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Pigment Composition of Macroalgae from a Norwegian Kelp Forest

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Marine Coastal Development
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Abstract

Variation in pigment composition between seasons in 10 different species of macroalgae was examined using high precision liquid chromatography (HPLC). In addition, a study on the variation of pigment quantity and quality with tissue age was performed on the kelp *Saccharina latissima.* All species contained Chlorophyll *a* (Chl *a*), and all Phaeophytes contained the accessory chlorophylls, Chl c_{1+2} in addition to the carotenoids fucoxanthin, violaxanthin, antheraxanthin and zeaxanthin. In the two Rhodophytes examined, the major pigments in addition to Chl *a* was β,β-carotene and zeaxanthin. Within each species, the light harvesting pigments follows the same tissue concentration trend as Chl *a*. No similar trend of seasonal variation in pigment composition was seen between species. In the Phaeophytes, the three Fucus species tended to have high pigment content, while *Ascophyllum nodosum* was recurring as the species with the lowest concentration of all pigments. There was no influence of tissue age in pigment content in the February samples of *S. latissima*, whereas age influence was seen both in May and September. The light harvesting pigments (LHPs) Chl *a*, fucoxanthin, Chl *c* and violaxanthin was detected in all seasons in *S. latissima*, while Zea was only detected in the May samples and antheraxanthin only in the September samples. In general for *S. latissima*, February showed the highest concentration of all LHP, while there was a decrease towards May and September. Shading and position in relation to light of macroalgae in the water column determines the pigment content in the wrack and kelp species studied. The limits for high (>1000 µg)/intermediate (500-1000 µg)/low content (<500 µg) of Chl *a* per gram wet weight, reveals that Phaeophytes and Rhodophytes have a highly variable Chl *a* content (101-1997 µg for Phaeophytes, 219-1303 µg for Rhodophytes) within PG, while the Chlorophytes generally showed less variation in [Chl *a*] with seasons (828-1656µg).

Key words: ecosystem understanding, pigment chemotaxonomy and function (photosynthesis and photoprotection), kelp forest zonation, seasonality in chemical composition of macroalgae and seaweeds

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Abbreviations and terms used

The following are definitions of terms and symbols used in this thesis.

Term (symbol)	Definition, unit
Chlorophytes	Green macroalgae
Eulittoral	Also called the intertidal zone, is the zone that is periodically immersed and submerged
Epigrowth	Organisms, both epiflora ("plant", i.e. micro- and macroscopic algae) and epifauna ("animal", including bacteria and other eukarytoic microbes) that inhabit the lamina of macroalgae
Interspecific	Between species
Intraspecific	Within species
LHC	Light harvesting carotenoid
LHP	Light harvesting pigment
Optical density (OD)	Same as absorbance, dimensionless
Phaeophytes	Brown macroalge
PPC	Photoprotective carotenoid
Rhodophytes	Red macroalgae
Sublittoral	Zone that span from one to a few meters below the mean low tide
Supralittoral	The splash zone above mean high tide in the littoral zone. Is submerged during tide variations above average range.
w.w.	Wet weight
Zonation	The positions of boundaries between species in the littoral zone. Species difference in preferences of living environment and competition between species leads to higher density of certain macroalgae and seaweed species at specific depths. This is referred to as kelp forest zonation.
λ	Wavelength, nm

Introduction

Kelp forest ecosystems are a vital part of the marine food web, along the Norwegian coast and worldwide. Macroalgae are important primary producers (Mann 1972a), and important ecosystem builders (Rueness 1998; Christie et al. 2009) providing a three dimensional habitat that is home, breeding ground and shelter for both the juvenile stages and adults of many important species of fish and crustaceans. In addition kelp provides a substrate for several different epigrowth species (Hagerman 1966; Schultze et al. 1990; Bologna and Steneck 1993; Hartvig 1995; Anderson et al. 1997; Rueness 1998; Lippert et al. 2001; Christie et al. 2003; Fredriksen et al. 2005; Norderhaug et al. 2005; Carlsen et al. 2007; Christie et al. 2009; Włodarska-Kowalczuk et al. 2009).

Kelp forest zonation

Extensive kelp and seaweed forests are found on rocky shores, from the high tide mark down to about 30 m of depth (Dayton 1985; Lüning 1990; Rueness 1998). The zonation of the different macroalgal species is defined by a variety of abiotic and biotic factors. Of abiotic factors, light regime (intensity, spectral composition and day length) is regarded as most important, followed by temperature, salinity, nutrient availability, and available substrate and wave exposure (Lüning 1990). Of biotic factors, competition for substrate, grazing and epigrowth are the most important (Kirk 1992; Lüning 1990). See Fig. 1 for an overview of the zonation of macroalgae in Brænnebukta in Trondheimsfjorden.

Light is an important, albeit limiting, factor for photosynthesis in the sublittoral zone (Fig. 1) (Lüning 1990; Kirk 1992). In the ocean the light intensity will decrease exponentially with depth due to attenuation in the water column. This attenuation also results in a change in the spectral quality of light with depth, as some wavelengths are attenuated more efficiently than others (Kirk 1992). Consequently, the light regime will vary as a function of depth, but also as a function of time, on a scale from seconds to seasons as the solar irradiance and concentration of attenuating substances changes. Within the kelp forest zonation, algae growing at different depths will be acclimated and adapted to life in different light climates, and also to other abiotic factors. For instance, one group of canopy algae, the Fucaceae, has adapted to withstand desiccation (dehydration resulting from air exposure at low tide) (Schonbeck and Norton 1978; Lüning 1990). Certain kelp species, like *Laminaria digitata* (Hudson) J.V. Lamouroux 1813, has mechanically adapted to the impact of the breaking waves, which characterises the exposed upper

sublittoral zone, by possessing flexible stipes and mechanically resistant thalli (Lüning 1990). The main canopy algae of the upper sublittoral at wave-exposed sites are *L. digitata* and other kelp species (Lüning 1990; Schultze et al. 1990), while *Saccharina latissima* (L.) C.E.Lane, C.Mayes, Druehl & G.W.Saunders 2006 dominates at more sheltered sites, and below the *L. digitata* zone (personal observation, Mann 1972b; Lüning 1990; Aamot et al. 2014).

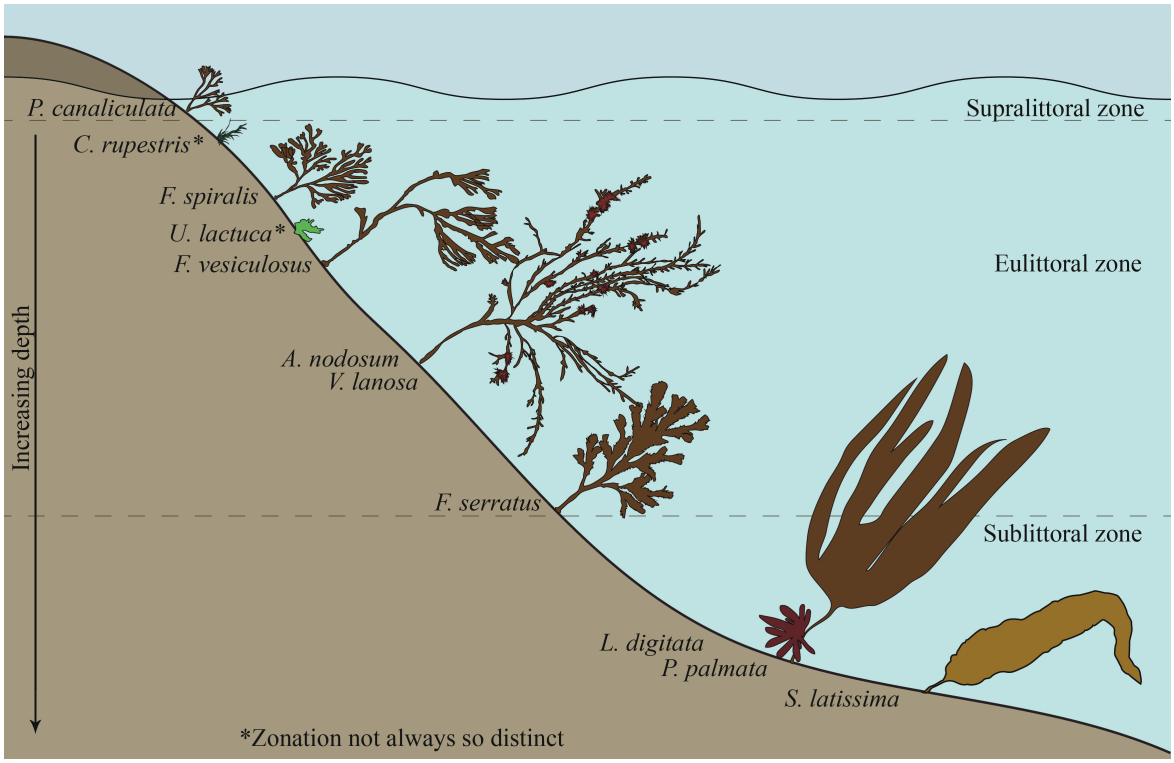

Fig. 1: Schematic drawing of the zonation of macroalgae in a semi-exposed pebble beach inside a mid-Norwegian fjord (Brænnebukta, Trondheimsfjorden, 63°26,827080' N, 010°19,868280' E). The dashed lines markes the upper and lower range of the tide. Zonation data from Lüning (1990) and personal observations (Illustration by Charlotte Hallerud).

Macroalgal species

For overview of macroalgal species investigated in this study, see Table 1.

Table 1: Overview of macroalgal species investigated in this study. Distribution range, age and preferences in abiotic factors from Printz (1953); Rueness (1976); Lüning (1990); Rueness (1998) and (Åsen 1980). Zonation data based on Lüning (1990) and personal observations. Pigment information from Rowan (1989).

Species	Distribution	Depth	Preferences	Life span	Major pigments
Brown algae – Class Phaeophyceae – Fucaceae All Fucaceae species studied exhibit apical growth of the thalli, thus the youngest tissue is found at the tips of the					

fronds (Rueness 1976; Rueness 1998)					
Pelvetia canaliculata (L.) Decaisne & Thuret 1845	European coastline - Portugal to N. Norway, not in Skagerrak	Supralittoral zone	Sheltered to moderately exposed, hard substrate, high tolerance of desiccation	4-5 years	Chl *a*, Fuco, Chl *c*, Viola, Anth, Zea
Fucus spiralis L. (1753)	Norwegian coastline	Upper littoral zone	Sheltered to moderately exposed rocky shores, high tolerance of desiccation,	Up to 4 years	
Fucus vesiculosus L. (1753)	Common along Norwegian coastline	Littoral zone	Rocky shores	4-5 years	
Ascophyllum nodosum (L.) Le Jolis 1863	Norwegian coastline	Littoral zone	Sheltered, hard substrate, rocky shores	Up to 20 years	
Fucus serratus L. (1753)	Norwegian coast	Transition between the littoral and sublittoral zone	Sheltered to moderately exposed, hard substrate	Perennial	
Brown algae– Class Phaeophyceae – Laminariaceae In general, the Laminariaceae are found below the Fucaceae in the tidal zonation					
Laminaria digitata	Arctic ocean and on both sides of the Atlantic ocean, France to Spitsbergen	Lower littoral and sublittoral zone (max 20 m)	Exposed to moderately exposed sites, hard substrate, strong currents	Perennial 10 years	Chl *a*, Fuco, Chl *c*, Viola, Anth, Zea
Saccharina latissima	North-Arctic down to Northern Portugal	Sublittoral zone (max 30 m)	Moderately to sheltered sites, often on unstable substrate such as boulders, mussels and rocks	2-5 years	
Red algae – Class Florideophyceae					
Palmaria palmata	Norwegian	Littoral and	Epiphytic species	N/A	Chl *a*, β,β-car, Zea

(L.) Weber & Mohr 1805	coastline	sublittoral zone (max 20 m)	growing on rock, mussels and other macroalgae,		
Vertebrata lanosa (L.) T.A. Christensen 1967	Norwegian coastline (Not Eastern Norway)	Mid-tidal zone	Epiphytic parasite commonly found on *Ascophyllum nodosum*, sheltered sites	Perennial	
Green algae – Class Ulvophyceae					
Ulva lactuca L. (1753)	All over Northern Europe	Littoral and sublittoral zone (max 20 m)	Sheltered to moderately exposed sites, rocky shores	Annual	Chl *a,* Chl *b,* Lut, Neo, Viola, Zea
Cladophora rupestris (L.) Kützing 1843	Atlantic coastline of Northern Europe	Littoral and sublittoral zone (max 20 m)	Surface of rocks	Perennial	

Kelp growth (Laminariaceae)

Kelp consists of a hapter (the attachment organ, holdfast), stipe and thallus/lamina (Rueness 1976). Lamina grows out from a specific tissue, called meristem – the growth zone (Newton 1931; Parke 1948; Mann 1972b; Kain 1979; Mann 1982). This tissue is located at the transition between stipes and lamina, while the oldest part of the kelp is found at the distal end where abrasion occurs (Fig. 2).

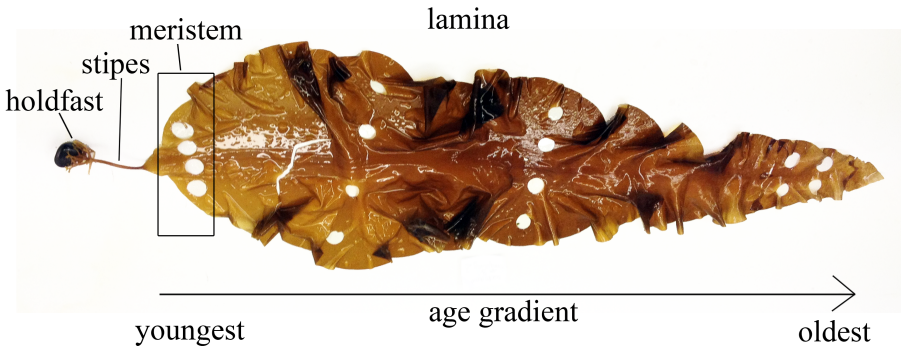

Fig. 2: Parts of a macroalgae, here illustrated with the species *Saccharina latissima.* The youngest part developes from the meristem and the oldest part is found at apex (Illustration by Charlotte Hallerud).

Chemical composition in brown algae (polysaccharides)

The individual algaes content of other substances than pigment could impact the pigment to wet weight ratio, and in general macroalgae has been found to show great variation in polysaccharide content. As photosynthesis continues at higher rates during the brighter months, polysaccharides build up in the kelp (Sakshaug and Sneli 2000).

Mannitol, a storage sugar, can make up as much as 30% of brown algae dry weight (Volesky et al. 1970). A study by Black (1948) on *Ascophyllum nodosum*, found that mannitol in this species makes up 6-12% of dry weight dependant on season and location, with the lowest content found in January and highest from July to October. A later study by Black (1949) found that the percentage of mannitol on a dry weight basis was "appriciatively" higher in *Fucus serratus* and *F. vesiculosus* than in *F. spiralis* and *Pelvetia canaliculata.* Black (1950a) also reported that mannitol constitutes 6-23% of dry weight in *Saccharina latissima*, and 4-27% in *Laminaria digitata*, dependant on season; lowest around April, increasing towards September. As in the land plants, macroalgae also use cellulose, a structural polysaccharide, to stiffen the cell walls, and Black (1950b) found that cellulose made up 4-5 % of lamina content in *S. latissima*, and 3-5% in *L. digitata*. Black (1950a) found that alginate, another structual polysaccharide, consitutes 5-14% of the dry weight in *S. latissima*, while it consitutes 8-19% in *L. digitata*. A report by Haug and Jensen (1954) for Norwegian Insitute of Seaweed Research looked at the same two species, and found that alginate constituted 16-18% of the dry weight content in *S. latissima* in May-Desember, and 33% of content in March. In *L. digitata*, alginate constituted 34% of content in Sep-Okt., and 45% in May-June. Black (1948) also reported that the alginate in *A. nodosum* was as high as 24-28%, with the lowest content in October, highest in April.

The Fucaceae contain fucoidan, a polysaccharide which is only found in small amounts in Laminariaceae. The amount of fucoidan in the Fucaceae was found to be dependant on degree of air exposure: in the shallow-growing *Pelvetia canaliculata* and *Fucus spiralis* fucoidan made up 18-24% total dry weight, while in the deeper growing *F. serratus* only 13% of dry weight was made up by fucoidan (Black 1954).

Pigments

There are three different groups of light harvesting and photoprotective pigments in macroalgae, i.e. the chlorophylls, carotenoids and phycobiliproteins (Rowan 1989; Jeffrey et al. 2005). The Phaeophytes (brown algae), Rhodophytes (red algae) and Chlorophytes (green algae) are separated into three classes based on their main taxa-specific (pigment-group, PG) pigments; this being fucoxanthin and chlorophyll *c* in Phaeophytes, phycobiliproteins in Rhodophytes and chlorophyll *b* in Chlorophytes (Rowan 1989). All Phaeophytes, Rhodophytes and Chlorophytes contain the light-harvesting pigment chlorophyll *a* (Rowan 1989).

Chlorophylls

All chlorophylls are lipid-soluble, and *in vivo* (bound up in a living cell) this group absorbs light in the blue and red part of the light spectrum (Lüning 1990; Brunet et al. 2011). Chlorophyll *a* is found in all photosynthetic macroalgae, while chlorophyll *b* is found in Chlorophytes, and the chlorophylls c_1 and c_2 are found in Phaeophytes (Rowan 1989). Both Chlorophyll *a* and *b* absorb violet-blue and orange-red light (absorption maximum of Chl *a* at 430 and 662 nm *in vitro*), albeit the absorption maximum of Chl *b* is shifted towards the middle of the spectrum (453 and 642 nm *in vitro*) (Rowan 1989; Johnsen and Sakshaug 2007 and references therein). The two types of Chl *c* (Chl c_{1+2}) absorbs mainly in the red part of the electromagnetic spectrum (orange; 585 nm, and bright red; 630-638 nm *in vitro*) (Rowan 1989).

Carotenoids

In vivo, the carotenoid absorption is highest in the blue and blue-green part of the visible spectrum (Rowan 1989). Fucoxanthin (Fuco) absorbs blue-green light (400-560 nm), specifically (Lüning 1990). Some of the carotenoids protect the photosynthetic reaction centre from excess light and are known as photoprotective pigments (Demmig-Adams and Adams III 1992). Carotenoids are separated into carotenes (hydrocarbons) and xanthophylls (containing oxygen) (Rowan 1989). The xanthophylls fucoxanthin, violaxanthin, antheraxanthin and zeaxanthin are found in the Phaeophytes, zeaxanthin is also found in the Rhodophytes, while lutein, neoxanthin, violaxanthin and zeaxanthin is found in the Chlorophytes (Rowan 1989). The carotene β,β-carotene is found in Rhodophytes (and to a certain extent in Phaeophytes and Chlorophytes) (Rowan 1989). A study by Johnsen et al. (1994) reported that the carotenoid peridinin, which absorbs approximetly at the same wavelengths as Fuco (400-550), was responsible for 60% (vs 5% for Chl *a*) of the light absorption in green light. It is therefore likely that the same is true for Fuco.

Phycobiliproteins

The water-soluble pigment-proteins characteristic for red macroalgae is called phycobiliproteins (Rowan 1989; Zhao et al. 2011). They absorb efficiently in the green to red part of the light spectrum (500-650 nm *in vitro*) (Rowan 1989). There are three major phycobiliprotein groups; allophycocyanins, phycocyanins, phycoerythrins (Rowan 1989; Jeffrey et al. 2005; Zhao et al. 2011). As the phycobiliproteins require water-soluble

solvents, in contrast to the lipid-soluble chlorophylls and carotenoids, this group has been left out of this study.

Factors affecting the pigment composition and content

The different species of macroalgae can exhibit considerable intraspecific variation in pigment composition, mainly because of photo acclimation; the regulation of the pigment content to maximize photosynthetic rate, and thus growth rate, in a variable light climate (Rodríguez et al. 2006; Johnsen and Sakshaug 2007). Several studies (Ramus et al. 1976a; Ramus et al. 1976b; Ramus 1983; Sakshaug and Holm-Hansen 1986) have shown that individual macroalgae can acclimate to the ambient light regime by adjusting the pigment ratio and total pigment content.

Light regime (irradiance, spectral irradiance and day length)

The amount of light available for the macroalgae in the littoral zone is dependent on depth and season. Several studies (Ramus et al. 1976a; Ramus et al. 1976b) have shown that macroalgae exhibit intraspecific variation in pigment content, depending on depth. (Colombo-Pallotta et al. 2006) showed that individual macroalgae can even adjust pigment content in different tissue to the ambient light climate of the specific tissue.

Shading by other macroalgae, or other parts of the macroalgae itself could also limit the light available for harvesting by the algae. Fig. 3 showed a simplified overview of shading by macroalgae. A study by Dean (1985) showed that the irradiance under a kelp canopy was reduced by as much as 70% compared to a nearby site cleared of kelp. In addition, Norton (1977) found that the canopy in a *Laminaria* forest could absorbs up to 89-97% of the available light.

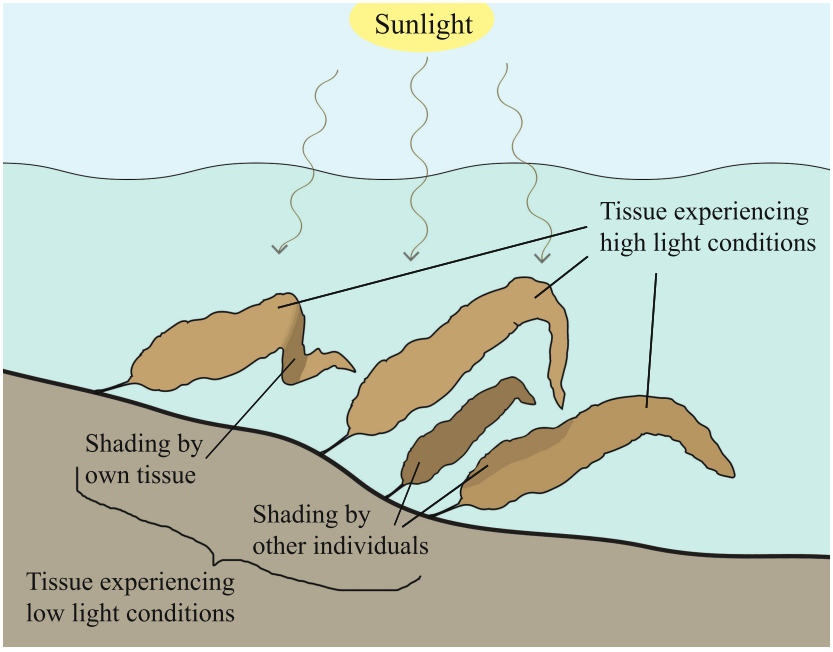

Fig. 3: Simplified overview of shading effect caused by macroalgae, here exemplified by *S. latissima* (Illustration by Charlotte Hallerud).

Norwegian coastal waters are often blue-green in appearance, because of a high concentration of suspended particles (phytoplankton and inorganic particles), and colored dissolved organic matter, cDOM (Jerlov 1976; Kirk 1994). The suspended particles will cause UV light and blue wavelengths to be strongly attenuated by scattering while cDOM will absorb much of the blue light, leaving the green and red wavelengths to penetrate deepest into the water column (Kirk 1994; Lüning 1990). The light scattering in water is high at shorter wavelengths relative to longer wavelengths and dependent on particle size (Sakshaug et al. 2009). cDOM are substances originating from breakdown of biological matter of terrestrial or marine origin, e.g. breakdown of macroalgal tissue to poly phenols (Lüning 1990; Kirk 1994).

There is a high degree of seasonal variation in the phytoplankton density in Trondheimsfjorden. Generally, the phytoplankton density is low during the winter season (November to February) (Sakshaug 1972). The spring bloom usually reaches a maximum in April, decreasing towards May, and in autumn the maximum density is observed in August and declines towards September (Sakshaug and Myklestad 1973). The amount of run-off from rivers into the fjord is also season dependant, and is associated with variation in particulate matter in the water. Characteristic for Trondheimsfjorden is that the run-off rate is low during winter, and increases during spring towards a maximum in May-June when thawing starts in the mountains (Sakshaug 1972). River run-off can contain high amounts of suspended material (Wassman et al. 2009).

A pronounced seasonal variation in abiotic factors, such as light climate, is found at high latitudes. Several studies (Mathieson et al. 1976; Kain 1979; Lüning 1979; Sjøtun 1993) have shown that physiological processes in macroalgae, e.g. growth, are dependent on season.

Nutrient availability

Chlorophylls contain nitrogen (Sheer 1991), and thus nitrogen is needed for chlorophyll synthesis. Inorganic nitrogen (N) is found to be limiting for macroalgae productivity in temperate waters (Lapointe et al. 1992). A study by Young et al. (2007) on several macroalgal species showed that internal N content declines as ambient N declines. The study also showed that ambient N (in the form of NO_3^-) is highest in concentration during winter, and declines during summer, so that less N is available in the water in early autumn (September).

Epigrowth (=epiflora and fauna)

Another factor contributing to variation in light climate for the macroalgae is epigrowth, flora and fauna living on the kelp, as this will deprive the host of light through shading (Andersen et al. 2011). Andersen et al. (2011) showed that the degree of shading from epigrowth was low during spring and summer, while high towards the end of summer and during autumn, while an unpublished study by Andersen et al. (2013) showed that shading caused by epiphytic cover can be substantial, and light can be reduced by as much as 11-91%, depending on the epiphytic species responsible (i.e. bryozoans and tunicates, respectively). Carlsen et al. (2007) found that bryozoans were the most common epifauna found on *Laminaria digitata* and *Saccharina latissima*. Personal observations confirmed this (Fig. 4). Figure 4 shows variation in epigrowth cover with season seen in this study. Epigrowth might also have other destructive effects damaging algal tissue, reduce the nutrient uptake ability and its ability to withstand breakage by waves (Hurd et al. 1994; Hurd et al. 2000; Hepburn et al. 2006; Krumhansl et al. 2011).

Fig. 4: Variation in epigrowth (bryozoan) coverage on kelp sampled in Brænnebukta at three different seasons (from left to right); spring, summer and fall (February, May and September). The epigrowth is visible as dark spots/areas.

Photo acclimation

Photo acclimation is the physiological response that accompanies changes in irradiance, and represents several processes which purpose it is to optimize several cellular activities such as photosynthesis and growth (Falkowski and LaRoche 1991; Anning et al. 2000; Raven and Geider 2003). Photo acclimation divided into short and long-term acclimation. Short-term photoacclimation (from minutes to hours) mainly involves regulation of the xanthophyll cycle (see below), while long-term photo acclimation (hours to days) involves changes in structure and composition of the photosystems. The latter is characterized by changes in pigment composition (up- or downregulation), photosynthetic parameters, enzymatic activities and cell volume and chemical composition (Brunet et al.

2011). Algae adapted to high light (HL) exhibit physiological characters such as lower chlorophyll content, high photosynthetic capacity and active photoprotective mechanisms, while algae adapted to low light (LL) typically exhibit the opposite (Ramus et al. 1977; Lichtenthaler and Babani 2004; Laisk et al. 2005). Due to the fact that LL acclimated species have to live for long periods of the year at or under the photosynthetic light compensation point (i.e. where photosynthetic rate equals respiration rate), they also exhibit slower growth to minimize cellular respiration (Lüning 1990), thinner thalli to reduce the ratio of non-photosynthetic to photosynthetic tissue and reduced synthesis of photosynthetic enzymes (Dring 1981).

Photoprotection

Certain pigments, e.g. carotenoids, can also function in photoprotection of the organism. Two different kinds of photoprotection have been defined: dynamic (reversible, common for photosynthetic organism exposed regularly to excess light) and chronic (irreversible, which might be harmful to the organism). The individual organisms acclimation state determines, to a great extent, at which light level photoprotection sets in (Figueroa et al. 2003; Brunet et al. 2011). For green macroalgae, the xanthophylls neoxanthin and lutein both participates in protecting the organism from photodamage by several mechanisms, e.g. protecting the Light Harvesting Complex proteins from photooxidative stress (unbalance between light harvesting and energy utilization, which leads to production of reactive oxygen species which can damage proteins and cause cell death) (Dall'Osto et al. 2006; Dall'Osto et al. 2007).

Another important part of the dynamic photoprotection is the xanthophyll cycle, which is found in Phaeophytes and Chlorophytes, and partly in Rhodophytes, constituting violaxanthin (Viola), antheraxanthin (Anth) and zeaxanthin (Zea) (Yamamoto et al. 1962; Raven and Geider 2003). When exposed to excess light conditions, the organism can synthesize Anth and Zea by a two-step de-epoxidation of the existing Viola (Yamamoto et al. 1962), see Equation 1. Viola and Anth are LHC, while Zea functions in protection of the organism against photooxidative damage (Havaux and Niyogi 1999).

$$\textit{Violaxanthin} \underset{\textit{epoxidation}}{\overset{\textit{de-epoxidation}}{\rightleftharpoons}} \textit{Antheraxanthin} \underset{\textit{epoxidation}}{\overset{\textit{de-epoxidation}}{\rightleftharpoons}} \textit{Zeaxanthin} \qquad (1)$$

Modified after (Yamamoto et al. 1962; Falkowski and Raven 2013)

Age

When the lamina grows and ages the composition of pigments might change, which might affect the individual parts of the tissues response to different environmental variables, e.g. light, temperature etc. (Valle 2014).

High Precision Liquid Chromatography (HPLC)

The introduction of pigment analysis in the late 1970's by using High Precision Liquid Chromatography (HPLC) simplified separation and identification of algal pigments (chlorophyll and carotenoids), and also made it more precise than previous methods (Abaychi and Riley 1979; Mantoura and Llewellyn 1983).

Aim

The aim of this study was to investigate the pigment composition in different pigment groups of macroalgal species, comprising Phaeophytes (brown), Chlorophytes (green) and Rhodophytes (red) found in a Norwegian kelp forest dominated by *Laminaria digitata*. HPLC was used to gain chemo-taxonomical and functional information of pigments from the taxa present from upper to lower tidal range, and to elucidate how placement within the kelp forest affects the pigment concentration and composition.

In addition to this, the effect of tissue age on pigment composition in lamina tissue from *Saccharina latissima* was explored (age gradient study).

Materials and methods

Experimental species

The species used in this study were selected from the range of species commonly found in the littoral zone in Brænnebukta, Trondheimsfjorden, to get a broad selection as possible for a semi-exposed habitat (Fig. 1). See Table 2 for a short description of where each species was found within the kelp forest in Brænnebukta.

Table 2: Overview of brown-, red- and green macroalgae in Brænnebukta, Trondheimsfjorden. See Fig. 1 for a visual overview.

Species	Zone
Brown algae	
Pelvetia canaliculata	Supralittoral. The highest growing species.
Fucus spiralis	Growing below *P. canaliculata*, in the upper eulittoral zone.
Fucus vesiculosus	Eulittoral zone, growing below *F. spiralis*.
Ascophyllum nodosum	Middle eulittoral zone, below *F. vesiculosus*.
Fucus serratus	Growing below *A. nodosum*.
Laminaria digitata	The shallowest living of the Laminariaceae found in Brænnebukta, just below *F. serratus*.
Saccharina latissima	Deepest growing species in this study. Found growing below *L. digitata* in Brænnebukta.
Red algae	
Palmaria palmata	Epiphyte on the stipes of *L. digitata*.
Vertebrata lanosa	Epiphyte on *A. nodosum*.
Green algae	
Ulva lactuca	Growing on rocks at the same depth as *Fucus serratus* and *Fucus vesiculosus*.
Cladophora rupestris	Supralittoral zone, at the same depth as *Pelvetia canaliculata* or higher up.

Sampling method

Macroalgae were collected in Brænnebukta in Trondheimsfjorden (63°26,827080’ N, 010°19,868280’ E) at three individual sampling dates throughout the year 2013, as listed in Table 2. See Figure 5 for location of Brænnebukta in Trondheimsfjorden.

Fig 5: Map of sampling area in Trondheims-fjorden. The red dot marks the sampling spot (see coordinates in text for details). The white arrow indicates the water inlet of the fjord.

Sampling was carried out by hand picking during low tide. At each sampling 9 different species of kelp were taken, as listed in Table 3. Three individuals of each species were collected, when this was possible. For *Vertebrata lanosa*, each cluster of growth on *Ascophyllum nodosum* is a separate individual, and too small to separate into 3 sub-replicates, thus 9 entire individuals were collected at each sampling. During the May sampling only two specimens of *Ulva lactuca* was found. Also, on the last sampling only two specimens of *Palmaria palmata* was found.

Table 3: Sampling dates and species collected at each sampling in Brænnebukta, 2013. Sampling depth and number of individuals collected of each species is also listed. Phytoplankton concentration in Trondheimsfjorden at sampling time is given, and the effect on available light for macroalgae and seaweeds is discussed in [Discussion].

Sampling date	*Temperature*	*Depth sampled*	*Scientific name*	*n*	*Light climate*
#1 Feb. 28th. Winter	4°C	0-4 m (low tide)	*Fucus serratus*	3	Phytoplankton bloom low*
			Fucus vesiculosus	3	
			Ascophyllum nodosum	3	
			Fucus spiralis	3	
			Pelvetia canaliculata	3	
			Laminaria digitata	3	
			Saccharina latissima	3	
			Vertebrata lanosa	9	
			Palmaria palmata	3	
			Cladophora rupestris	3	
#2 May 6th. Summer	12 °C	0-4 m (low tide)	*Fucus serratus*	3	Phytoplankton bloom moderate-high*
			Fucus vesiculosus	3	
			Ascophyllum nodosum	3	
			Fucus spiralis	3	
			Pelvetia canaliculata	3	

			Laminaria digitata	3	
			Saccharina latissima	3	
			Vertebrata lanosa	9	
			Palmaria palmata	3	
			Ulva lactuca	2	
#3 Sep. 3rd. Autumn	14 °C	0-4 m (low tide)	*Fucus serratus*	3	Phytoplankton bloom moderate*
			Fucus vesiculosus	3	
			Ascophyllum nodosum	3	
			Fucus spiralis	3	
			Pelvetia canaliculata	3	
			Laminaria digitata	3	
			Saccharina latissima	3	
			Vertebrata lanosa	9	
			Palmaria palmata	2	
			Cladophora rupestris	3	

*algeinfo.imr.no (web address #1)

Table 4: Weather data (Lade weather station), day length and sun-angle at time of (and before) sampling for the Trondheim region.

	# clear sky*	# days fair weather*	# days overcast*	# rainy days*	Average hours of daylight**	Average sun-angle
February	2	5	12	7	08h 50m	14.6°
April	1	5	12	8	14h 08m	36.6°
August	3	5	8	12	16h 30m	40.0°

* eklima.met.no (web address #2) **timeanddate.no (web address #3)

The samples were handled in dim light and as cold as possible to avoid high light and heat stress, which in combination with oxidation, low or high pH, degrades the chlorophylls and carotenoids before and during extraction (Rowan 1989). Tissue samples, in three replicates, were then cut out of each specimen, wrapped in individual packets of aluminium foil and frozen at -15 °C on the sampling day, until further use (Fig. 6 A-I).

A circular punch tool (15 mm in diameter) was used to cut out sub-samples from *L. digitata, S. latissima, P. palmata* and *U. lactuca*, while for the rest of the algal species, appropriate pieces were cut out using a scalpel.

Entire individuals of *S. latissima* and *Laminaria digitata* were stored for use in physiological experiments. The kelp was kept in nets in running seawater at about 3 m depth in a container outside Trondheim Biological Station.

Figure 6: Sub-replicate collection from each species of macroalgae. Each picture shows how the individual species was treated for sub-replicate collection. The numbers represents the code given to the individual samples during the experiment. A: *Ascophyllum nodosum*, B: *Fucus spiralis*, C: *Pelvetia canaliculata*, D: *Fucus vesiculosus*, E: *Fucus serratus*, F: *Palmaria palmata*, G: *Vertebrata lanosa*, H: *Ulva lactuca*, I: *Cladophora rupestris*.

Samples from *L. digitata* were taken as close to the meristem as possible, to insure that the youngest lamina tissue was used in the measurements (Fig. 7), because it is least affected by epigrowth (verified by personal observation, Fig. 4) and also gives the best representation of the passed months light climate (Valle 2014).

Fig. 7: An example on how the meristem samples where cut out from *L. digitata*. The meristem is the youngest part of the lamina and it is located close to the stipes.

Age gradient study on *Saccharina latissima*

Samples from three different individuals of *S. latissima* were taken from four different parts of the lamina; representing four different tissue ages for each individual, see Fig. 8. This procedure was repeated at all three samplings.

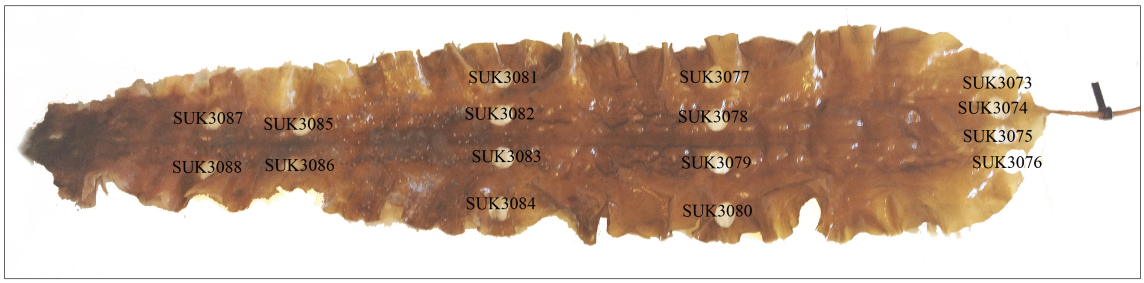

Fig. 8: An example on how the samples were cut out from *S. latissima*. Different parts of the lamina were sampled, to investigate differences in pigment composition in tissue of different age. Numbers indicate sample number dedicated to each sample before analysis.

Pigment extraction

For the HPLC analysis, four sub-samples of tissue (out of the 9 originally collected at each sampling, 3 from each specimen) were chosen from each species from each sampling period. One sub-sample was chosen from each of the three individuals of macroalgae, and a fourth one was chosen randomly.

The individual samples were transferred to a 10 mL glass test tube and weighed (Sauter AR 1014, August Sauter GmbH, Ebingen, Switzerland) to obtain wet weight (±0.000 g). 5 ml of 100% methanol (CH_4O) were then added, and the sample was crushed with a glass

rod. N_2 gas was blown into the test tube to eliminate air (O_2) and closed with a cap to avoid oxidation (Rowan 1989). The sample was then mixed using a test tube mixer (Vibrofix VF1, Janke & Kunkel GmbH & Co. KG, Staufen, Germany), and refrigerated in the dark at 4^{o}C for 24 hours for pigment extraction (Valle 2014). The extract was filtered through a single-use syringe with a 13 mm (diameter) syringe filter (0.2 um pore size) into a 2 ml HPLC vial in order to remove all particles.

High Precision Liquid Chromatography (HPLC)

The assessment of the pigment composition was done using a Hewlett Packard Agilent 1100 Series HPLC system. It was equipped with a quaternary pump system autosampler, Water Symmetry C_8 column, a Diode Array Absorbance Detector (190-950 nm) and a data program (ChemStation for LC 3D systems, Rev. B02.01.). The autosampler drew 77 µL from each sample vial and 23 µL distilled water (total volume of 100 µL). For overview of method see Zapata (2000) and Rodriguez et al. (2006). The column is the primary part of the HPLC system, within which the pigments are separated for identification. The individual pigments passes through the column at different times, according to their polarity and the polarity of the mobile phases. The pigments are then detected as peaks that arrive as a function of time.

The mobile phases in the column was as following: Solvent C was a mixture of methanol:acetonitrile:aqueous pyridine (0.25 M pyridine) in the ratio 50:25:25 (v:v:v), solvent D was acetonitril:acetone in the ratio 80:20 (v:v) (v=volume).

Absorbance spectra (= optical density (λ)) of each pigment peak were measured at 350-700 nm using a Diode Array Detector. Detection wavelengths for the chromatograms were 420, 440, 450 and 460 nm - 440 nm was used for further calculations, which detected both carotenoids and chlorophylls (Rodriguez et al. 2006).

Data analysis

µg pigments/g wet weight was calculated from the area under the absorption curve of the HPLC chromatograms detected at 440 nm, using equation 1. Calibration was done in absolute units from chromatogram peak area to µg pigment.

$$\mu g \text{ g w.w.}^{-1} = \frac{area * Rsf * V_e}{V_i} \quad (1)$$

Area: area of pigment peak from HPLC at 440 nm
Rsf: response factor calculated by calibration at 440 nm
V_e: extraction volume, (5) mL methanol used to extract pigments from tissue
V_i: injection volume, (77) µL extract injected in HPLC

Statistics

Statistical analysis of variation was done using Statgraphics Centurion version 16.2, from StatPoint Technologies, Inc., Warrenton, Virginia, USA, www.statgraphics.com.

One-Way ANOVA was used for statistical analysis of the variation between species within each season, and for variation between seasons within each species. Multifactor ANOVA was used for statistical analysis of the interaction between tissue age and season in *S. latissima*. The figures were made using SigmaPlot version 11.0, from Systat Software, Inc., San Jose, California, USA, www.sigmaplot.com. *P*-values are given in [results], while remaining results from statistical analysis is given in Appendix D.

The relative variability – coefficient of variation (CV, ±% of mean value), was calculated from the standard deviation (SD) using Microsoft Excel version 2011, from Microsoft, Redmond, Washington, USA. www.microsoft.com. The CV values are listed in Appendix A

Results

The pigment concentration in all species is denoted [pigment], and is given in µg pigment/g wet weight (w.w.). For clarity, the different algal groups are divided into pigment groups (PGs). All brown algae (Phaeophytes, PG1) contained accessory chlorophyll, Chl *c* (Fig. 10, Chl c_{1+2}, see appendix B), in addition to the carotenoids fucoxanthin (Fuco) (Fig. 9), violaxanthin, antheraxanthin and zeaxanthin (Fig. 11). The major light harvesting pigments (LHP) in PG1 were Chl *a*, Chl *c* and Fuco. In the two red algae (Rhodophytes, PG2) the major fat-soluble LHP in addition to Chl *a* was found to be β,β-carotene (Fig. 12), and the PPC zeaxanthin was also found (Fig. 13). Note that major phycobiliproteins making up a large fraction of the light-harvesting antenna for PSII in PG2 are water soluble (Zhao et al. 2011), and not extracted by the solvents used in this survey. Chlorophyll *b* was found in all green algae (Chlorophytes, PG3), along with lutein, violaxanthin, zeaxanthin and neoxanthin (Fig. 27). All species contained Chl *a* (Fig. 9).

Class- and interspecific differences

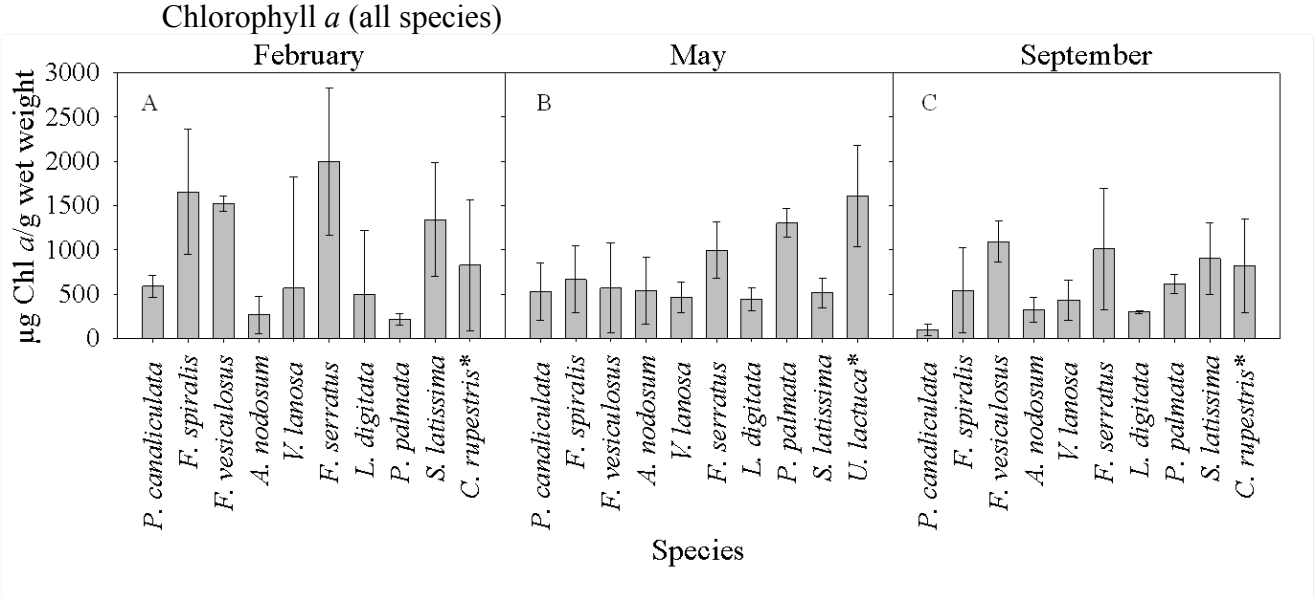

Fig. 9: Seasonal variation [Chl *a*] between brown-, green- and red macroalgae in Trondheimsfjorden. Note that the values for *S. latissima* are only average content in meristem samples.

In February, [Chl *a*] showed significant variation both between pigment groups (P=0.02) and between all species (P = 0.00, Fig. 9A). Brown algae (PG1) differed notably from green algae (PG3) (averaged [Chl *a*] of 1089 µg Chl *a*/g w.w. and 829, respectively). Highest [Chl *a*] in algal tissue (>1000 µg Chl a/g w.w.) was found in *Saccharina*

latissima and the three Fucus species, *F. spiralis, F. vesiculosus* and *F. serratus*. All had 6.7 times higher [Chl *a*] than the lowest [Chl *a*] found in *Ascophyllum nodosum* and *Palmaria palmata* (<500 µg Chl *a*/g w.w.).

There was also significant variation in [Chl *a*] in May, both between PGs (P=0.00), and between species (P=0.00). None of the PGs had the same [Chl *a*] in May. The [Chl *a*] was more similar between species in May than in February, with the highest [Chl *a*] (*U. lactuca*) being only 3.6 times higher than in the lowest (*Laminaria digitata*) (Fig. 9B). In May, [Chl *a*] had dropped compared to February in *S. latissima* and all Fucus-species (to mid-range), while [Chl *a*] in *L. digitata, Vertebrata lanosa* and *Pelvetia canaliculata* remained almost the same. *A. nodosum* had an increase to intermediate [Chl *a*], which was still among the lowest content found at this sampling.

The September samples did not show any significant variation between PGs (P=0.5), but there was a clear species variation (P = 0.002), with [Chl *a*] being 11 times higher in *F. serratus* and *F. vesiculosus* (highest), than in *P. canaliculata* (lowest) Fig. 9C). [Chl *a*] in *L. digitata* and *V. lanosa* remained almost the same as in previous months, while [Chl *a*] in *F. serratus* and *F. spiralis* remained similar to the May levels. *A. nodosum* again showed low content in September.

Phaeophytes (PG1)

Fucoxanthin

Although the [Fuco] in PG1 was roughly 4 times lower than [Chl *a*], the two show the same trend with regards to high/mid/low content. In February the [Fuco] in PG1 varied greatly (P=0.0006), albeit only a 3 time increase from lowest to highest content (Fig. 10A).

In May there was very even [Fuco], ranging only from 130 (*Ascophyllum nodosum*) to 262 (*F. serratus*) µg Fuco/g w.w. Fuco content had a marked drop from February to May in the three Fucus species and *Saccharina latissima*. Although the [Fuco] was more similar between species in May than February, the relationship between species was the same, and the variation in [Fuco] was still found to be significant (P=0.04) (Fig. 10B).

In September, the highest [Fuco] was found in *Fucus vesiculosus* (293 µg Fuco/g w.w.) and the lowest in *Pelvetia canaliculata* (24 µg Fuco/g w.w.), giving a 12-times difference between high and low [Fuco] (P=0.00, Fig. 10C). It is noteworthy that *A. nodosum* was among the species with the lowest concentrations of Fuco at all three samplings and *F. serratus* was among the highest, same as what was seen for Chl *a.*

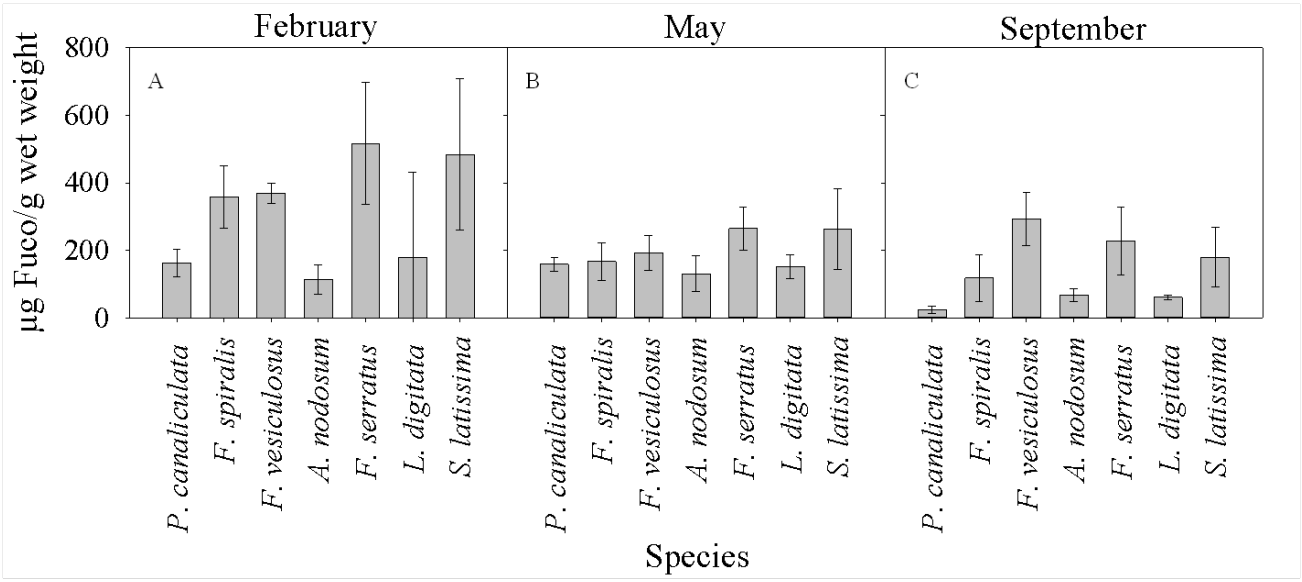

Fig. 10: Seasonal variation in [Fuco] between brown macroalgae, PG1, in Trondheimsfjorden. Note that the values for *S. latissima* are only average content in meristem samples.

Chlorophyll *c*

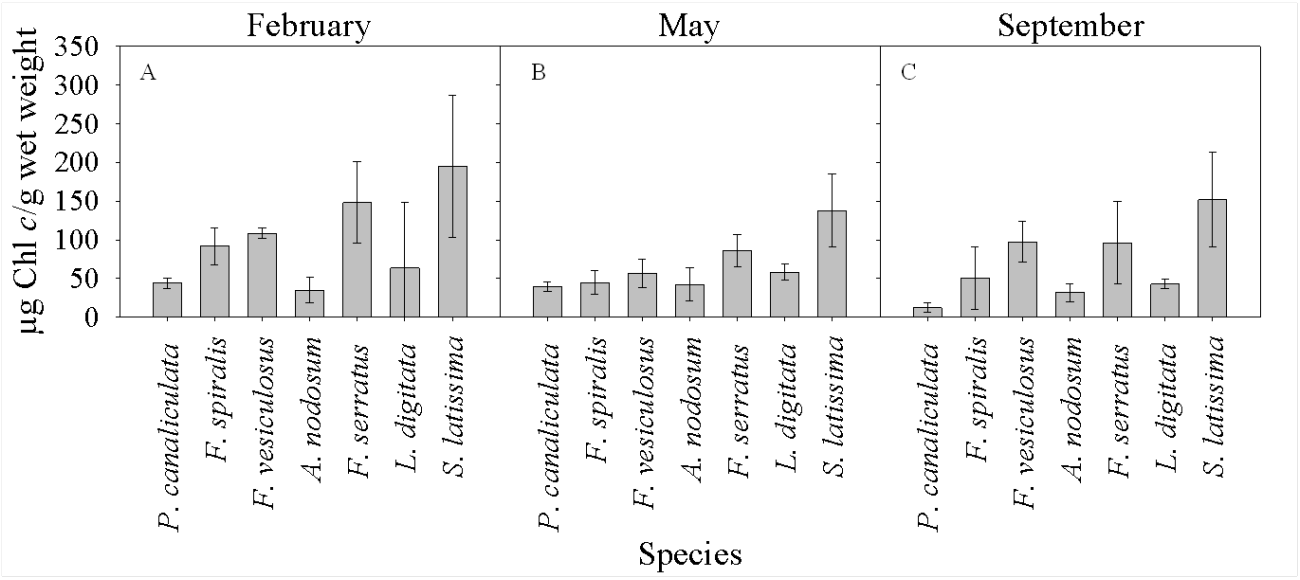

Fig. 11: Seasonal variation in [Chl *c*] between brown macroalgae, PG1, in Trondheimsfjorden. Note that the values for *S. latissima* are only average content in meristem samples.

The variation in [Chl *c*] also followed the trend of [Chl *a*] but with lower amplitude (10 times lower concentration), with some exceptions. There was a clear interspecific variation in [Chl *c*] in all months, but the relationship between species was the same in all seasons (February P=0.0002, May P=0.00, September P=0.00). Concentration of Chl *c* dropped with season in all species, and the highest [Chl *c*] was always found in *S. latissima*, and the lowest in *Ascophyllum nodosum*, with e.g. 5.6 times difference between the two in February (Fig. 11A-C). *Pelvetia canaliculata* was together with *Ascophyllum*

nodosum found to have low [Chl *c*] at all samplings. Also, all three Fucus species were found to have higher [Chl *c*] compared to the other species at all samplings.

The xanthophyll cycle (Violaxanthin, antheraxanthin and zeaxanthin)

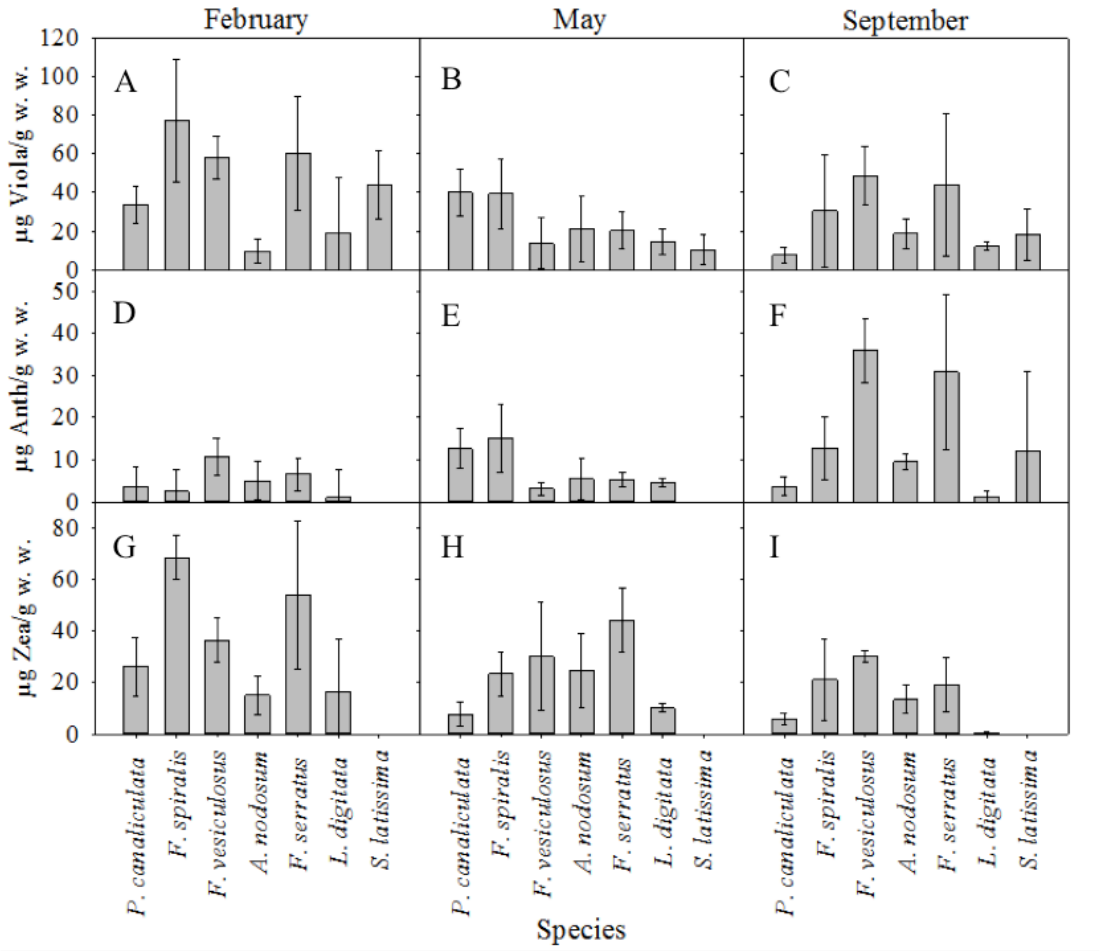

Fig. 12: Variation in pigment content in the xanthophyll cycle (Viola, Anth and Zea) between Phaeophytes, PG1. Note the differences in scale of the y-axes for the different pigments. Also, note that the values for *S. latissima* are only average content in meristem samples.

All three xanthophyll cycle pigments in PG1 showed significant interspecific difference within their month (*P*[Viola]= 0.0001, 0.0002, 0.015, *P*[Anth]=0.0002, 0.00, 0.005 and *P*[Zea]=0.00, 0.00, 0.00 in February, May and September, respectively (Fig. 12A-12I).

There was approximately 8-times difference between the highest and lowest [Viola] in February. *Fucus spiralis* had the highest [Viola] (77 µg Viola/g w.w.) closely followed by *F. vesiculosus, F. serratus* and *Saccharina latissima*. The lowest content was found in *Ascophyllum nodosum* (10 µg Viola/g w.w., Fig. 12A). In February [Anth] ranged from no Anth in *S. latissima* to 10.8 µg Anth/g w.w. in *F. vesiculosus.* [Zea] was highest in *F. spiralis* (68.3 µg Zea/g w.w.), closely followed by *F. serratus* (54 µg Zea/g w.w.). As with Anth, there was no Zea in *S. latissima* in February (Fig. 12G).

In May, [Viola] was highest in *Pelvetia canaliculata* and *Fucus spiralis* and lowest in *F. vesiculosus* and *L. digitata*, with an average of a 2.17 time increase from lowest to highest (Fig. 12B). *P. canaliculata* and *F. spiralis* had the highest [Anth] in May, with 13 and 15 µg Anth/g w.w., respectively. As in February, *Saccharina latissima* did not contain any Anth or Zea at all in May (Fig. 12E). *F. serratus* had the highest [Zea] in May (23 µg Zea/g w.w., Fig. 12H).

In September, [Viola] ranged from 7.7 µg Viola/g w.w. in *P. canaliculata* to 48.7 µg Viola/g w.w. in *F. vesiculosus* (Fig. 12C). [Anth] was highest in *F. vesiculosus* and *F. serratus* (36 to 31 µg Anth/g w.w., respectively, Fig.12F). The lowest content was found in *L. digitata* (1.3 µg Anth/g w.w.), yielding a 30-time difference between highest and lowest [Anth] in September. It is also noteworthy that this was the only sampling in which Anth was present in *S. latissima*, and in intermediate concentration at that. [Zea] was also present in September (Fig. 12I), with a clear difference between *F. vesiculosus* (highest content, at 30.2 µg Anth/g w.w.) and *L. digitata* and *S. latissima* (lowest content, at 0.2 and 0 µg Zea/g w.w., respectively). There was a large drop in [Zea] in *L. digitata* with season, (from 16 to almost 0 µg Zea/g w.w.) from February to September.

For comparison, the total concentration xanthophyll cycle (XC) pigments (ΣViola+Anth+Zea) in the different brown algae species is listed in Table 5. *S. latissima* is not listed here, as the seasonal sum for the different tissue ages in this species is listed in Table 9.

Table 5: Seasonal sum of pigments that constitutes the xanthophyll cycle in PG1 (Viola, Anth, Zea)

Species	February	May	September
P. canaliculata	63.4	60.4	17.3
F. spiralis	148.2	77.5	64.5
F. vesiculosus	105.3	46.9	114.8
A. nodosum	29.7	51.5	41.6
F. serratus	120.9	69.8	93.8
L. digitata	36.8	29.6	13.8
S. latissima	132.3	21.0	91.5

The ratio between Fuco and Chl *a* in all brown algae examined is listed in Table 6.

Table 6: Seasonal differences in Fuco:Chl *a* ratio in the different brown algae species.

Species	February	May	September
P. canaliculata	0.276	0.301	0.243
F. spiralis	0.217	0.249	0.217
F. vesiculosus	0.242	0.337	0.268
A. nodosum	0.424	0.242	0.210
F. serratus	0.258	0.266	0.225
L. digitata	0.359	0.342	0.204

Rhodophytes (PG2)

β,β-carotene

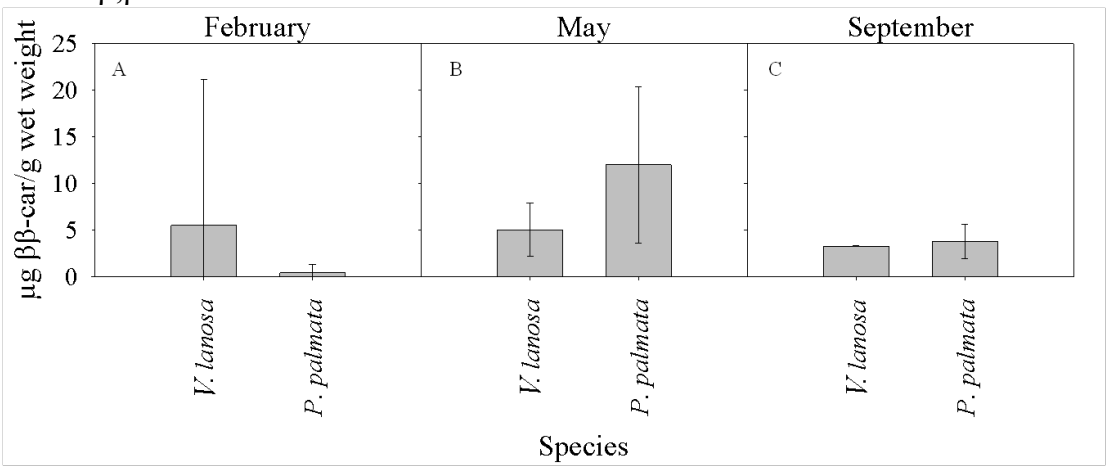

Fig. 13: Seasonal variation in [β,β-Car] between red algae.

No difference was detected between the two Rhodophytes (red algae) in February (*P*=0.2), May (*P*=0.16) or September (*P*=0.78) (Fig. 13 A-C) in the concentration of β,β-carotene.

Zeaxanthin

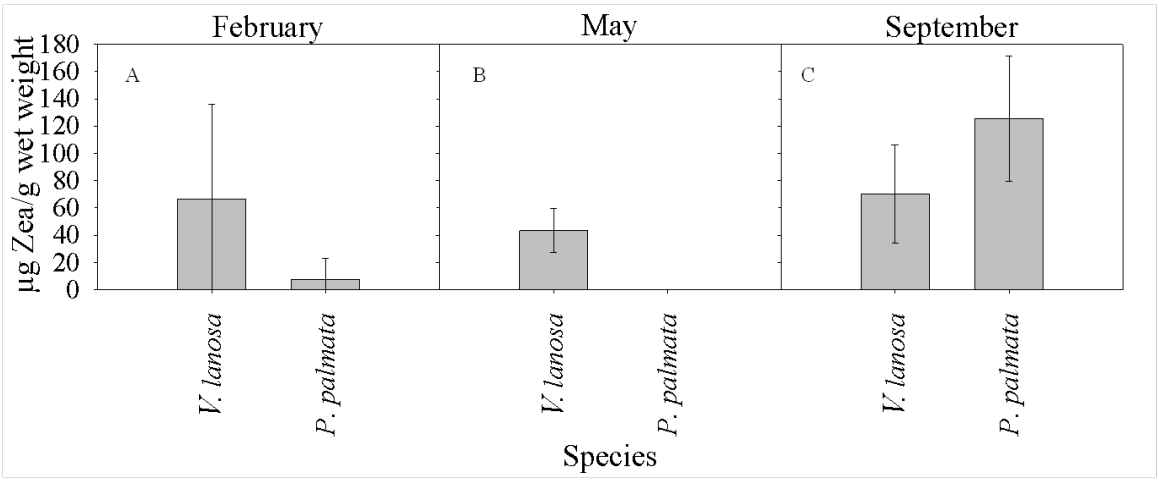

Fig. 14: Seasonal variation in [Zea] between red algae.

There was no significant difference between [Zea] in the two Rhodophytes in February (*P*=0.15) or September (*P*=0.11) (Fig 14 A and C), while in May there was significant difference (*P*=0.0017) with [Zea] ranging from 0 µg Zea/g w.w. in *P. palmata* to 43,3 µg Zea/g w.w. in *V. lanosa* (Fig 14B).

Green algae (PG3)

Only one Chlorophyte species was investigated each season, rendering comparison of intraspecific variation impossible.

Species-specific variation

The main result from the species-specific study was that within each species, all the different LHPs follow the same trend with regards to increase and decrease between seasons.

Phaeophytes (PG1)

For the 6 species of Phaeophytes tested it is noteworthy that none of them showed the same trend through the seasons. Also, intraspecifically, all LHP followed the same trend through the seasons.

Pelvetia canaliculata

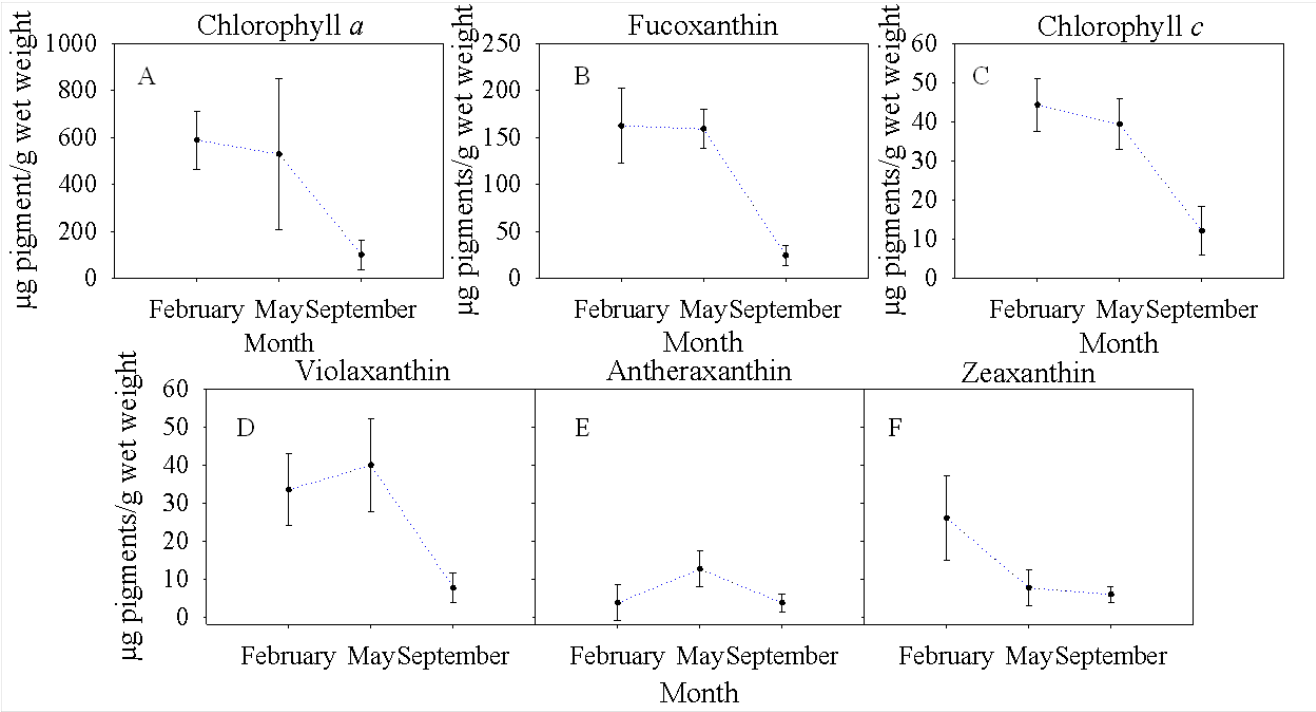

Fig. 15: Seasonal variation in pigment content in *Pelvetia canaliculata.*

In *P. canaliculata*, all LHP showed the same trend, i.e. September had on average 79% lower values of LHP than both February and May. The mean [Chl *a*] (P=0.01), [Fuco] (P=0.0001), [Chl *c*] (P=0.0001) and [Viola] (P=0.002) all showed significant variation between seasons (Fig. 15A-D). This was also true for the mean [Anth] and [Zea]. With regards to [Anth] (P=0.02), the May samples had 70% higher concentration than both in February and September (Fig. 15E). In February [Zea] was 73% higher than May and September (Fig. 15F).

Fucus spiralis

Again in *F. spiralis* all LHP showed the same intraspecific trend; in general there was a decrease in pigment content from February to September. The LHP concentration was approximately 53% higher in February than in May, and the May concentration was ~20% higher than in September.

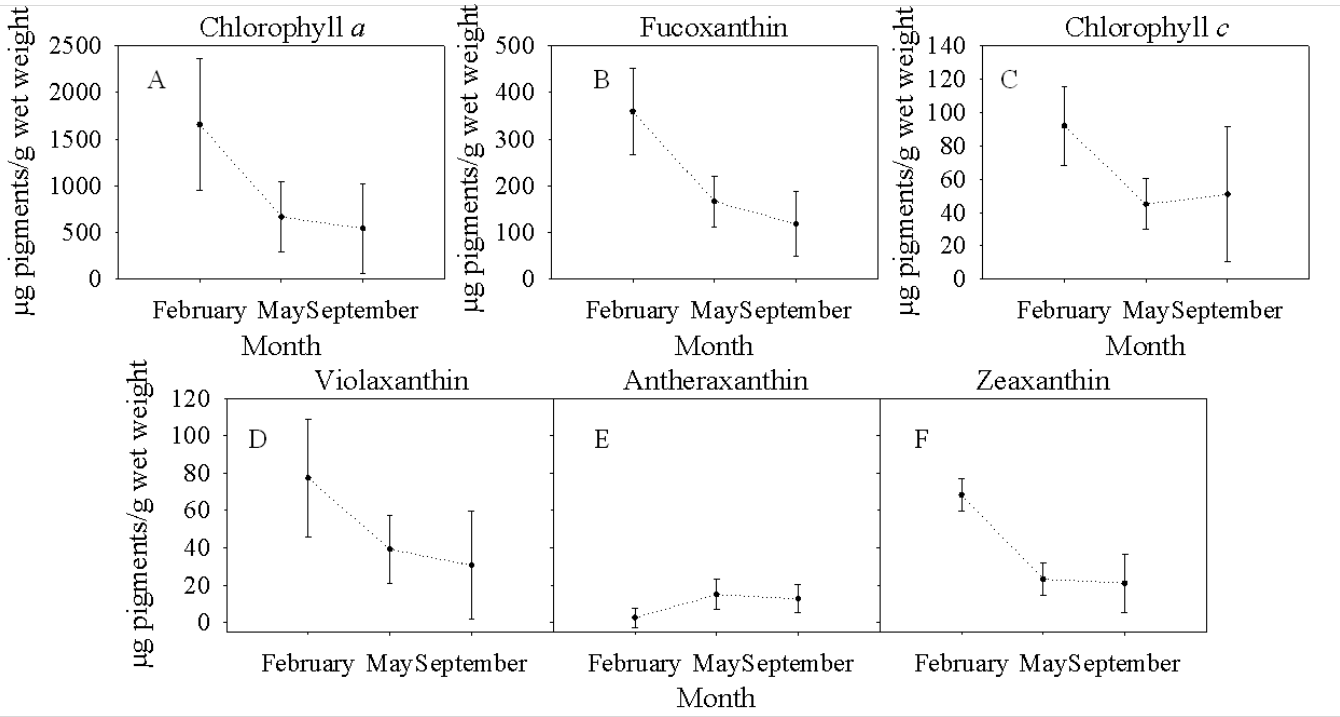

Fig. 16: Seasonal variation in pigment content in *Fucus spiralis*.

The mean [Chl *a*] (P=0.03) and [Fuco] (P=0.003) content showed significant intraspecific variation between the seasons (Fig. 16A, 16B) while the mean [Chl *c*] (P=0.09, Fig. 16C) and [Viola] (P=0.08, Fig. 16D) did not. [Anth] and [Zea] was not found to have any significant variation between seasons (P=0.07 and P=0.0004, respectively, Fig. 16E and F). Still, [Zea] showed the same trend as the LHPs, with a general decrease from February to May (66%), and a further decrease to September (8%).

Fucus vesiculosus

A clear intraspecific variation in mean LHP content between seasons was found, and every LHP in *F. vesiculosus* followed the same trend, i.e. a decrease from February to May (52±8%), and an increase from May to September (72±19%). [Chl *a*] (*P*=0.008, Fig 17A), [Fuco] (*P*=0.006, Fig. 17B) and [Chl *c*] (*P*=0.01, Fig. 17C) all showed significant variation.

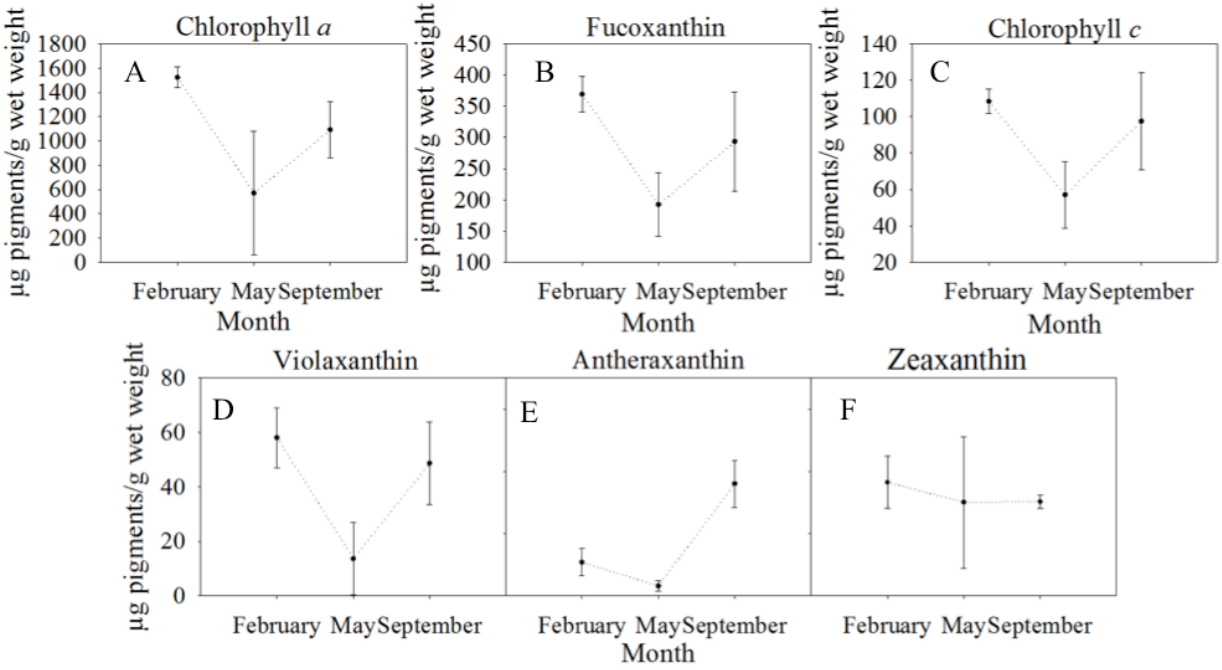

Fig. 17: Seasonal variation in pigment content in *Fucus vesiculosus.*

[Viola] also had a marked variation between seasons (*P*=0.003, Fig. 17D), with the same overall trend as the other LHP, but with a more profound increase and decrease than the other species (76% decrease from February to May, and 250% increase from May to September). Fig. 17E shows that [Anth] also had a clear variation between seasons (*P*=0.000), with a decrease from February to May (72%), and a 12-times increase from May to September. [Zea] showed no noteworthy difference between seasons (*P*=0.74, Fig. 17F).

Ascophyllum nodosum

There was no notable variation between seasons for this species (Fig. 18A-F). For all pigments the May data showed the highest variation (See appendix A for CV values for each).

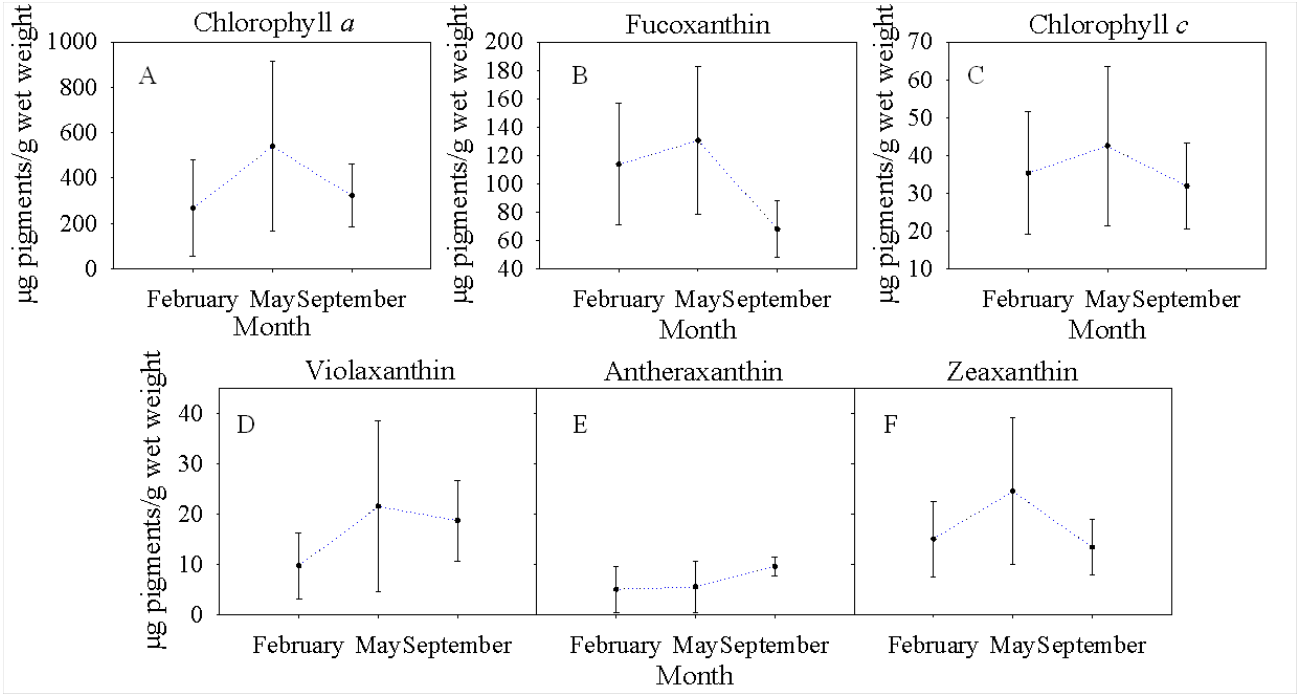

Fig. 18: Seasonal variation in pigment content in *Ascophyllum nodosum*

Fucus serratus

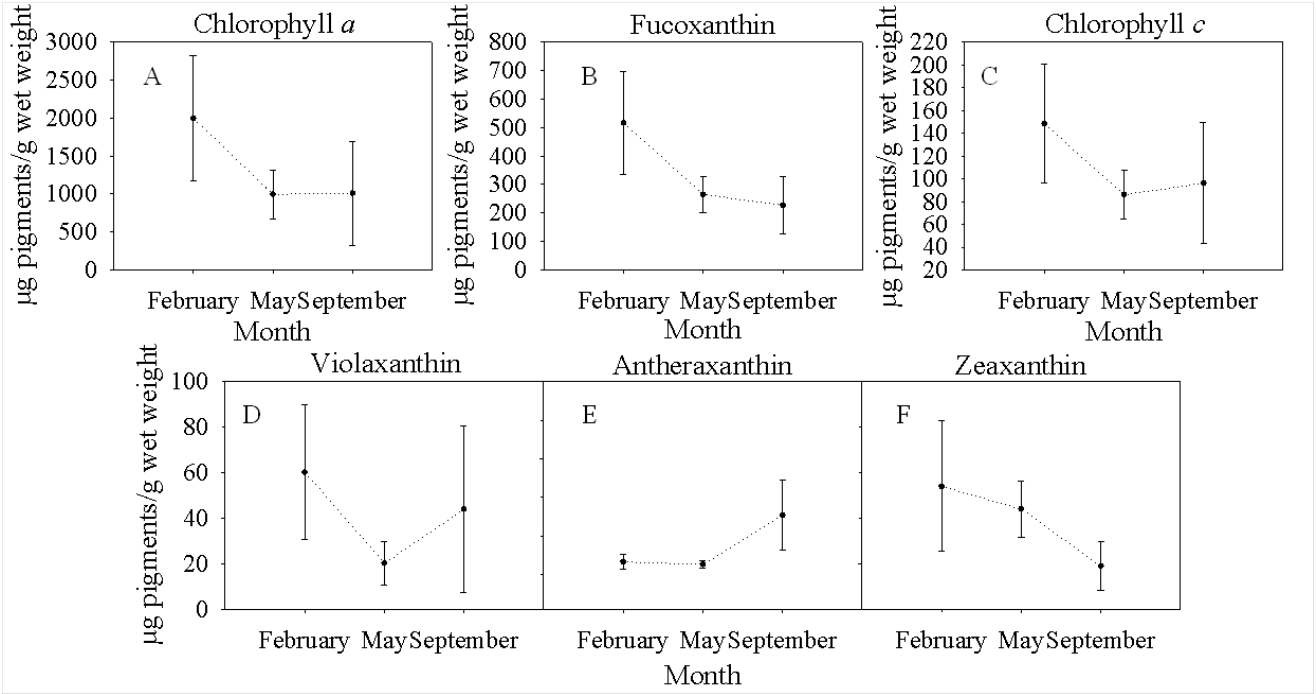

Fig. 19: Seasonal variation in pigment content in *Fucus serratus*.

For *F. serratus*, it was noted that the LHP [Chl *a*], [Fuco] and [Chl *c*] followed the same trend between seasons, while [Viola] did not. Also, there was no significant variation

between seasons with regards to either mean [Chl *a*] (*P*=0.09), [Chl *c*] (*P*=0.16), [Viola] (*P*=0.18) or the [Zea] (*P*=0.07) (Fig. 19A, C, D and F). [Fuco] showed a clear variation between seasons (*P*=0.02), with 2 times higher [Fuco] in February than both May and September (Fig. 19B). There was also a significant difference in [Anth] (*P*=0.015), with a 5 times higher [Anth] in September, compared to both February and May (Fig. 19E).

Laminaria digitata

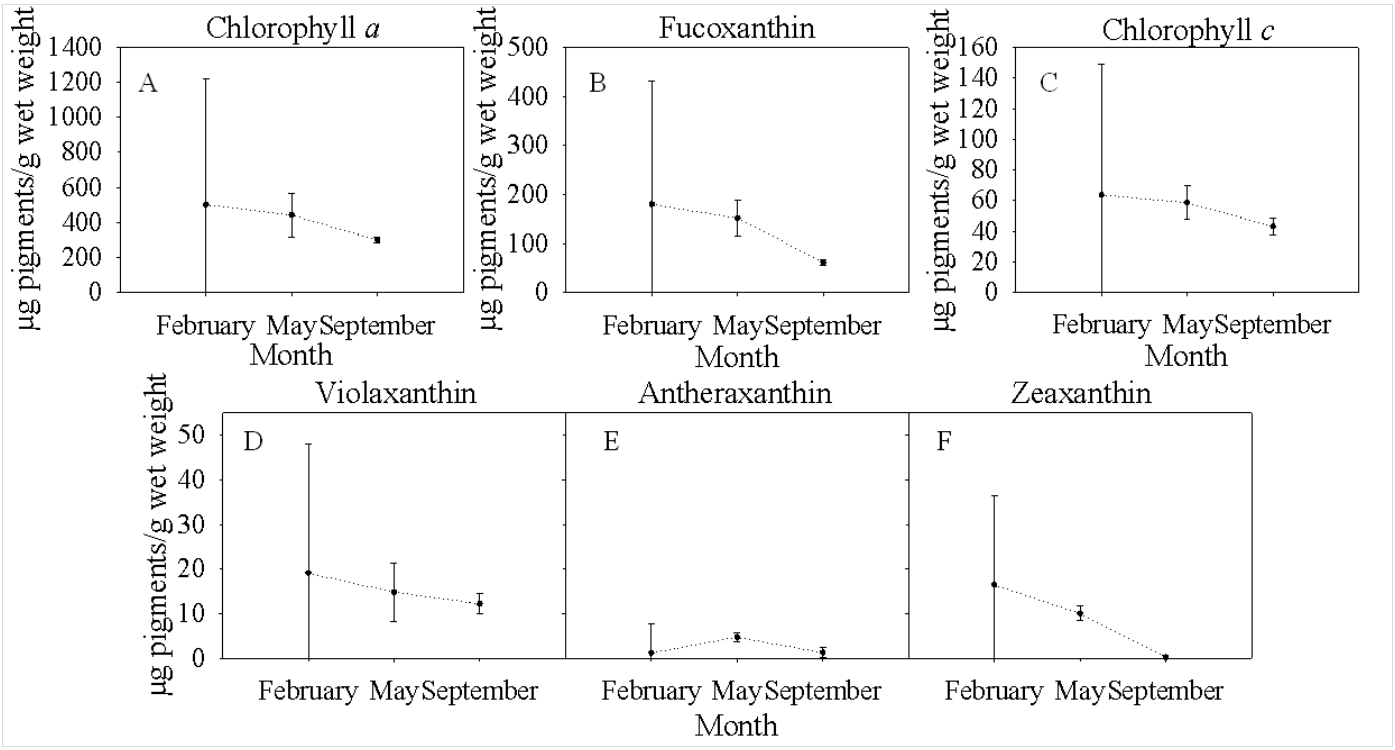

Fig. 20: Seasonal variation in pigment content in *Laminaria digitata.*

[Chl *a*] (*P*=0.099), [Chl *c*] (*P*=0.0856) and [Viola] (*P*=0.313) content in the *L. digitata* samples showed no notable variation between season (only a mean decrease of 13.5±7% from February to May, and 26±6% from May to September, Fig. 20A, C and D) [Fuco] had a clear variation between seasons (*P*=0.003), with September differing from February and May, the September samples contained approximately 60-66% % less [Fuco] than the other seasons (Fig. 20B).

There was a significant variation in [Anth] between seasons, with the May samples containing 4 times more than both February and September (Fig. 20E). There was also significant variation between seasons in [Zea], with a decrease from February to May (39%), and further decrease from May to September (98%) (Fig. 20F) (*P*=0.000). It is noteworthy that the data from February had high SD, which was caused by a high variation in the pigment content between the different individuals.

Saccharina latissima (the age study)

An age gradient study was performed on the tissue from the lamina of *S. latissima*. The data was divided into intraseasonal variation; the variation within each sampling season (between individuals and tissue of different age within individuals), and seasonal variation; the variation in pigment content between seasons (between individuals and overall variation in tissue of different age). The length of each *S. latissima* individual and an environmental description is provided in Table 7.

Table 7: Lamina length and description of surrounding environment (including shadowing effect by other individuals) for the *S. latissima* specimens collected at sampling 1 (February), 2 (May) and 3 (September).

Sampling #	*Kelp #*	*Length in cm*	*Environmental description*
1	1	45	
1	2	41.5	Attached to rope, not shadowing
1	3	31	
2	4	62	
2	5	69	Dense kelp forest, partly shadowing
2	6	65.5	
3	7	66	
3	8	57	Open sandy area, not shadowing
3	9	49	

Intraseasonal variation in pigment content

A high degree of variation was detected between the 3 specimens of *S. latissima* sampled each season. The February samples showed no influence of age on pigment content, whereas age influence was seen both in May and September. The LHPs Chl *a*, Fuco, Chl *c* and Viola was detected in all seasons, while Zea was only detected in the May samples and Anth only in the September samples.

February (Winter)

Variation between specimens in February

In *Saccharina latissima*, there were significant differences in mean pigment content between individuals in [Chl *a*] (P=0.002) and [Chl *c*] (P=0.003) (Fig. 21A-C, G-I) in February. With regards to [Fuco], individual #1 and #2 was similar to each other (28% CV), and ind#3 was significantly different from the other two individuals (P=0.0004, Fig 21D-F). Fig. 22A-C shows mean [Viola], which had significant differences between specimens (P=0.000). No Anth or Zea was detected in any of the individuals sampled in February.

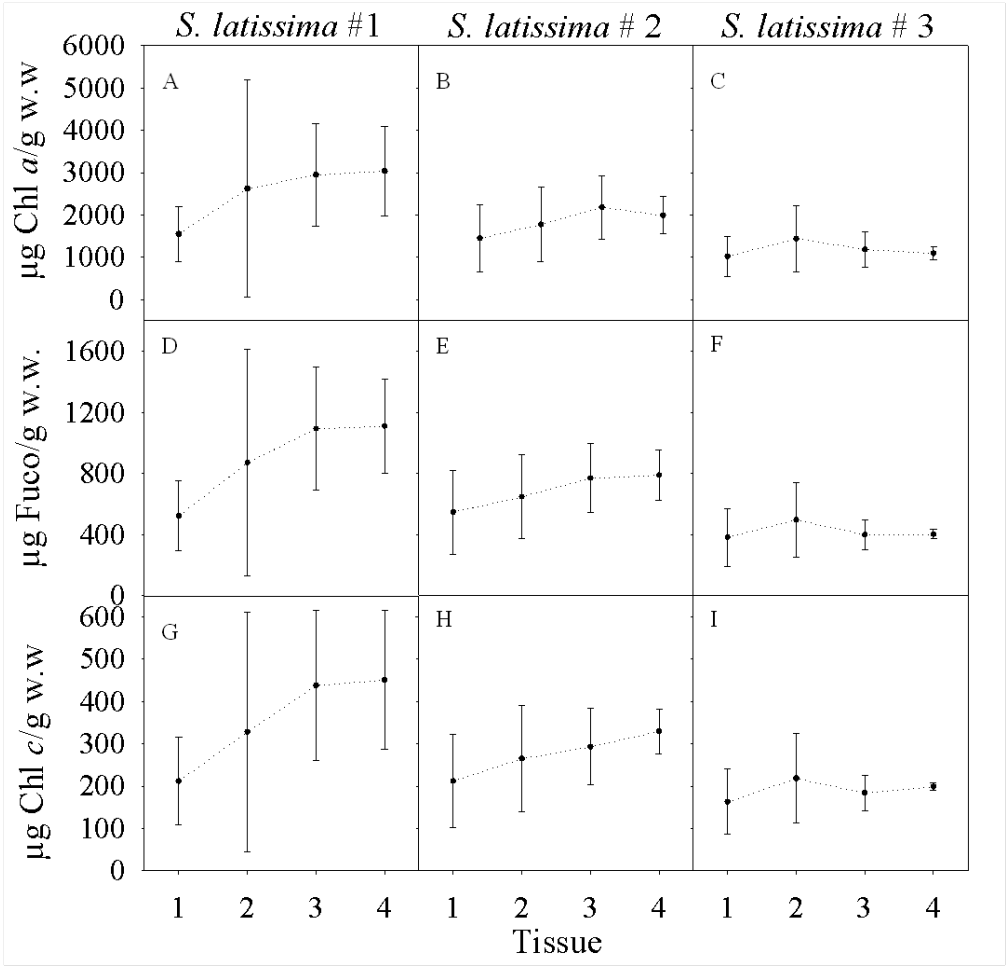

Fig. 21: Variation in Chl *a*, Fuco and Chl *c* content as a function of lamina age in *S. latissima* in February.

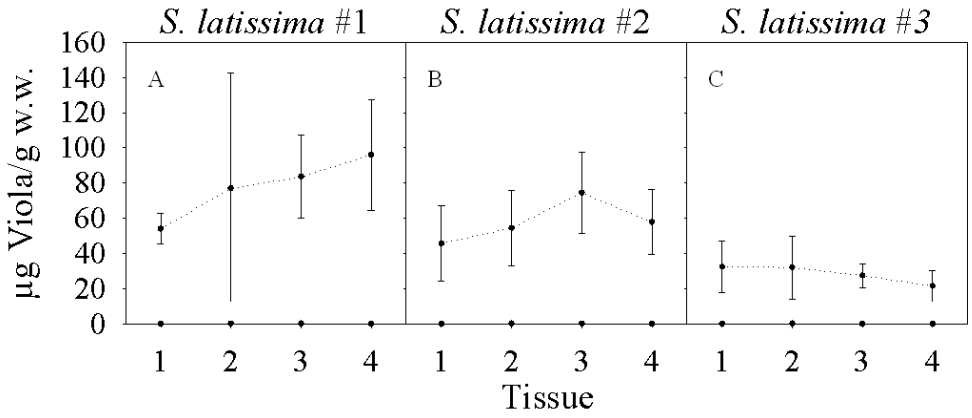

Fig. 22: Variation in Viola content, as a function of lamina age in *S. latissima* in February. No Anth or Zea was found in either specimen in February.

Variation with tissue age within specimens in February

There were no significant differences in any of the investigated pigments between tissues of different age for any of the specimens (Chl *a*: *P*=0.23, Fuco: *P*=0.11, Chl *c*: *P*=0.08, Viola *P*=0.38).

May (Summer)

Variation between specimens in May

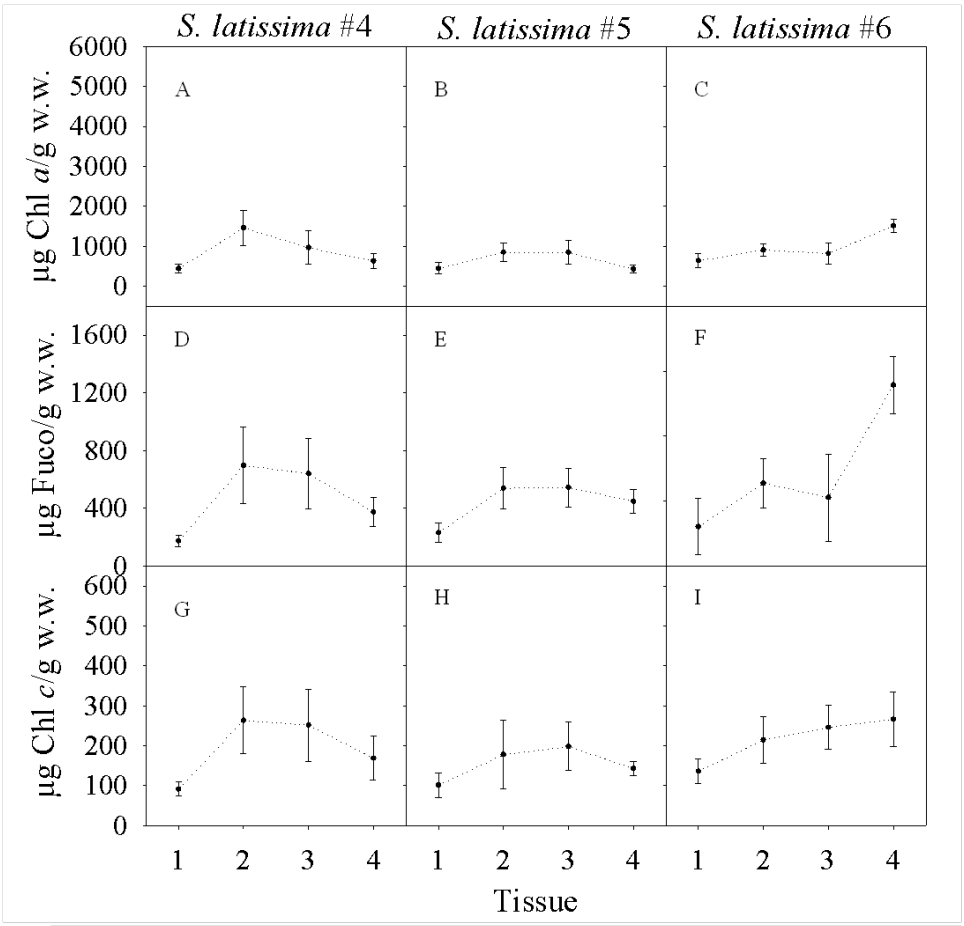

Fig. 23: Variation in pigment content as a function of lamina age in *S. latissima* in May.

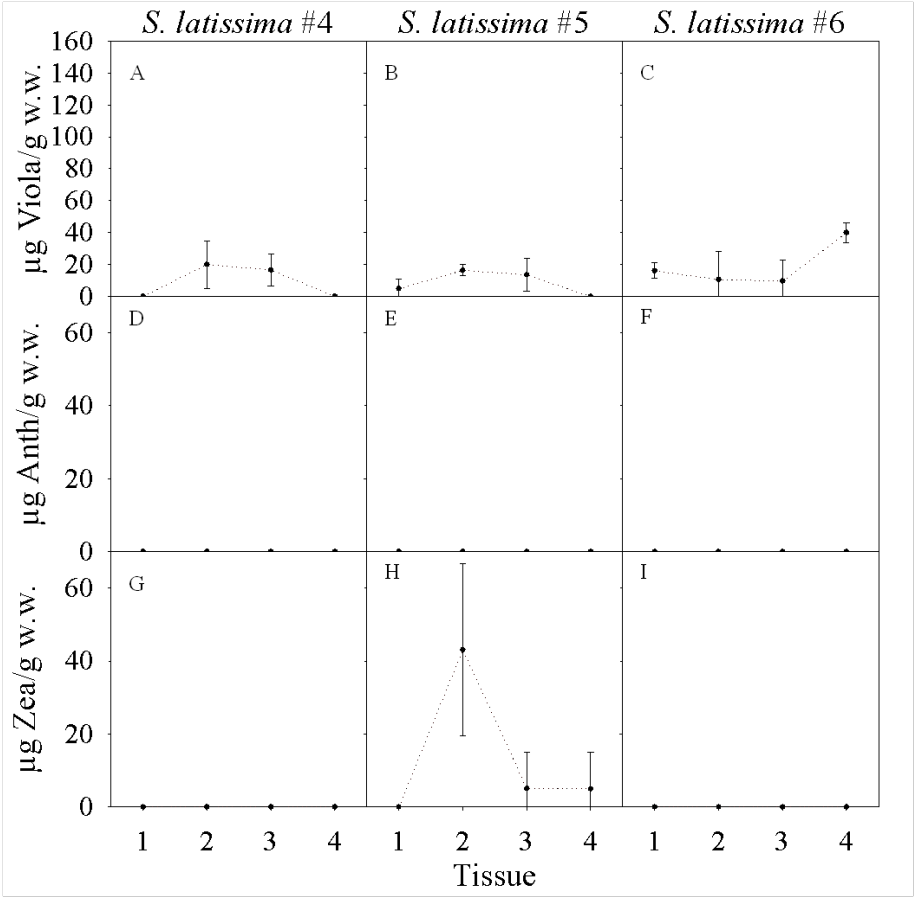

Fig. 24: Variation in pigment content in the xanthophyll cycle, as a function of lamina age in *S. latissima* in May.

[Chl *a*] showed significant differences between specimens (*P*=0.03), especially between #5 and #6, while #4 showed intermediate [Chl *a*] (Fig. 23A-C). [Fuco] showed no significant difference between specimens, albeit just barely (36% CV, *P*=0.051, Fig. 23D-F), but [Chl *c*] showed a clear variation between specimens (*P*=0.005), and (Fig. 23G-I).

The mean [Viola] also showed significant differences between specimens (*P*=0.04), with ind#6 standing out from the other two in that it contained higher amounts (Fig. 24A-C). There were notable differences in [Zea] between specimens (*P*=0.002) (Fig. 24G-I). Anth was not found in any of the *S. latissima* sampled in May (Fig. 24D-F).

Variation with tissue age within specimens in May

In May there was a significant influence of age on [Chl *a*] (*P*=0.003). [Fuco] and [Chl *c*] also showed a clear influence of tissue age on the pigment content (*P*=0.000 and *P*=0.0003, respectively). No influence on tissue age was seen in [Viola] (*P*=0.4). [Zea] showed significant influence of age (*P*=0.01), albeit only one specimen was found to contain this pigment.

September (Autumn)

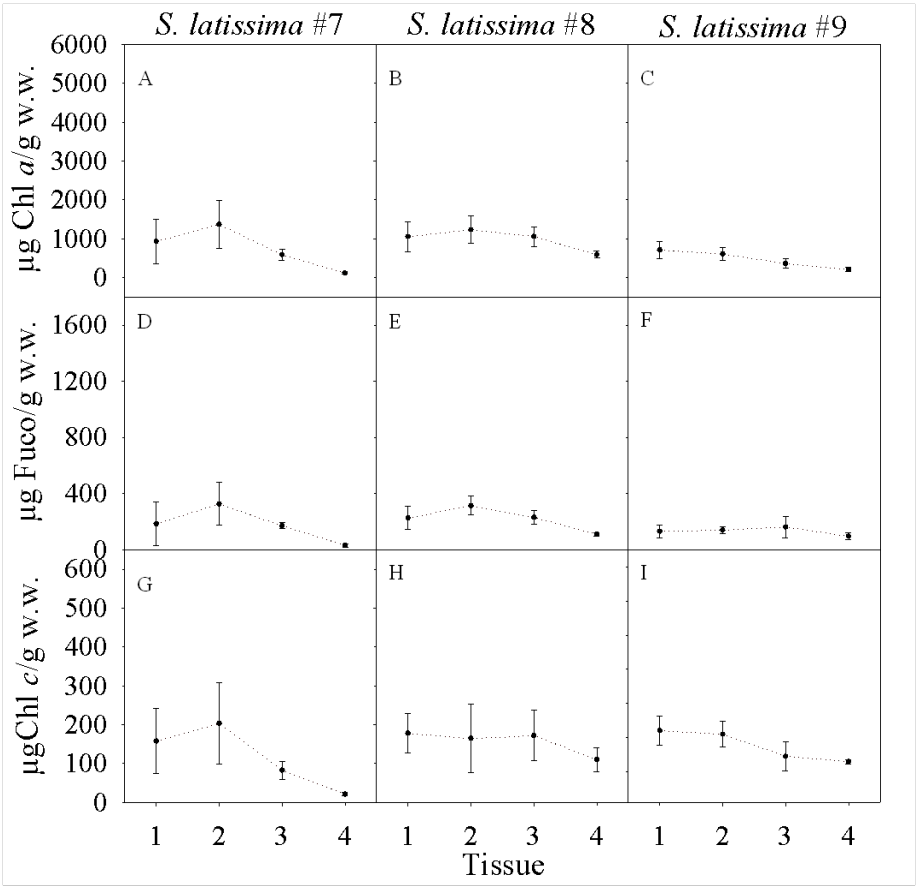

Fig. 25: Variation in pigment content as a function of lamina age in *S. latissima* in September.

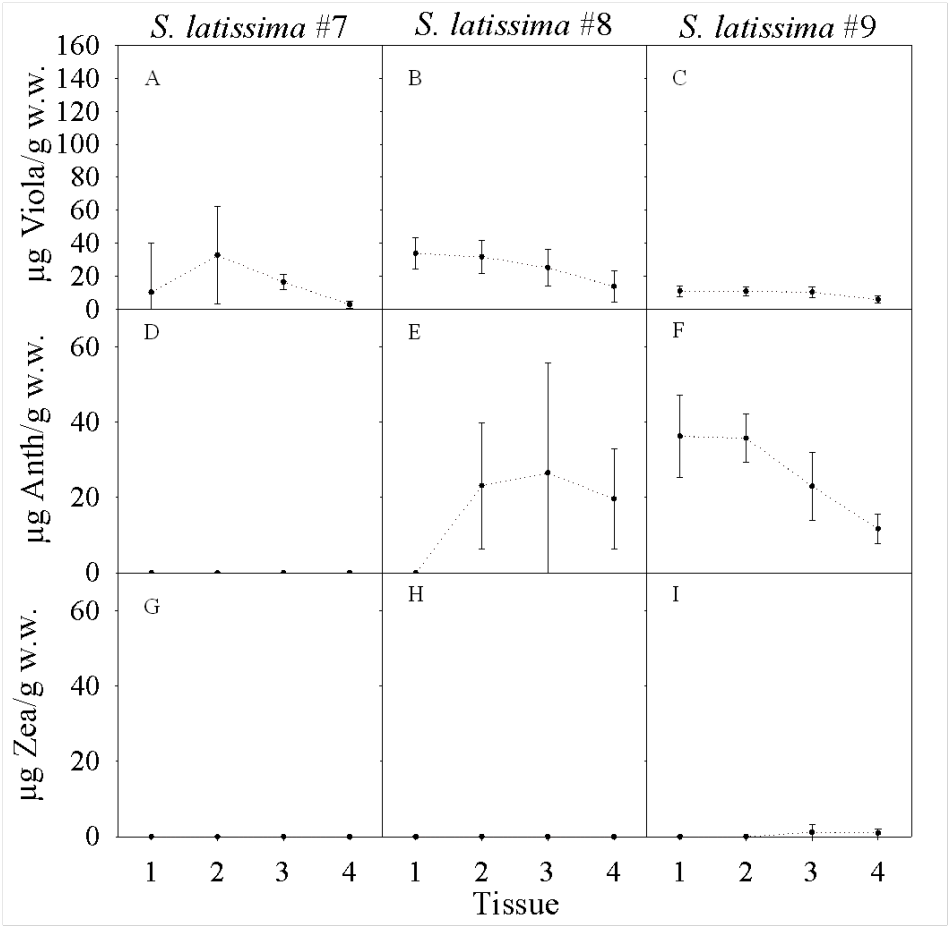

Fig. 26: Variation in pigment content in the xanthophyll cycle, as a function of lamina age in *S. latissima* in September.

Variation between specimens in September

A high biological variation in [Chl *a*] was found in September (CV 54%, *P*=0.0003), with ind#9 being most different from the other two (Fig. 25A-C). The mean [Fuco] (*P*=0.009) and [Chl *c*] (*P*=0.001) also showed significant variation between specimens (CVFuco: 49%, CVChl *c*: 53%) (Fig. 25D-I).

There was a difference between specimens in [Viola] in September, as ind#9 contained less Viola than ind#7 and #8 (*P*=0.0006) (Fig. 26A-C). The mean [Anth] also showed a difference between specimens (*P*=0.000) (Fig. 26D-F). There was no variation in [Zea] with respect to specimen (*P*=0.07) or (Fig. 26G-I).

Variation with tissue age within specimens in September

There was a significant influence of age on [Chl *a*] (*P*=0.000). There was also a clear influence of age for both Fuco and Chl *c* (*P*=0.000, *P*=0.0001, respectively). [Viola] also showed significant influence of age in May (*P*=0.005), but no age influence was seen in the [Anth] (*P*=0.32) or [Zea] (*P*=0.43).

Seasonal variation in *Saccharina latissima*

A high degree of biological variation was found between seasons. In general, February showed the highest concentration of all LHP, while there was a decrease towards May and September, these months showed fairly similar [LHP] (e.g. Chl *a* had a 58% decrease from February to May and September). The [Chl *a*] shows clear seasonal variation (*P*=0.000), with a total seasonal variation of 63%. In general there was a decrease from February to May and September (Fig.21A-C, 23A-C and 25A-C). The [Fuco] also showed a clear seasonal variation (a total variation of 62%) (*P*=0.000), (Fig. 21D-F, 23D-F and 25D-F). It is noteworthy that ind#6 (from May) had a more pronounced variation in [Fuco] between tissues of different age than other individuals. The [Chl *c*] content showed seasonal variation (*P*=0.000) (51% CV) (Fig. 21G-I, 23G-I and 25G-I9).

With regards to the [Viola] there was once again a high degree of seasonal variation (CV 90%) (*P*=0.00). Tissue from February differed most, with 4.5 times higher [Viola] than May, and 3.2 times more than September (Fig. 22A-C, 24A-C and 26A-C). The [Anth] also had clear seasonal variation (219% CV) (*P*=0.000), wherein the September samples had 15 times more than the other two samplings (22D-F, 24D-F and 26D-F). Mean [Zea] also had clear seasonal variation (471% CV) (*P*=0.005), with a 44-time increase from February to May, and then a 22-time mean decrease from May to September (Fig. 22G-I, 24G-I and 26G-I).

Interaction between season and tissue age

There was not found any interaction between season and age on [Chl *a*] (*P*=0.06). No interaction between season and age on [Viola] (*P*=0.45), nor any interaction between season and age on [Anth] (*P*=0.65). On the other hand, there was a significant interaction between season and age (*P*=0.048) on [Fuco]. In addition, there was a significant interaction between season and age (*P*=0.002) on [Chl *c*]. Finally, a clear interaction between season and age was seen on [Zea] (*P*=0.005).

The total XC pigment content in tissue of *Saccharina latissima* is listed in Table 8.

Table 8: Total xanthophyll cycle-pigment content in tissue of *S. latissima*

Sampling	Feb.			May			Sep.		
# / Tissue	#1	#2	#3	#4	#5	#6	#7	#8	#9
1	54.1	45.8	32.4	0.0	4.9	16.1	10.3	33.9	47.3
2	77.1	54.4	32.1	20.0	59.4	10.6	32.7	54.9	46.6
3	83.7	74.5	27.5	16.6	18.5	9.7	16.4	51.7	34.4
4	96.0	57.9	21.5	0.0	5.0	40.0	2.9	33.4	18.5

Rhodophytes (PG2)

Vertebrata lanosa

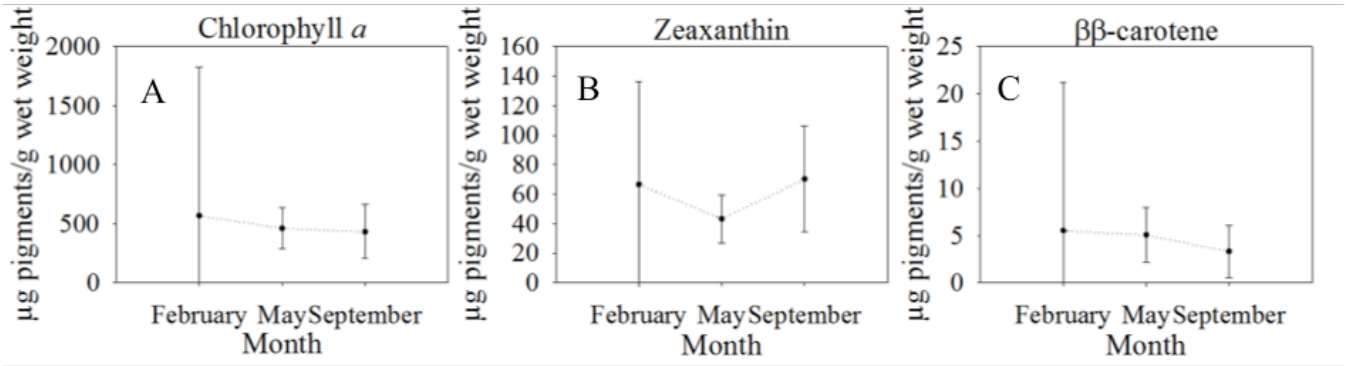

Fig. 27: Seasonal variation in pigment content in *Vertebrata lanosa.*

None of the main pigments in this species showed any clear variation between seasons (Fig. 27).

Palmaria palmata

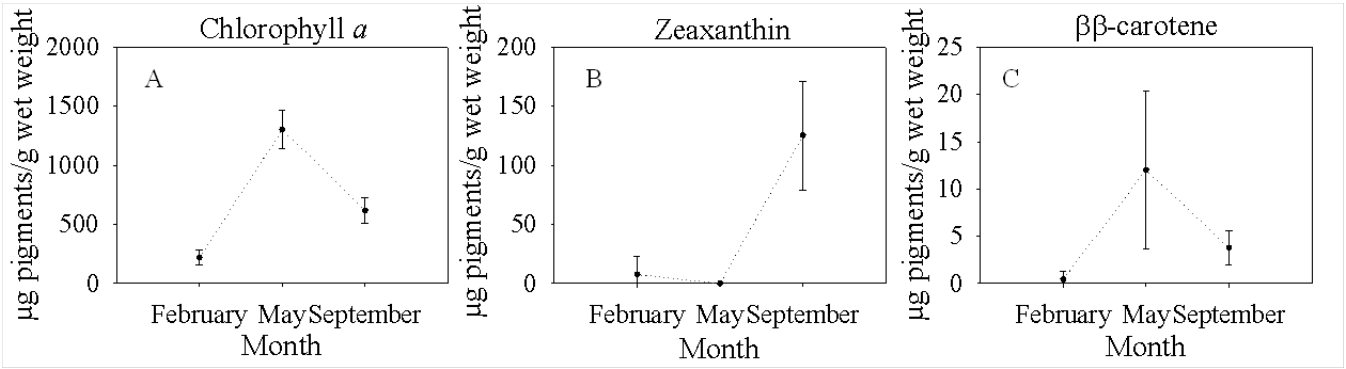

Fig. 28: Seasonal variation in pigment content in *Palmaria palmata.*

[Chl *a*] was significantly different between seasons (P=0.000). As seen in Fig. 28A, the [Chl *a*] in *P. palmata* was lowest in February, highest in May and intermediate in September. [β,β-Car] showed the same seasonal trend as Chl *a*, also with a significant variation (P=0.025) (Fig. 28B). [Zea] showed a notable variation between seasons (P=0.0002), the September samples contained on average 31.25 times more [Zea] than the other two samplings (Fig. 28C).

Chlorophytes (PG3)

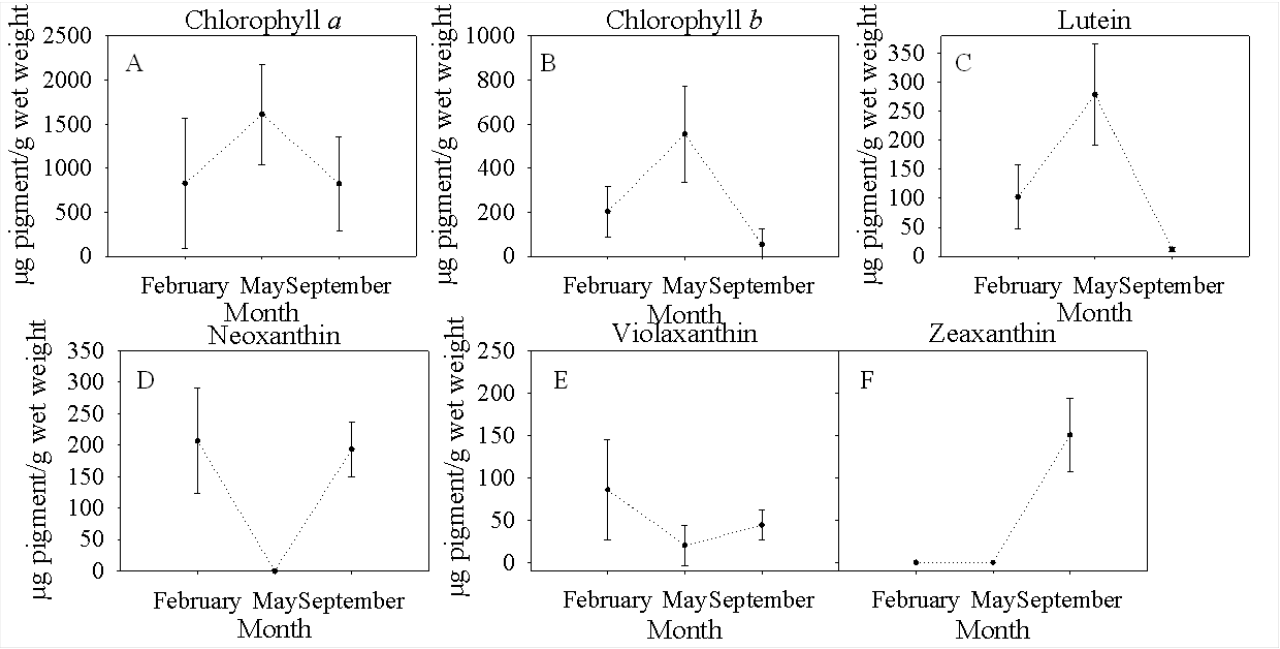

Fig. 29: Seasonal variation in pigment content in the Chlorophytes.

The main pigments detected in PG3 were the LHPs Chl *a*, Chl *b*, Lut and Viola, and the PPCs Neo and Zea. For *C. rupestris*, February and September were the only two months sampled. The pigments [Chl *a*] (*P*=0.99), [Chl *b*] (*P*=0.07), [Neo] (*P*=0.78) and [Viola] (*P*=0.23) did not show any significant seasonal variation (Fig. 29A, B, D and E). As seen in Fig. 29C the mean [Lut] was significantly higher in February than in September. (*P*=0.02). [Zea] did also differ between seasons, with clearly more in September. Albeit, it was an increase from 0 to 150 µg Zea/g w.w. from February to September (*P*=0.000) (Fig. 29F).

Notes on Ulva lactuca

The [Chl *a*] (*P*=0.17) and [Viola] (*P*=0.09) content in *U. lactuca* in May was not significantly different from the content in *C. rupestris* in the other months (Fig. 29A and E). On the other hand, the [Chl *b*], [Lut] and [Neo] (*P*=0.001) content in *U. lactuca* in May was significantly different from *C. rupestris* in the other months (Fig. 29B, C, D). The [Chl *b*] in May was 10 times higher than February, and 3 times higher than September (*P*=0.003). The [Lut] was 3 times higher than February, and 23 times higher than September (*P*=0.0004). In *U. lactuca* in May no [Neo] was detected, while it was found in *C. rupestris* other months. [Zea] in *U. lactuca* in May was the same as *C. rupestris* in February, but significantly different from [Zea] in September (*P*=0.000). No Zea was found in February and May at all (Fig. 29F).

Discussion

This study showed that macroalgae have a high degree of both intraspecific and interspecific biological variation in pigment content, and that pigment content and composition vary with season. In addition, in some seasons, pigment content in *Saccharina latissima* was found to be a function of the age of the lamina tissue.

Class- and interspecific differences (Phaeophytes)

A general trend for the species comparison in Phaeophytes, is that the three Fucus species tended to have high pigment content, while *Ascophyllum nodosum* was recurring as the species with the lowest concentration of all pigments. Of the two kelp species, *Laminaria digitata* generally had low pigment content compared to the Fucus species and *Saccharina latissima*, the latter showing a high degree of variation in pigment content but generally found among the "high-containing" species.

One possible explanation for the low pigment content in *A. nodosum* compared to the other species investigated is that as this species is quite stiff and compact in stature, it might contain more of other components, e.g. polysaccharides (Black 1948). One study has shown that *A. nodosum* had high alginate content compared to other macroalgal species studied (Black 1948; Black 1950a). As the pigment content is micrograms per gram wet-weight of tissue (w.w.), high alginate content, or a high content of other molecules, would mean a lower pigment-to-wet weight ratio. This could explain the "low" pigment content observed in *A. nodosum* in this study. In addition, another on-going study on the elemental composition of macroalgae has found a low content of all major elements in *A. nodosum* compared to other species (Kleiven 2014, personal communication). It is also possible that this species, and other similarly "stiff" species (e.g. *L. digitata*) have a high content of water in the vacuoles of the cells, resulting in a high wet weight and furthermore a low pigment-to-wet weight ratio.

The three Fucus species were found to have high mannitol content in a study by Black (1949), and it is somewhat surprising to see that their pigment to wet weight ratio were among the highest when comparing the species investigated in this study. Extensive research of the available literature that mentions these species resulted in little information on the content of other components in these species. It could therefore be that these species has a low content of components other than mannitol,

(e.g. water) and that pigments therefor make up a large fraction of total wet weight. This is seen as a high pigment to wet weight ratio in this study.

Compared to other species, *Laminaria digitata* was regularly found among the low pigment containing species. Personal observation found *L. digitata* to be a very stiff species, which is somewhat confirmed by several other studies finding high content of polysaccharides in this species compared to *S. latissima* (Black 1950a; Black 1950b). This might explain the observed low pigment content, but as *S. latissima* in the aforementioned studies was found to have similar (albeit somewhat lower) polysaccharide content, these two species should have had a more similar pigment-to-wet weight ratio. Personal observations noted that *S. latissima* has a far thinner lamina than *L. digitata*, indicating that some other component (e.g. water) constitutes the observed difference in lamina thickness, and therefore the observed difference in pigment content in relation to wet weight. More data is therefore needed on the relationship between chemical composition and pigment content in these species also, to make any conclusions on the determining factor for pigment content.

Species-specific variation (Phaeophytes)

Pelvetia canaliculata

P. canaliculata had a high LHP content in February and May compared to September, which would mean it was low light (LL) acclimated in February and May, and high light (HL) acclimated in September. This species is located in the supralittoral zone, and almost exclusively receives direct sunlight. As mentioned in the introduction, the light climate of previous weeks determines the pigment content and composition. As seen in Table 4, the algae received low irradiance caused by low sun-angle and short day-length in February and so “high” concentration of light harvesting pigment content is to be expected in accordance with LL acclimation (Ramus et al. 1977; Brunet et al. 2011). In addition, weather data shows that between 1/3 and 1/2 of the month was cloudy/overcast, causing low irradiances and this can cause LL-acclimated cells with high content of LHP’s (Table 4). It is important to point out that while pigment content in *P. canaliculata* might be low compared to other species in the different seasons, it is how the pigment content within the species changes with season that indicate the acclimation status. May is usually a brighter month relative to February, but high pigment content was also found in May. The weather data showed that April, the month before sampling was carried out, had the same amount of overcast days as February, although because of increased day length

this month still would provide more irradiance in total. There was a greater variation in Chl *a* content in May (seen as high SD for Chl *a*), which could mean that the individuals were to few to reveal the general acclimation status for the species. Other individuals could have shaded some of the individuals that were sampled, and as such the experienced light climate of the different replicates could vary greatly. The last sampling was carried out early in September, and as August had few overcast days, the decrease in LHP from February and May observed in these experiments was as expected (see Table 4).

Fucus spiralis

This species was found high in the eulittoral zone, which means it was exposed to air and direct sunlight regularly. As expected, high LHP content was observed in February, indicating LL acclimation. In contrast, for the May and September samples the content of all pigments, with the exception of Anth, was significantly lower, indicates that HL acclimation. The Zea content was remarkably high in February. This could be due to errors in handling, e.g. exposure of sample to light before extraction, driving de-epoxidation of Viola to Zea. Although, the xanthophyll cycle pigment content might not be representative, as handling (storing tissue in dark bags) would affect the xanthophyll cycle (as the epoxidation can happen in seconds) and thus the results. If a LL acclimated organism was exposed to high light during handling this could possibly result in a reaction.

Fucus vesiculosus

Growing slightly above the middle eulittoral zone (Fig. 1), this species would most likely be less affected by particles in the water attenuating light, and be more exposed to direct sunlight. The results showed high LHP content in February, indicating LL acclimation. As shown in Table 4, the difference in light reaching the sea surface between February and the two other sampling dates is caused by day length and changing sun angle. February had shorter days and lower sun angle, and relative to each other it is expected that the individuals collected in February then be acclimated to lower light than the two following samplings. Again, as expected, the samples from May have lower LHP content, indicating HL acclimation due to higher irradiances caused by longer days and higher sun angle. Although, note the high CV of the Chl *a* content which indicates a high degree of biological variation between replicates. The LHP content increase in September, compared to February and May. This is surprising, as the weather and sun data indicates a light climate very similar to

that before the May sampling. One explanation could be that the samples experienced shading by other algae, or by some of its own tissue.

Ascophyllum nodosum

This species is placed in the middle of the eulittoral zone, being submerged in seawater 50% of the diurnal cycle. Overall, this species showed lower pigment content compared to other species, with no significant seasonal variation. As it is placed in the middle of the zonation, it would be expected to have similar pigment content to the other species collected from this zone. One explanation for the low pigment content could be found in the chemical composition of this alga, as discussed earlier. As noted by Black (1948), this species contained relatively high amounts of alginate (albeit, this is compared to Laminariaceae). Personal observations did confirm that the specimens of this species are rigid, which might indicate high polysaccharide content. If polysaccharides make up a large fraction of the total wet weight compared to pigments, this could lead to low pigment-to-wet weight ratio. This might also be caused by water content in the cells, as previously discussed. Generally, *A. nodosum* showed a high degree of variation in May, ranging between 40%CV for Fuco and 90%CV for Anth (CV listed in Appendix A) for all pigments in May, indicating a high degree of variation between replicates. This could be a result of the replicates from this sampling being collected from individuals exposed to varied degrees of shading.

Fucus serratus

This species show a similar trend as *F. spiralis*, with high LHP content in February, relative to May and September. This was as expected, LL acclimation in February (shorter days and lower sun-angle giving a low total irradiance per day) and HL acclimation in May and September (longer days, higher sun-angle). The only exception is Viola, which increased slightly towards September. The reason for this could be that the individuals sampled contained Anth or Zea at sampling time (which would not be unexpected after longer periods of exposure to HL (Ramus et al. 1977; Brunet et al. 2011), and that this was transformed to Viola before extraction. It could also be that the specimens had produced Viola for later transformation to Zeaxanthin if needed.

Laminaria digitata

L. digitata had fairly similar pigment content between sampling periods (with a mean decrease of 13.5±7% from February to May, and a further mean decrease of 26±6% from May to September), compared to the wrack species, which showed a more pronounces though highly variable seasonal variation. There was high variation for all pigments in February (see appendix A for CV), which indicates high degree of biological variation. This could be because the Laminariaceae grow at high rates during winter (Lüning 1979), and rapid growth could lead to variation in pigment content between individual kelps, if these individual kelps grow at different rates. It is also a deeper living species, which is almost always submerged except from spring tide. In this experiment, February received lower irradiances, and phytoplankton blooms in May and September could make less light available for this deeper lying species (Table 3 and 4), which could explain this species stable pigment content compared to more shallow-lying species.

Note that *Laminaria digitata* is in fact part of a species complex that may comprise three hard-to-distinguish species, namely *L. digitata, L. hyperborea* and *Saccharina groenlandica* (Lund 2014, personal communication).

Summary of species specific study

The variation in pigment composition was found to be species specific, as no similar trend in variation between seasons was seen between the different species examined. This suggests that immediate light climate for individual algal tissue is the most important, determining factor for pigment content, i.e. shading by other individuals or own thallus/lamina might also have a great influence on pigment content and composition. If seasonal light regime was the major determining factor for pigment content and composition, a mutual trend for all species in the changes of pigment content and composition with season and depth should have been found.

Furthermore, the LHP content follows the same trend as Chl *a* within all Phaeophytes examined. This was expected, since the majority are bound together in the same light harvesting complexes.

Ratio between Fuco and Chl *a* (Fuco:Chl *a*)

Fuco is responsible for more light harvesting than Chl *a* (60% vs. 5%) in green coastal waters in Trondheimsfjorden, since the water is blue-green and Fuco absorbs green light (450-540) (Jerlov 1976; Kirk 1994; Johnsen et al. 1994)

The ratio of Fuco:Chl *a* in *Pelvetia canaliculata* and *Fucus spiralis* was fairly stable, and since it was positioned in the upper part of the littoral zone, exposed to air and direct sunlight and not as effected by light conditions in the water as deeper positioned species might be, this was relatively expected. For both species the highest Fuco:Chl *a* ratio was found in May when spring bloom takes place, which could make the water greener. This could affect light regime for the algae in the periods of submersion, triggering Fuco up-regulation, which further could explain this change in ratio. The same Fuco:Chl *a* ratio-change was found in *F. vesiculosus*, but with even higher ratio-change. The reason for the higher ratio-change could be that it is position further down in the littoral zone and thus more affected by the light climate in the water.

As for *Ascophyllum nodosum*, it obtained a higher ratio in February than May and September (almost twice as much). This indicates that this species somehow experienced a "greener" light climate this month, since Fuco absorbs in the green part of the light spectrum and to take full advantage of the available light the specimens might have adjusted its pigment composition accordingly (Johnsen et al. 1994). A possible explanation for this could be the low angle of the sun (Table 4) causing light to be heavily attenuated when hitting the sea surface, possibly resulting in a change in the "colour" of the light available for the macroalgae (Kirk 1992; Sakshaug et al. 2009).

F. serratus and *Laminaria digitata* showed approximately the same trend, were the same ratio was found in February and May, and a lower ratio in September. The reason for this could be that for these deeper lying species the light climate would be slightly different as less light penetrates. If then the light quality was on the "greener" end of the spectrum before the first two samplings, while it shifted to a slightly "bluer" light before the last sampling that could impact the observed pigment ratio. The phytoplankton data in Table 3 confirms that there was less phytoplankton in the water in September than May. In February on the other hand, there was even less phytoplankton, so the ratio should have been even lower. The reason for this not

being the case could be the lower angle of the sun, changing the colour of the incoming light, as previously discussed.

Saccharina latissima

Intraseasonal variation in pigment content

With regards to overall LHP content, in February and September these pigments follow the same trend as Chl *a* within each specimen (although no specimen is exactly the same). This was also the case in May, but with a greater variation in pigment content within specimen, between tissue of different age – a more intensive variation, with steeper increase and decrease in Fuco and Chl *c* than Chl *a.* This greater variation could be due to higher light leading to greater differences between tissue placed in direct sunlight and tissue placed in shadow. This coincides with the notes on environment provided in table 8, as the individuals from May where the only ones sampled in a dense kelp forest experiencing shading, while the individuals from other two samplings were collected in more open areas.

In general, little Antheraxanthin and Zeaxanthin was found in *S. latissima*, which could be due to this species being a deeper growing species, where lower amounts of light reaches, yielding less need for photoprotection. This could also be caused by the dark light handling, which as previously mentioned could trigger a transformation of these pigments to Violaxanthin. As little Violaxanthin was found as well, this could indicate that this species has little need for the photoprotective capacity of the XC.

Effect of tissue age on pigment content

The effect of tissue age on pigment content was found in varying degree between pigments and seasons. In February, there was no influence of age on either pigment. In May, Chl *a*, Fuco, Chl *c* and Zea showed significant differences, while Viola did not. Anth was not found this month. In September Chl *a*, Fuco, Chl *c* and Viola showed influence of age, while Anth and Zea did not. The varying results in this study showed no uniform trend in pigment content throughout lamina tissue, as would be expected if ageing of tissue had a direct influence on the pigment content. This indicates that age is in fact not a determining factor, and that the different tissue ages in *Saccharina latissima* acclimates to the immediate light climate, and rather that the observed variation is determined by the sampled specimens not being positioned in the water the same way (Table 8).

As showed by Colombo-Pallotta et al. (2006), macroalgal tissue can adjust pigment content to surrounding light regime, thus it is possible that this is also the case for *S. latissima*, and the tissue had in fact acclimated to its position in the water (shading by other individuals, by other parts of self, rocks etc.). Overall, in February all individuals showed an increase in light harvesting pigment content from meristem towards older tissue. Ind#1, which showed higher variation in all tissue ages than the other two individuals from the same sampling (Ref. Appendix A4), was the longest of the three collected in February (45 cm), which could also partly explain the higher variation in pigment content. Fig. 30 is provided to illustrate the variation in pigment content found throughout the lamina in *S. latissima*, both longitudinally and latitudinally. This indicates that better control over environmental variables such as light could be necessary to better understand the variation in pigment content with tissue age in macroalgae, both for conservation and cultivation purposes.

The similar trend in ind#1 and ind#2 suggests that these two were positioned similarly in the water. Since the meristem tissue in these two is relatively HL acclimated, while the older tissue were more LL acclimated, this suggests that the individuals might have been positioned upside-down, possible hanging downwards from the rope from which they were collected.

In May, less pigment was found throughout the lamina of the different specimens of *S. latissima* compared to February. This suggests that overall the individuals sampled were more HL acclimated in May, which would be expected in an early summer month. The individuals from May were the longest collected (lamina of 62-69 cm), and they were collected in a dense kelp forest, partly shadowing each other. The trend in pigment content was not the same between any of the specimens, which suggests that they all had different positions in the water, and different amounts of shading. Ind#4 seems to be HL acclimated in tissue 1 and 4, and LL acclimated in tissue 2 and 3 (relative to each other). This could be because the middle part of the lamina was shaded more than the two ends. Ind#5 showed less variation throughout the lamina (e.g. 51%CV and 37%CV for Chl *a* in Ind#4 and #5, respectively), with generally low content, except for Zea content in which the content was high in tissue 2. This suggests that it was HL acclimated.

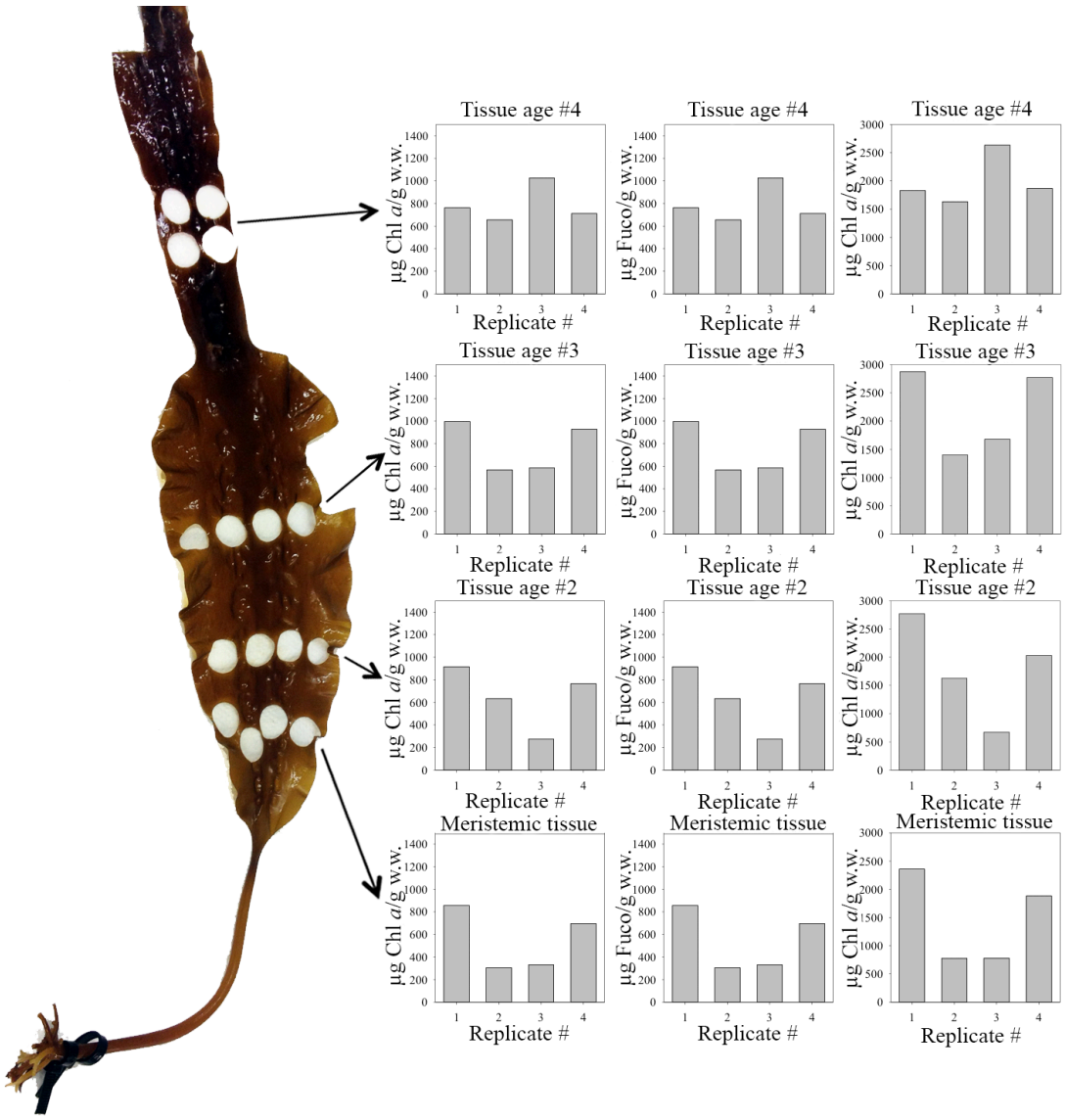

Fig. 30: Variation in pigment content throughout lamina of *Saccharina latissima* ind#2. Note the variation between replicates from the same tissue age.

In September, the overall pigment content in tissue 2 was higher than 1, then it decreased towards age tissue 4. The specimens sampled in September showed the highest total variation of all pigments, compared to the other two samplings (e.g. 54%CV for Chl *a*) One possible explanation for the slightly higher variation seen in the specimens sampled in September could be epigrowth shadowing the tissue, leaving large parts of the lamina to be LL acclimated, and also denying it nutrients (Hurd et al. 1994), which could make it difficult to synthesise pigments.

Seasonal variation in pigment content in *Saccharina latissima*

A high degree of seasonal variation was found in *S. latissima*. Overall, the highest pigment content was found in February, compared to May and September. This is as expected when looking at weather and daylight data (Table 4), with the samples from

the darker month (February) showed LL acclimatization, and samples from the brighter months (May and September) showed HL acclimatization.

A study by Kvernvik (2014, unpublished data) found that the same specimens were LL acclimated with regards to photosynthetic parameters in February.

Interaction between season and age in *S. latissima*

The results of the statistical interaction test suggest that influence of age on pigment content is dependent on season. The interaction between season and age varied between pigments (interaction was found in Fuco, Chl *c* and Zea, but not in Chl *a*, Viola and Anth). If tissue age had been a determining factor for pigment content, a similar trend throughout the lamina should have been found in all specimens. The varying results of the age study indicate that something else is more determining for pigment content than tissue age.

Interspecific variation between the Rhodophytes (red algae)

No significant variation was found between the two Rhodophytes with regards to β,β-carotene. This could be due to this pigments function as a precursor for other pigments through pigment synthesis (Rowan 1989), if the β,β-carotene is continuously transformed to other pigments (e.g. zeaxanthin), this could result in little β,β-carotene to be detected.

With regards to zeaxanthin (Zea) there was found significant variation between the two Rhodophytes in May, but not February and September. Albeit, *Palmaria palmata* didn't contain any Zea at all in May. It is noteworthy that when comparing the highest Zea content in the Rhodophytes to the highest in the Phaeophytes, it is almost twice as high in the former (125 µg Zea/g w.w. in *P. palmata* in September, 68 µg Zea/g w.w. in *Fucus spiralis* in February, Fig. 14 and 12). Although this variation could just be due to season, more data is needed to say anything about differences between pigment groups.

V. lanosa showed a very high variation in February (e.g. 221%CV for Chl *a*). This indicates a high degree of variation between individuals (all samples were taken from different individuals) and it seems likely that more data is needed before any conclusive remarks can be made. When looking at the Chl *a* content in all species

(Fig. 8), what is interesting is that this *V. lanosa* seems to contain similar amounts of Chl *a* as its host (*A. nodosum*) at all samplings. Also, similarly to *A. nodosum*, the pigment content in *V. lanosa* varied little between seasons. This could be a "coincidence", the red algae being LL acclimated and the brown algae containing high amounts of sugars.

Most research that has previously been done on V. lanosa has been in relation to the more economically attractive *A. nodosum* (which have been used as animal food for decades, ref. Rueness 1976), and more data is needed for any conclusive remarks on the variation in pigment content in *V. lanosa*.

For *P. palmata*, there is significant variation in LHPs between seasons. The specific individuals from this species used in this experiment were found on the stipes of *L. digitata*, where it most likely experienced a high degree of shading. As mentioned in the introduction, a *Laminaria* forest could absorb as much as 89-97% of the available light. If this shading was applied in varying degrees, this could be the explanation for the variation seen between seasons. In September the Zea content was high, whereas the LHP content was intermediate to low (compared to content from the other samplings), meaning the individuals sampled during this sampling might have experienced HL conditions.

Compared to other species with regards to Chl *a* content (Fig. 9), the Rhodophytes generally contained low concentrations, indicating LL acclimation. But, more information on the changes in composition of lipid-soluble vs. water-soluble pigment composition and content in Rhodophytes between seasons is needed (Rowan 1989; Brunet et al. 2011).

Xanthophyll cycle for all species in general

As the xanthophyll cycle (XC) is a second-to-minutes process, controlled directly by exposure to light (Brunet et al. 2011), concentration estimates of these pigments could easily have been affected by sample handling. All samples were transported and handled in as little light as possible, and this exposure to LL conditions might be enough to induce epoxidation towards Viola, leading to low concentrations or no trace of [Anth] and [Zea] at the point of pigment extraction. Total xanthophyll-pigment concentration could still say something about the photoprotective capacity of the organism. When looking at the total XC pigment content in Table 5, it is evident that

the total amount of XC pigments was not constant between seasons in either species. Albeit, the reason for this could easily be individual differences between specimens caused by different acclimation status, which means that a different approach might be necessary when studying the content of the XC pigments.

Green algae (Ulvophyceae)

Because of the low number of samples from the Chlorophytes, these results must be seen as an example of which pigments could be found in this PG. More study is needed to say anything about trend.

Conclusions for the brown algae (Phaeophytes)

- Position of the tissue in relation to light regime is more important than age with respect to pigment content and composition in *Saccharina latissima*.
- Shading and position in relation to light of macroalgae in the water determines the pigment content in the wrack and kelp species studied.
- The limits for high (>1000 µg)/intermediate (500-1000 µg)/low content (<500 µg) of Chl *a* per gram wet weight, reveals that Phaeophytes and Rhodophytes have a highly variable Chl *a* content (101-1997 µg for Phaeophytes, 219-1303 µg for Rhodphytes) within PG, while the Chlorophytes generally showed less variation in [Chl *a*] with seasons (828-1656µg). Albeit, more data is needed for the latter PG.

Future perspective

- A more extensive study on the effect of shading vs. effect of tissue age on pigment content in *Saccharina latissima* is needed to understand this species.
- Further information is needed on the relationship between chemical composition (e.g. polysaccharides) of the macroalgae and the pigment composition to say anything about what is defined as “high” and “low” content for the individual species.
- A different approach with improved methods could be needed for a look into the pigment content in the XC, as this is a fast reacting cycle.
- In the interest of enhanced knowledge about pigment content, either for conservation or cultivation purposes, a study in a more controlled environment, especially with regards to light and shadowing, might be necessary.

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Appendix

Appendix A

Appendix A: Coefficient of variation (CV), presented as fractions (1.00 =100%)
A1: CV values (calculated from SD and mean pigment content) for Phaeophytes. A value of 1.00 = 100%. S1-3 indicates sampling number.

	Chl *c*	Fuco	Viola	Anth	Zea	Chl *a*
P. canaliculata S1	0.15	0.24	0.28	1.24	0.43	0.21
P. canaliculata S2	0.16	0.13	0.31	0.37	0.62	0.61
P. canaliculata S3	0.51	0.42	0.52	0.62	0.35	0.64
F. spiralis S1	0.26	0.26	0.41	2.00	0.13	0.43
F. spiralis S2	0.34	0.33	0.46	0.54	0.37	0.56
F. spiralis S3	0.80	0.59	0.95	0.59	0.75	0.88
F. vesiculosus S1	0.06	0.08	0.19	0.40	0.23	0.05
F. vesiculosus S2	0.32	0.27	0.97	0.51	0.70	0.89
F. vesiculosus S3	0.27	0.27	0.31	0.21	0.07	0.21
A. nodosum S1	0.46	0.38	0.67	0.90	0.50	0.79
A. nodosum S2	0.50	0.40	0.79	0.91	0.59	0.70
A. nodosum S3	0.36	0.30	0.43	0.20	0.41	0.43
F. serratus S1	0.35	0.35	0.49	0.59	0.53	0.41
F. serratus S2	0.25	0.24	0.47	0.34	0.28	0.32
F. serratus S3	0.55	0.44	0.83	0.60	0.56	0.68
L. digitata S1	1.34	1.40	1.51	2.00	1.22	1.43
L. digitata S2	0.19	0.24	0.44	0.25	0.16	0.28
L. digitata S3	0.13	0.10	0.18	1.15	2.00	0.06

A2: CV values (calculated from SD and mean pigment content) for Rhodophytes. S1-3 indicates sampling number.

	Zea	*Chl a*	*β,β-Car*
V. lanosa S1	1.04	2.21	2.84
V. lanosa S2	0.37	0.38	0.57
V. lanosa S3	0.51	0.52	0.01
P. palmata S1	2.00	0.29	2.00
P. palmata S2	-	0.13	0.70
P. palmata S3	0.37	0.17	0.48

A3: CV values for (calculated from SD and mean pigment content) Chlorophytes. S1-3 indicates sampling number.

	Viola	*Lutein*	*Zea*	*Chl b*	*Chl a*	*Neo*
C. rupestris S1	0.69	0.54	-	0.57	0.89	1.06
C. rupestris S3	0.41	0.25	0.29	1.31	0.64	-
U. lactuca S2	1.16	0.31	-	0.40	0.35	0.24

A4: CV values for *S. latissima*

	Specimen	*CV Chl a*	*CV Fuco*	*CV Chl c*	*CV Viola*	*CV Anth*	*CV Zea*
February	#1	0.27	0.30	0.31	0.27	-	-
	#2	0.17	0.16	0.18	0.21	-	-
	#3	0.16	0.12	0.12	0.18	-	-

	Tot Feb	0.38	0.39	0.35	0.44	-	-
May	#4	0.51	0.52	0.41	1.16	-	-
	#5	0.37	0.33	0.28	0.87	-	1.51
	#6	0.39	0.26	0.27	0.75	-	2.79
	Tot May	0.43	0.36	0.33	0.91	-	-
September	#7	0.71	0.68	0.69	0.82	-	-
	#8	0.28	0.38	0.20	0.35	0.69	-
	#9	0.49	0.21	0.60	0.26	0.44	1.16
	Tot Sep	0.54	0.49	0.53	0.64	0.99	2.34
	Total variation	0.63	0.62	0.51	0.90	2.19	4.71

Appendix B

Appendix B: Average pigment content in µg pigment/g w.w.
B1: Average pigment content in all species except *S. latissima* (see B2). S1-3 indicates sampling number. Column named "Anth" is in reality Lutein in the green algae.

Av. pigment	Chl *c*	Fuco	Viola	Anth	Zea	Chl *b*	Chl *a*	β,β -Car	Neo
F. vesiculosus S1	108.37	369.16	58.05	10.82	36.46	0.00	1523.67	7.06	
F. vesiculosus S2	57.04	192.43	13.73	3.14	30.06	1.47	570.88	5.71	
F. vesiculosus S3	97.49	293.26	48.67	35.97	30.20	1.89	1092.35	2.16	
L. digitata S1	63.69	179.92	19.11	1.21	16.47	0.00	501.35	3.59	
L. digitata S2	58.64	151.67	14.85	4.73	10.05	0.00	443.22	2.28	
L. digitata S3	43.18	61.16	12.21	1.31	0.25	0.00	300.33	0.45	
V. lanosa S1		25.99	0.00	0.00	66.71	0.00	567.66	5.52	
V. lanosa S2		65.83	6.45	0.00	43.29	0.00	461.81	5.06	
V. lanosa S3		23.17	0.58	3.22	70.28	14.83	432.67	3.30	
A. nodosum S1	35.32	113.54	9.71	5.03	14.99	0.56	267.56	1.21	
A. nodosum S2	42.50	130.47	21.49	5.51	24.52	1.68	538.57	4.44	
A. nodosum S3	31.88	67.89	18.65	9.56	13.39	0.00	322.74	1.00	
C. rupestris S1		14.97	85.86	102.46	0.00	202.83	828.75	2.58	206.69
C. rupestris S3		16.98	44.26	12.02	150.38	54.29	823.97	0.00	193.26
U. lactuca S2		0.00	20.05	278.95	0.00	555.16	1610.25	25.96	
F. spiralis S1	91.88	359.08	77.35	2.56	68.33	0.00	1656.59	10.27	
F. spiralis S2	45.08	166.62	39.27	15.05	23.23	2.09	668.40	2.03	
F. spiralis S3	50.89	118.13	30.68	12.74	21.06	0.00	543.63	0.00	
F. serratus S1	148.55	516.30	60.28	6.64	54.01	0.00	1997.85	8.85	
F. serratus S2	86.04	265.31	20.32	5.32	44.13	0.00	997.53	6.05	
F. serratus S3	96.26	227.48	43.98	30.83	19.04	0.00	1011.24	2.15	
P. canaliculata S1	44.34	162.49	33.56	3.76	26.11	0.00	589.33	1.57	
P. canaliculata S2	39.39	159.15	40.04	12.66	7.73	0.00	528.71	1.75	
P. canaliculata S3	12.15	24.59	7.66	3.72	5.94	0.00	101.07	1.45	
P. palmata S1		20.74	0.00	17.89	7.67	0.00	219.57	0.44	
P. palmata S2		0.00	0.00	258.40	0.00	0.00	1303.50	12.02	
P. palmata S3		0.00	0.00	0.00	125.35	0.00	619.00	3.79	

Appendix B2: Average pigment content in *S. latissima*

Sampling 1	Av. pigment	Chl *c*	Fuco	Viola	Anth	Zea	Chl *a*
	Suk 1.1	211.6	522.8	54.1	0.0	0.0	1552.4
	Suk 1.2	328.4	870.4	77.1	0.0	0.0	2625.9

	Suk 1.3	437.9	1093.2	83.7	0.0	0.0	2946.7
	Suk 1.4	450.3	1109.1	96.0	0.0	0.0	3037.1
	Suk 2.1	212.1	547.5	45.8	0.0	0.0	1449.4
	Suk 2.2	265.0	647.3	54.5	0.0	0.0	1775.4
	Suk 2.3	292.8	769.5	74.5	0.0	0.0	2181.5
	Suk 2.4	329.6	788.9	57.9	0.0	0.0	1991.2
	Suk 3.1	162.9	382.6	32.4	0.0	0.0	1020.7
	Suk 3.2	218.4	496.7	32.1	0.0	0.0	1444.1
	Suk 3.3	184.2	398.8	27.5	0.0	0.0	1183.7
	Suk 3.4	198.8	402.1	21.5	0.0	0.0	1095.4
Sampling 2	Suk 1.1	91.3	173.5	0.0	0.0	0.0	446.8
	Suk 1.2	263.7	696.9	20.0	0.0	0.0	1466.4
	Suk 1.3	251.6	640.4	16.6	0.0	0.0	971.4
	Suk 1.4	168.9	373.0	0.0	0.0	0.0	632.6
	Suk 2.1	101.0	231.0	4.9	0.0	0.0	448.9
	Suk 2.2	178.2	539.2	16.3	0.0	43.1	856.0
	Suk 2.3	198.1	544.3	13.5	0.0	5.0	852.3
	Suk 2.4	142.7	447.1	0.0	0.0	5.0	432.2
	Suk 3.1	136.0	382.5	16.1	0.0	0.0	642.7
	Suk 3.2	214.7	597.6	10.6	0.0	0.0	909.9
	Suk 3.3	246.3	582.4	9.7	0.0	0.0	821.9
	Suk 3.4	266.5	746.6	40.0	0.0	0.0	1514.7
Sampling 3	Suk 1.1	158.0	183.9	10.3	0.0	0.0	935.9
	Suk 1.2	203.4	326.4	32.7	0.0	0.0	1374.5
	Suk 1.3	82.6	170.1	16.4	0.0	0.0	590.8
	Suk 1.4	20.9	32.0	2.9	0.0	0.0	117.7
	Suk 2.1	178.0	226.1	33.9	0.0	0.0	1059.1
	Suk 2.2	164.6	314.2	31.8	23.1	0.0	1236.3
	Suk 2.3	171.9	231.2	25.1	26.5	0.0	1060.0
	Suk 2.4	109.8	109.7	13.8	19.6	0.0	594.1
	Suk 3.1	120.1	130.8	11.0	36.3	0.0	716.3
	Suk 3.2	109.8	140.2	10.8	35.8	0.0	611.7
	Suk 3.3	44.5	161.9	10.3	23.0	1.1	362.9
	Suk 3.4	28.9	96.2	5.8	11.7	1.0	210.3

Appendix C

Appendix C: Standard deviation (SD)

C1: SD for all species except *S. latissima* (see appendix C2). S1-3 indicates sampling number. Column named "Anth" is in reality Lutein in the green algae.

SD	Chl *c*	Fuco	Viola	Anth	Zea	Chl *b*	Chl *a*	β,β -Car	Neo
F. vesiculosus S1	6.65	28.72	10.96	4.34	8.35		83.50	4.42	
F. vesiculosus S2	18.28	51.46	13.26	1.59	21.15	0.41	508.68	8.82	
F. vesiculosus S3	26.73	79.53	15.16	7.62	2.07	0.14	230.69	1.92	
L. digitata S1	85.10	251.65	28.86	0.00	20.09		715.21	5.73	
L. digitata S2	10.98	35.78	6.55	1.20	1.63		124.80	8.82	
L. digitata S3	5.71	6.25	2.23	1.51	0.50		17.42	0.04	
V. lanosa S1		14.46			69.49		1257.18	15.69	
V. lanosa S2		20.72	2.01		16.17		176.27	2.88	
V. lanosa S3		17.03	0.90	2.12	35.93		226.55	0.04	
A. nodosum	16.16	42.98	6.53	4.55	7.56	1.12	211.84	1.04	
A. nodosum	21.08	52.48	17.02	5.02	14.51	1.14	374.55	3.48	
A. nodosum	11.44	20.03	7.97	1.89	5.44		139.12	0.92	

C. rupestris S1	2.47	6.59	59.06	55.02		116.51	737.35	2.74	83.52
C. rupestris S3		4.50	18.14	3.01	43.70	70.89	530.05	0.00	43.81
U. lactuca S2			23.34	87.41		219.30	570.63	6.29	
F. spiralis S1	23.84	92.00	31.73	5.12	8.58		707.53	7.23	
F. spiralis S2	15.36	54.66	18.23	8.09	8.49	219.30	373.64	1.50	
F. spiralis S3	40.50	69.53	29.10	7.49	15.75		480.99	0.00	
F. serratus S1	52.53	180.68	29.37	3.91	28.65		828.04	4.63	
F. serratus S2	21.17	63.45	9.56	1.83	12.29		320.59	4.11	
F. serratus S3	53.29	100.47	36.60	18.36	10.57		684.01	2.63	
P. canaliculata S1	6.77	39.60	9.48	4.67	11.22		124.06	0.58	
P. canaliculata S2	6.41	20.50	12.33	4.70	4.82		320.77	1.50	
P. canaliculata S3	6.25	10.40	3.95	2.30	2.06		64.27	0.83	
P. palmata S1		4.91		12.74	15.34		63.91	0.87	
P. palmata S2				34.48			163.44	8.38	
P. palmata S1					46.06		107.31	1.83	

Appendix C2: Standard deviation (SD) for *S. latissima*

SD	Chl *c*	Fuco	Viola	Anth	Zea	Chl *a*
Suk 1.1	103.5	230.2	8.8			653.6
Suk 1.2	283.6	741.9	65.3			2570.6
Suk 1.3	178.0	402.7	23.8			1208.7
Suk 1.4	164.0	305.7	31.5			1061.2
Suk 2.1	110.4	274.4	21.6			798.2
Suk 2.2	126.0	274.4	21.6			875.6
Suk 2.3	90.3	224.5	23.0			751.5
Suk 2.4	53.1	163.7	18.5			440.2
Suk 3.1	77.6	188.9	14.7			478.6
Suk 3.2	105.2	245.5	17.9			779.1
Suk 3.3	42.1	96.4	6.8			413.3
Suk 3.4	8.6	30.5	8.9			145.0
Suk 1.1	17.9	39.9				111.8
Suk 1.2	84.0	265.7	15.0			443.2
Suk 1.3	90.5	245.0	10.2			419.1
Suk 1.4	54.9	99.0				192.5
Suk 2.1	31.6	68.7	5.9			144.4
Suk 2.2	86.2	145.1	3.5		23.5	241.1
Suk 2.3	61.0	134.8	10.2		10.1	292.5
Suk 2.4	18.2	81.9			9.9	102.4
Suk 3.1	30.8	121.0	4.9			174.0
Suk 3.2	59.1	245.5	17.9			152.7
Suk 3.3	54.8	107.2	12.9			268.7
Suk 3.4	68.1	91.9	6.3			175.4
Suk 1.1	83.7	153.4	29.7			569.1
Suk 1.2	105.9	153.4	29.7			618.6
Suk 1.3	23.3	21.6	4.6			145.7
Suk 1.4	4.3	12.1	2.2			27.5
Suk 2.1	51.3	83.1	9.7			386.4
Suk 2.2	89.1	67.7	10.0	16.8		357.1
Suk 2.3	65.1	50.4	10.9	29.0		254.9
Suk 2.4	30.8	13.2	9.3	13.2		91.1
Suk 3.1	42.4	43.5	3.2	11.0		225.4
Suk 3.2	38.8	22.8	2.9	6.4		156.2
Suk 3.3	42.4	77.6	3.1	9.1	2.2	118.8
Suk 3.4	6.1	24.1	2.1	3.8	1.1	54.6

Appendix D

Appendix D: Results from the statistical tests (ANOVA)
Unless otherwise stated, all test are One-Way ANOVA

Pigment group (PG) comparison (Chl *a* content):

ANOVA Table for ChlA – Feb (Season_1)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4.12888E6	2	2.06444E6	4.17	0.0217
Within groups	2.22516E7	45	494479.		
Total (Corr.)	2.63805E7	47			

ANOVA Table for ChlA – May (Season_2)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4.01559E6	2	2.00779E6	15.03	0.0000
Within groups	6.0124E6	45	133609.		
Total (Corr.)	1.0028E7	47			

ANOVA Table for ChlA - Sep (Season_3)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	260103.	2	130052.	0.62	0.5433
Within groups	9.46392E6	45	210309.		
Total (Corr.)	9.72403E6	47			

Species comparison (Chl *a*)

ANOVA Table for ChlA - Feb

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1.53046E7	9	1.70051E6	5.83	0.0000
Within groups	1.10758E7	38	291470.		
Total (Corr.)	2.63805E7	47			

ANOVA Table for ChlA - May

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	6.3059E6	9	700655.	7.15	0.0000
Within groups	3.72208E6	38	97949.6		
Total (Corr.)	1.0028E7	47			

ANOVA Table for ChlA - Sep

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4.55097E6	9	505663.	3.71	0.0020
Within groups	5.17306E6	38	136133.		
Total (Corr.)	9.72403E6	47			

Species comparison (Fuco)

ANOVA Table for Fuco - Feb

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	808800.	6	134800.	5.60	0.0006
Within groups	698039.	29	24070.3		
Total (Corr.)	1.50684E6	35			

ANOVA Table for Fuco - May

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	102523.	6	17087.2	2.50	0.0452
Within groups	198416.	29	6841.92		
Total (Corr.)	300939.	35			

ANOVA Table for FucoSep

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	242486.	6	40414.4	7.83	0.0000
Within groups	149702.	29	5162.15		
Total (Corr.)	392189.	35			

Species comparison (Chl *c*)

ANOVA Table for Chl *c* - Feb

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	139654.	6	23275.6	6.43	0.0002
Within groups	105032.	29	3621.79		
Total (Corr.)	244686.	35			

ANOVA Table for ChlC - May

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	61240.6	6	10206.8	9.99	0.0000
Within groups	29633.8	29	1021.85		
Total (Corr.)	90874.4	35			

ANOVA Table for ChlC - Sep

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	98990.4	6	16498.4	8.34	0.0000
Within groups	57402.5	29	1979.4		
Total (Corr.)	156393.	35			

Species comparison (Viola)

ANOVA Table for ViolaFeb

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	13901.2	6	2316.87	6.80	0.0001
Within groups	9882.41	29	340.773		
Total (Corr.)	23783.6	35			

ANOVA Table for Viola - May

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	5354.7	6	892.45	6.52	0.0002
Within groups	3969.24	29	136.87		
Total (Corr.)	9323.94	35			

ANOVA Table for Viola - Sep

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	6326.24	6	1054.37	3.24	0.0147
Within groups	9442.15	29	325.591		
Total (Corr.)	15768.4	35			

Species comparison (Anth)

ANOVA Table for Anth - Feb

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	434.0	6	72.3333	6.43	0.0002
Within groups	326.017	29	11.242		
Total (Corr.)	760.017	35			

ANOVA Table for Anth - May

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	953.229	6	158.871	12.79	0.0000
Within groups	360.308	29	12.4244		
Total (Corr.)	1313.54	35			

ANOVA Table for Anth - Sep

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4251.08	6	708.513	3.91	0.0055
Within groups	5253.19	29	181.144		
Total (Corr.)	9504.26	35			

Species comparison (Zea)

ANOVA Table for Zea - Feb

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	19565.3	6	3260.88	27.16	0.0000
Within groups	3481.42	29	120.049		
Total (Corr.)	23046.7	35			

ANOVA Table for Zea - May

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	7933.98	6	1322.33	14.10	0.0000
Within groups	2720.64	29	93.815		
Total (Corr.)	10654.6	35			

ANOVA Table for Zea - Sep

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4140.88	6	690.147	16.75	0.0000
Within groups	1195.03	29	41.208		
Total (Corr.)	5335.92	35			

Species specific study

P. canaliculata

Chl *a* by Season

ANOVA Table for Chl a by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	566666.	2	283333.	6.94	0.0150
Within groups	367245.	9	40805.1		
Total (Corr.)	933912.	11			

Fuco by Season

ANOVA Table for Fuco by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	49499.7	2	24749.9	35.40	0.0001
Within groups	6292.93	9	699.214		
Total (Corr.)	55792.6	11			

Chl *c* by Season

ANOVA Table for Chl c by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2402.73	2	1201.37	28.49	0.0001
Within groups	379.577	9	42.1753		
Total (Corr.)	2782.31	11			

Viola by Season

ANOVA Table for Viola by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2345.25	2	1172.62	13.66	0.0019
Within groups	772.662	9	85.8514		
Total (Corr.)	3117.91	11			

Anth by Season

ANOVA Table for Anth by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	211.822	2	105.911	6.50	0.0179
Within groups	146.607	9	16.2897		
Total (Corr.)	358.429	11			

Zea by Season

ANOVA Table for Zea by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	998.322	2	499.161	9.76	0.0056
Within groups	460.325	9	51.1472		
Total (Corr.)	1458.65	11			

F. spiralis

Chl *a* by Season

ANOVA Table for Chl *a* by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2.97457E6	2	1.48728E6	5.12	0.0328
Within groups	2.61473E6	9	290525.		
Total (Corr.)	5.5893E6	11			

Fuco by Season

ANOVA Table for Fuco by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	129938.	2	64968.9	11.97	0.0029
Within groups	48831.8	9	5425.75		
Total (Corr.)	178770.	11			

Chl *c* by Season

ANOVA Table for Chl *c* by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	5208.86	2	2604.43	3.20	0.0893
Within groups	7331.57	9	814.619		
Total (Corr.)	12540.4	11			

Viola by Season

ANOVA Table for Viola by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4936.3	2	2468.15	3.39	0.0801
Within groups	6561.07	9	729.007		
Total (Corr.)	11497.4	11			

Anth by Season

ANOVA Table for Anth by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	352.595	2	176.297	3.57	0.0721
Within groups	444.005	9	49.3339		
Total (Corr.)	796.6	11			

Zea by Season

ANOVA Table for Zea by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	5698.22	2	2849.11	21.74	0.0004
Within groups	1179.69	9	131.076		
Total (Corr.)	6877.91	11			

F. vesiculosus

ChlA by Season

ANOVA Table for ChlA by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1.82098E6	2	910488.	8.56	0.0083
Within groups	956913.	9	106324.		
Total (Corr.)	2.77789E6	11			

Fuco by Season

ANOVA Table for Fuco by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	62914.6	2	31457.3	9.63	0.0058
Within groups	29401.1	9	3266.79		
Total (Corr.)	92315.8	11			

Chl *c* by Season

ANOVA Table for Chl *c* by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	5849.66	2	2924.83	8.04	0.0099
Within groups	3274.67	9	363.852		
Total (Corr.)	9124.33	11			

Viola by Season

ANOVA Table for Viola by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	4369.05	2	2184.52	12.50	0.0025
Within groups	1573.36	9	174.818		
Total (Corr.)	5942.41	11			

Anth by Season

ANOVA Table for Anth by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2360.09	2	1180.04	44.70	0.0000
Within groups	237.618	9	26.4019		
Total (Corr.)	2597.7	11			

Zea by Season

ANOVA Table for Zea by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	107.572	2	53.7858	0.31	0.7411
Within groups	1562.22	9	173.58		
Total (Corr.)	1669.79	11			

A. nodosum

Chl *a* by Season

ANOVA Table for Chl *a* by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	164120.	2	82060.0	1.20	0.3442
Within groups	613578.	9	68175.4		
Total (Corr.)	777698.	11			

Fuco by Season

ANOVA Table for Fuco by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	8379.43	2	4189.72	2.51	0.1358
Within groups	15001.8	9	1666.87		
Total (Corr.)	23381.2	11			

Chl *c* by Season

ANOVA Table for Chl *c* by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	235.282	2	117.641	0.42	0.6687
Within groups	2514.81	9	279.423		
Total (Corr.)	2750.09	11			

Viola by Season

ANOVA Table for Viola by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	303.695	2	151.848	1.16	0.3576
Within groups	1182.89	9	131.432		
Total (Corr.)	1486.58	11			

Anth by Season

ANOVA Table for Anth by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	50.045	2	25.0225	1.53	0.2684
Within groups	147.435	9	16.3817		
Total (Corr.)	197.48	11			

Zea by Season

ANOVA Table for Zea by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	291.312	2	145.656	1.47	0.2805
Within groups	892.517	9	99.1686		
Total (Corr.)	1183.83	11			

F. serratus

Chl *a* by Season

ANOVA Table for Chl *a* by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2.63236E6	2	1.31618E6	3.14	0.0922
Within groups	3.76898E6	9	418775.		
Total (Corr.)	6.40133E6	11			

Fuco by Season

ANOVA Table for Fuco by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	197135.	2	98567.7	6.32	0.0193
Within groups	140330.	9	15592.2		
Total (Corr.)	337465.	11			

Chl *c* by Season

ANOVA Table for Chl c by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	9003.81	2	4501.9	2.23	0.1631
Within groups	18142.8	9	2015.87		
Total (Corr.)	27146.6	11			

Viola by Season

ANOVA Table for Viola by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	3232.26	2	1616.13	2.11	0.1769
Within groups	6884.42	9	764.935		
Total (Corr.)	10116.7	11			

Anth by Season

ANOVA Table for Anth by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1650.11	2	825.053	6.94	0.0150
Within groups	1070.54	9	118.949		
Total (Corr.)	2720.65	11			

Zea by Season

ANOVA Table for Zea by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2601.03	2	1300.52	3.60	0.0709
Within groups	3249.61	9	361.068		
Total (Corr.)	5850.65	11			

L. digitata:

Chl a by Season

ANOVA Table for Chl a by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	85596.0	2	42798.0	3.01	0.0997
Within groups	127937.	9	14215.2		
Total (Corr.)	213533.	11			

Fuco by Season

ANOVA Table for Fuco by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	30786.5	2	15393.2	11.47	0.0034
Within groups	12082.4	9	1342.49		
Total (Corr.)	42868.9	11			

Chl *c* by Season

ANOVA Table for Chl c by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	910.212	2	455.106	3.27	0.0856
Within groups	1252.56	9	139.173		
Total (Corr.)	2162.77	11			

Viola by Season

ANOVA Table for Viola by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	96.2917	2	48.1458	1.33	0.3129
Within groups	326.837	9	36.3153		
Total (Corr.)	423.129	11			

Anth by Season

ANOVA Table for Anth by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	31.9817	2	15.9908	4.95	0.0355
Within groups	29.095	9	3.23278		
Total (Corr.)	61.0767	11			

Zea by Season

ANOVA Table for Zea by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	535.385	2	267.693	50.26	0.0000
Within groups	47.9375	9	5.32639		
Total (Corr.)	583.322	11			

Chlorophytes

Chl *a* by Season

ANOVA Table for Chl *a* by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1.63867E6	2	819333.	2.14	0.1740
Within groups	3.45103E6	9	383448.		
Total (Corr.)	5.0897E6	11			

Chl *b* by Season

ANOVA Table for Chl *b* by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	529405.	2	264702.	11.91	0.0030
Within groups	200038.	9	22226.4		
Total (Corr.)	729442.	11			

Lutein by Season

ANOVA Table for Lutein by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	147461.	2	73730.5	20.71	0.0004
Within groups	32045.5	9	3560.61		
Total (Corr.)	179506.	11			

Neo by Season

ANOVA Table for Neo by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	107002.	2	53500.9	18.04	0.0007
Within groups	26683.8	9	2964.86		
Total (Corr.)	133686.	11			

Viola by Season

ANOVA Table for Viola by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	8859.96	2	4429.98	3.05	0.0977
Within groups	13093.1	9	1454.79		
Total (Corr.)	21953.1	11			

Zea by Season

ANOVA Table for Zea by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	60300.4	2	30150.2	47.30	0.0000
Within groups	5737.39	9	637.487		
Total (Corr.)	66037.8	11			

C. rupestris

Chl *a* by Season

ANOVA Table for Chl a by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	45.6754	1	45.6754	0.00	0.9919
Within groups	2.47403E6	6	412338.		

Total (Corr.)	2.47407E6	7			

Chl *b* by Season

ANOVA Table for Chl b by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	44104.5	1	44104.5	4.74	0.0722
Within groups	55779.2	6	9296.53		
Total (Corr.)	99883.7	7			

Lutein by Season

ANOVA Table for Lutein by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	16362.4	1	16362.4	10.77	0.0168
Within groups	9116.15	6	1519.36		
Total (Corr.)	25478.6	7			

Neo by Season

ANOVA Table for Neo by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	361.805	1	361.805	0.08	0.7851
Within groups	26683.8	6	4447.29		
Total (Corr.)	27045.6	7			

Viola by Season

ANOVA Table for Viola by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	3456.96	1	3456.96	1.81	0.2271
Within groups	11459.2	6	1909.87		
Total (Corr.)	14916.2	7			

Zea by Season

ANOVA Table for Zea by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	45225.3	1	45225.3	47.30	0.0005
Within groups	5737.39	6	956.231		
Total (Corr.)	50962.7	7			

P. palmata

Chl *a* by Season

ANOVA Table for Chl *a* by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2.40397E6	2	1.20198E6	85.23	0.0000
Within groups	126924.	9	14102.7		
Total (Corr.)	2.53089E6	11			

β β-Car by Season

ANOVA Table for BB by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	283.482	2	141.741	5.74	0.0247
Within groups	222.108	9	24.6786		
Total (Corr.)	505.589	11			

Zea by Season

ANOVA Table for Zea by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	39491.9	2	19746.0	25.12	0.0002
Within groups	7073.76	9	785.973		
Total (Corr.)	46565.7	11			

V. lanosa

Chl *a* by Season

ANOVA Table for Chl *a* by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	40377.0	2	20188.5	0.14	0.8735
Within groups	1.32308E6	9	147009.		
Total (Corr.)	1.36346E6	11			

β β-Car by Season

ANOVA Table for BB-car by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	11.0717	2	5.53583	0.25	0.7846
Within groups	199.935	9	22.215		
Total (Corr.)	211.007	11			

Zea by Season

ANOVA Table for Zea by Season

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1717.32	2	858.661	0.40	0.6794
Within groups	19147.8	9	2127.54		
Total (Corr.)	20865.1	11			

Saccharina latissima

Feb: Multifactor ANOVA - Chl a

Analysis of Variance for Chl a - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	1.46806E7	2	7.34032E6	7.49	0.0017
B:Age	4.43588E6	3	1.47863E6	1.51	0.2263
RESIDUAL	4.11748E7	42	980351.		
TOTAL (CORRECTED)	6.02913E7	47			

May: Multifactor ANOVA - Chl a

Analysis of Variance for Chl a - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	896206.	2	448103.	3.70	0.0330
B:Age	1.98472E6	3	661575.	5.47	0.0029
RESIDUAL	5.08151E6	42	120988.		
TOTAL (CORRECTED)	7.96244E6	47			

All F-ratios are based on the residual mean square error.

Sep: Multifactor ANOVA - Chl *a*

Analysis of Variance for Chl *a* - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	2.10377E6	2	1.05188E6	9.83	0.0003
B:Age	3.96462E6	3	1.32154E6	12.35	0.0000
RESIDUAL	4.49314E6	42	106980.		
TOTAL (CORRECTED)	1.05615E7	47			

All F-ratios are based on the residual mean square error.

February: Multifactor ANOVA - Fuco

Analysis of Variance for Fuco - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	1.84325E6	2	921623.	9.56	0.0004
B:Age	610455.	3	203485.	2.11	0.1133
RESIDUAL	4.04937E6	42	96413.5		
TOTAL (CORRECTED)	6.50307E6	47			

All F-ratios are based on the residual mean square error.

May: Multifactor ANOVA - Fuco

Analysis of Variance for Fuco - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	165144.	2	82572.2	3.16	0.0525
B:Age	926562.	3	308854.	11.83	0.0000
RESIDUAL	1.09609E6	42	26097.4		
TOTAL (CORRECTED)	2.1878E6	47			

All F-ratios are based on the residual mean square error.

Sep: Multifactor ANOVA - Fuco

Analysis of Variance for Fuco - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	62033.7	2	31016.9	5.21	0.0095
B:Age	199181.	3	66393.6	11.15	0.0000
RESIDUAL	250025.	42	5952.98		
TOTAL (CORRECTED)	511239.	47			

All F-ratios are based on the residual mean square error.

February: Multifactor ANOVA - Chl *c*

Analysis of Variance for Chl *c* - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	220410.	2	110205.	6.71	0.0030
B:Age	118300.	3	39433.4	2.40	0.0812
RESIDUAL	689773.	42	16423.2		
TOTAL (CORRECTED)	1.02848E6	47			

All F-ratios are based on the residual mean square error.

May: Multifactor ANOVA - Chl *c*

Analysis of Variance for Chl *c* - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	55503.2	2	27751.6	5.97	0.0052
B:Age	107536.	3	35845.2	7.71	0.0003
RESIDUAL	195282.	42	4649.56		
TOTAL (CORRECTED)	358320.	47			

All F-ratios are based on the residual mean square error.

Sep: Multifactor ANOVA - Chl *c*

Analysis of Variance for Chl *c* - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	51554.1	2	25777.1	7.69	0.0014
B:Age	88543.0	3	29514.3	8.81	0.0001
RESIDUAL	140712.	42	3350.29		
TOTAL (CORRECTED)	280809.	47			

All F-ratios are based on the residual mean square error.

Feb: Multifactor ANOVA - Viola

Analysis of Variance for Viola - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	19729.8	2	9864.89	14.39	0.0000
B:Age	2144.88	3	714.962	1.04	0.3836
RESIDUAL	28800.6	42	685.729		
TOTAL (CORRECTED)	50675.3	47			

All F-ratios are based on the residual mean square error.

May: Multifactor ANOVA - Viola

Analysis of Variance for Viola - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	1101.23	2	550.615	3.50	0.0391

B:Age	495.855	3	165.285	1.05	0.3796
RESIDUAL	6598.35	42	157.104		
TOTAL (CORRECTED)	8195.44	47			

All F-ratios are based on the residual mean square error.

Sep: Multifactor ANOVA - Viola

Analysis of Variance for Viola - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	2268.18	2	1134.09	8.99	0.0006
B:Age	1894.17	3	631.391	5.01	0.0047
RESIDUAL	5297.66	42	126.135		
TOTAL (CORRECTED)	9460.01	47			

All F-ratios are based on the residual mean square error.

Sep: Multifactor ANOVA - Anth

Analysis of Variance for Anth - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	5860.71	2	2930.36	16.66	0.0000
B:Age	630.545	3	210.182	1.19	0.3234
RESIDUAL	7387.91	42	175.903		
TOTAL (CORRECTED)	13879.2	47			

All F-ratios are based on the residual mean square error.

May: Multifactor ANOVA - Zea

Analysis of Variance for Zea - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	1877.62	2	938.808	7.22	0.0020
B:Age	1603.26	3	534.42	4.11	0.0121
RESIDUAL	5464.42	42	130.105		
TOTAL (CORRECTED)	8945.29	47			

All F-ratios are based on the residual mean square error.

Sep: Multifactor ANOVA - Zea

Analysis of Variance for Zea - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Ind#	2.89815	2	1.44908	2.82	0.0710
B:Age	1.46214	3	0.487381	0.95	0.4261
RESIDUAL	21.5904	42	0.514058		
TOTAL (CORRECTED)	25.9507	47			

All F-ratios are based on the residual mean square error.

Interseasonal *S. latissima*

Multifactor ANOVA - Chl a

Analysis of Variance for Chl a - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Season	3.70231E7	2	1.85115E7	35.71	0.0000
B:Age	4.00809E6	3	1.33603E6	2.58	0.0565
INTERACTIONS					
AB	6.37713E6	6	1.06286E6	2.05	0.0634
RESIDUAL	6.843E7	132	518409.		
TOTAL (CORRECTED)	1.15838E8	143			

All F-ratios are based on the residual mean square error.

Multifactor ANOVA - Fuco

Analysis of Variance for Fuco - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Season	5.98538E6	2	2.99269E6	52.91	0.0000
B:Age	995880.	3	331960.	5.87	0.0009
INTERACTIONS					
AB	740317.	6	123386.	2.18	0.0486
RESIDUAL	7.46591E6	132	56559.9		
TOTAL (CORRECTED)	1.51875E7	143			

All F-ratios are based on the residual mean square error.

Multifactor ANOVA - Chl c

Analysis of Variance for Chl c - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Season	601242.	2	300621.	29.32	0.0000
B:Age	91567.7	3	30522.6	2.98	0.0339
INTERACTIONS					
AB	222810.	6	37135.1	3.62	0.0023
RESIDUAL	1.35323E6	132	10251.8		
TOTAL (CORRECTED)	2.26885E6	143			

All F-ratios are based on the residual mean square error.

Multifactor ANOVA - Viola

Analysis of Variance for Viola - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Season	51958.0	2	25979.0	53.75	0.0000
B:Age	1728.98	3	576.328	1.19	0.3152
INTERACTIONS					
AB	2805.93	6	467.655	0.97	0.4498
RESIDUAL	63795.8	132	483.302		
TOTAL (CORRECTED)	120289.	143			

All F-ratios are based on the residual mean square error.

Multifactor ANOVA - Anth

Analysis of Variance for Anth - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Season	6882.77	2	3441.39	34.29	0.0000
B:Age	210.182	3	70.0606	0.70	0.5548
INTERACTIONS					
AB	420.364	6	70.0606	0.70	0.6516
RESIDUAL	13248.6	132	100.368		
TOTAL (CORRECTED)	20761.9	143			

All F-ratios are based on the residual mean square error.

Multifactor ANOVA - Zea

Analysis of Variance for Zea - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Season	602.249	2	301.125	5.40	0.0056
B:Age	519.571	3	173.19	3.10	0.0289
INTERACTIONS					
AB	1085.15	6	180.858	3.24	0.0053
RESIDUAL	7366.52	132	55.807		
TOTAL (CORRECTED)	9573.49	143			

All F-ratios are based on the residual mean square error.