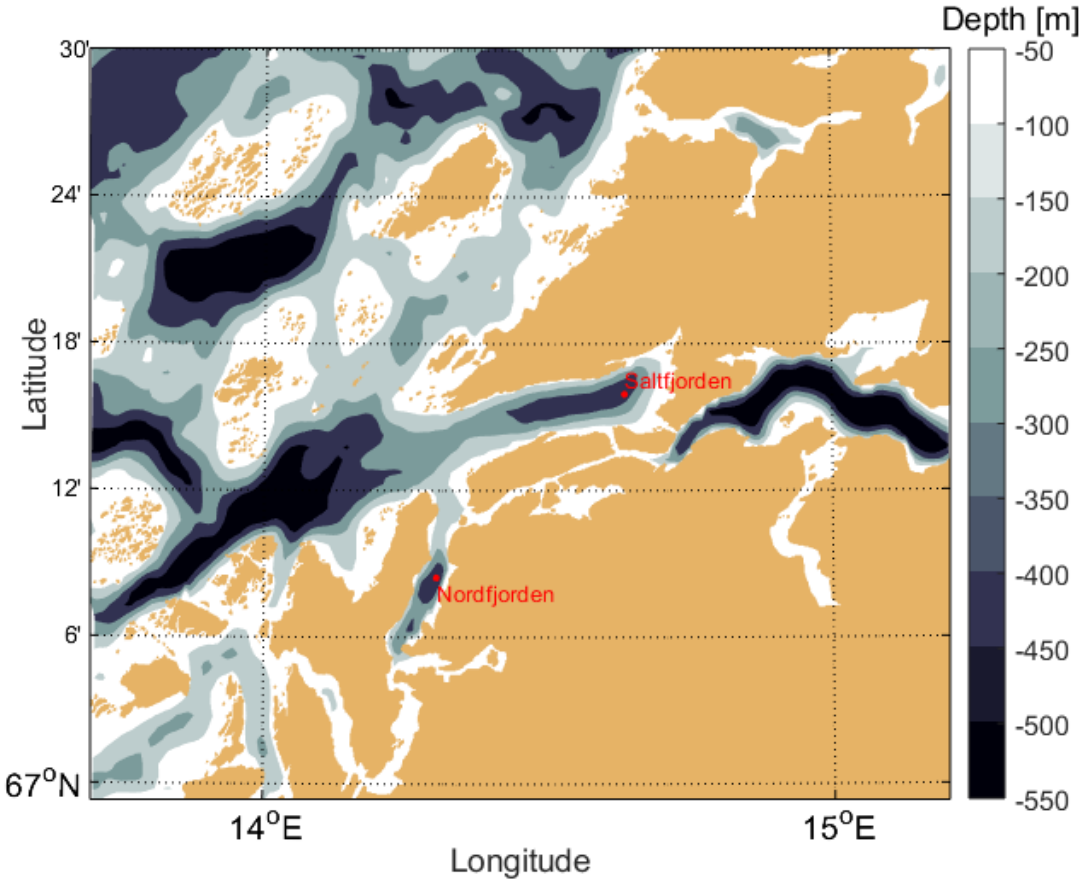

Figure 5. Map of sampling stations in Nordfjorden and Saltfjorden.

Saltfjorden is a fjord located to the south of Bodø, connected to the Norwegian sea through Vestfjorden located between Lofoten and Salten. Nordfjorden lies at the mouth of Saltfjorden, extending southward. Basin depth of both fjords measures approximately 350 m (Kartverket, n.d). These locations were chosen for studying seasonal progression of the *Calanus* spp. community because of their easy access from Mørkvedbukta research station (Nord University) by boat. In addition, *Calanus* spp. abundance in Saltfjorden has previously been reported to be relatively high (Skreslet et al., 2000).

Hydrographical data was collected using a conductivity, temperature and depth profiler (CTD) (model SD204, SAIV A/S, Norway). Measurements were recorded for every meter from the surface to 50 m. This was done for every sampling date except for June 12, because of an instrument malfunction.

Copepods for pigment analysis were sampled by vertical net hauls (towing speed 0.5 m s^{-1}) using a WP2 net (mesh size 200 µm, diameter 57 cm, KC Denmark). A Nansen release mechanism with a drop messenger was used to close the plankton net at 20 m. The animals were flushed into 30 l containers half full of filtered seawater. The animals were gently handled until processing on land. Because both fjords where sampled on the same day, zooplankton collected from the first fjord were kept on the stern of the boat for up to 3 h. The air temperature varied from -10 °C on March 4 to 16°C on April 25, and the containers were kept away from direct sunlight. On land, the containers with the copepods were stored in an environmental chamber holding 4°C until further processing. The copepods were gently filtered through a 200 µm sieve and transferred to petri dishes. The aim was to collect three replicates of 30 *Calanus* spp. CVs for investigation of seasonal development of astaxanthin concentration, but this was only achieved on one sampling date (May 15), due to relatively low abundances of CVs on the other dates. Females and CIVs were collected when few CVs were present.

Seawater for phytoplankton pigment analysis was collected from 5m and 30m with a 7.5 L Niskin bottle, which was closed at the desired depth using a drop messenger. The water was stored in 25 l plastic cans covered with black plastic bags to restrict light exposure. The water samples were processed on land within 5 h of sampling. The water was filtered through glass microfiber GF/F filters (diameter 90 mm, pore size 0.7 µm, Whatman®), using a vacuum pump. Volume of water filtered varied from 10.0 l in March to 2.0 l in April, as a result of increased particle concentrations in the water, as particles tended to clog up the pores in the filters. The filters were not dried completely to avoid destruction of the phytoplankton cells, in contrast to the filters from 2018 which were relatively dry. The filters were individually wrapped in aluminum foil, placed in zip lock plastic bags, and stored in a -40°C freezer until pigment extraction.

Calanus abundances from April 4, May 15, June 12 and July 12 were estimated microscopically by Solberg (2019) as part of her bachelor's thesis. The copepods were collected using the same WP2 net as described above, from the two depth layers (10-0 m and 40-20 m). The samples were preserved in a borax-buffered 4 % formalin in seawater solution,

before enumeration and identification of the different copepod stages. At least 500 individuals from each sample were identified.

Table 3. Sampling dates, fjords, sampling depths, *Calanus* spp. stage (AF = adult female, CIV = copepodite stage 4, CV = copepodite stage 5), and number of sample replicates x number of individuals in each replicate.

Date	Fjord	Depth (m)	Stage	N
March 4	Nordfjorden	-	-	-
	Saltfjorden	10-0	AF	1 x 20
March 19	Nordfjorden	10-0	AF	1 x 10
		40-20	AF	1 x 30
	Saltfjorden	10-0	AF	1 x 5
		40-20	AF	1 x 30
April 4	Nordfjorden	40-20	AF	1 x 18
	Saltfjorden	10-0	AF	1 x 30
		40-20	AF	1 x 18
April 25	Nordfjorden	10-0	CIV	3 x 30
		40-20	CIV	3 x 30
	Saltfjorden	10-0	CV	3 x 30
		40-20	CV	2 x 30 + 1 x 20
May 15	Nordfjorden	10-0	CV	3 x 30
		40-20	CV	3 x 30
	Saltfjorden	10-0	CV	3 x 30
		40-20	CV	3 x 30
May 29	Nordfjorden	10-0	CV	1 x 30
		40-20	CV	1 x 30
	Saltfjorden	10-0	CV	1 x 20
		40-20	CV	1 x 25
June 12	Nordfjorden	10-0	CV	1 x 10
		40-20	CV	1 x 30
	Saltfjorden	40-20	CV	1 x 30
June 25	Nordfjorden	40-20	CIV	1 x 30
June 26	Saltfjorden	40-20	CV	1 x 30
		40-20	CIV	1 x 21

Table 3. (*continued*)

Date	Fjord	Depth (m)	Stage	N
July 12	Nordfjorden	10-0	CV	1 x 30
		40-20	CV	1 x 30 + 1 x 7
	Saltfjorden	40-20	CV	1 x 7

2.2 High Performance Liquid Chromatography (HPLC)

The method used for separation and identification of copepod and phytoplankton pigments was HPLC, a method which separates compounds based on different affinities for a stationary and a mobile phase. Separation takes place in the column, where samples are injected along with the mobile phase. When using a C_{18} column, compounds are separated based on polarity and molecular shape (Garrido et al., 2011, p. 170). The most polar, and smallest, compounds will leave the column first together with the polar mobile phase. Non-polar compounds will have greater affinity for the stationary phase inside the column and will leave the column at a later time. When leaving the column, the compounds absorbance is measured to quantify concentration in the injected sample. The compounds can then be recognized based on retention time and wavelength of maximum absorption. See Garrido et al. (2011) for a detailed review on HPLC for phytoplankton pigments.

The copepod and phytoplankton pigments were analyzed at Nord University in Bodø, using an HPLC (1100 series, Agilent) equipped with a quaternary pump, thermostatted column compartment and diode array detector, according to the method by Egeland (2012). Detection wavelengths were 390, 420, 450 and 480 nm. Two identical C18 columns were used (ACE, 4.6 x 250 mm, with 5 µm packing), for better separation. The composition of the mobile phase changed from start to finish (gradient elution). The mobile phase was introduced at a constant flow rate of 0.5 ml min^{-1} in the following order (minutes, % solvent A, % solvent B, % solvent C, % solvent D); (0, 19.9, 80.0, 0.0, 0.1), (60, 0.0, 69.9, 30.0, 0.1), (100, 0.0, 30.0, 50.0, 20.0), (110, 0.0, 0.0, 40.0, 60.0), (120, 0.0, 99.9, 0, 0.1), (130, 19.9, 80.0, 0.0, 0.1). Solvent A was 1 M ammonium acetate, solvent B was methanol, solvent C was acetone and solvent D was hexane.

Phyto- and zooplankton pigments from summer 2018 were extracted and analyzed 12 and 7 months after collection, respectively. Phyto- and zooplankton pigments from spring

2019 were analyzed one to three months after collection, while phyto- and zooplankton pigments from Saltfjorden and Nordfjorden were analyzed throughout the sampling period.

2.2.1 Copepod pigments

The copepods were removed from the aluminum foil with a steel spatula and crushed in acetone to extract the pigments from the body tissues. The lab was kept dark during processing to avoid pigment degradation from light exposure. The acetone pigment extract and the pale copepods were then filtered through a small glass filter funnel with porosity of 4, to prevent any animal parts from entering the HPLC system. The acetone in the pigment extract was evaporated to dryness using a rotary evaporator (Heidolph instruments). The temperature of the bath did not exceed 30°C to avoid pigment degradation. After evaporation, the dry pigments were flushed with nitrogen to avoid degradation from exposure to oxygen, before 0.60 ml of acetone was added to the flask. The pigment extract was then transferred to quartz cuvettes (micro cell type 115-QS, light path 10 mm, Hellma®). Pigment absorption was measured in a UV-visible spectrophotometer (Varian Cary 100, Agilent). The pigment extract was then transferred from the cuvette to the flask and evaporated with the rotavapor. The pigment left in the flask was flushed with nitrogen after evaporation. 0.20 ml of acetone was added to the flask and then transferred to a 2 ml HPLC vial with 250 µl insert and flushed gently with nitrogen. A volume of 50.0-150 µl was injected into the HPLC from each vial, depending on concentration measured with the spectrophotometer.

The amount of astaxanthin in the sample replicates was determined from the formula:

$$mg\ astaxanthin = \frac{volume\ (mL) \times absorbance}{absorption\ coefficient\ (l\ g^{-1}\ cm^{-1})}$$

The absorption coefficient, 206 l g^{-1} cm^{-1} for astaxanthin in acetone, was retrieved from Egeland (2011 p. 732). Astaxanthin concentrations were calculated as µg individual $copepod^{-1}$ (µg $ind.^{-1}$).

The different forms of astaxanthin were grouped into free astaxanthin, astaxanthin monoesters and astaxanthin diesters, based on retention time (free astaxanthin retention time = 64 min, astaxanthin monoesters retention time = 82-95 min, astaxanthin diesters retention time 95-110 min) and peak shape, where peak shape for astaxanthin diesters was more narrow

than astaxanthin monoesters and free astaxanthin, due to less hydrogen bonding to the stationary phase.

2.2.2 Phytoplankton pigments

Filters were individually removed from the freezer, cut into 1 mm pieces, and placed in glass media bottles containing acetone. The volume of acetone was 2.0-3.0 ml for the 25 mm filters, and 8.0 ml for the 90 mm filters. It was made sure that the filters were completely covered with acetone to ensure that all pigments were extracted from the filters. For the 90 mm filters, seawater had to be accounted for in the total volume, since the filters were frozen with a substantial amount of water (which was not the case for the 25 mm filters). Ideally, each filter should be weighed after filtration, but this was not done. Instead, the weight of seawater was measured in one filter, then converted to ml seawater using a density of 1.0265 g/cm^3. The solvent for extraction was then estimated to consist of 70 % acetone. The filters in acetone were flushed with nitrogen before being placed back in the freezer. After 24 h the pigment extract was filtered using syringe filters (GHD membrane, diameter 25 mm, 0.2 µm pore size, Acrodisc®), transferred to HPLC vials and flushed with nitrogen. A volume of 150-400 µl was injected into the HPLC from each vial.

Pigments were recognized based on absorption spectra (spectral shape and wavelength) and retention time from known standards (CaroteNature, or isolated pigments donated by prof. S. Liaaen-Jensen Norges tekniske-naturvitenskapelige universitet, Trondheim). Pigment concentrations in the samples were determined by setting up a 5-point calibration curve with known standards. The pigment standards identities were confirmed, and concentrations measured, with spectrophotometry. Absorption coefficients for the pigment standards were retrieved from Egeland et al. (2011). Phytoplankton phyla were identified based on pigment signatures from Jeffrey et al. (2011). Because of uncertainty regarding concentrations calculated based on extinction coefficients, all concentrations will be reported with two significant figures.

The HPLC method did not separate chlorophyll c_1 and c_2 or zeaxanthin and lutein, which will be referred to as chlorophyll c_1+c_2 and zeaxanthin/lutein respectively. *Cis*- and *trans*-forms were combined when calculating concentrations. Concentrations were expressed as µg l^{-1} seawater.

2.3 Data analysis

R (R Core Team, 2016) was used for all statistical analysis and the R package ggplot2 (Wickham, 2009) was used to produce figures. Non-parametric tests were used due to non-normal distribution of the data, unequal variances, and relatively small sample sizes. Significance was accepted at $p < .05$, and highest level of significance set to $p = .001$.

Depths were categorized into surface (20-0 m), mid (40-20 m) and deep (below 100 m). Samples from station 1, summer 2018, were put in the surface category. Kruskal-Wallis one-way analysis of variance tests (Kruskal & Wallis, 1952) were used to test for differences in copepod astaxanthin concentration between depths and stages, and percentage of astaxanthin forms between depths and stages. A Wilcoxon signed rank test (Wilcoxon & Wilcox, 1964) was used to test for differences in copepod astaxanthin concentration between surface and mid depths from the cruise in spring 2019, where no deep samples were collected.

Principal Component Analysis (PCA) (Jackson, 2005) on scaled data was performed to assess relationships between copepod astaxanthin and phytoplankton pigment concentrations from summer 2018. Phytoplankton pigment concentrations from 5 m (10 m from station 9), and copepod astaxanthin concentration from surface and mid depths were used in the PCA. Spearman's correlations (Spearman, 1904) were used to establish any significant correlations between copepod astaxanthin and phytoplankton pigment concentrations.

Spearman's rank correlations were used to evaluate potential relationships between copepod astaxanthin concentration and percentage of astaxanthin forms from summer 2018 and spring 2019. Only astaxanthin concentration in *Calanus* spp. CVs were used in the correlation tests. Spearman's rank correlations were also used when evaluating relationships between chlorophyll *a* concentration and accessory pigments from Saltfjorden and Nordfjorden. Copepod astaxanthin concentration from the fjords were not used in any statistical tests, because of the low number of samples containing CVs. Changes in copepod astaxanthin concentration and astaxanthin forms with starvation was calculated as percentage change compared to the control groups. Differences in copepod astaxanthin concentration with time of day from the cruises in 2018 and 2019 were inspected visually.

Tables with correlation test scores and *p*-values are given in appendix A.

3.0 Results

3.1 Cruise, summer 2018

3.1.1 Astaxanthin concentration in Calanus spp.

Three forms of astaxanthin were identified in *Calanus* spp.; free astaxanthin, including its *cis* form (see appendix B fig. 15a & b for absorption spectra), astaxanthin monoesters and astaxanthin diesters (fig. 7). Esterified astaxanthin was always the dominant form, ranging from 82-96 % of total astaxanthin (fig. 8). Total astaxanthin content ranged from 0.021-0.41 µg ind.$^{-1}$ in *Calanus* spp. CVs collected outside the coast of Vesterålen and Tromsø, summer 2018 (fig. 6). The least pigmented copepods were residing in the deep layer (below 100 m), were total astaxanthin content averaged (mean ± SD) 0.038 ± 0.009 µg ind.$^{-1}$. Copepods with the highest pigmentation were collected from surface and mid layers at the northernmost stations on Tromsøflaket (stations 7, 9, 10 and 11), where total astaxanthin ranged from 0.14-0.41 µg ind.$^{-1}$. A significant difference was found between astaxanthin concentration from the three depth layers ($\chi^2 = 8.8$, $df = 2$, $p < .001$), due to the low values found in copepods from the deep layer (see appendix C, fig. 16 for boxplot).

Total astaxanthin concentration in the copepods was negatively correlated with percentage of free astaxanthin (*rho* = -0.762, $p < .001$) and astaxanthin monoesters (*rho* = -0.824, $p < .001$), and positively correlated with percentage of astaxanthin diesters (*rho* = 0.860, $p < .001$). There were significant differences in astaxanthin forms between the depth layers, free astaxanthin ($\chi^2 = 13.5$, $df = 2$, $p = 0.001$), astaxanthin monoesteres ($\chi^2 = 32.0$, $df = 2$, $p < 0.001$), and astaxanthin diesters ($\chi^2 = 32.0$, $df = 2$, $p < 0.001$), due to the higher percentage of free astaxanthin and astaxanthin monoesters, and lower percentage of astaxanthin diesters in copepods from the deep layers (see appendix C, fig. 17 for boxplots).

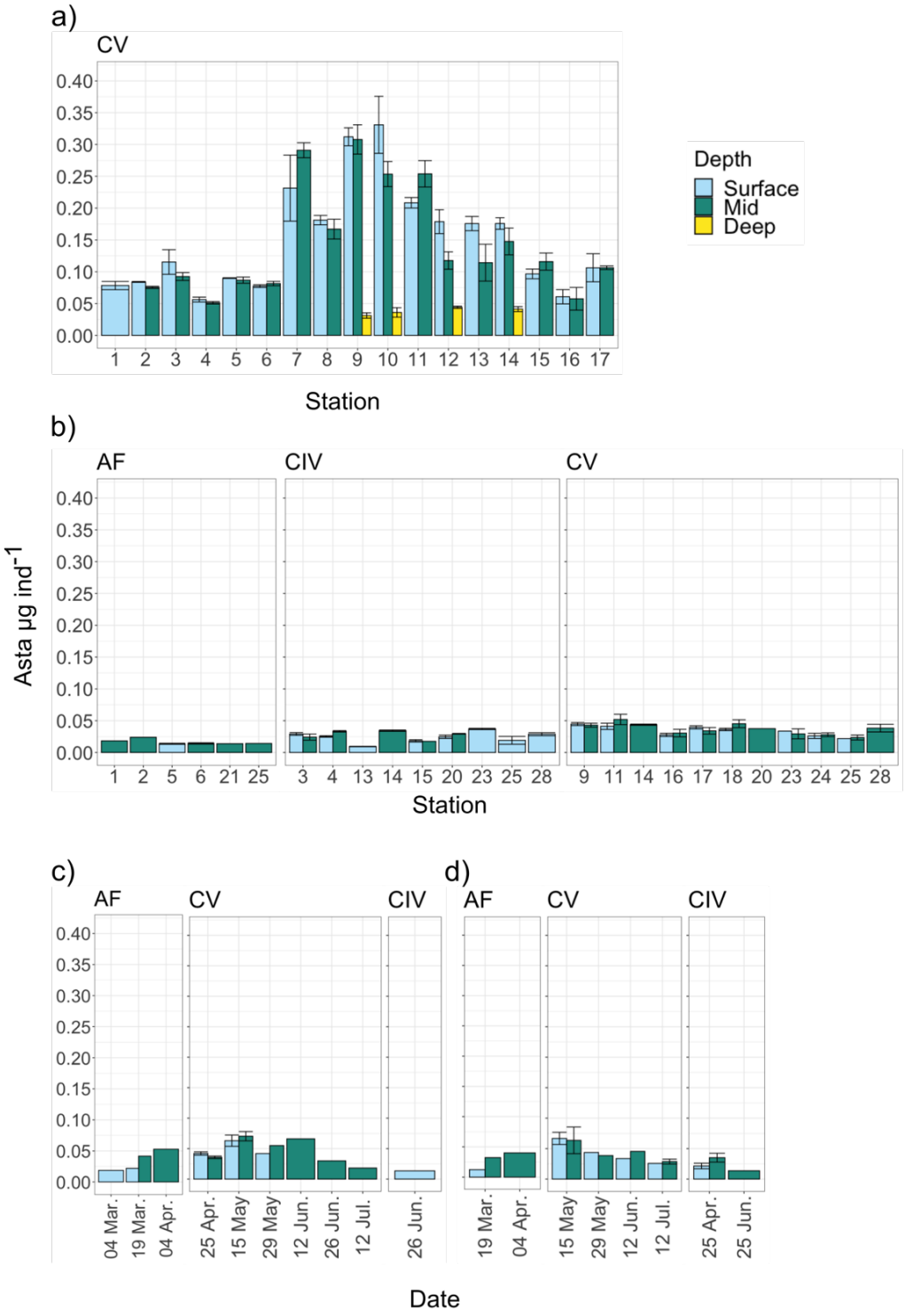

Figure 6. Concentration of astaxanthin in *Calanus* spp. at different depths. a) Summer 2018, all stages CV, b) spring 2019, stages AF, CIV and CV, and from c) Saltfjorden and d) Nordfjorden, from late winter to summer 2019, stages AF, CV and CIV. Bars represent the mean (± SE) of three replicate samples, except for stations and dates were N = 1 or 2 (see Methods for N, table 1, 2 and 3).

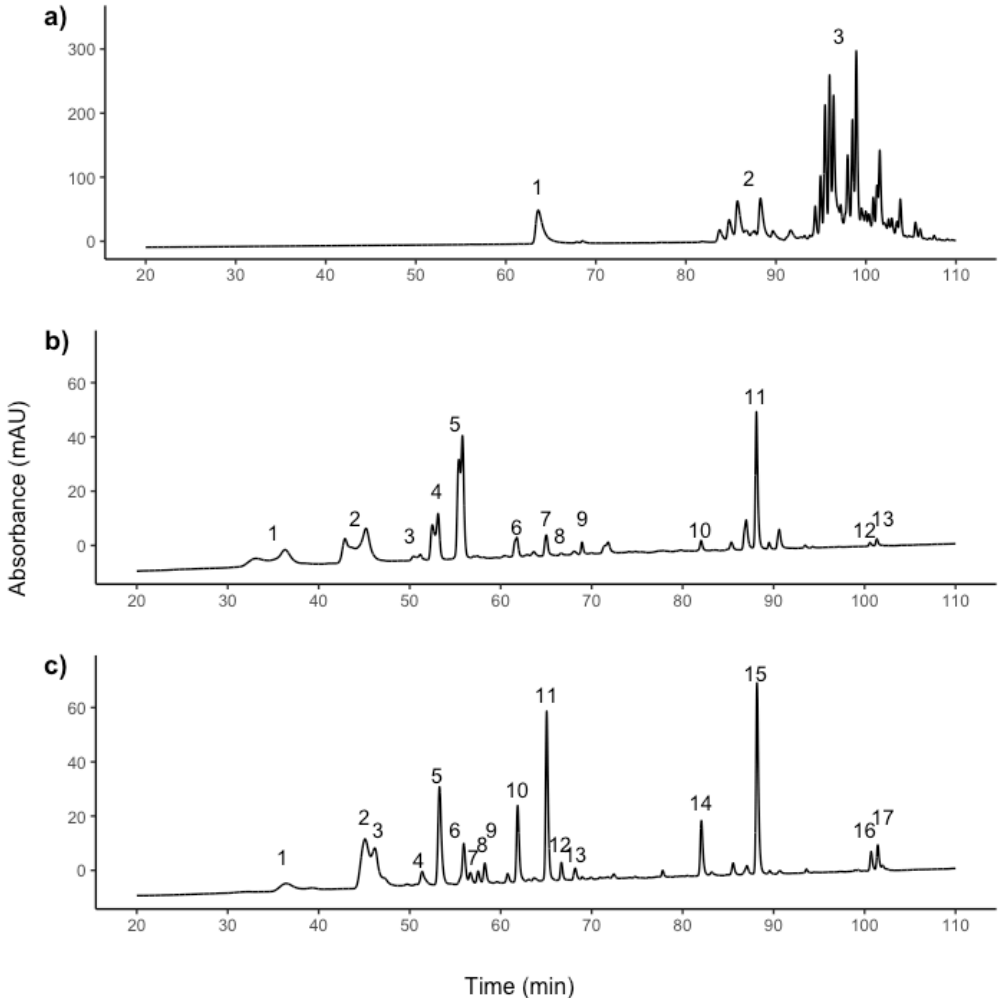

Figure 7. Chromatograms with retention time (minutes) and absorbance (mAU). a) *Calanus* spp. pigments from summer 2018, station 10. 1: free astaxanthin, 2: astaxanthin monoesters, 3: astaxanthin diesters. b) phytoplankton pigments from summer 2018, station 10. Pigments identified; 1: chl c_3, 2: chl c_2+c_1, 3: but-fuco, 4: fuco, 5: hex-fuco, 6: diadino, 7: allo, 8: diato, 9: zea/lut, including *cis*-zea/lut, 10: chl *b*, 11: chl *a*, 12: βε-car, 13: ββ-car. c) phytoplankton pigments from 5 m depth, Nordfjord, May 15. 1: chl c_3, 2: chl c_1+c_2, 3: peri, 4: but-fuco, 5: fuco, 6: hex-fuco, 7: c-neo, 8: pras, 9: viola, 10: diadino, 11: allo, 12: diato, 13: zea/lut, including *cis*-zea/lut, 14: chl *b*, 15: chl *a*, 16: βε-car, 17: ββ-car.

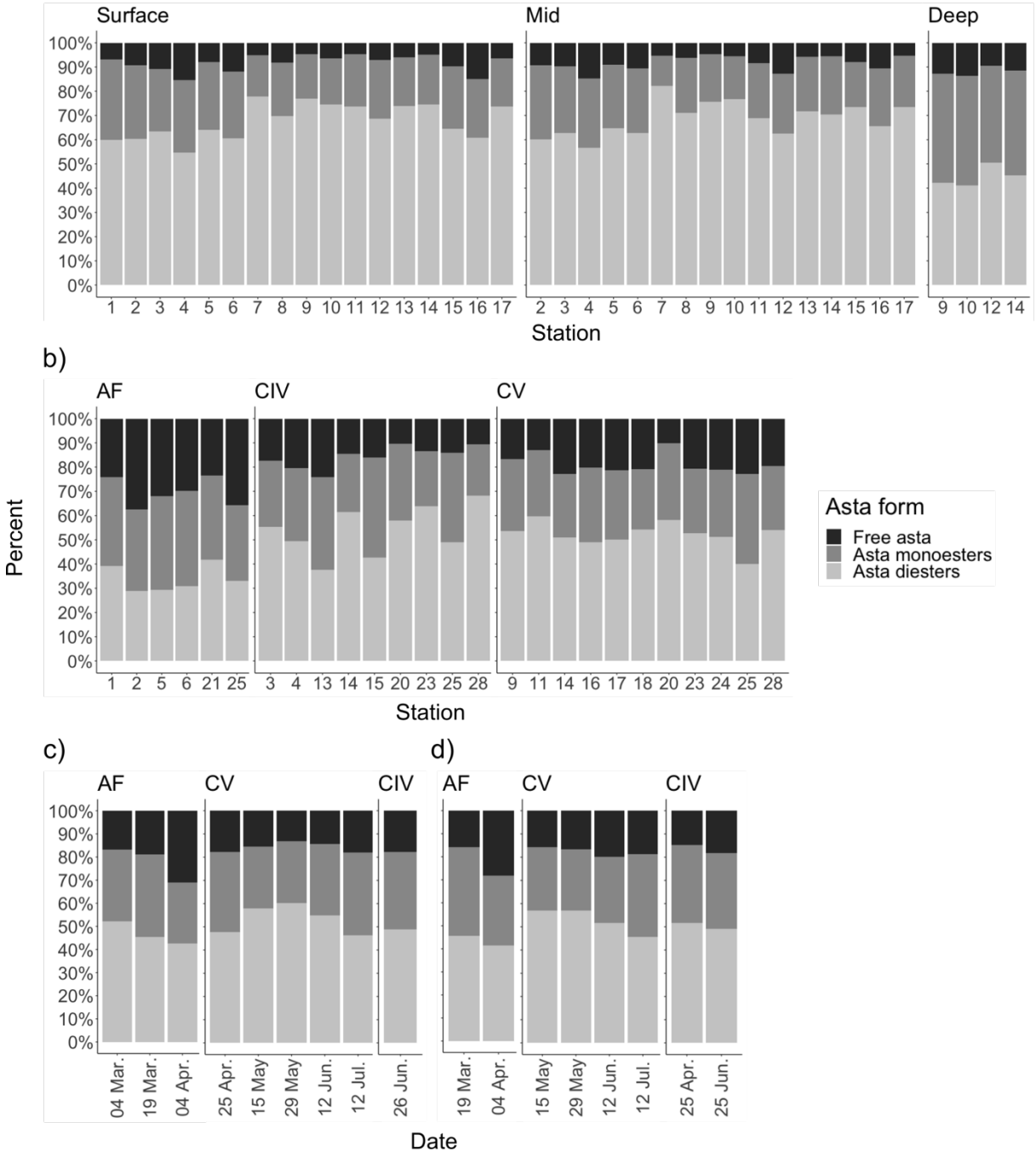

Figure 8. Percentage of astaxanthin forms in *Calanus* spp. collected a) summer 2018, all stages CV, b) spring 2019, stages AF, CV and CIV c) Saltfjorden, stages AF, CV and CIV and d) Nordfjorden, stages AF, CV and CIV. Each bar represents means from 1-6 replicate samples (see Methods for N, table 1, 2 and 3).

3.1.2 Variation in pigmentation with time of day

There was no apparent pattern in *Calanus* spp. astaxanthin concentration with time of day (fig. 9). Highly pigmented individuals were collected both at morning and evening stations.

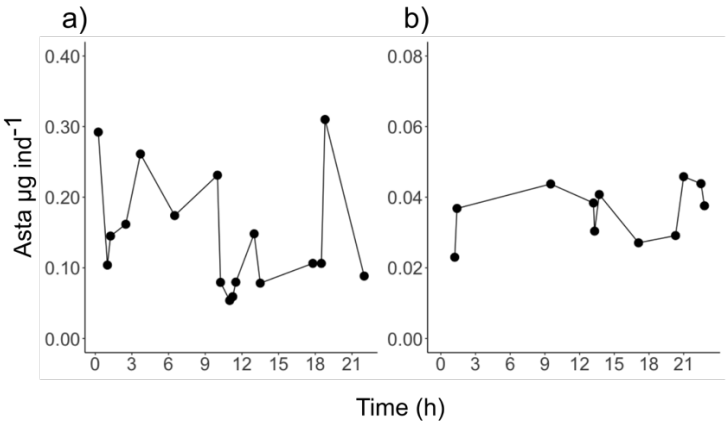

Figure 9. Astaxanthin concentration *Calanus* spp. CVs (µg ind.$^{-1}$) with time of day from a) summer 2018 and b) spring 2019. Each point represents the mean of 1-6 replicate samples (for N see Methods, table 1 and 2). Note the different scales on the y-axis.

3.1.3 Phytoplankton pigments

A total of 13 phytoplankton pigments were identified (fig. 7), excluding chlorophyll degradation products, such as chlorophyllide *a*, pheophorbide *a*, pheophytin *a*, pyropheophorbide *a* and pyropheophytin *a*, which were present in all samples. Unidentified pigments accounted for between 3-14 % of total pigments (calculated from peak area). The pigments 9′-*cis*-neoxanthin, violaxanthin and prasinoxanthin were categorized as unidentified as they could only be recognized based on retention time, and not absorption spectra. Pigments with low retention times (chlorophyll c_3, chlorophyll c_1+c_2, 19′-butanoyloxyfucoxanthin, fucoxanthin, 19′-hexanoyloxyfucoxanthin and diadinoxanthin) showed an abnormal double peak-shape. When calculating concentrations, these double peaks were treated as one, as absorption spectra indicated that both peaks were identical pigments (see appendix B, fig. 15c-h for absorption spectra).

Highest concentrations of chlorophyll *a* (> 2.0 µg l^{-1}) were found at stations 8, 9, 10 and 12 on Tromsøflaket, and total carotenoids (> 1.5 µg l^{-1}) at stations 9, 10 and 12 (fig. 10). At stations were water from deeper depths were filtered, β,β-carotene and zeaxanthin/lutein

generally had higher concentrations in the deeper layers (20-50 m) than at surface (0-10 m). At station 8, concentration of β,β-carotene was almost doubled from the surface (0.025 µg l^{-1}) to 50 m (0.046 µg l^{-1}).

The first principal component explained 57 % of the variation in the data and the second principal component explained 27 %, to a combined total of 84 %. The PCA biplot revealed positive correlations between all pigments (fig. 11). The strongest relationship between copepod astaxanthin concentration and phytoplankton pigment was with chlorophyll *a* concentration, which were significantly correlated (*rho* = 0.63, *p* = .011). It is worth noting that at station 11, *Calanus* spp. pigmentation was high (> 2.0 µg in^{-1}), while chlorophyll a concentration was relatively low (chlorophyll *a* <1.0 µg l-1). The biplot illustrates a clustering of pigments chlorophyll *b*, alloxanthin, fucoxanthin, β,ε-carotene and β,β-carotene on one end, and chlorophyll c_1+c_2, chlorophyll c_3, 19′-hexanoyloxyfucoxanthin, 19′-butanoyloxyfucoxanthin, diadinoxanthin, diatoxanthin and zeaxanthin/lutein on the other. In addition to chlorophyll *a*, copepod astaxanthin concentration was significantly correlated with alloxanthin (*rho* = 0.58, *p* = .024) and zeaxanthin/lutein (*rho* = 0.58, *p* = .024), representing variables from two different clusters (see appendix A, table 5, for all correlation tests).

The two pigment clusters could be grouped together based on stations where peak concentrations occurred. At station 10 and 12, high concentrations of 19′-hexanoyloxyfucoxanthin, chlorophyll c_3 and 19′-butanoyloxyfucoxanthin were found, indicating the presence of haptophytes, while zeaxanthin/lutein indicates the presence of either cyanobacteria or green algae. At station 8, high concentrations of chlorophyll *b*, β,ε-carotene and β,β-carotene were found, which indicates the presence of green algae, while alloxanthin indicates the presence of cryptophytes. The high concentration of fucoxanthin at station 9 indicates the presence of diatoms, although this pigment is present in many phytoplankton phyla from the red algal lineage.

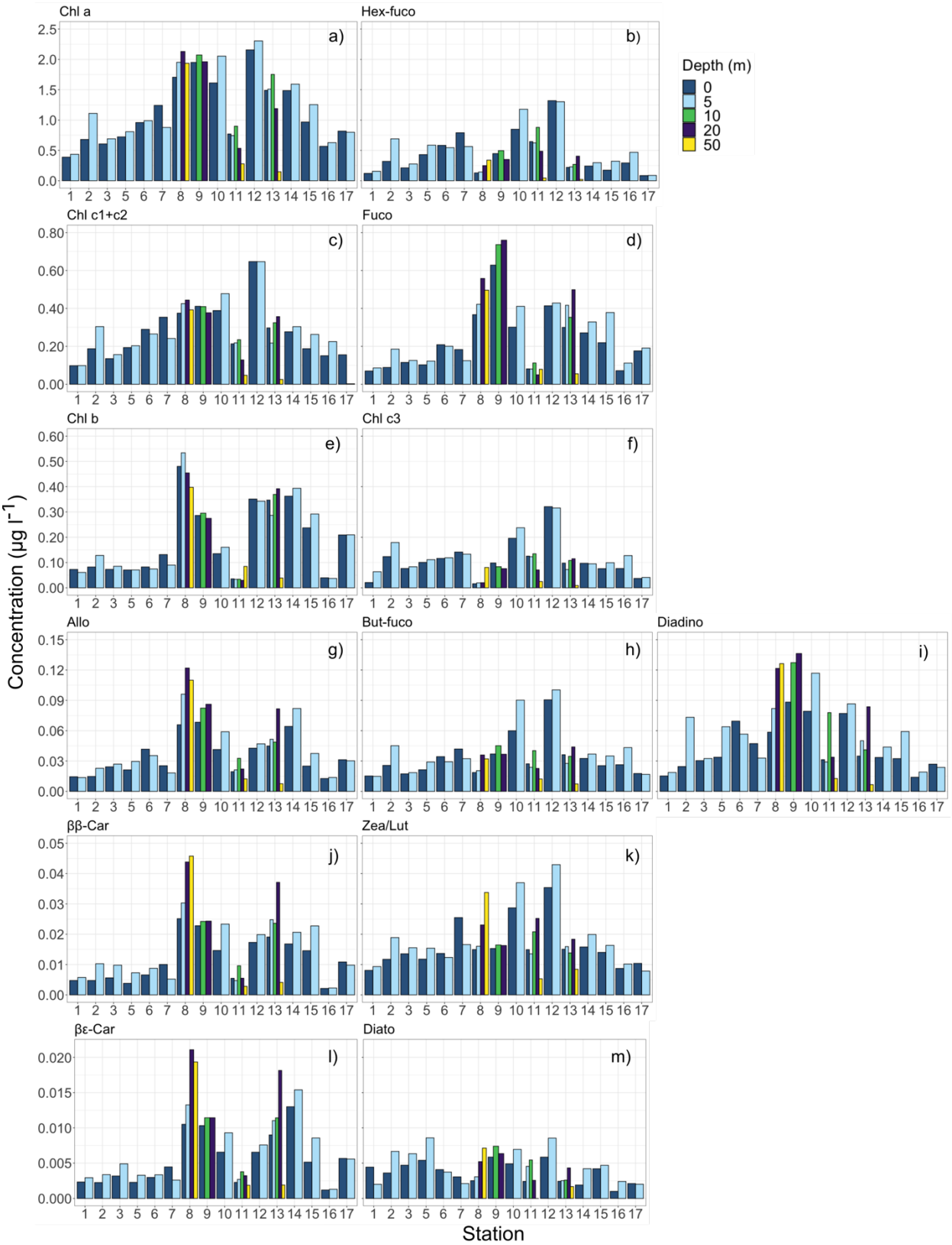

Figure 10. Concentration of phytoplankton pigments collected from different depths during summer 2018. Each bar represents the concentration from one sample. a) Chl *a*, b) hex-fuco, c) chl c_2+c_2, d) fuco, e) chl *b*, f) chl c_3, g) allo, h) but-fuco, i) diadino, j) ββ-car, k) zea/lut, l) βε-car, m) diato. Note the different scales on the y-axis.

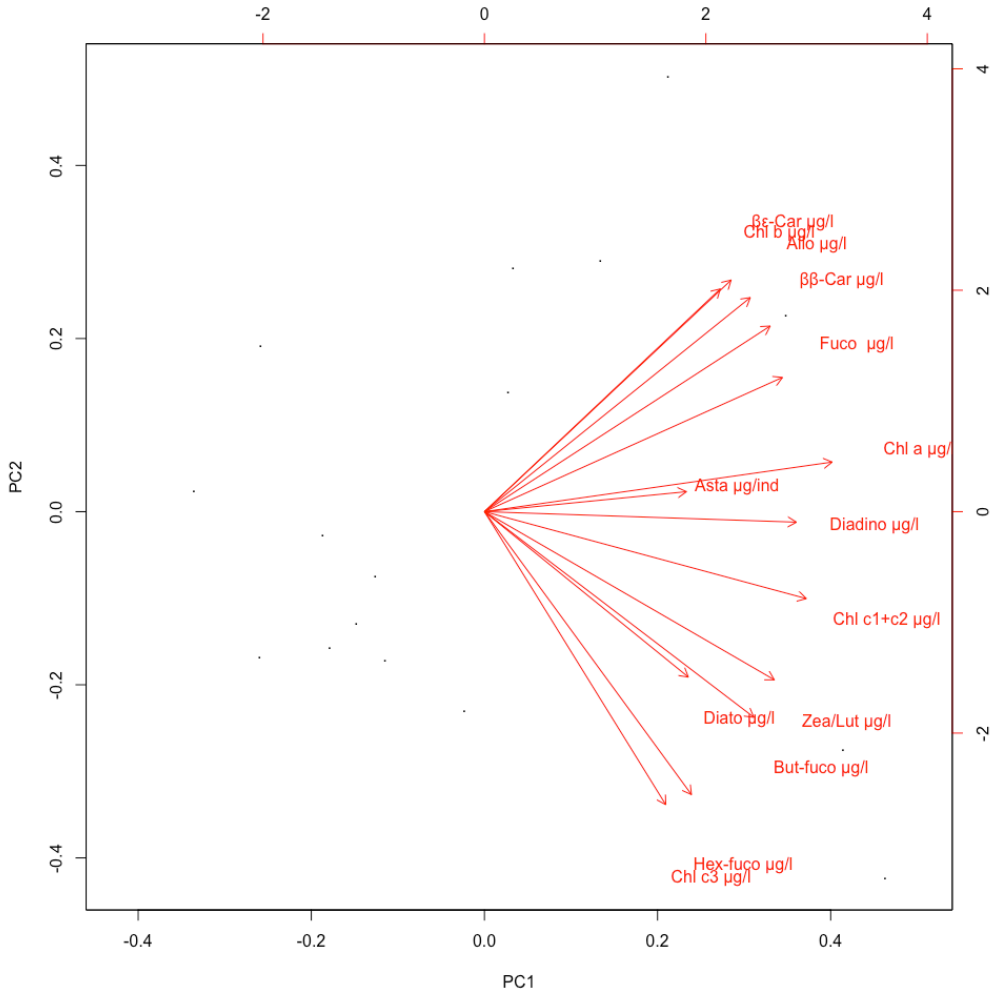

Figure 11. PCA biplot of surface phytoplankton pigments and astaxanthin in *Calanus* spp. CVs from summer 2018. Each dot represents individual samples (16 in total).

The phytoplankton community composition differed somewhat between stations on- and off-shelf, with a higher contribution of haptophytes in the off-shelf stations (see appendix D, fig. 20a).

3.2 Cruise, spring 2019

3.2.1 Astaxanthin concentration in Calanus spp.

Average astaxanthin concentration in *Calanus* spp. ranged from 0.014-0.068 µg ind.$^{-1}$ in CVs, from 0.0090-0.038 in µg ind.$^{-1}$ in CIVs, and from 0.012-0.024 µg ind.$^{-1}$ in females. A

significant difference was found between the means of total astaxanthin in the three different developmental stages (χ^2=36.2, *df*=2, $p < .001$) (see appendix C, fig. 18a, for boxplot). The highest concentrations were found in CVs at station 11, located on the continental shelf, and lowest concentrations were found in CIVs at station 13, located off-shelf (fig. 6). There was no significant difference in astaxanthin concentration in CVs from surface and mid depth layers ($p = .438$) (appendix C, fig. 18b, for boxplot).

Percentage of esterified astaxanthin dominated in all three stages (fig. 8). There was significant differences in free astaxanthin ($\chi^2 = 33.4$, $df = 2$, $p < .001$) astaxanthin monoesters ($\chi^2 = 17.2$, $df = 2$, $p < .001$) and astaxanthin diesters ($\chi^2 = 24.2$, $df = 2$, $p < .001$) between stages, mostly due to differences between females compared to CIVs and CVs (see appendix C, fig. 19, for boxplots). Copepod astaxanthin concentration was negatively correlated with percentage of free astaxanthin (*rho* = -0.394, $p = .005$) and positively correlated with percentage of astaxanthin diesters (*rho* = 0.387, $p = .005$). There was no correlation between copepod astaxanthin concentration and percentage of astaxanthin monoesters (*rho* = -0.107, $p = .458$).

3.2.2 Variation in Astaxanthin with time of day

No clear pattern was observed for variation in *Calanus* spp. CV pigmentation with time of day (fig. 9).

3.2.3 Change in astaxanthin with starvation

Astaxanthin concentration in *Calanus* spp. CVs starved for 24 h in the dark was on average 28 % lower than controls (table 4). Pigment loss was not as evident in the 12 h light:dark treatment, with on average 12 % lower in starved animals than controls. Percentage of astaxanthin diesters decreased in both these treatments, while the percentage of free astaxanthin and astaxanthin monoesters increased. Animals starved for 24 h in light showed the opposite pattern; astaxanthin concentration and percentage of astaxanthin monoesters and diesters were slightly higher, while free astaxanthin was lower than controls. Slight loss of pigmentation was detected in the antennules and around oil sac in starved individuals (fig 12).

Table 4. Astaxanthin concentration and percentage of astaxanthin forms in *Calanus* spp. CVs starved for 24 h with different light treatments, compared to control groups (mean ± SD). N = number of samples x individuals in each replicate.

Treatment	N	Asta (µg ind.$^{-1}$) **Change**	Free asta (%) **Change**	Asta monoesters (%) **Change**	Asta diesters (%) **Change**
12 h light:dark					
Control	15 x 30	0.045 ± 0.002	17 ± 5	28 ± 2	55 ± 5
Starved	2 x 20 + 1 x 18	0.040 ± 0.001	23 ± 3	34 ± 6	43 ± 9
		-12 %	**+31 %**	**+24 %**	**-22 %**
24 h dark					
Control	15 x 30	0.035 ± 0.005	20 ± 1	29 ± 2	51 ± 2
Starved	3 x 20	0.025 ± 0.008	21 ± 1	33 ± 1	46 ± 2
		-28 %	**+4 %**	**+15 %**	**-10 %**
24 h light					
Control	15 x 30	0.032 ± 0.007	21 ± 0	27 ± 1	53 ± 1
Starved	2 x 20 + 1 x 19	0.037 ± 0.005	17 ± 1	28 ± 1	55 ± 2
		+13 %	**-19 %**	**+5 %**	**+5 %**

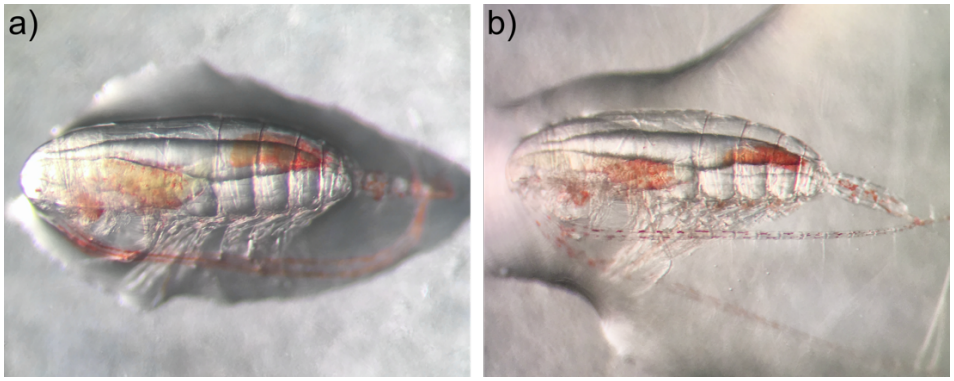

Figure 12. *Calanus* sp. from spring 2019, station 14. a) Initial pigmentation b) after 24 h of starvation.

3.3 Seasonal fjord study

3.3.1 Temperature and Salinity

Temperature and salinity varied seasonally (fig. 13). Temperature at mid depths were higher than the surface layer in late winter, and lower mid-spring to mid-summer in both fjords, while salinity was greater at mid depths throughout the seasons. Salinity was negatively correlated with chlorophyll *a* concentration in Saltfjorden (*rho* = -0.729, *p* = .002) and Nordfjorden (*rho* = -0.544, *p* = .03), while temperature was positively correlated with chlorophyll *a* concentration in Saltfjorden (*rho* = 0.735, *p* = .002) but not in Nordfjorden (*rho* = 0.194, *p* = .47).

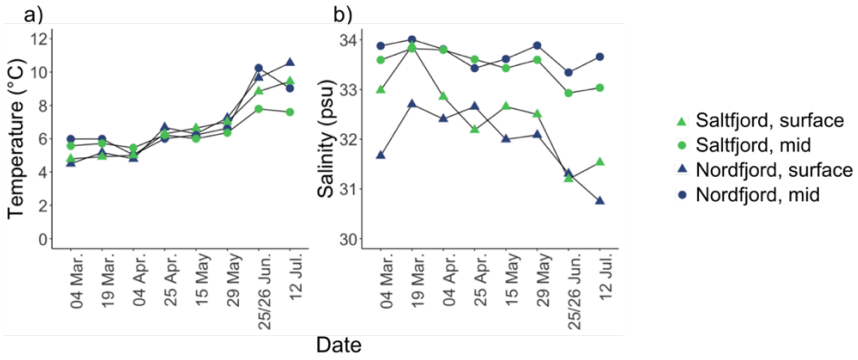

Figure 13. Temperature and salinity from surface (10-0 m) and mid (40-20 m) layers in Saltfjorden and Nordfjorden from late winter to mid-summer 2019. Sampling dates are identical for both fjords except from June 25 (Nordfjorden) and June 26 (Saltfjorden). Each point represents the mean of 10 values for surface (10-0 m) and 20 values for mid (20-40 m) depths.

3.3.2 Seasonal development of astaxanthin in Calanus spp. populations

Astaxanthin concentration in *Calanus* spp. from the Saltfjorden population ranged from 0.019-0.053 µg ind.$^{-1}$ in females and 0.018-0.085 µg ind.$^{-1}$ in CVs (fig. 6). In the Nordfjorden population, astaxanthin concentration ranged from 0.012-0.040 µg ind.$^{-1}$ in females, 0.013-0.048 µg ind.$^{-1}$ in CIVs and 0.025-0.10 µg ind.$^{-1}$ In CVs. Astaxanthin concentrations in CVs were highest in mid-May, then decreased towards July in both fjords.

Percentage of the three different astaxanthin forms varied seasonally and between stages in both populations (fig. 8). Astaxanthin diesters was the dominant, and most dynamic, form of astaxanthin in all stages, ranging from 41-52 % in females, from 48-54 % in CIVs

and from 45-63 % in CVs from both fjords. Percentage of astaxanthin diesters and monoesters decreased in females from March to April, while percentage of free astaxanthin increased. Percentage of astaxanthin diesters and monoesters in CVs increased from April to May, then decreased towards July.

3.3.3 Phytoplankton pigments

16 phytoplankton pigments were identified from Saltfjorden and 17 from Nordfjorden (fig. 7), excluding chlorophyll *a* degradation products which were present in minor amounts. Unidentified pigments accounted for between 0-5 % of total pigments. Since peridinin overlaps with chlorophyll c_1+c_2, there must be a sufficient amount in the sample for the pigment to be identified, which was only the case for the Nordfjorden samples.

There were often large variations in concentration of pigments between surface and mid depths in the fjords (fig. 14). In Nordfjorden, the highest concentrations of chlorophyll *a* was found in April, with 4.7 µg l^{-1} at 5 m, and 2.9 µg l^{-1} at 30 m. Chlorophyll *a* maximum was reached two months later in Saltfjorden, with 3.9 µg l^{-1} at 5 m and 1.4 µg l^{-1} at 30 m.

In Saltfjorden, chlorophyll c_1+c_2 showed the strongest correlation with chlorophyll *a* concentration (*rho* = 0.981, $p < .001$), followed by diadinoxanthin (*rho* = 0.96, $p < .001$) and fucoxanthin (*rho* = 0.94, $p < .001$), indicating that phytoplankton blooms were mostly dominated by algae from the red algal lineage, such as diatoms. This was also the case in Nordfjord; concentrations of chlorophyll *a* showed the strongest correlations with chlorophyll c_1+c_2 (*rho* = 0.987, $p < .001$), diadinoxanthin (*rho* = 0.969, $p < .001$) and fucoxanthin (*rho* = 0.959, $p < .001$).

Although the progression of the phytoplankton community differed between Saltfjorden and Nordfjorden, the contribution of each pigment to total pigment concentrations were similar on May 15 (see appendix D, fig. 20b & c). Diatoms, as indicated by fucoxanthin, contributed less to the total phytoplankton community compared to the other dates. Concentrations of chlorophyll *b*, β,ε-carotene, alloxanthin, 19′-butanoyloxyfucoxanthin, 19′-hexanoyloxyfucoxanthin, diatoxanthin, 9′-*cis*-neoxanthin, prasinoxanthin, violaxanthin and zeaxanthin/lutein all peaked in mid-May in both fjords, indicating a diverse phytoplankton community with green algae, cryptophytes and haptophytes. In addition, dinoflagellates, as indicated by peridinin, were present in Nordfjorden from May. Highest concentrations of β,β-carotene in the surface were found in May in Nordfjorden and in June in Saltfjorden.

Figure 14. Phytoplankton pigments (µg l^{-1}) collected from surface (5 m) and mid (30 m) depths from Saltfjorden and Nordfjorden. Sampling dates are identical for both fjords except from June 25 (Nordfjorden) and June 26 (Saltfjorden). Each point represents values from one replicate sample. Note the different scales on the y-axis. a) Chl *a*, b) chl *b*, c) chl c_1+c_2, d) fuco, e) peri, f) chl c_3, g) allo, h) but-fuco, i) diadino, j) hex-fuco, k) ββ-car, l) βε-car, m) diato, n) c-neo, o) pras, p) viola, q) zea/lut.

4.0 Discussion

4.1 Spatial and temporal variations in astaxanthin concentration in *Calanus* spp.

Large variations were found in the astaxanthin content in *Calanus* spp. High values (up to 4.1 µg ind.$^{-1}$) were found in CVs from Tromsøflaket, summer 2018 (fig. 6). Such high copepod pigmentation was not encountered during the cruise in spring 2019, nor throughout the productive season in Saltfjorden and Nordfjorden and have not been reported before for marine copepods (to the authors knowledge). Astaxanthin concentration in CVs from spring 2019 (mean 0.037 µg ind.$^{-1}$) were comparable to the lowest values from summer 2018, which were found in CVs from below 100 m (mean 0.038 µg ind.$^{-1}$). These results indicate that the copepods accumulate astaxanthin during the productive season, with increasing concentrations toward summer. However, in Saltfjorden and Nordfjorden, astaxanthin concentration in the copepods increased from winter to spring, and then decreased slightly towards summer. The lower values found in the copepods in summer might be explained by the occurrence of a second generation that was sampled and did not yet accumulate much astaxanthin. There were two peaks in nauplii abundance, one in April and one in June, in both fjords (Solberg, 2019), which supports this assumption. Samplings should have been extended towards fall to investigate if astaxanthin concentration increased with development in this later generation.

Other studies have reported astaxanthin concentrations of 0.1-0.2 µg mg dry weight^{-1} in *C. finmarchicus* CVs from Disko bay (Hylander et al., 2015), which translates to 0.03-0.05 µg ind.$^{-1}$ using an estimated dry weight of 250 µg ind.$^{-1}$, retrieved from Bergvik et al. (2012). *C. pacificus* females have been reported with values ranging from 0.0114-0.0924 µg ind.$^{-1}$ (Juhl et al., 1996). These values are similar to the concentrations found in *Calanus* spp. collected from Norwegian coastal waters in spring.

Based on values from spring 2019, CVs contained on average 30 and 57 % more astaxanthin per copepod compared to CIVs and females, respectively. The large differences in pigmentation between CVs and females were most likely due to differences in sampling locations, as females were generally collected at off shelf stations where the seasonal progression of the phytoplankton bloom often lags behind compared to coastal areas (Broms & Melle, 2007). The copepods collected at coastal stations would thus have had more time to synthesize and accumulate astaxanthin from precursor molecules. In addition, females could accumulate astaxanthin to a lesser extent than CVs, as they generally do not enter diapause

and thus needs less antioxidant protection of lipid reserves. In the fjords, astaxanthin content in females increased while astaxanthin diesters decreased from March to April. This could be due to energy transfer (carbon and lipids) to the eggs, which would decrease the females lipid content and thus fraction of esterified astaxanthin (Hirche, 1996b).

Diurnal variations in pigmentation were not found in *Calanus* spp. collected off the coast of Northern Norway, from either summer 2018 or spring 2019 (fig. 9). These results, together with previous studies (Hylander et al., 2015), corroborate the assumption that *Calanus* spp. CVs do not exhibit synchronized feeding activity during the productive season in the Arctic and Subarctic (Basedow et al., 2010). Since both cruises took place at a time with little variation between day and night (approx. 19 h of daylight in May and 24 h daylight in June), it would be interesting to examine if diurnal changes exist earlier in spring or in fall, when the nights are longer.

4.2 Astaxanthin in *Calanus* spp. with regards to phytoplankton community and biomass

Astaxanthin accumulation in copepods have previously been reported to be dynamic and related to recent feeding history (Juhl et al., 1996; Sommer et al., 2006). Concentration of astaxanthin in *Calanus* spp. from summer 2018 was positively correlated with chlorophyll *a* concentration, which suggest an increased accumulation of astaxanthin when phytoplankton biomass is high. However, highly pigmented individuals were found at stations were chlorophyll *a* concentration was relatively low. This could be caused by a higher abundance of *Calanus* spp. at these stations, and thus larger grazing pressure on the phytoplankton. In CVs from Saltfjorden and Nordfjorden, highest concentrations were found on May 15 with average values of 0.067 and 0.065 µg ind.$^{-1}$, respectively. Although the chlorophyll *a* concentration, and thus phytoplankton biomass, was almost three times higher in Nordfjorden than Saltfjorden on May 15 (fig. 14), astaxanthin concentration per copepod was similar (fig. 6). Chlorophyll *a* concentration in both fjords reached higher values than any station from Tromsøflaket summer 2018. This demonstrates that phytoplankton biomass is not enough to predict where astaxanthin concentration in *Calanus* spp. will be highest. Interestingly, also the highest abundances of *Calanus* spp. were found on May 15, with 200 ind. m^{-3} from 40-20 m in Saltfjorden and 3 000 ind. m^{-3} from 10-0 m in Nordfjorden (Solberg, 2019). These differences in abundance could be attributable to an earlier development of the spring bloom in Nordfjorden (fig. 14), caused by an earlier stratification of the water column due to

meltwater (fig. 13). This is in agreement with previous studies showing that phytoplankton biomass largely determines the recruitment of *C. finmarchicus* (Plourde et al., 2008).

Although no abundance data is available from summer 2018 so far, considerably higher concentrations of *Calanus* spp. from surface net samples were observed at most stations compared to samples from the fjords. *C. finmarchicus* swarms, with abundances > 1 000 ind. m^{-3} (Basedow et al., 2019; Wishner et al., 1988) and grazing rates up to 219 ng chl *a* $ind.^{-1}$ day^{-1} (Meyer-Harms et al., 1999), would exert substantial grazing pressure on the phytoplankton community. Phytoplankton productivity may thus have been high at stations from summer 2018, while grazing pressure from the copepods would control phytoplankton biomass. Alternatively, the most pigmented individuals from summer 2018, could have recently been advected into an area with higher phytoplankton biomass, which would cause an immediate increase in astaxanthin concentration. If astaxanthin is as dynamic as previously reported (Juhl et al., 1996), the individuals in a swarm would not be able to sustain high pigmentation while rapidly depleting the phytoplankton standing stock.

No difference in astaxanthin concentration, or astaxanthin forms, in *Calanus* spp. from surface (10-0 m) and mid (40-20 m) depths were found (see Appendix C, fig. 16 and fig. 18 for boxplots). Astaxanthin concentration in the copepods from Nordfjorden was similar at surface and mid depths (fig. 6), while chlorophyll *a* concentration was almost four times higher at surface than mid depths (fig. 14). Ideally, astaxanthin content in the copepods should have been compared to concentration of chlorophyll *a* at different depths from each station from summer 2018. However, these results indicate that the copepods are capable of retaining its pigment for some time regardless of phytoplankton biomass. The significantly lower astaxanthin concentration, including astaxanthin diesters, in the copepods from below 100 m, indicate that *Calanus* spp. metabolizes the esters when starved. It thus seems likely that astaxanthin accumulation is related to lipid metabolism, as suggested by Schneider et al. (2016).

From summer 2018, most of the variance in astaxanthin content of the copepods could be explained by the phytoplankton pigments. Significant correlations were found between astaxanthin concentration in *Calanus* spp. and alloxanthin and zeaxanthin/lutein concentrations, indicative of cryptophytes and cyanobacteria or green algae, respectively. Interestingly, the precursor molecule β,β-carotene was not significantly correlated to astaxanthin concentration in the copepods. There is no obvious explanation for a positive

relationship between cryptophytes and astaxanthin in *Calanus* spp., as cryptophytes generally do not contain precursor molecules (neither does dinoflagellates with cryptophyte endosymbionts) (Jeffrey et al., 2011, pp. 45-55). The positive relationship between zeaxanthin/lutein and astaxanthin concentration *Calanus* spp. could be explained by the copepods ability to synthesize astaxanthin from zeaxanthin. However, these results should be interpreted with care, as these correlations could be caused by collinearity with chlorophyll *a*. Zeaxanthin/lutein concentration peaked concurrently with astaxanthin concentration in *Calanus* spp. from Saltfjorden and Nordfjorden, while β,β-carotene did not. It has previously been shown that copepods fed diets with different carotenoid compositions differed in astaxanthin concentrations (Caramujo et al., 2012). *Calanus* spp. could thus possibly have a higher assimilation efficiency for zeaxanthin compared to β,β-carotene, which would explain the concurrent peaks of zeaxanthin concentration and astaxanthin in *Calanus* spp.. However, more research is required to verify this.

Previous studies have shown that astaxanthin concentration in copepods were highest when the community was dominated by chlorophytes, dinoflagellates and diatoms (Andersson et al., 2003). Chlorophytes and diatoms could be present at the Tromsøflaket-stations, as indicated by high concentrations of chlorophyll *b* and fucoxanthin (fig. 10), although microscopic analysis would be necessary for accurate identification, since many phyla contain these pigments (Jeffrey et al., 2011, pp. 45-55). Dinoflagellates, however, were probably not abundant at any station, as indicated by the absence of peridinin.

Selective feeding by *C. finmarchicus* complicates the interpretation of the results, as a high concentration of one particular phylum does not necessarily mean they are ingested by the copepods. Diatoms and dinoflagellates have been shown to be selected by *C. finmarchicus* (Meyer-Harms et al., 1999), which are not known to contain any of the precursor molecules as major accessory pigment (Jeffrey et al., 2011, pp. 45-55). In addition, *C. finmarchicus* are not strictly herbivorous, as they are able to grow and reproduce on a diet of heterotrophs such as ciliates and microzooplankton (Ohman & Runge, 1994). Large ciliates have been found to be preferred over phytoplankton (Nejstgaard et al., 1997). A high proportion of heterotrophs in the diet could be responsible for the low astaxanthin concentrations in the copepods from Saltfjorden and Nordfjorden. On the other hand, it has been shown that copepods, including *C. finmarchicus*, increase gut evacuation rate with increasing phytoplankton biomass (Kiørboe & Tiselius, 1987; Pasternak, 1994). Holeton et al. (2009) suggested this led to decreased pigmentation in copepods, due to decreased assimilation efficiencies. This could

explain the relatively low pigmentation in the copepods in Saltfjorden and Nordfjorden when phytoplankton biomass was high. However, if this were the case, copepods from Saltfjorden would be more pigmented than those form Nordfjorden on May 15, which was not the case. Still, it would be interesting to investigate the association between phytoplankton biomass, gut evacuation rate, and pigmentation in *C. finmarchicus*, to establish if there is a non-linear relationship between phytoplankton concentration and astaxanthin content in the copepods.

4.3 Potential factors regulating astaxanthin accumulation in *Calanus* spp.

Astaxanthin has previously been proposed to function as a "sunscreen" molecule, protecting against UVR. Van Nieuwerburgh et al. (2005) proposed that copepods shielded by dense phytoplankton blooms require less astaxanthin due to reduced UVR exposure. My results do not indicate decreased pigmentation in *Calanus* spp. with increasing phytoplankton biomass. The lack of difference between copepod astaxanthin concentration from surface and mid depths also disputes this theory. However, light exposure could very well be involved in astaxanthin synthesis and metabolism. Pigmentation in *Calanus* spp. starved for 24 h in the dark decreased on average 26 % compared to controls, while pigmentation was on average 12 % higher in starved individuals in light (table 4). This was mainly due to decreases and increases in astaxanthin diesters, respectively. This is in accordance with previous studies (Sommer et al., 2006), which demonstrates a possible influence of illumination on pigmentation, where absence of light enhances pigmentation loss. Juhl et al. (1996) reported a 20-40 % decrease in pigmentation after 4 h of starvation in *C. pacificus* which had previously been fed with a high concentration of phytoplankton, while animals fed a low concentration of phytoplankton showed little difference in pigmentation compared to starved individuals. The reason for the comparatively low percentage of pigment loss in starved *Calanus* spp. CVs could be due to their initial low levels of astaxanthin. However, these results can only serve as a suggestion for further research, as sample sizes were small (three replicates for each treatment) with few individuals in each replicate. Based on personal observations, it appeared as pigmentation in the antennules decreased with starvation in the copepods (fig. 12). Redness of antennules have been used to separate the species *C. finmarchicus* (usually pale antennules) and *C. glacialis* (red antennules) (Choquet et al., 2018). It would therefore be interesting to investigate if antennule pigmentation is determined by feeding activity, rather than genetic differences.

One explanation for the low astaxanthin values encountered in Saltfjorden and Nordfjorden copepods, could be that these individuals do not form large scale swarms, and could therefore be more susceptible to predation if intensely colored. The disadvantage of intense pigmentation has previously been demonstrated (Brüsin et al., 2016; Byron, 1982; Hairston, 1979), and could likely be an evolutionary driver for astaxanthin accumulation in *Calanus* spp. Pigmentation in copepods has previously been suggested to be a trade-off between UVR and predation threats (Hansson, 2000, 2004; Hylander et al., 2009). As the risk of being eaten is reduced for swarming individuals (Milinski, 1977), *C. finmarchicus* in surface swarms could perhaps benefit from containing high amounts of pigment, without the associated costs of increased predation pressure due to conspicuousness.

4.4 Conclusion

Astaxanthin concentration in *Calanus* spp. varied temporarily and spatially, with highest values in CVs during spring in the fjords and summer off the coast of Northern Norway. Especially high values where found in copepods collected at Tromsøflaket in June. Such high values could be a rare occurrence, since highly pigmented copepods were only encountered at this particular area in summer 2018 and have not been reported in the literature elsewhere. However, swarming behavior could possibly stimulate astaxanthin synthesis by reducing the costs of being conspicuous. Despite phytoplankton biomass being higher in the fjords, astaxanthin content in the copepods was relatively low. Although phytoplankton biomass seemed to be of minor importance in determining astaxanthin variability in this study, phytoplankton pigments explained much of the variance in copepod astaxanthin content. A phytoplankton community rich in zeaxanthin appeared to promote astaxanthin accumulation, while phytoplankton containing β,β-carotene did not. Earlier evidence on the influence of UVR on the synthesis of astaxanthin was supported by this study, but the detailed process is not conclusive and has yet to be determined.

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Appendix A

Correlation tables.

Table A5. Correlation coefficients and *p*-values between *Calanus* spp. astaxanthin (µg ind.$^{-1}$) and phytoplankton pigments (µg l^{-1}), and percentage of astaxanthin forms from summer 2018. Correlations consisted of 16 datapoints, where pigments were concentrations from one replicate collected from 5 m, and astaxanthin µg ind.$^{-1}$ was mean values of 6 sample replicates with 30 ind., collected from surface and mid depths.

SUMMER 2018		
Asta µg ind.$^{-1}$		
Variable	*rho*	*p*-value
Chl *a* µg l^{-1}	0.626	.011
Chl *b* µg l^{-1}	0.497	.052
Chl c_1+c_2 µg l^{-1}	0.468	.070
Chl c_3 µg l^{-1}	0.076	.780
ββ-Car µg l^{-1}	0.462	.074
βε-Car µg l^{-1}	0.485	.059
Allo µg l^{-1}	0.568	.024
But-fuco µg l^{-1}	0.265	.321
Diadino µg l^{-1}	0.482	.061
Diato µg l^{-1}	0.247	.355
Fuco µg l^{-1}	0.491	.056
Hex-fuco µg l^{-1}	0.203	.450
Zea/Lut µg l^{-1}	0.568	.024
Free asta %	-0.762	< .001
Asta monoesters %	-0.824	< .001
Asta diesters %	0.860	< .001

Table A6. Correlation coefficients and *p*-values between *Calanus* spp. astaxanthin (µg ind.$^{-1}$) and percentage of astaxanthin forms from spring 2019. Correlations consisted of 11 datapoints where each variable was the mean of 1-6 replicates with 30 copepods (see methods for N, table 2).

SPRING 2019		
Asta µg ind.$^{-1}$		
Variable	*rho*	*p*-value
Free asta %	-0.394	.005
Asta monoesters %	-0.107	.458
Asta diesters %	0.387	.005

Table A7. Correlation coefficients and *p*-values between chl *a* and accessory pigments (µg l^{-1}), and temperature (°C) and salinity (psu), from Nordfjorden and Saltfjorden. Correlations consisted of 18 datapoints from 9 dates (16 datapoints and 8 dates in correlations with temperature and salinity).

NORDFJORDEN			SALTFJORDEN	
Chl *a*				
Variable	*rho*	*p*-value	*rho*	*p*-value
Chl *b* µg l^{-1}	0.768	< .001	0.766	< .001
Chl c_1+c_2 µg l^{-1}	0.987	< .001	0.981	< .001
Fuco µg l^{-1}	0.959	< .001	0.942	< .001
Allo µg l^{-1}	0.807	< .001	0.682	.002
But-fuco µg l^{-1}	0.687	.687	0.285	.251
Diadino µg l^{-1}	0.969	< .001	0.963	< .001
Hex-fuco µg l^{-1}	0.618	.006	0.535	.022
Chl c_3 µg l^{-1}	0.644	.004	-0.005	.983
ββ-Car µg l^{-1}	0.919	< .001	0.930	< .001
βε-Car µg l^{-1}	0.820	< .001	0.587	.010
Diato µg l^{-1}	0.923	< .001	0.898	< .001
c-Neo µg l^{-1}	0.406	.094	0.398	.102
Pras µg l^{-1}	0.491	.038	0.652	.003
Viola µg l^{-1}	0.687	.002	0.883	< .001
Zea/Lut µg l^{-1}	0.795	< .001	0.879	< .001
Peri µg l^{-1}	0.405	.096	-	-
Temperature (°C)	0.194	.470	0.735	.002
Salinity (psu)	-0.544	.032	-0.729	.002

Appendix B

Absorption spectra.

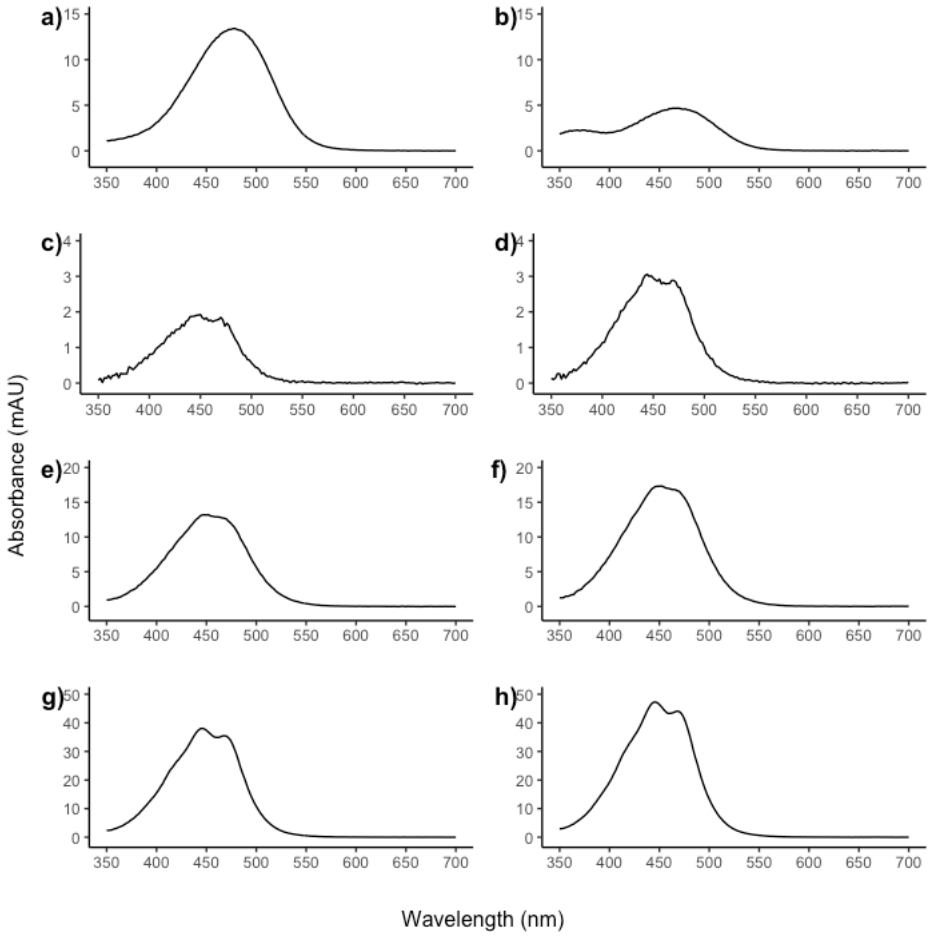

Figure B15. Absorption spectra of *Calanus* pigments: a) astaxanthin b) *cis*- astaxanthin, and double peaks from phytoplankton pigments, summer 2018: c) but-fuco peak 1, d) but-fuco peak 2, e) fuco peak 1, f) fuco peak 2, g) hex-fuco peak 1, h) hex-fuco peak 2.

Appendix C

Boxplots.

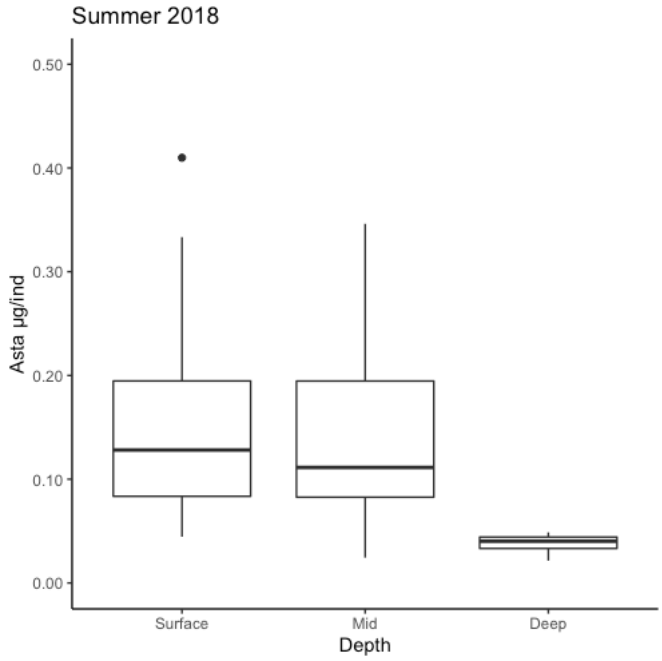

Figure C16. Astaxanthin concentration in *Calanus* spp. CVs with depth, collected summer 2018. *n* surface = 51, *n* mid = 48, *n* deep = 12.

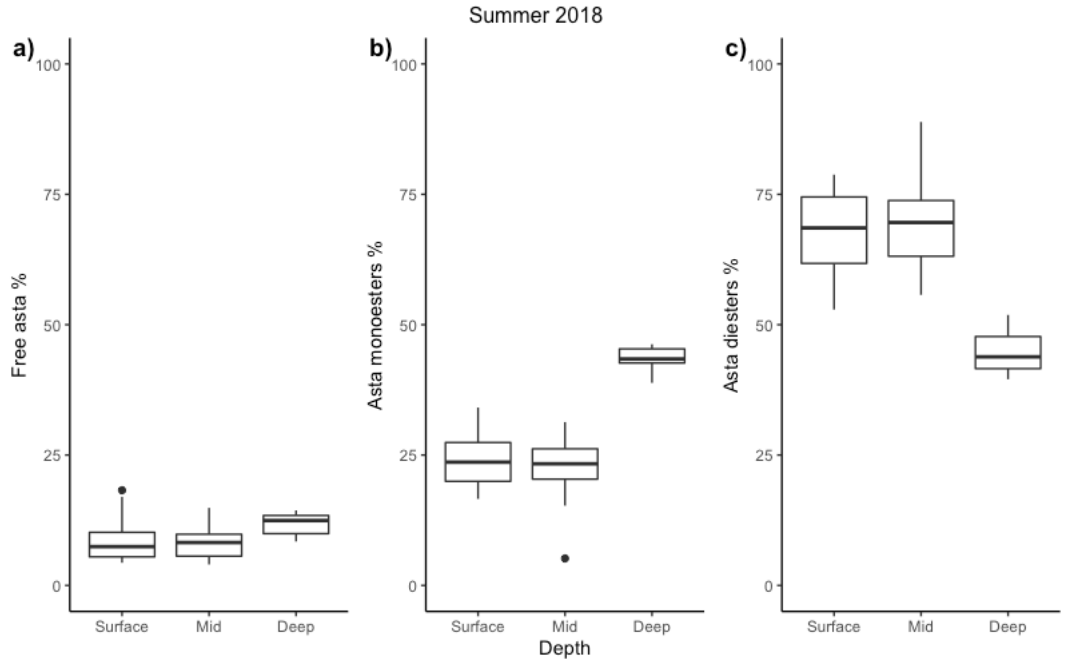

Figure C17. Percentage of astaxanthin forms with depth, collected summer 2018. *n* surface = 51, *n* mid = 48, *n* deep = 12.

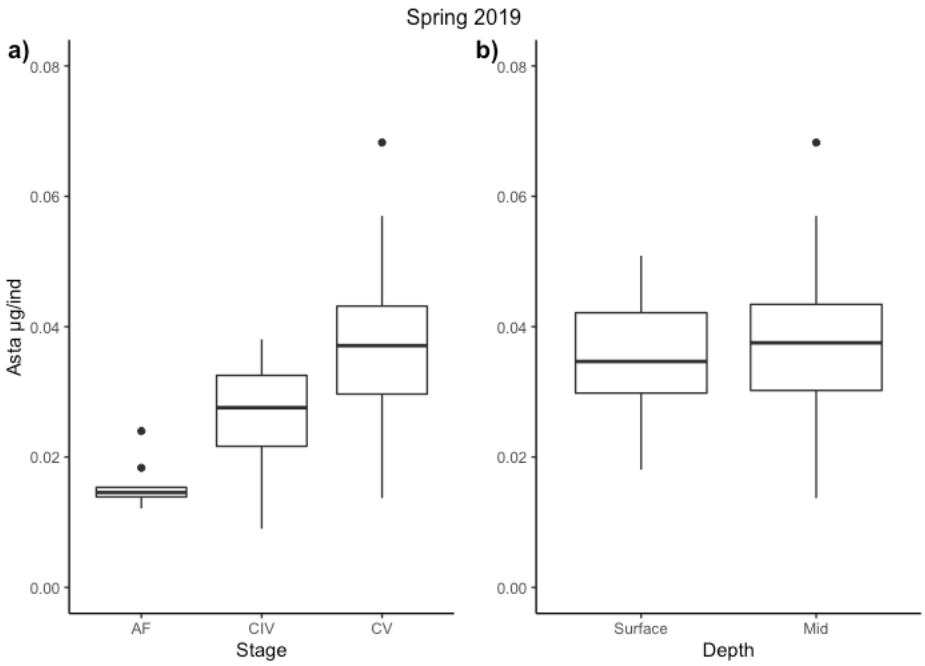

Figure C18. Astaxanthin in *Calanus* spp. a) with developmental stages, AF, CIV and CV. b) with depth. All stages CVs. See Methods for N, table 2.

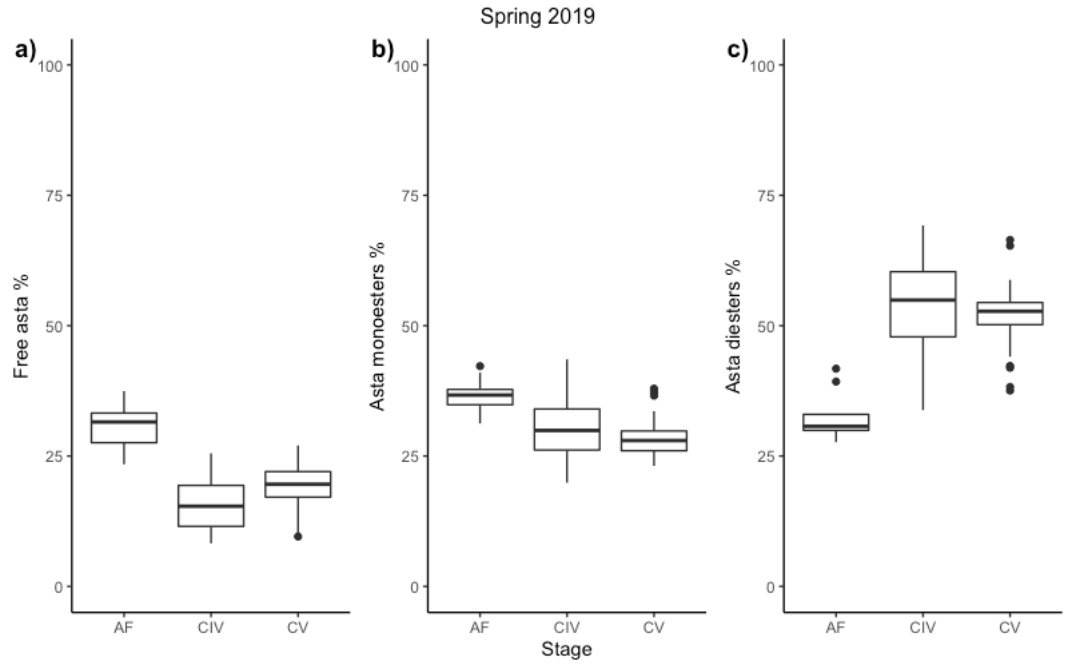

Figure C19. Percentage of astaxanthin forms in *Calanus* spp. with developmental stage, collected spring 2019. See Methods for N, table 2.

Appendix D

Percentage of phytoplankton accessory pigments.

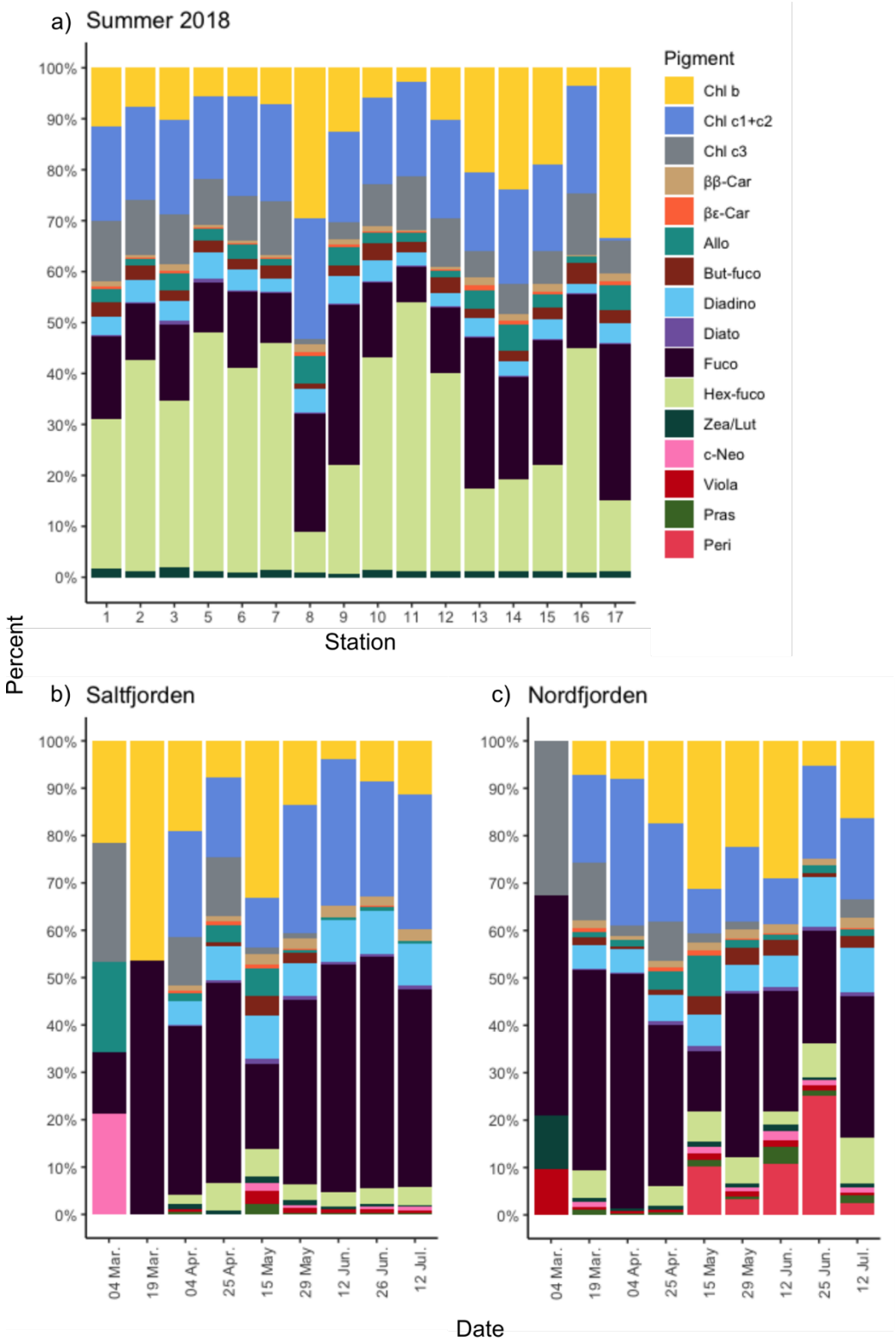

Figure D20. Phytoplankton accessory pigment composition, expressed as % of total accessory pigment concentrations (µg l-1) at 5 m depth, from a) summer 2018, b) Saltfjorden and c) Nordfjorden.