OIL CONTENT AND QUALITY IN TROPICAL SEAWEEDS

Thesis submitted by

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STATEMENT ON THE CONTRIBUTIONS OF OTHERS

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ABSTRACT

The potential of seaweeds as feedstock for oil-based bioproducts was investigated, and the results support seaweeds as a biomass source for oil-based bioproducts. Seaweeds have not traditionally been perceived as a suitable feedstock for oil-based bioproducts because of their low content of lipids and fatty acids. In contrast to this perception the first major outcome of this thesis was the provision of new benchmark species of seaweed for oil-based bioproducts, selected because of their high oil contents combined with high proportions of omega-3 polyunsaturated fatty acids (PUFA(n-3)), which are the target fatty acids for high value products in health and nutrition. The three key species of seaweed identified were *Spatoglossum macrodontum*, *Dictyota bartayresii* and *Derbesia tenuissima* with high total lipid contents (~ 12 % dry weight (dw)) and high total fatty acid contents (4 – 8 % dw). These species also had a high proportion of PUFA(n-3) which were ~ 20 % of total fatty acids (TFA) in *S. macrodontum* and *D. bartayresii* and over 30 % of TFA in *D. tenuissima*. The second major outcome of this thesis was then the identification and quantification of natural variability of fatty acids within the key species of seaweed which can be exploited for further improvements in the content and composition of fatty acids. For *S. macrodontum*, the content of TFA (55 – 83 mg g^{-1} dw) and the proportion of PUFA(n-3) (16 – 25 % of TFA) varied substantially (~ 50 %) on a temporal scale. For *D. bartayresii*, the content of TFA (45 – 55 mg g^{-1} dw) varied slightly (~ 20%) and the proportion of PUFA(n-3) (16 – 24 % of TFA) varied substantially (~ 50 %) on a temporal scale. There was also spatial variation for *D. bartayresii* which was ~ 50 % for the content of TFA (36 – 54 mg g^{-1} dw) but less than 10 % for the proportion of PUFA(n-3) (18 – 20 % of TFA). The third major outcome of this thesis was then the demonstration that environmental parameters are drivers for fatty acid variability in these species. The first line of evidence was from seasonal field-based studies on *S. macrodontum* and *D. bartayresii* which showed a

higher proportion of PUFA(n-3) in winter when water temperature and light availability were at their annual minimum (~ 40 – 50 % higher in winter). In a second line of experimental evidence for *D. tenuissima*, colder water temperature was identified as the major driver (explained ~ 40 % of the total variability) to improve the proportion of PUFA(n-3) by ~ 20 % in this species. In a similar manner, high light intensity reduced the quality of the biomass by increasing saturation. The fourth major outcome of this thesis was the identification of a relationship between biotic parameters (plant size and life cycle stage) and fatty acids. In *D. bartayresii*, plants with a larger thallus length had significantly higher contents of TFA and slightly higher proportions of PUFA(n-3) and in *S. macrodontum* older plants in their "decline phase" had a more saturated fatty acid profile than younger plants in their "growth phase". Both the environmentally and biotic driven variability in fatty acids can be exploited through culture and harvest strategies to improve the fatty acid content and quality in these feedstocks. The fifth major outcome of this thesis was for the first time the provision of evidence for the genotypic variability of fatty acids within species of seaweed which is the basis for selective breeding to improve the yield of target fatty acids. First, there was substantial spatial variability (~ 40 – 60 %) in the content of fatty acids between the sampling locations of *D. bartayresii*, suggesting genotypic differences between populations. The second line of evidence was from experimental data on isolates of *D. tenuissima* where the content of TFA ranged from 34 to 55 mg g^{-1} dw and 49 % of the variation was genotypic (between isolates). The proportion of PUFA(n-3) ranged from 31 to 46 % of TFA with a strong interactive effect of genotype and water temperature. In two isolates, the proportion of PUFA(n-3) increased by 20 % under cultivation at low temperature while in a third isolate temperature had no effect. Increases in PUFA(n-3) occurred with a stable content of TFA and high growth rates, leading to net increases in PUFA(n-3) productivity in two isolates. And last, the sixth major outcome of this thesis was the identification of fatty acid variability within individual plants. The content of TFA and to a lesser degree in the composition of fatty acids varied substantially within plants of *S. macrodontum*

(TFA: 21 – 106 mg g^{-1} dw) and *D. bartayresii* (TFA: 40 – 57 mg g^{-1} dw) with a higher content of TFA and a higher proportion of PUFA(n-3) in the upper sections compared to the base. Overall, this thesis provides the basic framework on which to develop strategies for the domestication of seaweeds for the production of oil-based bioproducts in a similar manner to the past improvements in the oil yield of terrestrial oil crops and also microalgae. The most important domestication steps identified in this thesis were species selection and permanent improvements in the content and composition of fatty acids through selective breeding with *D. tenuissima* being the prime target species for this process.

LIST OF ABBREVIATIONS

ALA	C18:3(n-3), α-linolenic acid
C	carbon
DHA	C22:6(n-3), docosahexaenoic acid
dw	dry weight
EPA	C20:5(n-3), eicosapentaenoic acid
FAME	fatty acid methyl esters
fw	fresh weight
GC-MS	gas chromatography mass spectrometry
JCU	James Cook University
MUFA	monounsaturated fatty acid
N	nitrogen
PUFA	polyunsaturated fatty acid
PUFA(n-3)	polyunsaturated omega-3 fatty acid
PUFA(n-6)	polyunsaturated omega-6 fatty acid
SDA	C18:4(n-3), stearidonic acid
SGR	specific growth rate
SE	standard error
SFA	saturated fatty acid
TAG	triacylglycerol
TFA	total fatty acid

TABLE OF CONTENTS

Statement on the contributions of others ..... **ii**

Acknowledgements ..... **iii**

Abstract ..... **iv**

List of abbreviations ..... **vii**

Table of contents ..... **viii**

List of Tables ..... **xii**

List of Figures ..... **xiii**

List of appendices ..... **xv**

SUPPLEMENTARY TABLES XV
SUPPLEMENTARY FIGURES XV
PUBLICATIONS XVI

CHAPTER 1

General Introduction **1**

1.1. SEAWEED AS A BIOMASS FEEDSTOCK 1
1.1.1. General definition, ecology and classification *1*
1.1.2. Historic use of seaweed *3*
1.1.3. Modern use of seaweed *4*
1.1.4. Future use of seaweed *6*
1.2. OIL-BASED BIOPRODUCTS 10
1.2.1. Lipids and fatty acids *10*
1.2.2. Functional foods and nutraceuticals *12*
1.2.3. Animal feeds *13*
1.2.4. Biodiesel *14*
1.2.5. Oleochemicals *16*
1.3. SEAWEED BIOMASS AND OIL PRODUCTION 17
1.4. AIMS AND CHAPTER SUMMARY 18

CHAPTER 2

Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bioproducts **20**

2.1. INTRODUCTION 20
2.2. MATERIALS AND METHODS 23
2.2.1. Field sites *23*
2.2.2. Sampling procedures *24*

2.2.3. Total lipid analysis *24*
2.2.4. Fatty acid extraction and analysis *25*
2.2.5. Statistical analysis *26*
2.3. RESULTS 27
2.3.1. Total lipids *27*
2.3.2. Fatty acids *27*
2.3.3. Conspecific variation *35*
2.4. DISCUSSION 37
2.4.1. Total lipid content *38*
2.4.2. Fatty acids *39*
2.4.3. Conspecific variation *40*

CHAPTER 3

Seasonal and within-plant variation in fatty acid content and composition in the brown seaweed *Spatoglossum macrodontum* (Dictyotales, Phaeophyceae) 43

3.1. INTRODUCTION 43
3.2. MATERIALS AND METHODS 45
3.2.1. Species selection and field site *45*
3.2.2. Biomass and fatty acid analysis *46*
3.2.3. Biomass increase estimates *47*
3.2.4. Proximate and ultimate analysis *47*
3.2.5. Statistical analysis *48*
3.3. RESULTS 48
3.3.1. Plant sizes and biomass productivity *48*
3.3.2. Total fatty acid content *50*
3.3.3. Fatty acid composition *52*
3.3.4. Proximate and ultimate analysis *55*
3.4. DISCUSSION 58
3.4.1. Plant sizes and biomass seasonality *58*
3.4.2. Fatty acids *59*
3.4.3. Conclusion *65*

CHAPTER 4

Spatial, seasonal and within-plant variation in total fatty acid content and composition in the brown seaweeds *Dictyota bartayresii* and *Dictyopteris australis* (Dictyotales, Phaeophyceae) 66

4.1. INTRODUCTION 66
4.2. MATERIALS AND METHODS 69
4.2.1. Study organism and field sites *69*
4.2.2. Biomass sampling and preparation *69*
4.2.3. Fatty acid analysis *71*
4.2.4. Statistical analysis *71*

4.3. RESULTS 72
4.3.1. Plant sizes *72*
4.3.2. Total fatty acid content – plant sizes *73*
4.3.3. Total fatty acid content – spatial and seasonal variation *74*
4.3.4. Total fatty acid content – within-plant variation *76*
4.3.5. Fatty acid composition – plant sizes *77*
4.3.6. Fatty acid composition – spatial and seasonal variation *78*
4.3.7. Fatty acid composition – within-plant variation *81*
4.4. DISCUSSION 84
4.4.1. Distribution and plant sizes *84*
4.4.2. Total fatty acid content and composition *85*
4.4.3. Seasonal and spatial variability in fatty acids *87*
4.4.4. Within-plant fatty acid variation *90*
4.4.5. Biomass application *92*

CHAPTER 5

Environmental effects on growth and fatty acids in three isolates of *Derbesia tenuissima* (Bryopsidales, Chlorophyta) 93

5.1. INTRODUCTION 93
5.2. MATERIALS AND METHODS 96
5.2.1. Study organism and biomass collection *96*
5.2.2. Species identification *96*
5.2.3. Experimental design *98*
5.2.4. Acclimation and growth trials *99*
5.2.5. Carbon, nitrogen and fatty acid analysis *100*
5.2.6. Growth rates *100*
5.2.7. Statistical analysis *100*
5.3. RESULTS 101
5.3.1. Species and isolate identification *101*
5.3.2. Specific growth rate *105*
5.3.3. Carbon to nitrogen ratio *105*
5.3.4. Total fatty acid content *108*
5.3.5. Fatty acid composition *109*
5.4. DISCUSSION 115
5.4.1. Species identification and morphology *115*
5.4.2. Growth and internal nitrogen *116*
5.4.3. Total fatty acid content and fatty acid composition *118*
5.4.4. Conclusion *122*

CHAPTER 6

Synthesis and discussion 124

6.1. GENERAL STATEMENT 124
6.2. SPECIES SELECTION AND PROFILING 125

6.2.1. Total content of lipids and fatty acids *125*
6.2.2. Fatty acid composition *127*
6.3. FATTY ACID VARIABILITY WITHIN SPECIES 131
6.3.1. Variability – Environmental drivers *132*
6.3.2. Variability – Biotic drivers *135*
6.3.3. Variability – Genotypic drivers *136*
6.3.4. Variability within the thallus *137*
6.4. SUITABILITY FOR OIL-BASED APPLICATIONS 139
6.4.1. Cultivation methods *139*
6.4.2. Economic potential *141*
6.5. FUTURE RESEARCH DIRECTIONS 144
6.6. CONCLUSION 146

REFERENCES 147

APPENDIX 179

SUPPLEMENTARY TABLES 179
SUPPLEMENTARY FIGURES 191

LIST OF TABLES

Table 2.1. Fatty acid profiles and total lipid content of selected seaweed species.. 35

Table 3.1. Elemental analysis of different plant sections and whole plants collected in September 2012..... 55

Table 3.2. Average content of fatty acids of *Spatoglossum macrodontum* (whole plants) collected over one year and average of seasonal fatty acid content of *Spatoglossum macrodontum* compared with typical fatty acid profiles of traditional oil feedstock plant species. 56

Table 4.1. Climate data for the sampling locations (Kissing Point, Nelly Bay, Orpheus Island)..... 70

Table 4.2. Average TFA content, the average proportion of the broad fatty acid groups (SFA, MUFA, PUFA(n-3), PUFA(n-6)) and selected fatty acids of particular interest across locations and between summer and winter.. 83

Table 5.1. Key morphological features of three isolates of *Derbesia tenuissima*. 103

Table 5.2. Output of 4-way factorial ANOVA testing the effects of temperature, light intensity and nitrogen concentrations in three isolates of *Derbesia tenuissima* on the SGR, carbon to nitrogen ratio (C:N ratio), TFA, SFA, MUFA, PUFA(n-3) and PUFA(n-6).. 104

Table 6.1. A comparative profile of the contents of total lipids and fatty acids of selected seaweeds, microalgae and terrestrial oil crops. 130

LIST OF FIGURES

Figure 1.1. Model of a triacylglycerol (TAG) with three fatty acids 12

Figure 2.1. Mean total lipid and mean total fatty acid content of seaweed orders and species.. 29

Figure 2.2. Polynomial (2^{nd} order) relationships between total lipid content and fatty acid content for different seaweed taxa (brown, green and red seaweed). 30

Figure 2.3. Principal component analysis (PCA) of seaweed species samples based on fatty acid profiles. Score loading bi-plot of saturated and PUFA (n-3) fatty acids..... 32

Figure 2.4. Mean content of the saturated fatty acids C14:0 and C16:0 in different seaweed orders and species. 33

Figure 2.5. Mean content of selected essential PUFA(n-3) in different seaweed orders and species. 34

Figure 2.6. Principal component analysis of fatty acid composition for *Dictyota bartayresii* triplicate samples from different locations (Kissing Point, Nelly Bay, Orpheus Island) and sampling times. Bi-plot of fatty acid loadings for *Dictyota bartayresii*. Principal component analysis of fatty acid composition of *Dictyota dichotoma* triplicate samples from two different locations (Kissing Point, Nelly Bay) and sampling times. Bi-plot of fatty acid loadings for *Dictyota dichotoma*.. 37

Figure 3.1. Average length and fresh weight of *Spatoglossum macrodontum* plants collected over one year..... 50

Figure 3.3. Correlation of mean total fatty acids (TFA), saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA) and PUFA(n-3) with water temperature (°C). 53

Figure 3.4. Proportion of SFA, MUFA and PUFA of TFA content of whole plants and plant sections (tips, midsections, base sections) of *Spatoglossum macrodontum* sampled over one year.. 54

Figure 4.1. Average fresh weight and plant thallus length as annual averages of *Dictyota bartayresii* from different locations (Nelly Bay, Orpheus Island, Kissing Point) and *Dictyopteris australis* from Nelly Bay. Monthly averages of *Dictyota bartayresii* and *Dictyopteris australis* from Nelly Bay. 73

Figure 4.2. Relationship between TFA content with individual plant thallus lengths and plant fresh weights in all individual plants of *Dictyota bartayresii* and *Dictyopteris australis*..... 74

Figure 4.3. Average content of TFA in 'whole plants' and plant sections (upper section, lower section) in *Dictyota bartayresii* and 'whole plants' and plant sections (tips, midsection, base section) in *Dictyopteris australis* as annual averages and monthly averages..... 76

Figure 4.4. Relationship between the proportion of PUFA(n-3) with plant thallus length and plant fresh weight in all individual plants of *Dictyota bartayresii* and *Dictyopteris australis*..... 78

Figure 4.5. Average proportion of fatty acids groups (SFA, MUFA, PUFA(n-3), PUFA(n-6)) as annual averages of *Dictyota bartayresii* from different locations (Nelly Bay, Orpheus Island, Kissing Point) and *Dictyopteris australis* from Nelly Bay. Monthly averages of *Dictyota bartayresii* and *Dictyopteris australis* from Nelly Bay..... 80

Figure 4.6. Multidimensional Scaling (MDS) showing the differences in average fatty acid composition in whole plants and plant sections of *Dictyota bartayresii* across locations and *Dictyopteris australis* from Nelly Bay..... 82

Figure 5.1. Maximum likelihood tree of *Derbesia* and *Bryopsis* rbcL sequence data... 102

Figure 5.2. Histological photographs of three isolates of *Derbesia tenuissima*..... 103

Figure 5.3. Mean specific growth rate (SGR) in three isolates of *Derbesia tenuissima* grown at different temperatures, different nitrogen concentrations and different light conditions..... 106

Figure 5.4. Mean of the carbon to nitrogen ratio (C:N ratio) in three isolates of *Derbesia tenuissima* grown at different temperatures, different nitrogen concentrations and different light conditions..... 107

Figure 5.5. Mean content of TFA in three isolates of *Derbesia tenuissima* grown at different temperatures, different nitrogen concentrations, and different light conditions. 109

Figure 5.6. Mean composition of the broad fatty acid groups (SFA, MUFA, PUFA(n-3), PUFA(n-6)) in three isolates of *Derbesia tenuissima* grown at different temperatures, different nitrogen concentrations, and different light conditions..... 111

Figure 5.7. Correlations of the content of TFA, SFA and PUFA(n-3) with the specific growth rate (SGR) and the carbon to nitrogen ratio (C:N ratio). 112

Figure 5.8. Multidimensional Scaling (MDS) showing the differences in average fatty acid composition of three isolates of *Derbesia tenuissima* grown at different levels of temperature, nitrogen and light with MDS ordinates and overlayed vector loadings of individual fatty acids..... 113

LIST OF APPENDICES

Supplementary Tables

Table A2.1. Total lipid content of seaweeds. 179

Table A2.2. Fatty acid compostion of analysed brown seaweeds. 180

Table A2.3. Fatty acid composition of all investigated *Dictyota bartayresii* plants (every replicate from all samples of this species). 184

Table A2.4. Fatty acid composition of all investigated *Dictyota dichotoma* plants (every replicate from all samples of this species). 185

Table A3.1. Average content of fatty acids of *Spatoglossum macrodontum* plant sections (tips, midsections, base sections) collected over one year.. 186

Table A4.1. Average TFA content, the average proportion of the broad fatty acid groups (SFA, MUFA, PUFA(n-3), PUFA(n-6)) and individual fatty acids across locations and between summer and winter..... 188

Supplementary Figures

Figure A3.1. Shematic drawing of typical *Spatoglossum macrodontum* plant with plant sections used in this Chapter (tips, midsection, base sections) 191

Figure A3.2. Correlation of plant fresh weight and content of TFA, MUFA, SFA, and PUFA 192

Figure A4.1. Shematic drawing of typical *Dictyopteris australis* plant with plant sections used in this Chapter (tips, midsections, base sections) 193

Figure A4.2. Shematic drawing of typical *Dictyota bartayresii* plant with plant sections used in this Chapter (upper sections, lower sections) 194

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Gosch BJ, Lawton RJ, Paul NA, de Nys R, Magnusson M (2015b) Environmental effects on growth and fatty acids in three isolates of *Derbesia tenuissima* (Bryopsidales, Chlorophyta). Algal Research 9:82-93

CHAPTER 1

General Introduction

1.1. Seaweed as a biomass feedstock

1.1.1. General definition, ecology and classification

The term 'seaweed' refers to the multicellular and macroscopic eukaryote algae (macroalgae) found in the marine environment. Seaweeds can be found in every climate zone from the arctic to the tropics (Bolton 1994) and while there are free-floating forms in the open ocean (e.g. *Sargassum* spp., Laffoley et al. 2011) the highest diversity and density of biomass is found in shallow coastal habitats where seaweeds are attached to the substratum with access to nutrients and light (Lobban and Harrison 1997). Because of highly efficient photosynthesis and a high capacity to absorb nutrients from the water column, seaweeds are the most productive photosynthetic organisms rivalled only by microalgae (Stephens et al. 2013a). In many coastal habitats, seaweed biomass dominates the substratum and a large quantity of this biomass is recycled through grazing and biological decomposition emphasizing its important role in aquatic ecosystems (Lobban and Harrison 1997). There is a large variation in the morphology of seaweeds with many filamentous, foliose and branching forms utilising a wide range of habitats in the littoral zone (Lobban and Harrison 1997). This diversity in morphologies is a reflection of high taxonomic diversity but is also a result of phenotypic and genotypic plasticity within species (Hwang et al. 2005; Wright 2005).

There are more than 8000 identified species of seaweed (Guiry and Guiry 2014; Lűning 1990) with the highest taxonomic diversity being found in Japan, South Africa and Australia (Kerswell 2006). Australia in particular is a seaweed biodiversity hotspot with a high degree of endemism, attributed to Australia's long geographical isolation, unique pattern of ocean currents and heterogeneity of rocky shore habitats (Phillips 2001). There

are three distinct lineages of eukaryote algae that can be broadly divided based on their phylogenetic origin, and the associated distinct pigment coloration, into the red, green and brown seaweeds (Lobban and Harrison 1997; Cock et al. 2010). The red seaweeds are the multicellular and marine red algae (Rhodophyta), with fossil records dating back to the mesoprotoerozoic about 1.2 billion years ago (Butterfield 2000). A common feature of red seaweeds is the distinct red coloration caused by the pigments phycoerythrin and phycocyanin (Clayton and King 1990). There are over 6000 extant species, making it the most diverse group of seaweed (Guiry and Guiry 2014), including the commercially important *Porphyra* spp. (Nori), *Palmaria palmata*, *Chondrus crispus* and *Gracilaria* spp. The green seaweeds are the multicellular and marine forms of the green algae (Chlorophyta) and their green color is caused by a pigmentation of chlorophyll a and b that is similar to land plants and emphasizes their close evolutionary relationship (Bhattacharya and Medlin 1998). Green seaweeds have a high diversity of over 1500 species (Guiry and Guiry 2014) with many opportunistic species such as *Ulva* spp. and *Cladophora* spp. that can form large blooms also referred to as green tides (Liu et al. 2009; Smetacek and Zingone 2013). There are over 1800 species of brown seaweeds (Guiry and Guiry 2014) within the class Phaeophyceae and there are no unicellular and only few freshwater species within this class. They are genetically most distant to red and green algae (Cock et al. 2010; Moreira et al. 2000) and it is estimated that they evolved relatively recently in evolutionary terms with the earliest evidence for diversification from the mid Jurassic approximately 175 million years ago (Silberfeld et al. 2010). They differ from the other lineages of seaweed in having fucoxanthin as a pigment which provides them with their distinct brown coloration (Clayton and King 1990). There are many edible species within the brown seaweeds including the commercially important kelps *Saccharina japonica* (Kombu) and *Undaria pinnatifida* (Wakame).

1.1.2. Historic use of seaweed

Considering the abundance of seaweed biomass and the high diversity of species in nearly every coastal environment, it is not surprising that seaweeds have been utilised in many ways by coastal people. Food is one of the oldest and most widespread uses of seaweeds with first evidence from pre-historic populations (Dillehay et al. 2008). The consumption of seaweed has been particularly important in Japan and China where it has been a staple food since the 4th and 6th century (McHugh 2003) and remains a popular and significant part of the traditional diet. Although there have been local traditions of seaweed consumption in parts of coastal Europe, its significance has diminished (Mouritsen et al. 2013) and has only recently been re-introduced through Asian cuisine and the increased awareness of the nutritional value of seaweeds (Mouritsen et al. 2012; Mouritsen et al. 2013). The presence of large quantities of easily available biomass in coastal regions has also provided an ideal feedstock for agricultural applications as animal feeds and fertilizers. One of the oldest non-food applications of seaweed biomass is as a fertilizer where seaweeds are applied to crops and gardens to promote plant growth (Craigie 2011). While seaweed mulches and fertilizers were widespread in coastal Europe during pre-industrial times (Craigie 2011), their use has declined with the introduction of chemical substitutes and only regained a revival as liquid fertilizers based on seaweed extracts (Craigie 2011). Seaweed biomass has also been used as animal feed with livestock being driven onto exposed seaweed fields at low tide (Chapman and Chapman 1980; Jewell 1978). The industrial scale production of seaweed based animal feeds began in the 1960s in Norway where washed up kelps were processed into seaweed meals (Chapman and Chapman 1980; McHugh 2003) and seaweeds continue to be used in animal feed supplements (Holdt and Kraan 2011).

In addition to the utilisation of seaweeds as whole products either for human consumption, fertilizer, or as animal feed, there have also been early attempts to utilise the nutritional and biochemical properties of species for human health. For example, there

is evidence that prehistoric societies used seaweeds for medicinal purposes (Dillehay et al. 2008) and the powder and extracts of the brown seaweed *Sargassum* have been utilised in traditional Chinese medicine for more than 2000 years to treat diseases including goitre (Liu et al. 2012). However, the first records of the extraction of specific compounds from seaweed to utilise the biochemical potential of seaweed biomass directly were the extraction of complex polysaccharides from kelps. The gelling properties of alginic acid from kelps were discovered in 15th century Japan and industrial production of alginic acid started in 1923 (Okazaki 1971). The historic use of seaweeds established the traditional perspective of seaweeds as part of a staple eastern diet and the feedstock for phycocolloids. However, with the current awareness of resource constraints in a growing and increasingly affluent population and the application of modern biotechnologies to seaweed production, the modern use of seaweeds is changing rapidly.

1.1.3. Modern use of seaweed

In 2012, approximately 20 million tonnes of seaweed was harvested with 90 % of the biomass being produced in culture and only a small and decreasing proportion derived from wild harvests (McHugh 2003; FAO 2014). Although the production of seaweed has experienced rapid growth of more than 100 % in the last decade (FAO 2014), annual production is still dominated by products of relatively low value in the food and phycocolloid sector with only a small degree of diversification and growth towards the expanding markets of high value health products and animal feeds (FAO 2014; Paul et al. 2012). Approximately 9 million tonnes of the annual production is for direct human consumption and is sold as dried seaweed derived predominately from the two kelps *Saccharina japonica* (Kombu) and *Undaria pinnatifida* (Wakame), and the red seaweed *Porphyra* spp. (Nori) (FAO 2014). Only a marginal proportion of the global seaweed harvest is consumed fresh (Paul et al. 2012), with prominent examples being the green seaweeds *Ulva* spp. (sea lettuce, Aonori) (Kim et al. 2011) and *Caulerpa* spp. (sea grapes) (Paul et al. 2014).

The main driver for the recent expansion of the seaweed industry, however, has been the expansion of the phycocolloids industry (Bixler and Porse 2011). Phycocolloids are complex sugars (polysaccharides) extracted from red and brown seaweeds and can be broadly categorized as agar, alginate and carrageenan with applications as gelling agents and emulsifiers in the food and cosmetics industries (Burey et al. 2008), but also for medical purposes such as wound dressings (Kraan 2012; Thomas 2000). In 2009, nearly 50 % of the total seaweed biomass harvest was utilised for the extraction of approximately 86,100 tonnes of phycocolloids of which 11 % was agar, 31 % alginates and 58 % carrageenan, predominately from the red seaweeds *Gracilaria* and *Kappaphycus* and the brown seaweed *Saccharina* (Bixler and Porse 2011). Apart from phycocolloids, the proportion of non-food applications is marginal with small industries utilising approximately 10,000 tonnes of seaweed biomass for the production of liquid fertilisers and mulches for gardening and agriculture (McHugh 2003) with an estimated market size of US$ 5 million annually (Pulz and Gross 2004). Further, approximately 100,000 tonnes of seaweed biomass is harvested annually for the production of seaweed meals mainly as a feed additive for livestock (Evans and Critchley 2014) and an even smaller proportion is used in aquaculture, either as the main feed or as a feed additive (Bolton et al. 2009). Despite a long history of seaweed use in traditional medicines, and the analysis and confirmation of functionally effective compounds for health (Dillehay et al. 2008; Liu et al. 2012), there are only a few products utilising this potential commercially (Hafting et al. 2012). One of the commercially utilised biochemical compounds is the sulphated polysaccharide fucoidan derived from brown seaweeds which is marketed as a nutraceutical for the improvement of the immune system, preventing and supporting the treatment of cancers, and health in general (Ale et al. 2011; Carvalho and Pereira 2015).

1.1.4. Future use of seaweed

The majority of seaweed-derived products are typical commodities and accordingly have a lower economic value per unit biomass than niche products (Hafting et al. 2012; Paul et al. 2012). Further expansion of the size and value of the seaweed market depends on the growth of existing markets for food and phycocolloids, and more importantly on the development of new species and new high value products (Hafting et al. 2012). These higher value products will be based around functional applications of seaweed biochemicals. Seaweeds produce a range of functional compounds that provide generic nutritional and health benefits including polysaccharides, fibre, protein and oils (Holdt and Kraan 2011; Wijesekara et al. 2011), and many biochemical compounds with specific anti-inflammatory, antibiotic, antiviral and antioxidant properties (Holdt and Kraan 2011; Ibañez and Cifuentes 2013; Jiménez-Escrig et al. 2012; Jung et al. 2013; McCauley et al. 2014; Sabeena and Jacobsen 2013). The potential of these nutritional profiles has, however, not been fully exploited as a bioresource, in particular as new high value nutritional and health products marketed as functional foods and nutraceuticals. This is in stark contrast to the commercialisation of terrestrial plants and increasingly also microalgae for a wide range of health products as functional foods and nutraceuticals (Bigliardi and Galati 2013; Borowitzka 2013; del Río-Celestino et al. 2008).

Functional foods are generally defined as whole or processed foods that contain added functional or health-promoting ingredients on top of their basic nutritional value (Doyon and Labrecque 2008). There are a wide variety of functional foods on the market with added vitamins, minerals and antioxidants, but also essential amino acids and fatty acids (Bigliardi and Galati 2013; Khan et al. 2013). Overall, the market for functional foods has been estimated to be US$ 168 billion in 2012 (Khan et al. 2013) with strong projected growth (Bigliardi and Galati 2013; Hafting et al. 2012). While functional foods are aimed to be healthy versions of traditional foods with similar taste and appearance (Verbeke 2006), there is also an increasing drive to market the functional ingredients directly as

nutraceuticals in the form of tablets, powders and extracts. These nutraceuticals also differ from functional foods in having specific therapeutic claims towards the cure or prevention of diseases (Kalra 2003). Estimates for the nutraceutical market size range between US$ 80 and 130 billion for 2014 (Dutta et al. 2014) with a projected growth to over US$ 200 billion in 2018 (Dutta et al. 2014). Nutraceuticals include many products to supplement essential amino acids, vitamins and antioxidants derived from terrestrial plants (Kaur and Das 2011), eukaryote microalgae (Borowitzka 2013; Carvalho and Pereira 2015) and filamentous cyanobacteria (*Spirulina* spp.) (Borowitzka 2013; Carvalho and Pereira 2015).

Of particular interest, however, is the supplementation of essential omega-3 fatty acids that are currently derived from fish oils, but also increasingly from microalgae, with a projected market size of US$ 34.7 billion in 2016 (Quotes 2012; Adarme-Vega et al. 2014). However, omega-3 fatty acids derived from seaweed biomass have not been utilized to date. Analysis of the biochemical composition of seaweed biomass is restricted to a small fraction of the total number of seaweed species, but even in these few species, the biochemical composition is diverse with a rich composition of polysaccharides, proteins, oils and other bioactive compounds similar in diversity and complexity to terrestrial plants and microalgae (Kumari et al. 2010; McDermid and Stuercke 2003; Viera et al. 2005; Zhang et al. 2010). However, the vast diversity of seaweed species and their biochemical composition of proteins, fibre and oils have not been utilised as high value products, and the potential of oils is discussed in more detail in section 1.2.

Another avenue for growth of the seaweed industry is the expansion of markets for livestock and aquaculture feeds. The global market for livestock feeds is estimated at US$ 370 billion at a global production of approximately 1 billion tonnes (IFIF 2014). However, there is increasing concern about the sustainability of meat production as a large proportion of feeds are based on cereals and soybean which compete directly with

food crops for arable land and also have a low feed conversion efficiency (Alexandratos and Bruinsma 2012; Godfray et al. 2010). Seaweed biomass provides an alternative to traditional animal feed ingredients as it does not compete with traditional food crops and also has higher biomass productivity per unit land area (Stephens et al. 2013a). The suitability of seaweed biomass in livestock feeds has been experimentally demonstrated for the major groups of livestock (cattle, pigs, chicken, sheep) (Chapman and Chapmann 1980; Hansen et al. 2003; Kulshreshtha et al. 2014) and there is a traditional industry that utilises over 100,000 tonnes of seaweed biomass (mainly *Ascophyllum nodosum*) for livestock and increasingly also pet animal feeds (Evans and Critchley 2014). A prominent feed product produced from *Ascophyllum nodosum* is Tasco® which is marketed as a prebiotic functional feed for animal health and improved production performance (Arcadian Seaplants 2015). The inclusion of seaweed biomass in feeds can benefit animal health (Hwang et al. 2014; Kulshreshtha et al. 2014; O'Sullivan et al. 2010) and meat quality (Carrillo et al. 2009; Díaz-Chirón et al. 2009; Hwang et al. 2014), and the inclusion of some species can reduce methane emission in cattle production (Machado et al. 2014).

In contrast to livestock feeds, seaweed biomass is a largely untapped bioresource for inclusion in aquaculture feeds. Aquaculture (finfish, crustaceans, molluscs) is the fastest growing sector of primary industries with an annual production of over 70 million tonnes worth US$ 138 billion (FAO 2014). The traditional and primary ingredient of aquaculture feeds are fish meals and oils (Naylor et al. 2009), however these are problematic as there is a growing discrepancy between an expanding demand of aquaculture feeds and a declining supply of fish meals and oils (FAO 2012; Globefish 2014). A major issue in the aquaculture industry is therefore the replacement or supplementation of these fish-derived ingredients with alternative sources of protein and oils. Soybean and rapeseed are common fish meal and fish oil replacements (Naylor et al. 2009), but they also compete with traditional food crops and have a lower proportion of omega-3 fatty acids with

negative implications for fish product quality (Dubois et al. 2007; Naylor et al. 2009; Nichols et al. 2014a). The inclusion of seaweed biomass, either as complete biomass or extracted and refined components, in aquaculture feeds has been successful in experimental feeding trials for selected aquaculture species (Pereira et al. 2012; Wilke et al. 2014). Notably, the highest rates of inclusion (15 – 30 %) of unrefined seaweed biomass are beneficial to herbivorous fishes (Stadtlander et al. 2013) while seaweed inclusion rates between 2 – 5 % without adverse effects on growth are reported for a range of other species (Nakagawa et al. 1997; Ragaza et al. 2013). To date, seaweed has mainly been used at a commercial scale for polyculture with abalone (Bolton et al. 2009). However, the rich biochemical composition of proteins and oils and the potential to produce seaweed biomass in polyculture with aquatic animal production, while also providing a bioremediation function (Buschmann et al. 2009), makes seaweed a promising and sustainable source for feed ingredients.

Seaweed biomass also has potential as a renewable bioresource in the bioenergy and biomaterials industries. While petroleum based fuels and materials dominate the energy, transport and manufacturing sectors, there is increasing demand for alternative and renewable solutions (Gavrilescu and Chisti 2005). To date, only terrestrial crops have been commercially utilised for biofuels (Issariyakul and Dalai 2014) or for the production of bio-polymers (Desroches et al. 2012), but their use for industrial non-food applications and the associated competition with traditional food crops is increasingly seen as problematic (Foley et al. 2011; Tan et al. 2009). While there has been abundant research on the use of microalgae as a feedstock for biofuels (Mata et al. 2010a; Sharma et al. 2012; Stephens et al. 2013a,b), high production costs and technical difficulties prevent commercial production (Borowitzka 2013; Stephens et al. 2010a). An alternative bioresource feedstock is seaweed biomass with high productivities in culture (Capo et al. 1999; Bolton et al. 2009; Mata et al. 2010b) and production on non-arable land or in large-scale offshore cultivation thereby avoiding competition with food crops. Seaweed

biomass can be converted by fermentation to bioethanol (John and Anisha 2011; Kraan 2013) and biogas (Bruhn et al. 2011), and by hydrothermal liquefaction (Anastasakis and Ross 2011; Neveux et al. 2014a) and pyrolysis (Budarian et al. 2011; Ross et al. 2008; Rowbotham et al. 2012) to liquid bio-oils. There is also the potential to extract oils from seaweed biomass with subsequent transesterification of fatty acids to methyl esters for biodiesel (Maceiras et al. 2011; Demirbas and Demirbas 2011). Seaweed biomass can also be converted to biochar with applications in agriculture for soil improvement or as solid fuel, and is effective for carbon sequestration (Bird et al. 2011; Rowbotham et al. 2012). In addition, there is potential to substitute petroleum-based building blocks in the chemical industry with renewable raw materials for the manufacture of chemical compounds and in particular renewable, non-toxic and biodegradable bio-polymers (Gandini 2008; Lligadas et al. 2010).

1.2. Oil-based bioproducts

1.2.1. Lipids and fatty acids

Oils provide an ideal platform to further extend the product portfolio of seaweed biomass beyond foods and phycocolloids. Seaweed biomass is a novel choice as a bioresource for renewable oils with high biomass productivity and a high species diversity that is essentially untapped in terms of developing scalable bioresources for oil-based products. Lipids are the major source of ‘oil’ in seaweeds and can be defined as hydrophobic and lipophilic molecules including fats, oils and waxes, and in the broader sense any biomolecule with lipophilic properties such as sterols, terpenes and some vitamins (Kumari et al. 2013b; Thomson 1996). Together with proteins and fibre, lipids are frequently reported in seaweeds as part of the broad nutritional profile (Hwang et al. 2008; McDermid and Stuercke 2003; Viera et al. 2005; Zhang et al. 2010) and are rarely targeted as a valuable stand-alone bioresource.

Lipids have important structural functions in the membranes and in particular the photosynthetic complex of algae, which includes seaweeds (Kumari et al. 2013b). There are three major groups of lipids consisting of triacylglycerol (TAG) (Figure 1.1), phospholipids and glycolipids. The TAGs are non-polar lipids and function as energy reserve and storage products (Kumari et al. 2013b; Sharma et al. 2012). Phospholipids are polar lipids and mainly found in the extra-chloroplast membranes forming the lipid bi-layer of cells but specific forms such as phosphatidylglycerol are associated with the thylakoid membranes within the chloroplasts (Thompson 1996). Glycolipids are also polar and predominately found within the chloroplast as part of the thylakoid complex and are therefore the major lipid class associated with photosynthesis (Thompson 1996). Triacylglycerols consist of three esterified fatty acids bound to a glycerol backbone (Figure 1.1). Phospholipids and glycolipids have a similar structure to TAG but have a phosphate and carbohydrate group attached, respectively, and different numbers of fatty acids (Christie 2003; Kumari et al. 2013b). The basic structural components of lipids are the fatty acids as aliphatic hydrocarbon chains with a carboxylic head group and varying degrees of saturation (Christie 2003; Kumari et al. 2013b). Based on the degree of saturation, fatty acids can be broadly classified as saturated fatty acids (SFA) with no double bond, monounsaturated fatty acids (MUFA) with one double bond and polyunsaturated fatty acids (PUFA) with multiple double bonds along the hydrocarbon carbon chain (Christie 2003; Bell and Tocher 2008; Kumari et al. 2013b). Typically, fatty acids are designated as CX:Y(n-z), where X is the total number of carbon, Y is the number of double bonds, and z is the position of the ultimate double bond from the terminal methyl group (Bell and Tocher 2008) (Figure 1.1). For example, the nutritionally important PUFA of the "omega-3" category (PUFA(n-3)) have their last double bond along the hydrocarbon carbon chain at the third position from the terminal methyl group. The terms "omega-3" and "PUFA(n-3)" are subsequently used synonymously. Fatty acids in algae are predominately produced in the chloroplasts as saturated or monounsaturated fatty acids and then are further transformed through saturation and desaturation and

various degrees of chain elongation to more unsaturated fatty acids such as long-chained PUFA (≥ 20 carbons) (Bell and Tocher 2008; Pereira et al. 2003). Although most algae also contain fatty acids in their free form (Kumari et al. 2013b) the majority are incorporated into more complex triacylglycerols, phospholipids or glycolipids.

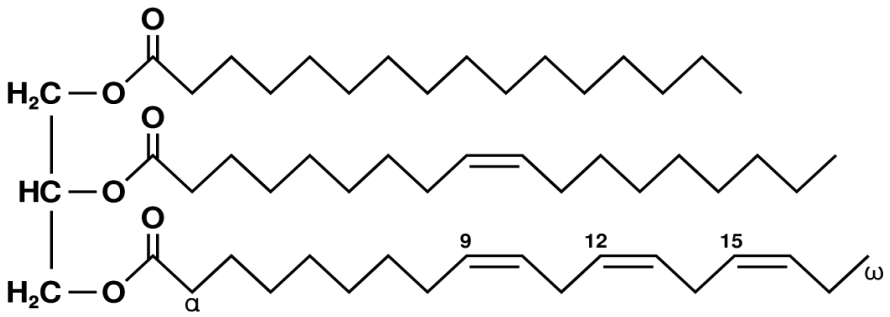

Figure 1.1. Model of a triacylglycerol (TAG) with three fatty acids with the bottom fatty acid being an omega-3 fatty acid (source: Wikipedia).

1.2.2. Functional foods and nutraceuticals

Functional foods are traditional foods with added functional compounds (Doyon and Labrecque 2008) and nutraceuticals are defined as functional compounds with health promoting properties usually marketed as powder, extracts, tablets or capsules (Kalra 2003). The PUFA(n-3) are of particular interest as a functional ingredient. There is increasing awareness of the health benefits of a diet rich in PUFA(n-3) which includes improved cardiovascular health (Russo 2009), reduced severity of certain mental health disorders (Su et al. 2003) and the redistribution of body fat as demonstrated in animal trials (Poudyal et al. 2012, 2013). However, the typical western diet is deficient in PUFA(n-3) with an unfavorably high n-6/n-3 ratio in most animal oils (Simopoulos 2008) and vegetable oils (Dubois et al. 2007). Traditionally these PUFA(n-3) are derived from marine fish oils and marketed in the form of functional foods and nutraceuticals with a projected market of US$ 34.7 billion in 2016 (Adarme-Vega et al. 2014). However, fish oils as a source for PUFA(n-3) are problematic as fish stocks are increasingly depleted (Pauly et al. 2005) and current demand for fish oil already exceeds supply (Globefish 2014). Furthermore, there are quality concerns with high loads of mercury in some fish

species (Kris-Etherton et al. 2002), 'fishy' taste to some oils and increasing customer preference for vegetarian dietary products (Leitzmann 2014). Although some terrestrial crops such as borage, chia seed and walnuts have high contents of PUFA(n-3) they generally lack the long-chained (≥ 20 carbons) omega-3 fatty acids such as EPA (C20:5(n-3), eicosapentaenoic acid) or DHA (C22:6(n-3), docosahexaenoic acid) (Adarme-Vega et al. 2014; del Río-Celestino et al. 2008; Dubois et al. 2007; Simopoulos 2002), have low productivities of oil, and also compete with traditional food crops for arable land (Foley et al. 2011). Although there is already a range of omega-3 functional foods and nutraceuticals available derived from terrestrial plants and microalgae (Spolaore et al. 2006; Trautwein 2001), seaweed biomass is a largely untapped resource. Considering the market value of US$ 140 kg^{-1} for algal derived omega-3 oils (Borowitzka 2013) and a projected global market for EPA and DHA of 135,000 – 190,000 tonnes in 2015 (Borowitzka 2013; Frost and Sullivan 2010), the economic potential for seaweed based omega-3 oils is substantial.

1.2.3. Animal feeds

In a similar manner to health and nutrition in humans, oils and in particular omega-3 fatty acids have applications for livestock and aquaculture animals. Although the major focus for novel livestock and aquaculture feeds is on the supply of protein (Naylor et al. 2009), there is increasing evidence that oils, and in particular the omega-3 fatty acids, can improve the growth and health of farmed animals. Although many of the long-term health effects associated with omega-3 supplementation in human nutrition are not applicable in farmed animals where the focus is on short production cycles, the inclusion of PUFA(n-3) in the diet of livestock can be beneficial with evidence for reduced inflammation in pigs (Bahar et al. 2012) and improved reproductive performance in pigs and cattle (Mateo et al. 2009; Petit et al. 2002). Omega-3 fatty acids also play a major role in the rearing of aquaculture species and are critical functional feed ingredients for the larvae of many species (Muller-Feuga 2000; Tocher 2010; Tocher 2015). However, the major focus is the

enhancement of meat and animal product quality, defined by a lower n-6/n-3 ratio, through omega-3 fatty acid supplementation (Díaz-Chirón et al. 2009; Wood et al. 2004).

Traditional sources of omega-3 fatty acids for livestock feeds have been terrestrial plants such as flaxseed and linseed which are good sources of ALA (C18:3(n-3), α-linolenic acid) (Dubois et al. 2007; Fraeye et al. 2012; Howe et al. 2002), but the most common source for the addition of the nutritionally important long-chained PUFA(n-3) is fish oils (Fraeye et al. 2012; Howe et al. 2002). For aquaculture species the common sources for omega-3 fatty acids are fish meals and fish oil (Naylor et al. 2009; Tacon and Metian 2008) and increasingly microalgae (Milledge 2011). As described, fish oils are unsustainable due to declining fish stocks (Globefish 2014; Pauly et al. 2005) and the high technological standard required for the production of microalgae is considered a substantial expense in aquaculture businesses (Borowitzka 2013). Similar to functional foods and nutraceuticals, seaweed biomass has the potential to replace or complement terrestrial oil crops and fish oils as the source for omega-3 fatty acids in livestock and aquaculture feeds. To date the omega-3 enrichment through the inclusion of seaweeds in feeds has been demonstrated for ruminant products (meat) (Hwang et al. 2014; Díaz-Chirón et al. 2009), pork (Moroney et al. 2015), poultry (meat, eggs) (Carrillo et al. 2009; Islam et al. 2014) and aquaculture species such as flounder and salmon (Ragaza et al. 2013; Wilke et al. 2014).

1.2.4. Biodiesel

In contrast to the high value health and nutritional applications, there is also potential to produce biofuels and biomaterials from seaweed oils. The production of biodiesel from renewable sources to replace fossil-derived fuels has gained much attention. Biodiesel can be produced from plant and animal oils through the transesterification of the triacylglycerols to fatty acid methyl esters (Meher et al. 2006). Common feedstocks for the commercial production of biodiesel are soybean and rapeseed (Issariyakul and Dalai

2014), and waste oils (e.g. used cooking oil) (Mandolesi et al. 2013). However, many of these traditional oil crops require large areas of land due to their low oil productivity and also compete with traditional food crops and are therefore seen as problematic as a future replacement of fossil fuels (Tan et al. 2009). There has been an abundance of research on microalgae as a novel feedstock for the production of biodiesel because algae have higher photosynthetic efficiencies and biomass productivities than terrestrial oil crops (Mata et al. 2010a; Stephens et al. 2013a) and do not compete with traditional food crops as they can be cultivated on non-arable land. Although traditionally not considered as a feedstock for biodiesel, seaweed biomass has similar productivities to microalgae (Bolton et al. 2009; Capo et al. 1999; Mata et al. 2010b) and can be produced on non-arable land in land-based intensive cultivation (Magnusson et al. 2014; Mata et al. 2015) and also in large-scale offshore cultivation (Kraan 2013). Importantly, the cultivation of seaweeds requires only a basic level of technology, with corresponding lower costs of production than microalgae (Paul et al. 2012). However, the major rationale for seaweed not being considered as a feedstock for biodiesel is the presumably low oil content.

For biodiesel, as for any other oil-based bioproduct, the oil quality of a feedstock is an important factor with implications for the quality of the fuel. Importantly, there are a range of industrial standards for biodiesel from renewable sources, regulating the minimum requirements for oil quality and in particular the degree of saturation (see Figure 1.1) (Knothe 2006). Generally, a feedstock with a more saturated fatty acid profile is preferred as this leads to higher oxidative stability and higher ignition quality (cetane number), and produces an overall higher quality product (Hu et al. 2008; Knothe 2008). Feedstocks with a high proportion of the monounsaturated fatty acid (MUFA) C18:1 can further improve the biodiesel quality by enhancing the low temperature properties and kinetic viscosity, and reducing the emissions of hydrocarbons and CO_2 (Knothe 2008). In contrast, a feedstock rich in polyunsaturated fatty acids (PUFA) results in an oxidatively less stable and lower quality biofuel. In particular the proportion of the PUFA C18:2 and

C18:3 are critical as these fatty acids are particularly susceptible to oxidation and the proportion of C18:3 has been regulated to 12 % of the total fatty acids (TFA) according to the European biodiesel quality standard EN 14214 (Knothe 2006). Highly unsaturated fatty acids (≥ 4 double bonds) have also been banned based on European quality standards and therefore exclude fish oils as feedstock for biodiesel (Knothe 2006).

1.2.5. Oleochemicals

In addition to biodiesel, oils from plant sources are becoming a focus within the chemical industry as a renewable platform for the synthesis of chemical compounds and in particular renewable, non-toxic and biodegradable bio-polymers (Gandini 2008; Lligadas et al. 2010). Therefore the manufacture and synthesis of bio-polymers such as polyurethanes from renewable vegetable oils have gained increasing attention in recent years. These bio-polymers have wide applications as foams, adhesives, sealants and also biomedical implants (Biermann et al. 2006; Petrović 2008). The basic building blocks of polyurethanes are the polyols and isocyanates and these are predominately produced from petroleum-based oils. However, the polyol component in particular has been successfully substituted from renewable sources with commercial products available from major chemical producers including Dow Chemical, BASF and Bayer (Desroches et al. 2012). To date, approximately 13 % of the 150 million tonnes of total annual production of vegetable oil (Desroches et al. 2012) is utilised by the chemical industry as a feedstock for polymers, solvents and chemical building blocks with the market for polyurethanes alone being estimated at US$ 30 – 35 billion annually (Petrović 2008). The global production of polyurethanes in 2010 was 14 million tonnes and strong growth to 18 million tonnes in 2016 is projected (Nohra et al. 2013; Petrović 2008). While the production of bio-polymers from various vegetables oils such as palm oil, rapeseed and soybean oil gains momentum, seaweed and algal biomass in general has not been considered as a feedstock for oleochemcial products despite superior biomass productivities.

1.3. Seaweed biomass and oil production

While there are significant developed and developing markets for high quality marine oils, seaweed has not been perceived as a suitable feedstock for oil-based products because of its presumably low content of "oils" (total lipids, total fatty acids). The total lipid content for the majority of reported seaweeds is in the range of 3 – 5 % dry weight (dw) (Montgomery and Gerking, 1980; McDermid and Stuercke 2003) which is very low compared to microalgae (~ 10 – 50 %, Griffiths and Harrison 2009; Huerlimann et al. 2010), seeds of terrestrial oil crops (~ 20 – 50 %, Issariyakul and Dalai 2014) and oil fish (~ 10 %, Aro et al. 2000; Zlatanos and Laskaridis 2007). However, only about 200 of the estimated 8000 species of seaweed have been analyzed for their total lipid contents and even fewer for their content and composition of fatty acids. Notably, some species have total lipid contents above 10 % dw making them potential feedstock species for oil-based bioproducts (Montgomery and Gerking 1980; McDermid and Stuercke 2003; Polat and Ozogul 2008). The identification of target species with high contents of total lipids and specifically high contents and high quality compositions of fatty acids is the first step in developing novel species of seaweed for the production of oil-based bioproducts. The second step is then the identification and quantification of natural variability in the content and composition of fatty acids within a species as this is the basis to improve the yield of target fatty acids through environmental manipulation and also selection of genotypes with advantageous attributes. While the process of domestication has been a key tool to improve the efficiency of terrestrial oil crops (Byrum et al. 1997; Downey and Craig 1964; Khush 2001), and there have been enormous advances in the cultivation of microalgae for oil production (Grima et al. 1995; Sharma et al. 2012; Rodolfi et al. 2009; Wijffels and Barbosa 2010), this process is still in its infancy in seaweeds (Robinson et al. 2013).

Therefore the overarching aim of this thesis is the identification of novel species of seaweed with high total lipid and fatty acid contents and fatty acid compositions (oil quality) suitable for oil-based applications. Furthermore, this thesis aims to identify and

quantify variability in the content and composition of fatty acids as this is the basis for further improvements through refined culture strategies and strain selection.

1.4. Aims and Chapter summary

Chapter 2 investigates the potential of seaweeds from Northern Queensland, Australia, as feedstock for oil-based products as functional foods, nutraceuticals and biodiesel. The first aim was to identify novel target species suitable for oil-based bioproducts through the quantification of total lipid content, total fatty acid content and fatty acid composition for more than 40 species of seaweed. The second aim was to investigate the relationship between the total lipid content and the fatty acid fraction as the critical parameter for oil-based bioproducts for each species and the broad taxonomic groups of brown, red and green seaweeds. The third aim was to investigate the variation of total fatty acids and fatty acid composition within a species from different locations and sampling periods for two of the species with a high fatty acid content to identify the potential for manipulating environment, or selecting genotypes, to optimize yields and initiate selective breeding to further improve and optimize fatty acid content and composition.

Chapter 3 investigates in detail the variation of fatty acid content and composition and the drivers thereof for *Spatoglossum macrodontum* with an emphasis on specific monounsaturated fatty acids and PUFA(n-3) and their potential for use as fine chemicals, functional foods and nutraceuticals. This brown seaweed has been identified as a potential feedstock for oil-based products in Chapter 2 because of its high fatty acid content. The first aim was to quantify the monthly variation of the total fatty content and composition for the period of one year and correlate this with monthly changes in biomass size and water temperature as the key biological and environmental drivers for fatty acids. The second aim was to investigate the variation of fatty acids between thallus sections (tips, midsection, base section) of individual plants. Together this data provides the fundamental basis on which to develop culture and harvest strategies for this species.

Chapter 4 investigates the variation of total fatty acid content and composition and the drivers thereof in further details for *Dictyota bartayresii* and *Dictyopteris australis* with emphasis on PUFA(n-3) and their potential for oil-based bioproducts such as functional foods and nutraceuticals. These brown seaweeds have been identified as potential candidates for oil-based products in Chapter 2 because of their high total lipid and fatty acid contents. The first aim was to quantify the broad seasonal (summer *vs.* winter) and monthly variability in the content and composition of the fatty acids of both species and also the spatial variation in *D. bartayresii*. Variation in fatty acids was correlated with variation in biomass to investigate biomass changes as potential driver for fatty acids. The second aim was to analyze the variation in fatty acids for different parts of the thallus. Although changes in biomass have been identified and quantified as a driver for variation in fatty acids, the effects of the key environmental and genotype components of the variation could not be established beyond correlation.

Chapter 5 investigates empirically the effects of the key environmental parameters temperature, light and nitrogen on the growth and fatty acid content and composition in three isolates of the green seaweed *Derbesia tenuissima* with the aim to not only quantify but also portion the environmental and the genotype component of the variation. This green seaweed has been identified in Chapter 2 as a potential feedstock for oil-based products because of its high fatty acid content with a high proportion of PUFA(n-3) and its suitability for functional foods and nutraceuticals has been discussed in detail. This Chapter provides fundamental empirical data to further exploit the growth and fatty acid production capacity of this novel seaweed.

Chapter 6 provides a synthesis of the data and recommendations for future research.

CHAPTER 2

Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bioproducts [1]

2.1. Introduction

Algal biomass offers an innovative contribution to the challenge of providing sustainable bioenergy resources (Brennan and Owende 2010). Algae have high productivities, can be cultured on non-arable land and can utilise waste-streams as a nutrient source. Their rapid growth through photosynthesis provides for the capture and recycling of carbon dioxide (CO_2), and algal biomass provides for bioenergy generation through a diversity of processes including direct combustion, gasification, saccharification and fermentation, and thermochemical processing (Singh and Olsen 2011; Demirbas 2011; Nigam and Singh 2011). However, the major focus for research, development and commercialisation is the cultivation of algae for the production of oil-based products. While algal oils have applications as biodiesel, through the transesterification of lipids to replace fossil-derived fuels, and as feedstock for industrial chemicals, the main emphasis is on high value products as functional foods, nutraceuticals and fish oil replacement in animal feeds.

The vast majority of research on algal oils has concentrated on microalgae, where some species contain in excess of 30 % total lipid based on dry weight (dw) (Renaud et al. 1999; Chisti 2007; Huerlimann et al. 2010). However there are significant technical challenges in the cost and complexity of cultivation and harvesting microalgae at an industrial scale (Ginzburg 1993; Mata et al. 2010a; Stephens et al. 2010a,b). In contrast,

[1] Adapted from: Gosch BJ, Magnusson M, Paul NA, de Nys R (2012) Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bioproducts. GCB Bioenergy 4:919-930

marine and freshwater macroalgae are simpler to produce and are particularly suited to low technology cultivation methods with a well-established multi-billion dollar industry producing more than 20 million tonnes per annum (FAO 2014). However, none of this production targets oil. This is in part because macroalgae are typically perceived as unsuitable for the production of oil-based products as most species have low total lipid contents of less than 5 % dw (e.g. Montgomery and Gerking 1980; McDermid and Stuercke 2003; Kumari et al. 2010). However, this paradigm of low total lipid content for macroalgae is challenged by some species which have a total lipid content above 15 % dw, in particular within the order Dictyotales which consistently have a total lipid content between 11 % and 20 % dw (Montgomery and Gerking 1980; McDermid and Stuercke 2003).

Although the total lipid content has been determined for a number of seaweed species, this is normally in the context of gross chemical composition (% lipid, carbohydrate, protein etc.) for nutritional profiling of species for use in aquaculture (Viera et al. 2005; Zhang et al. 2010) or human consumption (McDermid and Stuercke 2003; Hwang et al. 2008). However, quantification of the fatty acid component of the total lipid extract (generally up to 24-carbon [C24] long aliphatic hydrocarbon chains with a carboxylic head group and varying degrees of saturation), is the critical parameter in determining the yield and suitability of oils as biofuel feedstock, or nutraceutical or fish oil replacement. The limited information available for the fatty acid composition of seaweeds shows a similar range of fatty acids to microalgae, with carbon chains between C14-C24 (Khotimchenko et al. 2002; Kumari et al. 2010; Saito et al. 2010; Sánchez-Machado et al. 2004; Vaskovsky et al. 1996). Notably, the diversity of species investigated for quantitative fatty acid profiles is very low, approximated at less than 200 of the estimated 8000 species identified to date (Kumari et al. 2010; Kumari et al. 2013a; Lűning 1990; Sánchez-Machado et al. 2004; Schmid et al. 2014). This provides significant potential to identify novel high oil species, similar to those already identified within the Dictyotales.

Therefore, the first critical step in assessing seaweeds as a feedstock for oil-based bioproducts is to establish, qualitatively and quantitatively, their total lipid content and fatty acid profiles. This includes variation within individuals of the same species to identify the possible effects of genotype and environment on lipid quality and quantity. The subsequent step is to determine the extent to which environmental factors affect total lipid and fatty acid content, such as light (Hotimchenko 2002), temperature (Al-Hasan et al. 1991; Nelson et al. 2002) and nutrient regime (Hu et al. 2008; Livne and Sukenik 1992; Rodolfi et al. 2009). This then provides the basis to manipulate and select environments and/or genotypes to optimise lipid quantity and quality under intensive culture. The final step in this process is then to deliver intensive cultivation of elite macroalgae to provide high productivity of oil, based on biomass productivity (g dw m^{-2} day^{-1}) and fatty acid yield to meet productivity benchmarks (g (oil) m^{-2} day^{-1}) (Stephens et al. 2010a). As the first step in identifying target seaweed species for culture for oil-based products, I simultaneously quantified the total lipid content and the fatty acid profiles of a diversity of tropical seaweed species from North Queensland, Australia.

The primary objectives is to identify species with high total lipid contents and suitable fatty acid profiles, primarily for applications as nutraceuticals and alternatively as biofuel, as these are the two key drivers of demand for algal oil production. In addition, I evaluated the relationship between the total lipid content and the total fatty acid (TFA) content for each of the three main taxonomic groups of seaweeds (brown, green and red). Finally, I quantified conspecific variation in lipid profiles between and within locations and sampling periods to identify the potential for manipulating environment, or selecting genotypes, to optimise yields and initiate selective breeding for high lipid yielding traits.

2.2. Materials and Methods

2.2.1. Field sites

Four sites were selected in tropical North Queensland, Australia, to maximise species diversity and ensure multiple habitats, from intertidal rocky shores to mid-shelf coral reefs. Kissing Point (19.23°S, 146.79°E) is an intertidal rocky shore/ sedimentary habitat located on the mainland in Townsville. Samples were collected during low tides when seaweeds were exposed, as water at this site was consistently turbid from wind and wave action. Nelly Bay (19.16°S, 146.85°E) is located approximately 8 km from Townsville on Magnetic Island. Samples were collected from the reef flat and fringing reef approximately 100 m from the shoreline. This reef flat was dominated by seaweeds, with increasing coral cover toward the reef slope. Orpheus Island (18.61°S, 146.49°E) is an inshore island 50 km north of Townsville. The island is surrounded by fringing coral reefs and samples were taken from a floating and continuously submerged long line located 50 m from shore. Rib Reef (18.48°S, 146.87°E) is a mid-shelf coral reef located within the central Great Barrier Reef approximately 50 km east of Orpheus Island. The reef is approximately 5 km^2 in dimension. Seaweeds were mainly found on sandy flats and on rocks between the coral outcrops. Sampling was conducted by snorkel at low tide when water depth was between 1 and 2 m at the sampling areas. In addition to these field collections, some seaweed samples were also evaluated from the culture collections at the Marine and Aquaculture Research Facilities Unit (MARFU) at James Cook University (JCU), Townsville, where seaweeds were maintained in outdoor tank-based recirculation systems. The culture vessels were cylindrical polyethylene tanks with aeration (tumble culture) filled with approximately 60 L seawater. The following water quality parameters were measured at the time of harvest: salinity = 34.3 ppt, pH = 8.2, Nitrogen = 1.5 mg N L^{-1}. Nutrients were supplied by the addition of F/2 medium (Guillard and Ryther 1962).

2.2.2. Sampling procedures

The four sites provided predominantly different flora and the most apparent species at each of the sites were selected, with an effort to sample species of different taxonomic origin and also different morphologies (e.g. foliose and turfing varieties). Kissing Point (7th September 2010, 6th October 2010) and Nelly Bay (8th September, 21th September 2010, 5th October 2010, 27th October 2010, 1st November 2010) were sampled multiple times while Orpheus Island (3rd November 2010) and Rib Reef (3rd November 2010) were only sampled once. Samples from the culture facility (MARFU) were harvested on the 24th February 2011. To ensure that a representative measure of both lipid content and quality (i.e. fatty acid profiles) was made for each seaweed species, at least 5 individual plants per species, depending on availability, were haphazardly collected during each sampling period at each field site; constituting a sample. An individual was characterised by a single holdfast and was not connected to another plant with rhizomes or other tissue. There were many turfing algae that could not be identified below broader taxonomic groupings. Turfing species were treated as individuals if that seaweed mat was spatially isolated.

Individuals were held in seawater filled plastic bags and transported in an ice box to JCU, Townsville. Each individual was washed in freshwater to remove debris, epiphytes and animals. A small representative portion of each individual was preserved in 70 % ethanol and 4 % formalin for taxonomic identification, while the remainder of the material was freeze-dried and ground with an analytical mill through a 1 mm sieve. The seaweed powder was sealed in airtight jars and stored at -20°C.

2.2.3. Total lipid analysis

Total lipid content was analysed for three individuals of a sample that represented a unique species, following Folch et al. (1957). Approximately 200 mg (± 0.1 mg) of dried seaweed powder was placed into an 8 mL Teflon capped glass vial. To this, 5 mL

chloroform:methanol (2:1, v/v) mixture was added. The samples were then heated at 60°C for 1 hour followed by filtration to remove particulate matter using a vacuum pump through a Whatman GF/A filter (Whatman Plc, Maidstone, UK). Additional chloroform-methanol mixture was used to rinse the filter to recover all lipids. The filtered crude extract was then washed with 20 % of its volume in 0.9 % NaCl solution. After vortexing and standing for several minutes, an upper and lower phase established. The upper phase was siphoned out and the lower phase, containing the lipids, was evaporated under a gentle stream of nitrogen. The total weight of the lipid extract was then determined.

2.2.4. Fatty acid extraction and analysis

Fatty acids were analysed for three individuals of each collected sample. A direct transesterification method, adapted from Cohen et al. (1988) and Rodríguez-Ruiz et al. (1998), was used to simultaneously extract and esterify the fatty acids to fatty acid methyl esters (FAMEs) for analysis by GC-MS. This method is more efficient and rapid in recovering fatty acids than traditional solvent extraction methods followed by transesterification (Griffiths et al. 2010). Briefly, 2 mL freshly prepared methylation mixture (methanol, acetyl chloride, 20:1 (v/v)) and 300 µl internal standard solution (nonadecanoic acid ($C_{19}H_{38}O_{2;}$ > 99 %; Sigma-Aldrich, Castle Hill, NSW, Australia), 0.2 mg mL^{-1} in methanol) was added to approximately 50 mg (± 0.1 mg) of dried seaweed powder. The samples were heated at 100°C for 1 hour, then allowed to cool down before 1 mL of hexane was added. To ensure complete partitioning of FAMEs into the hexane phase, samples were heated again to 100°C for one minute, during which a single methanol/ hexane phase formed. Two mL of deionized water was then added to facilitate phase separation. The hexane (upper) phase containing the FAMEs was collected and filtered through a 0.2 µm PTFE syringe filter prior to injection on the GC column. All solvents were HPLC grade.

Fatty acid analysis was carried out on an Agilent 7890 GC equipped with a flame ionization detector (FID) and connected to an Agilent 5975C Electron Ionization (EI) Turbo Mass Spectrometer (Agilent Technologies Australia Pty Ltd), for identification of FAMEs. Separation was achieved on a DB-23 capillary column with a cyanopropyl stationary phase (60 m × 0.55 mm id × 0.15 µm) with helium as carrier gas in constant pressure mode. Injector and FID inlet temperatures were 150°C and 250°C, respectively (split injection, 1/50). Column temperature was programmed as outlined in David et al. (2002) which in brief ramped from 50 – 230°C. Fatty acids were quantified relative to peak areas of standards (Sigma Aldrich) corrected to the recovery of an internal standard (C19:0). The TFA content was determined as the sum of all FAMEs. Fatty acids are designated as CX:Y(n-z), where X is the total number of carbon, Y is the number of double bonds, and z is the position of the ultimate double bond from the terminal methyl group.

2.2.5. Statistical analysis

The broad variation of fatty acids between the main taxonomic groups (phylum, order, and species) was investigated using Principal Component Analysis (PCA) (Systat software Inc, Chicago, IL, USA) with the mean of three individual plants of a sample as the variables. The outcome was plotted in two dimensions (PCA1, PCA2). The score loading was analysed for each fatty acid, however, only SFA and PUFA(n-3) were identified in the bi-plot of PCA1 *vs*. PCA2. The intra- and inter- spatial (sites) and temporal variability of fatty acid composition of *Dictyota bartayresii* and *Dictyota dichotoma* were subsequently analysed in separate PCAs using the fatty acids of individual plants as variables to assess conspecific variation between and within environments (location, sampling period).

2.3. Results

2.3.1. Total lipids

Brown seaweeds (Phaeophyceae) typically had the highest total lipid content, followed by green (Chlorophyta) and red seaweeds (Rhodophyta) (Figure 2.1, Table A2.1). However, at lower taxonomic levels there is considerable variation in total lipid content between orders, and also species (Figure 2.1, Table A2.1). For example, within the brown seaweeds it is predominantly the order Dictyotales (79.8 mg g^{-1} dw ± 14.0 SE) which has a high total lipid content. Similarly, within the green seaweeds, the orders Bryopsidales (68.9 mg g^{-1} dw ± 10.5 SE) and Cladophorales (57.5 mg g^{-1} dw ± 12.4 SE) have the highest total lipid content (Figure 2.1). It is therefore these orders which are discussed in detail. There is also considerable variation in total lipid content within these orders. For the Dictyotales, *D. bartayresii*, *D. dichotoma* and *S. macrodontum* have a total lipid content greater than 100 mg g^{-1} dw, whereas *Lobophora variegata* and *Padina australis* have well below 50 mg g^{-1} dw (Figure 2.1, Table A2.1). Within the Bryopsidales, *Caulerpa sertularioides* (130.4 mg g^{-1} dw ± 8.4 SE) and *D. tenuissima* (121.4 mg g^{-1} dw ± 3.4 SE) have the highest total lipid content found in this Chapter. *Cladophora patentiramea* is the only species in the Cladophorales which approaches 100 mg lipid g^{-1} dw (Figure 2.1).

2.3.2. Fatty acids

TFA content, measured as FAME for each taxonomic group (brown, green and red seaweeds), correlated positively with the total lipid content with the line of best-fit being a polynomial (2^{nd} order) function (R^2 = 0.69, $y = -0.0173\ x^2 + 0.5175\ x$, Figure 2.2). Notably, the TFA content decreased with increasing total lipid content for all groups. In brown seaweeds the trend was least pronounced while it was strongest in green seaweeds. Red seaweeds had a similar trend to green seaweeds with the lowest levels of both lipids and fatty acids compared to the other groups.

The three seaweed orders with the highest TFA content were the Dictyotales followed by the Cladophorales and Bryopsidales (Figure 2.1, Table A2.2). These orders also contained the species with the highest TFA content. *S. macrodontum* (Dictyotales) (57.40 mg g^{-1} dw ± 0.87 SE) had an exceptionally high TFA content, essentially 50 % of the total lipid content (Figure 2.1). Other Dictyotales species, *D. bartayresii* and *D. dichotoma*, not only had a considerably lower TFA content compared to *S. macrodontum*, but also only contained 30 % fatty acids relative to total lipids. There were, however, high fatty acid to total lipid ratios in *P. australis* (67 %) and the closely related *Lobophora variegata* (58 %) (Figure 2.1). Within the Bryopsidales, only *D. tenuissima* had a TFA content above 30 mg g^{-1} dw, although this corresponded to a low fatty acid to total lipid ratio (32 %). *C. patentiramea* was the only species of the Cladophorales with a TFA content above 30 mg g^{-1} dw at 35 % of total lipids.

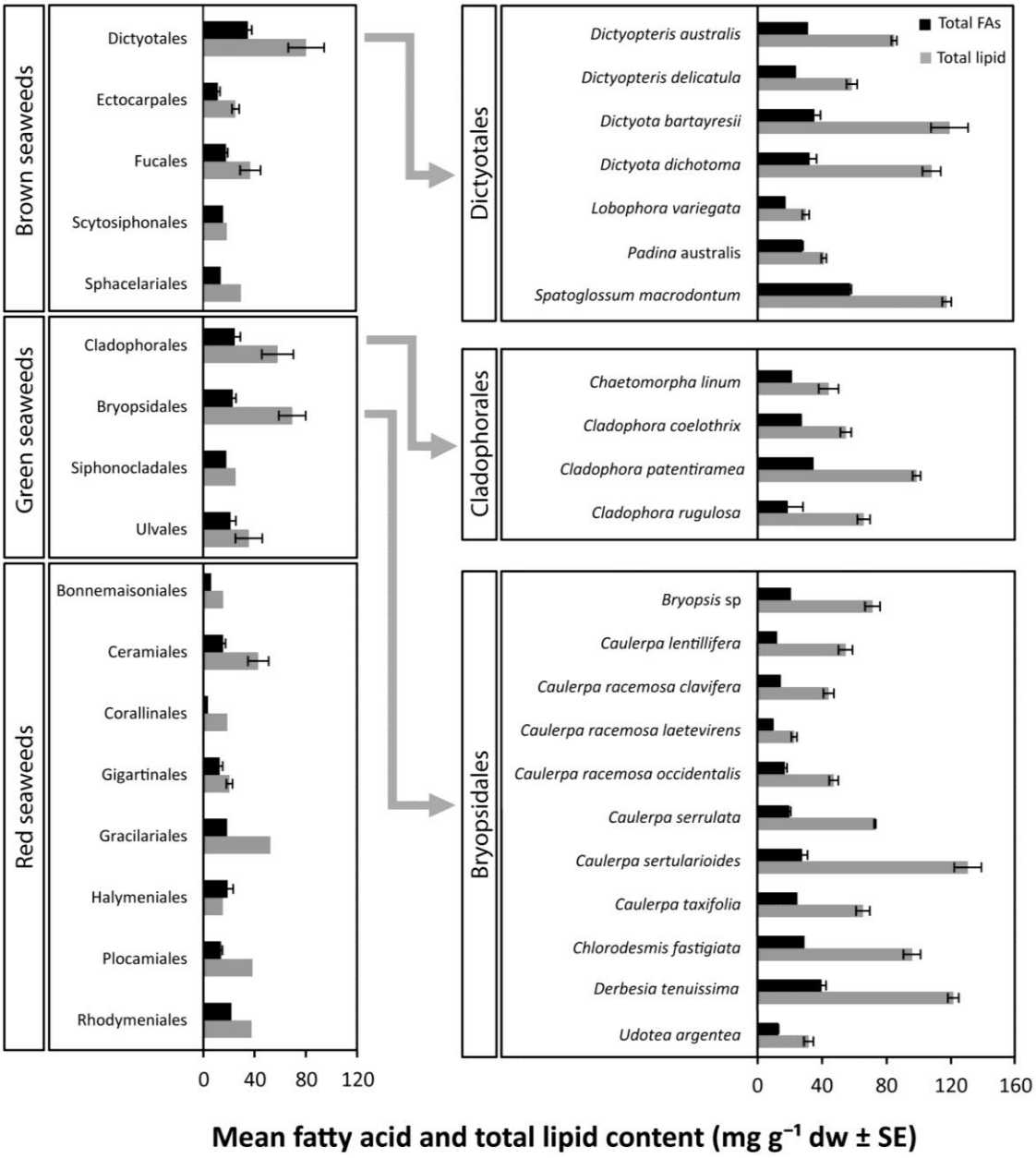

Figure 2.1. Mean total lipid (mg g^{-1} dw ± SE) and mean total fatty acid (TFA) content (mg g^{-1} dw ± SE) of seaweed orders (left) and species (right). All means are the means of samples except total lipid for species is expressed as the mean of a single set of triplicates.

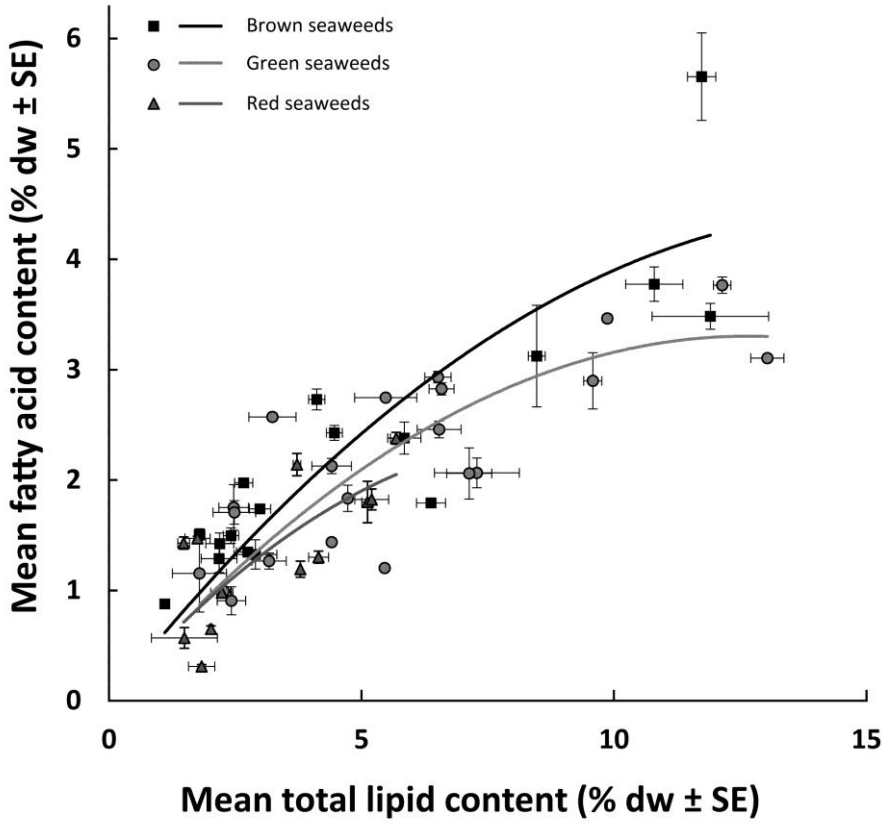

Figure 2.2. Polynomial (2nd order) relationships between total lipid content and total fatty acid (TFA) content for different seaweed taxa (brown, green and red seaweed). Individual data points represent mean values (% dw ± SE) for each species. Brown seaweed: $y = -0.0188\ x^2 + 0.5218\ x$, $R^2 = 0.76$; Green seaweed: $y = -0.0206\ x^2 + 0.5218\ x$, $R^2 = 0.64$; Red seaweed: $y = -0.0287\ x^2 + 0.5234\ x$, $R^2 = 0.53$. Functions are only predictive within the shown data range.

In a similar manner to total lipid and TFA contents, there was also considerable variation in fatty acid quality between taxonomic groups and species (Figure 2.3a, Table A2.2). Over 50 % of variation between samples (mean of 3 replicate individuals) was explained by two principal components. The first component (PCA1) separated the seaweed species primarily based on their TFA content (x-axis), and so separated the fatty acid rich species (right) from the fatty acid poor species (left) (Figure 2.3a). Fatty acid rich species such as *D. bartayresii*, *S. macrodontum* and *D. tenuissima* therefore had the highest PCA1 scores, with species of the red seaweeds generally had the lowest PCA1 scores, reflecting their lower TFA contents. On the second principal component axis (PCA2), the seaweed samples were separated into two main groups based on their fatty acid profiles, with the

brown and red seaweeds grouped together while the green seaweeds grouped out with higher PCA2 scores (Figure 2.3a). A second PCA separated the seaweed species primarily based on their saturated (SFA) and polyunsaturated fatty acids (PUFA). Green seaweeds were distinguished by their high quantities of PUFA, PUFA(n-3), and in particular by their high level of C18:3(n-3) (ALA) (Figure 2.3b). In contrast, brown seaweeds were characterised by their higher amount of SFA, in particular C14:0.

A more detailed investigation of fatty acids across taxonomic groups further clarified the relationship between fatty acid composition and taxonomy (Figure 2.4 and 2.5). The most abundant SFA in these tropical seaweeds was C16:0 across all taxonomic groups. The red seaweeds had the highest proportion of C16:0 relative to their TFA contents, followed by the green and brown seaweeds (Figure 2.4). Within the red seaweeds the Halymeniales (8.36 mg g^{-1} dw ± 0.79 SE) had the highest total C16:0 content. This was followed by the Dictyotales within the brown seaweeds (7.82 mg g^{-1} dw ± 0.78 SE). On a lower taxonomic level *S. macrodontum* (14.30 mg g^{-1} dw ± 1.00 SE) (Dictyotales) had the highest C16:0 content followed by *D. tenuissima* (11.96 mg g^{-1} dw ± 0.31 SE) (Bryopsidales). C14:0 was generally low in most taxonomic groups rarely exceeding 10 % of total SFA content. Exceptions were the Dictyotales where C14:0 reached 26 % of total SFA in *Dictyopteris australis*, 23 % in *S. macrodontum* and 21 % in *D. bartayresii*. *S. macrodontum* (4.95 mg g^{-1} dw ± 0.21 SE) had the highest total C14:0 content. The Dictyotales were also characterised by a high amount of C18:1 which reaches 18 % of TFA in *S. macrodontum* (Table 2.1).

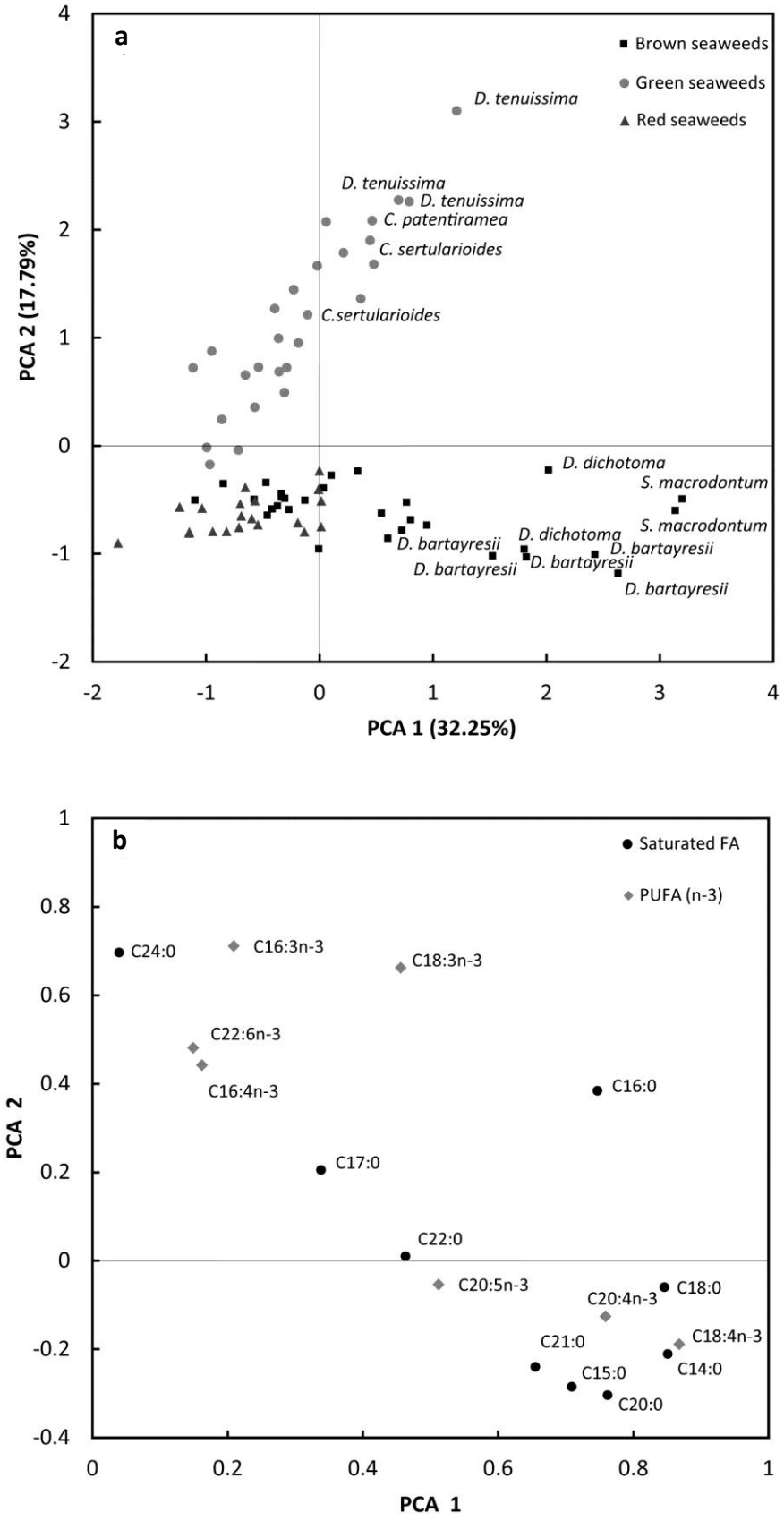

Figure 2.3. a Principal component analysis (PCA) of seaweed species samples based on fatty acid profiles. **b** Score loading bi-plot of SFA and PUFA(n-3).

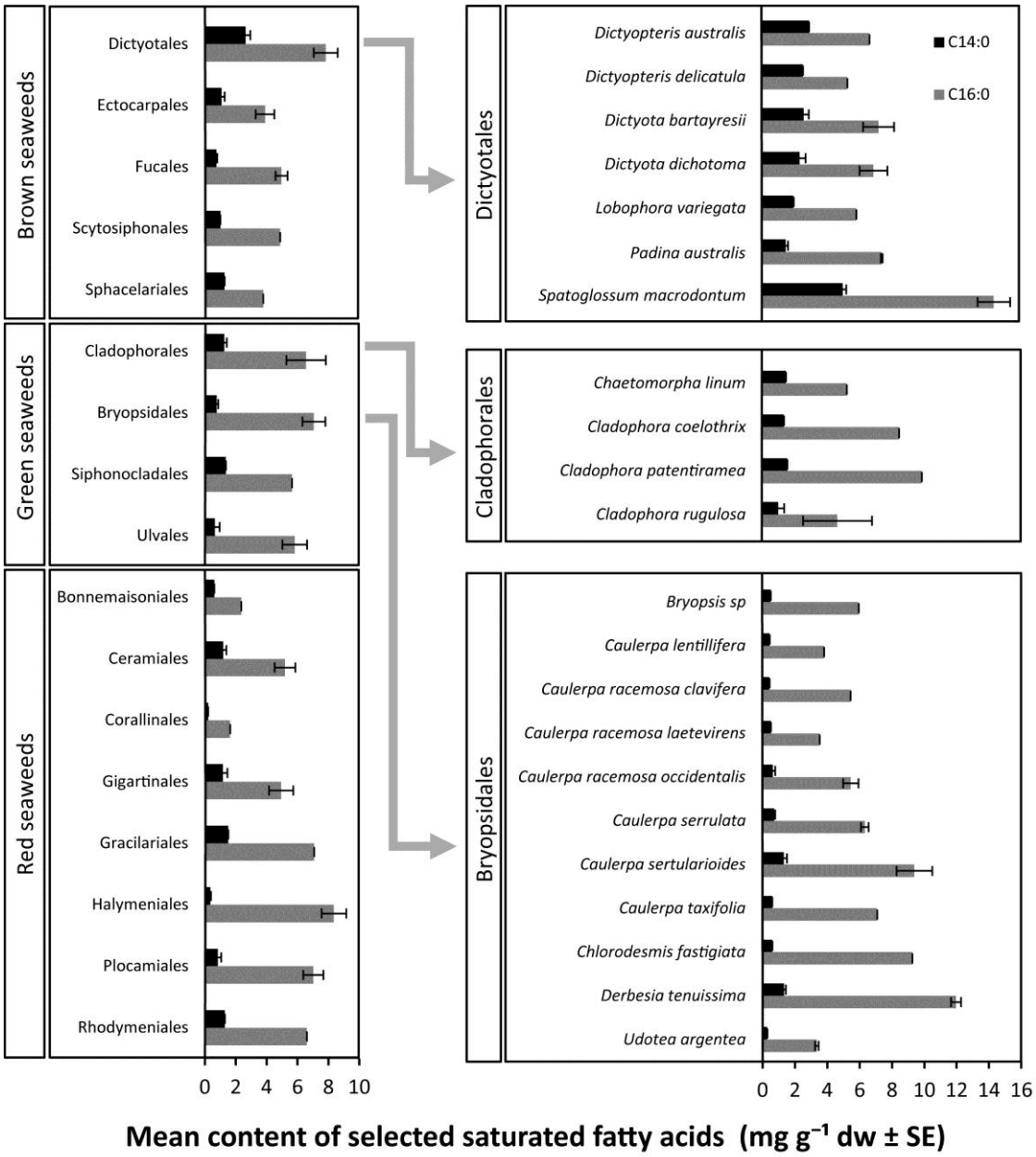

Figure 2.4. Mean content (mg g^{-1} dw ± SE) of the saturated fatty acids C14:0 and C16:0 in different seaweed orders (left) and species (right).

The PUFA(n-3) composition of these seaweeds was clearly related to taxonomy (Figure 2.5). Most notable were the high amounts of C18:3(n-3) (ALA) in green seaweeds, particularly the Bryopsidales. *D. tenuissima* (6.14 mg g^{-1} dw ± 1.40 SE) had the highest C18:3(n-3) content amongst all seaweeds. The Dictyotales also had high amounts of C18:3(n-3), in particular *S. macrodontum* (2.62 mg g^{-1} dw ± 0.04 SE). The PUFA C20:5(n-3) (EPA) was well represented in all taxa and was the dominant essential PUFA

in the red seaweeds with *Champia parvula* (3.30 mg g^{-1} dw) having the highest amount in the study. In the brown seaweeds, C20:5(n-3) was generally as abundant as C18:3(n-3) and the most abundant essential PUFA(n-3) in *D. bartayresii*, *D. dichotoma* and *Dictyopteris delicatula*. *S. macrodontum* (2.03 mg g^{-1} dw ± 0.06 SE) had the highest total C20:5(n-3) content within the Dictyotales. The essential PUFA C22:6(n-3) (DHA) was most common in the green seaweeds, and was generally low or below the detection threshold in the brown and red seaweeds.

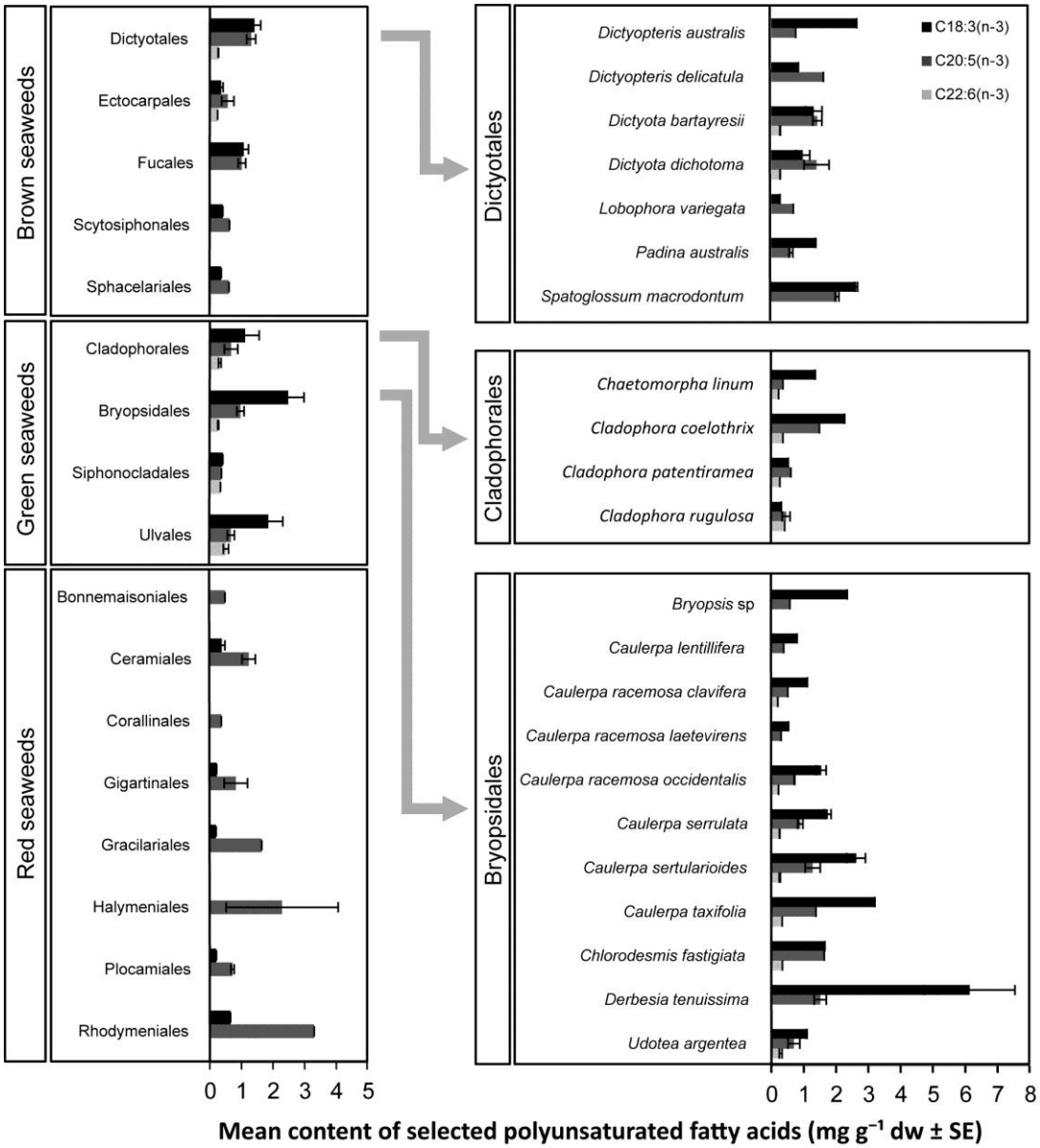

Figure 2.5. Mean content (mg g^{-1} dw ± SE) of selected essential PUFA(n-3) in different seaweed orders (left) and species (right).

Table 2.1. Fatty acid profiles (mg g^{-1} dw, mean of samples ± STDEV) and total lipid content (mg g^{-1} dw, mean ± STDEV, n = 3) of selected seaweed species. SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

	Dictyotales			Cladophorales	Bryopsidales	
	Dictyota bartayresii	*Dictyota dichotoma*	*Spatoglossum macrodontum*	*Cladophora patentiramea*	*Caulerpa sertularioides*	*Derbesia tenuissima*
C14:0	2.54 ± 0.70	2.28 ± 0.65	4.95 ± 0.30	1.53	1.32 ± 0.27	1.34 ± 0.17
C14:1	0.38 ± 0.12	0.30 ± 0.06	0.14 ± 0.10	0.23		0.30 ± 0.03
C15:0	0.34 ± 0.07	0.24 ± 0.08	0.32 ± 0.03	0.23	0.17 ± 0.05	0.24 ± 0.01
C16:0	7.17 ± 2.14	6.85 ± 1.49	14.30 ± 1.42	9.84	9.39 ± 1.56	11.96 ± 0.54
C16:1(n-9)	0.28 ± 0.02	0.21 ± 0.08	0.24 ± 0.00	0.48	0.37 ± 0.06	0.34 ± 0.04
C16:1(n-7)	0.62 ± 0.09	0.82 ± 0.37	1.89 ± 0.16	1.05	1.26 ± 0.28	1.91 ± 0.37
C16:1	2.21 ± 0.65	2.09 ± 1.50	0.38 ± 0.00	0.13	0.07 ± 0.01	0.24 ± 0.02
C16:2(n-6)	0.27 ± 0.06	0.22 ± 0.18	0.17 ± 0.12	0.39	0.61 ± 0.20	0.82 ± 0.38
C16:2(n-4)	0.05 ± 0.07	0.10 ± 0.11	0.11 ± 0.06	3.39		0.13 ± 0.05
C17:0	0.06 ± 0.09	0.14 ± 0.05	0.22 ± 0.01		0.15 ± 0.01	0.21 ± 0.05
C16:3(n-6)	0.42 ± 0.10	0.34 ± 0.09	0.23 ± 0.01	0.22	0.03 ± 0.05	0.16 ± 0.04
C16:3(n-3)	0.16 ± 0.08	0.25 ± 0.28	0.14 ± 0.06	0.07	1.75 ± 0.35	3.34 ± 1.71
C16:4(n-3)	0.31 ± 0.13	0.38 ± 0.28	0.18 ± 0.00	0.74	0.25 ± 0.02	0.43 ± 0.03
C18:0	0.78 ± 0.16	0.60 ± 0.11	0.74 ± 0.05	0.35	0.45 ± 0.06	0.50 ± 0.09
C18:1(n-9)	4.65 ± 1.38	4.43 ± 0.51	10.31 ± 0.91	4.48	1.28 ± 0.05	2.41 ± 0.32
C18:2(n-6) trans	0.42 ± 0.29	0.29 ± 0.04	0.26 ± 0.00			0.02 ± 0.04
C18:2(n-6) cis	0.92 ± 0.33	0.71 ± 0.08	1.54 ± 0.04	3.03	1.97 ± 0.58	2.75 ± 0.39
C18:3(n-6)	0.49 ± 0.17	0.49 ± 0.13	0.65 ± 0.04	3.31	0.39 ± 0.05	0.77 ± 0.19
C18:3(n-3)	1.31 ± 0.57	0.97 ± 0.38	2.62 ± 0.06	0.52	2.62 ± 0.41	6.14 ± 2.42
C18:4(n-3)	3.42 ± 1.21	2.51 ± 0.81	5.21 ± 0.52	0.45	0.52 ± 0.12	0.64 ± 0.13
C20:0	0.39 ± 0.13	0.33 ± 0.10	0.45 ± 0.00			0.10 ± 0.05
C21:0	0.80 ± 0.29	0.60 ± 0.41	0.22 ± 0.16		0.26 ± 0.04	
C20:3(n-6)	0.52 ± 0.33	0.47 ± 0.23	2.15 ± 0.09	0.15	0.26 ± 0.04	0.28 ± 0.09
C20:4(n-6)	2.57 ± 1.58	2.76 ± 1.75	4.47 ± 0.37	2.77	0.33 ± 0.04	1.17 ± 0.23
C20:4(n-3)	0.73 ± 0.24	0.54 ± 0.14	2.21 ± 0.01		0.36 ± 0.05	0.17 ± 0.11
C22:0	0.52 ± 0.11	0.49 ± 0.25	0.65 ± 0.06	0.16	0.45 ± 0.08	0.52 ± 0.14
C20:5(n-3)	1.42 ± 0.32	1.40 ± 0.67	2.03 ± 0.08	0.60	1.28 ± 0.33	1.51 ± 0.33
C24:0	0.01 ± 0.03	0.02 ± 0.04	0.30 ± 0.01	0.08	0.98 ± 0.16	0.90 ± 0.17
C22:6(n-3)	0.20 ± 0.10	0.18 ± 0.01	±	0.18	0.26 ± 0.03	
Other FAs	1.18 ± 0.47	1.21 ± 0.38	0.29 ± 0.16	0.26	0.81 ± 0.19	0.28 ± 0.24
Total FAs	35.15 ± 8.69	32.19 ± 7.76	57.40 ± 1.23	34.63	27.61 ± 4.85	39.58 ± 5.09
Total SFA	12.60 ± 3.12	11.55 ± 2.41	22.17 ± 1.32	12.19	13.17 ± 2.15	15.76 ± 0.53
Total MUFA	8.14 ± 1.88	7.85 ± 1.61	12.95 ± 1.17	6.37	2.98 ± 0.40	5.19 ± 0.61
Total PUFA	13.23 ± 4.61	11.58 ± 4.41	21.99 ± 1.09	15.82	10.65 ± 2.11	18.35 ± 4.25
PUFA (n-3)	7.57 ± 2.33	6.22 ± 2.28	12.41 ± 0.61	2.57	7.05 ± 1.31	12.23 ± 4.61
PUFA (n-6)	5.62 ± 2.52	5.27 ± 2.08	9.47 ± 0.42	9.86	3.60 ± 0.80	5.98 ± 0.33
Total lipid	119.10 ± 20.00	108.00 ± 9.90	117.30 ± 4.90	98.70 ± 4.30	130.40 ± 14.60	121.40 ± 5.90

FA fatty acids, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

2.3.3. Conspecific variation

The TFA content in *D. bartayresii* varied by up to 50 % between samples from different environments (different locations and sampling periods), while TFA content within samples (same location and time) varied only between 5 and 17 % (Table A2.3). For example, individuals from Orpheus Island had the lowest TFA content (22.46 mg g^{-1} dw ± 1.24 SE) while individuals from Kissing Point had the highest TFA content (42.56 mg g^{-1} dw ± 1.36 SE). Furthermore, the TFA content between two locations within Nelly Bay

collected on the same day but from opposite ends of the bay, approximately 500 m apart, differed by 27 % (South Nelly Bay, 8th September 2010: 32.04 mg g^{-1} dw ± 0.50 SE; North Nelly Bay, 8th September 2010: 43.88 mg g^{-1} dw ± 3.45 SE) (Table A2.3). In contrast, the TFA content between two sets of samples from the same site collected 6 weeks apart differed only by 8 % (North Nelly Bay, 21th October 2010: 34.82 mg g^{-1} dw ± 1.17 SE) (Table A2.3).

Principal component analysis also demonstrated a larger variation in fatty acid composition between samples from a different environment than between individual replicate plants (Figure 2.6ac). For *D. bartayresii*, 64.80 % of the variation in fatty acid composition between the individual plants can be explained by the first two principal components (Figure 2.6a). Differences in the TFA contents (PCA 1) explained over 40 % of the total variation, and the Orpheus Island individuals had clearly lower TFA contents than individuals from Kissing Point. Furthermore, the Orpheus Island individuals were characterised by a low PUFA(n-3) (PCA 2), and particularly low C18:3(n-3) level, compared to plants from other locations (Figure 2.6b).

The TFA content of *D. dichotoma* varied by 38 % between samples, but only by 8 – 27 % between individual replicate plants within a sample (Table A2.4). The Kissing Point sample had the lowest TFA content (23.32 mg g^{-1} dw ± 2.49 SE) while the two samples from Nelly Bay collected 7 weeks apart had similar high TFA contents of 35.52 mg g^{-1} dw ± 0.95 SE (8th September 2010) and 37.74 mg g^{-1} dw ± 1.56 SE (27th October 2010) (Table A2.4). Principal component analysis demonstrated that there was no clear pattern between location and fatty acid composition (Figure 2.6c). Plants from Kissing Point and Nelly Bay (8th September 2010) had a similar fatty acid composition while the plants from Nelly Bay collected on the 27th October 2010 were distinct. The main distinction was a particularly high PUFA(n-3) content and low C14:0 content in the Nelly Bay individuals sampled on 27th October 2010 (Figure 2.6d, Table A2.4).

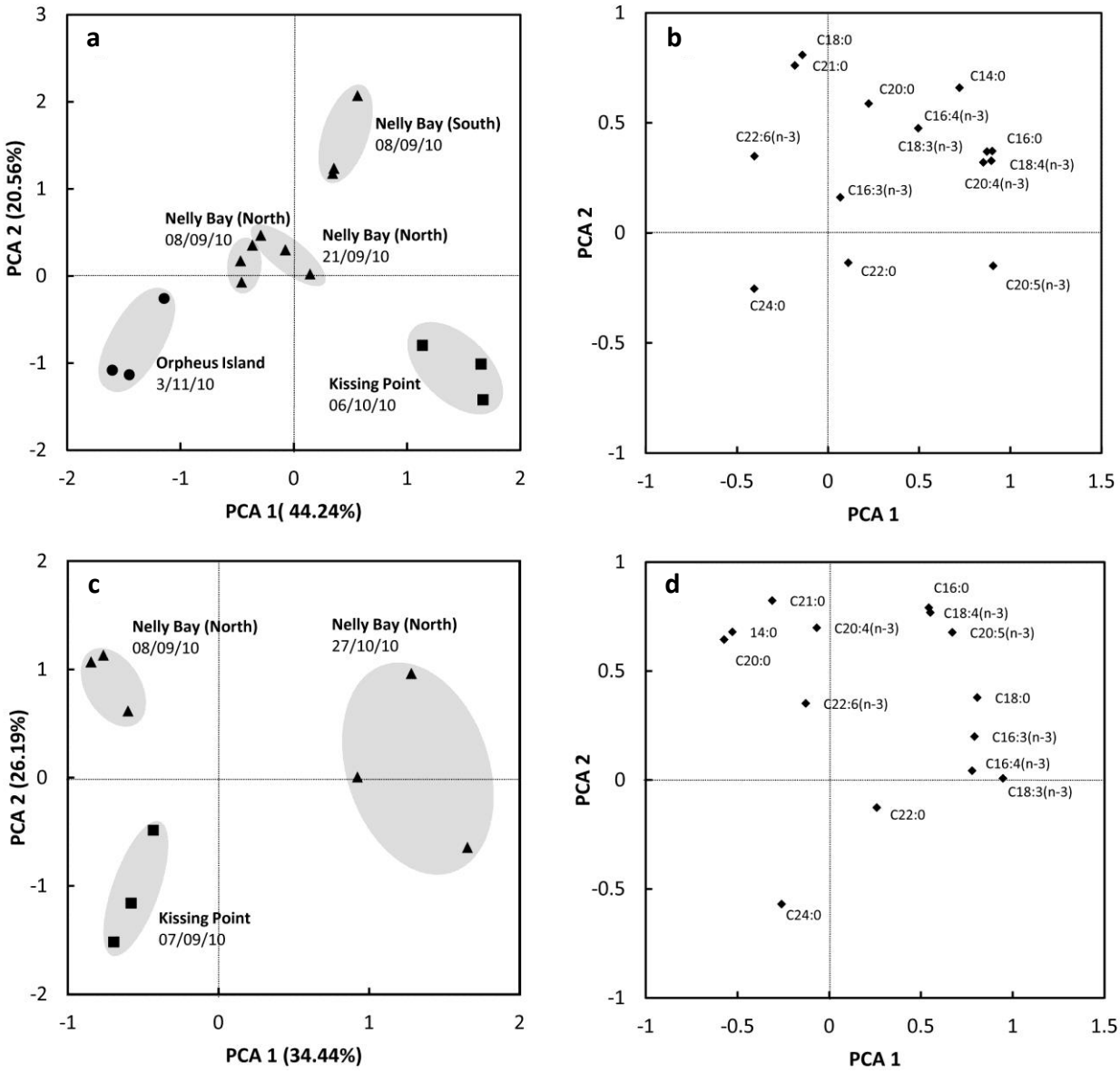

Figure 2.6. a Principal component analysis of fatty acid composition for *Dictyota bartayresii* triplicate samples from different locations (Kissing Point, Nelly Bay, Orpheus Island) and sampling times. **b** Bi-plot of fatty acid loadings for *Dictyota bartayresii*. **c** Principal component analysis of fatty acid composition of *Dictyota dichotoma* triplicate samples from two different locations (Kissing Point, Nelly Bay) and sampling times. **d** Bi-plot of fatty acid loadings for *Dictyota dichotoma*. Shaded areas only used to visualize the replicates of one sample.

2.4. Discussion

This study provides clear evidence that some seaweeds have a high total lipid and fatty acid content and that the paradigm that seaweeds are not suitable for oil-based products, because of their presumably low total lipid and fatty acid content, is clearly challenged with lipid contents exceeding those of many species of microalgae (e.g. Huerlimann et al.

2010; Mata et al. 2010a). Furthermore, the quantitative and qualitative yields of fatty acids of selected species makes them suitable targets for functional foods, nutraceuticals, and animal feeds, or alternatively as biofuel. In general, brown seaweeds are preferred because of their high fatty acid content, followed by green seaweeds, while red seaweeds appear to have little application for oil-based products. Two species are clearly identified as key targets from this study, *S. macrodontum* from the Dictyotales and *D. tenuissima* from the Bryopsidales. In addition to these primary key species, *Dictyota bartayresii*, *D. dichotoma*, *D. australis* and *C. sertularioides* are potential feedstock species for oil-based bioproducts because of their high oil contents. Notably, there is also important variation in total lipid content and fatty acid profiles within species due to environment and/or genotype. These data provide a basis for the optimisation of environmental culture conditions and selection of high oil-yielding genotypes to further enhance fatty acid yields under culture.

2.4.1. Total lipid content

Although the majority of investigated seaweed species in this Chapter have a total lipid content below 5 % dw, there are a number of species with a total lipid content greater than 10 % dw and these are therefore interesting candidates for oil-based products. Within the brown seaweeds, the Dictyotales and in particular *D. bartayresii*, *S. macrodontum* and *D. dichotoma* have the highest total lipid contents (10 – 12 % dw). *Dictyota* has been previously identified as a lipid rich genus, with consistently high total lipid contents of up to 20 % dw (Montgomery and Gerking 1980; McDermid and Stuercke 2003). No information on total lipid content is available for *S. macrodontum* and its potential for oil-based products is identified here for the first time. This may be a unique species rather than genus, as the congener *Spatoglossum asperum* has a total lipid content of less than 5 % dw (Kumari et al. 2010). *S. macrodontum* has a pan-tropical distribution, occurs in large stands, and is in some places an introduced “pest” species due to its high growth rate and reproductive ability (Skelton et al. 2007). These attributes, in

conjunction with the high levels of fatty acids reported here, make this species a primary target for low cost, high density culture for oil-based products. In a similar manner the total lipid content in *D. tenuissima* (12 – 13 % dw) is among the highest found for green seaweeds. While the Bryopsidales are a lipid rich order when compared to other green seaweeds, total lipids rarely exceed 5 % dw (McDermid and Stuercke 2003). This is the first report of lipid and fatty acid content for *D. tenuissima*, and fortuitously it also has rapid growth at high density under intensive culture (Magnusson et al. 2014; Mata et al. 2015), providing for optimised production for oil-based products.

2.4.2. Fatty acids

Although the total lipid content is a potential indicator for the suitability of seaweeds for oil-based products, it is the TFA content and quality that are most relevant to determine applicability for a specific end-use. Although there is a strong correlation between total lipid and TFA content in this Chapter, the relative proportion of fatty acids actually decreases with increasing total lipid content. Therefore, high total lipid contents in seaweeds are not necessarily good indicators for screening for high TFA content, and by virtue screening for the suitability of oil-based products. However, brown seaweeds have a stronger relationship between total lipid and TFA content than either green or red seaweeds.

Fatty acid profiles varied between taxonomic groups and green, red and brown seaweeds can all be distinguished by their fatty acid profile. Green seaweeds have a high level of PUFA(n-3) and in particular a high amount of C18:3(n-3) (ALA), whereas red seaweeds have high amounts of saturated fatty acids and some brown seaweeds are particularly rich in C14:0. Red seaweeds also have the highest level of C16:0 confirming previous studies (Vaskovsky et al. 1996). In addition, the essential fatty acid C20:5(n-3) (EPA) is abundant in the red seaweeds and is a common trait (Graeve et al. 2002; Khotimchenko et al. 2002). Finally, brown seaweeds generally have a lower level of C16:0, with the

Dictyotales being an exception. The Dictyotales are unique in also being rich in C14:0 with a high proportion of saturated fatty acids (Khotimchenko 1995).

For biofuel production, algae with a high proportion of saturated fatty acids are preferred as this leads to higher oxidative stability and higher ignition quality (cetane number), and produces an overall higher quality product (Hu et al. 2008; Knothe 2008). Feedstocks with a high proportion of the monounsaturated fatty acid (MUFA) C18:1 can further enhance biofuel quality by improving the low-temperature properties and kinematic viscosity, and reducing the emissions of hydrocarbon and CO_2 (Knothe 2008). In contrast, a high content of PUFAs results in less stable and lower quality biofuel. The results from this Chapter suggest that the Dictyotales are a suitable biomass target for biodiesel production because of their high total lipid, high fatty acid level and high proportion of SFA. In addition to having the highest quantities of fatty acids reported for a seaweed, *S. macrodontum* also has the highest quality of fatty acids for biofuel production with a fatty acid profile rich in saturated fatty acids and with C18:1 as the second most abundant fatty acid.

Algae with a high content of the essential PUFA(n-3) are preferred for human consumption or the production of nutraceuticals, functional foods, and fish oil replacements. The red alga *Champia parvula*, has an exceptional level of C20:5(n-3) (3.30 mg g^{-1} dw ± 0.11 SE), while *D. tenuissima* is rich in C20:5(n-3) and has a high level of C18:3(n-3). *D. tenuissima* is also suitable for biofuel production; however, its low saturated fatty acid profile may produce a lower quality product compared to *S. macrodontum*.

2.4.3. Conspecific variation

One of the major outcomes of this study is the identification of significant variation in the quantitative fatty acid profile amongst individuals within species for *D. bartayresii* and

D. dichotoma sampled from different sites, and at different times. The relative contribution of environment and genotype could not be partitioned in this sampling design, however, the potential for these effects are critical for cultivation strategies for seaweeds. The main environmental differences between the locations (inshore *vs.* offshore) and sampling periods (spring *vs.* summer) are water temperature and nutrient load and these factors affect the quantitative fatty acid profiles of seaweeds and algae more broadly. For example, seaweeds from cold water environments had higher PUFA contents compared to seaweeds from warmer waters (Graeve et al. 2002; Nelson et al. 2002). Experimental data also suggests that chilling prior to harvest can increase the levels of C20:4 and C20:5 in Phaeophyceae and Rhodophyta (Al-Hasan et al. 1991). Additionally, macroalgae grown in shade have higher total lipid content compared to algae grown in full light (Hotimchenko 2002). There is also evidence that nitrogen starvation increases lipid synthesis and so improves total lipid content of algae (Livne and Sukenik 1992; Hu et al. 2008). However, most data related to this effect has been obtained from microalgae and only limited evidence is available for macroalgae (e.g. Mulbry et al. 2008).

In addition to environmental factors directly influencing the fatty acid content and profile of seaweeds, it is also possible that genotypes with specific quantitative fatty acid profiles exist across environments. Relatively little information is available for the heritability of seaweed natural products (see Wright et al. 2004). However, if genotype or genotype × environment interactions exist, individual plants of a seaweed species with particularly high total lipid content and favourable fatty acid profile can be identified for selective breeding of seaweed strains with favourable properties for oil production. The next step in my research is to develop this concept for *S. macrodontum* and *D. tenuissima*, in combination with intensive culture for high biomass productivities. Combining the selection of traits for high quantitative fatty acid profile with high productivities provides opportunities for delivering macroalgal biomass for oil-based products. While the techno-

economic evaluation of macroalgal oil-based production has not reached the levels of sophistication for microalgae (Stephens et al. 2010a,b), many macroalgae have productivities that match or exceed those of microalgae on a dry weight per unit area basis (Capo et al. 1999; Mata et al. 2010b) and do not require complex and costly harvesting and drying systems (Paul et al. 2012). However, the production of biodiesel is not economically viable at this stage. For example, a seaweed biomass productivity of 20 g dw m^{-2} day^{-1} with an extractable total lipid (oil) content of 10 % gives a total lipid yield of 2 g (total lipid) m^{-2} day^{-1}, which is below the benchmark for economic biodiesel production in microalgae (5 g (oil) m^{-2} day^{-1}) (Stephens et al. 2010a). Although there is significant scale for improvements in both biomass productivity and lipid content, the difference in value between algal derived omega-3 oils (US$ 140 kg^{-1}, Borowitzka 2013) and crude oil for the fuel market (US$ 1.13 kg^{-1}, Borowitzka 2013) is two orders of magnitude and therefore utilising oils derived from seaweed in the fuel market, as opposed to the omega-3 market, is clearly not economically sound. However, there are a diversity of viable options to firstly deliver high value oil-based products from the lipid fraction of algal biomass, with the remaining biomass being converted to a range of bioenergy products (Brennan and Owende 2010; Demirbas 2011; Nigam and Singh 2011; Ross et al. 2008; Singh and Olsen 2011). This includes the production of liquid fuels with seaweed biomass being investigated for biocrude (bio-oil) production through hydrothermal liquefaction (Anastasakis and Ross 2011; Neveux et al. 2014a; Zhou et al. 2010) and pyrolysis (Budarin et al. 2011; Rowbotham et al. 2012). The primary focus for the utilisation of seaweed oils is therfore the production of high value products in health and nutrition.

CHAPTER 3

Seasonal and within-plant variation in fatty acid content and composition in the brown seaweed *Spatoglossum macrodontum* (Dictyotales, Phaeophyceae) [2]

3.1. Introduction

There is an increasing focus on marine biomass as a renewable source of oils for foods, nutraceuticals and increasingly as a feedstock for a complex range of products including polymers, paints and solvents (Biermann et al. 2006; Stengel et al. 2011). Of particular importance for these applications is the 'quality' of oil, where the quantity of specific fatty acids determines the application of biomass for oil-based products. For example, nutraceuticals require a feedstock rich in PUFA(n-3) (Gill and Valivety 1997) and, even more specifically, the chemical industry has demands for fatty acids such as C18:1(n-9) as precursors for biopolymers (Biermann et al. 2006; Biermann and Metzger 2008; Lligadas et al. 2010). Currently the diversity of oil feedstock crops is mainly limited to soybean, oil palm and rape seed which compete with food crops (Pimentel et al. 2009) and have a narrow range of PUFA(n-3) fatty acids (Dubois et al. 2007), limiting their use in health products. Oils from marine fish, which are traditionally utilized for the production of nutraceuticals, are seen as problematic as fish stocks are increasingly overfished and depleted (Pauly et al. 2005). Seaweed biomass is a novel choice as a bioresource for renewable oil because there is a broad biodiversity that is essentially untapped in terms of developing scalable bioresources for oil-based products. Furthermore, many species of seaweeds have high productivities under culture (Bolton et

[2] Adapted from: Gosch BJ, Paul NA, de Nys R, Magnusson M (2014) Seasonal and within-plant variation in fatty acid content and composition in the brown seaweed *Spatoglossum macrodontum* (Dictyotales, Phaeophyceae). Journal of Applied Phycology 27:387-398

al. 2009; Lawton et al. 2013a; Magnusson et al. 2014), some of which also have a relatively high oil content with a distinct and diverse composition of fatty acids (Chapter 2; Gosch et al. 2012) making them targets for more in-depth investigation.

Importantly, the fatty acid content and composition of conspecific individuals of seaweed can vary considerably depending on geographies and time of sampling (Chapter 2; Gosch et al. 2012; Hernández-Carmona et al. 2009; Nelson et al. 2002). Although there is potential for genotypic variation in the regulation of fatty acid content (Robinson et al. 2013), this spatial and temporal variation in fatty acid content and composition is generally linked to the environment, in particular nutrient availability (Gómez and Wiencke 1998; Gordillo et al. 2001) and water temperature (Al-Hasan et al. 1991; Floreto et al. 1993) but also with changes in light level (Hotimchenko 2002) or salinity (Kumar et al. 2010). On a smaller scale, fatty acids also vary in their content and composition within individual plants and this is potentially related to the morphological, functional and physiological differentiation of plant parts for growth, photosynthesis and energy storage (Gómez and Wiencke 1998; Lawrence and McClintock 1988; Stengel and Dring 1998). To date, only a limited number of species of brown seaweeds have been investigated for their internal fatty acid and lipid (oil) composition with no consistent pattern in content and composition emerging. For example, *Sargassum confusum* has higher lipids in its main axis than in blades, while the main axis in *Cystoseira hakodatensis* has the lowest lipid content (Terasaki et al. 2009). *Postelsia palmaeformis* has more than double the lipid content in its fronds than in the holdfast (Lawrence and McClintock 1988). Therefore, it is expected that within-plant fatty acid distribution is species-specific and depends on the morphology of the species as well as its physiological and ecological circumstances. This inherent variability constitutes a challenge for the aquaculture of seaweeds for oils because a stable supply of extractable oils and fatty acids is preferable. However, and more importantly, it also provides the opportunity to exploit this natural

variability and develop culture strategies that result in optimized yields of desired target fatty acids.

A critical first step, prior to the domestication of seaweeds for renewable oil-based products, is therefore the quantification of natural variation in TFA content and fatty acid composition, and an understanding of the drivers thereof. Subsequently, culture conditions and harvest strategies for this new target species can be designed to predict the optimal TFA content and fatty acid composition for applications in specific oil-based products. In this Chapter I quantified the variation in TFA content and fatty acid composition of wild collected *Spatoglossum macrodontum* (Dictyotales, Phaeophyceae) plants. This species was selected because it is particularly rich in TFA (Chapter 2; Gosch et al. 2012), with a wide distribution in the Pacific region. The first objective was to identify seasonal patterns in the content and composition of fatty acids within biomass and correlate this with plant size, and sea surface temperature. The second objective was to identify potential within-plant variation of the content and composition in fatty acids between sections (tips, midsections and base sections) of the seaweed. Together this information will provide the fundamental data on which to develop culture and harvest strategies.

3.2. Materials and Methods

3.2.1. Species selection and field site

S. macrodontum was selected for this study because it is particularly rich in fatty acids and found along the Northern Queensland coast (Chapter 2; Gosch et al. 2012). *S. macrodontum* has a broad distribution in the Pacific region including Queensland, Lord Howe Island, Samoa, French Polynesia, Hawaii and Japan (Guiry and Guiry 2014; Skelton et al. 2007). This species Samples were collected from Nelly Bay (19.16°S, 146.85°E), which is approximately 8 km from Townsville on Magnetic Island, Queensland, Australia. Samples were collected from the reef flat and fringing reef

approximately 100 m from the shoreline. The reef flat is dominated by seaweed with increasing coral cover toward the reef slope.

3.2.2. Biomass and fatty acid analysis

Individual plants of *S. macrodontum* were haphazardly collected from Nelly Bay, for fatty acid and biomass size analysis, each month (when present, see section 3.3) from November 2011 to November 2012. In July, sampling was conducted at two occasions: 'early July' and 'late July' and specific sampling dates are provided in Table 3.2. During each sampling period, 15 plants were collected except in November 2011 (n = 14) and 'early July' (n = 9). A plant was considered as an individual if it was clearly spatially isolated (> 1 m) from other plants. Plants of different sizes were collected and transported on ice to James Cook University, Townsville, where they were rinsed in freshwater to remove debris, epiphytes and fauna. The total length and biomass of each individual was measured to correlate TFA content and fatty acid composition with plant size. A representative section of each individual plant, defined as a cutting that includes the tips, mid- and base sections in a similar proportion to a whole plant, was removed to estimate TFA content and fatty acid composition of whole plants. Furthermore, to obtain a detailed understanding of the within-plant variation in fatty acid content and composition, three 1 cm^2 cuttings were taken respectively from the tips, mid- and base sections from each plant (Figure A3.1). All samples were frozen to -20°C, freeze-dried and milled to a fine powder. The seaweed powder was sealed in airtight jars and stored at -20°C until fatty acid analysis.

Fatty acids were analysed for each replicate section and each representative section. A direct transesterification method was used to simultaneously extract and esterify the fatty acids to fatty acid methyl esters (FAMEs) from 0.030 g dw subsamples for analysis by GC-MS (7890 GC, 5975C MS, DB-23 capillary column with 15 µm cyanopropyl stationary phase, 60 m length and 0.25 mm inner diameter (Agilent Technologies

Australia Pty Ltd.)), as described in detail in Chapter 2 (Gosch et al. 2012). TFA content was determined as the sum of all FAMEs. Fatty acids are designated as CX:Y(n-z), where X is the total number of carbon, Y is the number of double bonds, and z is the position of the ultimate double bond from the terminal methyl group.

3.2.3. Biomass increase estimates

Based on average fresh weight (fw) and plant length data of each sampling period (n = 15, exceptions: n = 14 for November 2011, n = 9 for 'early July' 2012), specific growth rates were calculated with the following equation: (1) Specific growth rate (SGR) (% day^{-1}) = 100 x [$\ln(W_f/W_i)$] / t; with W_f and W_i being the final and initial fresh weights or plant lengths. Length and fresh weight (fw) data of collected plants were used to create growth models (3^{rd} order polynomial functions) to characterise the seasonal changes.

3.2.4. Proximate and ultimate analysis

Ash and elemental composition (carbon (C), hydrogen (H), oxygen (O), nitrogen (N) and sulfur (S)) were analysed to compare the physiological state of whole plants and plant sections to fatty acid profiles in three random plants per section of the September collection period where plants reached their size maximum and are therefore of special interest for commercial applications. Because these analyses required more biomass than for fatty acid analysis alone (see section 3.2.2), subsamples of pooled replicate sections (n = 3 replicate cuttings) per plant were taken. Three random plants for each section and whole plant analysis were used. Ash content was determined by combustion in air at 550^{o}C (202C, SEM Ltd., Australia) and moisture content was measured on a moisture balance (MS70, A&D Company Ltd., Japan). Ultimate analysis (CHONS) was outsourced to OEA laboratory Ltd., UK.

3.2.5. Statistical analysis

The effect of plant size (fw) on TFA content and fatty acid composition (SFA, MUFA, PUFA) was analysed by correlations (Correlation, IBM SPSS version 21). The effects of sampling period on TFA content of whole plants was analysed by a one-way ANOVA and Tukey's HSD post-hoc tests with 15 individual plants (exceptions: n = 14 for November 2011, n = 9 for 'early July' 2012) for each sampling period (One-way ANOVA, Tukey's HSD, IBM SPSS version 21). Within-plant variation (tips, mid- and base sections) in TFA content and interaction with sampling period was analysed with a two-way factorial ANOVA and Tukey's HSD post-hoc tests with 15 individual plants per sampling period (exceptions: n = 14 for November 2011, n = 9 for 'early July' 2012) and the mean of three cuttings per plant for each section (Two-way ANOVA, Tukey's HSD, IBM SPSS version 21). TFA content, PUFA content and PUFA(n-3) content were correlated with water temperature data obtained from automated submerged water temperature loggers (NELFL 1, 2.4 m reef flat site 1) from the sampling site (AIMS 2014; Correlation, IBM SPSS version 21). Variation in carbon, nitrogen, ash and C/N between different plant sections (tips, mid- and base sections) (n = 3) and whole plants (n = 3), were analysed by one-way ANOVAs and Tukey's HSD post-hoc tests (One-way ANOVA, Tukey's HSD, IBM SPSS version 21).

3.3. Results

3.3.1. Plant sizes and biomass productivity

Although sampling was conducted from January to May on a monthly basis, no plants were found until June (Figure 3.1) when the first young plants were identified at an average length of 14.2 cm ± 0.9 SE and a fresh weight of 8.4 g ± 1.3 SE. After an initial rapid increase in length to approximately 29 cm during the first two months, plant length remained relatively stable until September while plant fresh weight increased from June to their peak weight (136.3 g ± 19.5 SE) in September. During this period plants

generally became ‘bushier’ as the number of branches increased. Both length and fresh weight decreased toward November 2012 (24.7 cm ± 1.9 SE, 61.5 g ± 9.0 SE) and were then similar to plants collected at the end of the previous growing season (November 2011) (23.4 cm ± 1.1 SE, 52.0 g ± 7.5 SE).

Based on the size averages (Figure 3.1), biomass of *S. macrodontum* changed from June to September at an average specific growth rate (SGR) of 2.49 % day^{-1} ± 0.99 SE with a range of SGR from -0.39 % day^{-1} (‘late July’ to August) to 3.78 % day^{-1} (‘early July’ to ‘late July’). From September to October plant biomass changed at an SGR of -0.37 % day^{-1} and then rapidly declined from October to November at an SGR of -2.27 % day^{-1}. Although plant length followed the same growth pattern as biomass, the increase of length was comparably low with an average SGR of 0.66 % day^{-1} ± 0.46 SE (range: -0.48 to 1.76 % day^{-1}) from June to September. Annual size variation of *S. macrodontum* can be best described by a line of best fit of polynomial (3rd order) functions for both length (R^2 = 0.85, $y = -0.126\ x^3 + 0.726\ x^2 + 3.147\ x + 10.5$) and fresh weight ($R^2$ = 0.87, $y = -2.450\ x^3 + 22.739\ x^2 + 31.672\ x + 15$) illustrating a phase of rapid growth from June to September (growth phase) followed by a period of relative stable biomass size (stationary phase) and a prominent ‘decline phase’ from October to November (Figure 3.1).

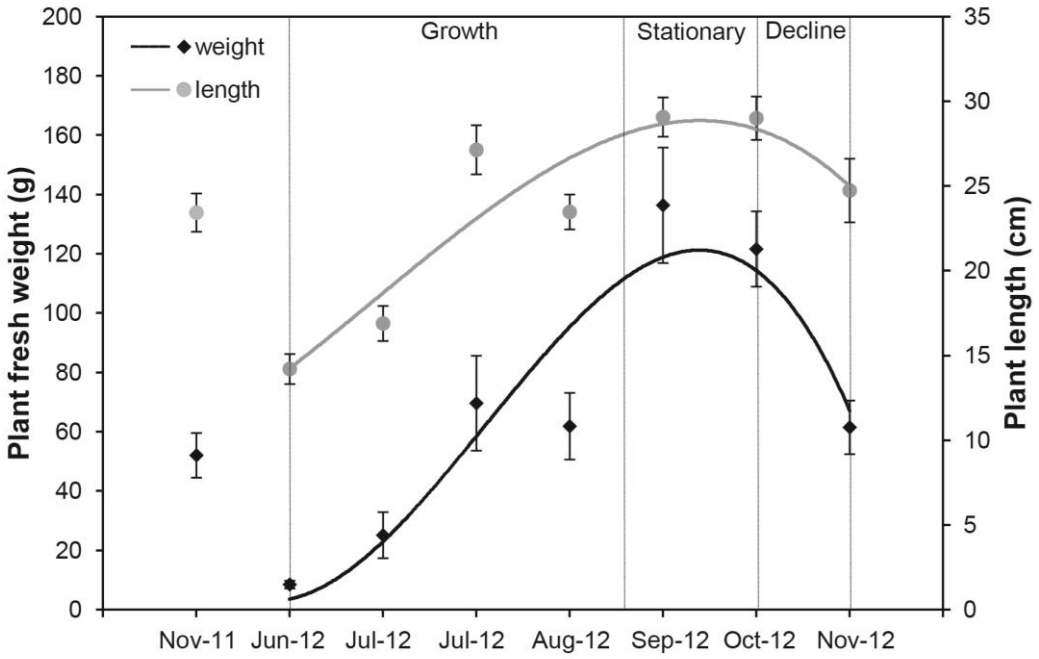

Figure 3.1. Average length (cm ± SE) and fresh weight (g ± SE) of *S. macrodontum* plants collected over one year. 15 plants per sampling period were used except November 2011 (n = 14) and 'early July' (n = 9). No samples found from January to May 2012. Specific sampling dates are provided in Table 3.2. Polynomial (3rd order) estimate of plant length (cm, grey line) (R^2 = 0.85, y = -0.126 x^3 + 0.726 x^2 + 3.147 x + 10.5) and plant fresh weight (g, black line) (R^2 = 0.87, y = -2.450 x^3 + 22.739 x^2 + 31.672 x + 15) of plants collected from June to November 2012. Vertical lines define growth phases.

3.3.2. Total fatty acid content

There was clear seasonal variation in TFA content in *S. macrodontum* (ANOVA: $F_{7,104}$ = 4.906, p < 0.001, Tukey's HSD) (Figure 3.2a, Table 3.2). TFA content in whole plants increased by over 20 % from 65.4 mg g^{-1} dw ± 3.5 SE in June to its annual TFA maximum of 82.7 mg g^{-1} dw ± 5.7 SE in 'late July'. This was followed by a rapid decline in average TFA content by over 30 % in August (56.2 mg g^{-1} dw ± 2.3 SE). For the remainder of the year, TFA content varied between 65.9 mg g^{-1} dw ± 3.3 SE in September and 55.5 mg g^{-1} dw ± 4.2 SE in November. There was a negative trend but no significant correlation between TFA content and water temperature at the sampling site (r = -0.50, p = 0.206, n = 8; Figure 3.3a), and no correlation between TFA content and plant size (fw) (r = -0.001, p = 0.991, n = 112).

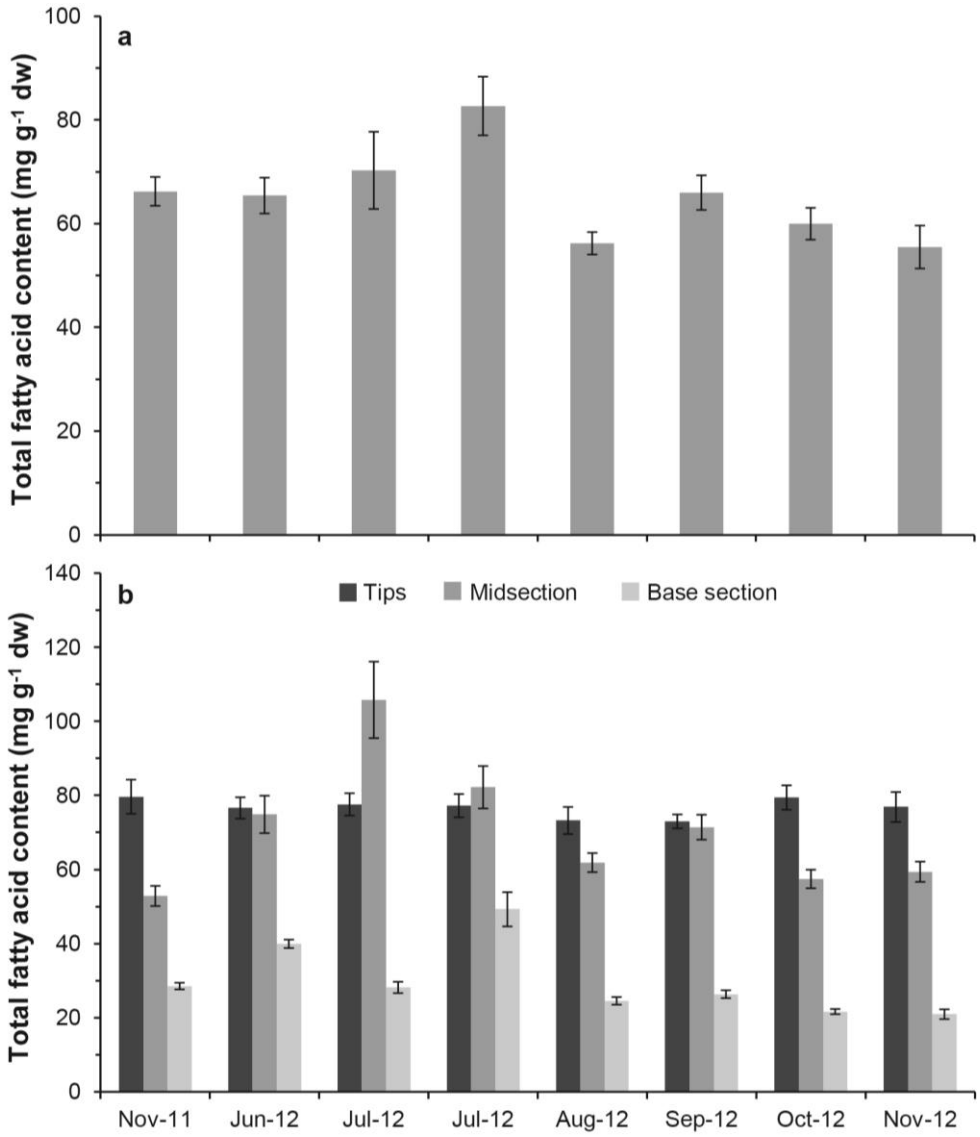

Figure 3.2. Seasonal variation in mean TFA content (mg g^{-1} dw ± SE) of *S. macrodontum* collected over the period of one year from November 2011 to November 2012 for a whole plants (one-way ANOVA: $F_{7,104} = 4.906$, $p < 0.001$) and b seasonal within-plant variation in TFA in the tips, mid- and base-sections (two-way ANOVA: $F_{21,424} = 5.232$, $p < 0.001$). 15 plants per sampling period were used except November 2011 (n = 14) and 'early July' (n = 9). Specific sampling dates are provided in Table 3.2.

There was also distinct within-plant variation in TFA content depending on sampling date (2-way ANOVA: Plant section × Sampling date, $F_{14, 319} = 7.781$, $p < 0.001$) (Figure 3.2b). TFA content was significantly different between all plant sections (Tukey's HSD: $p < 0.001$) with the base sections always having the lowest TFA content ranging from 20.9 mg g^{-1} dw ± 1.3 SE in November 2012 to 49.3 mg g^{-1} dw ± 4.6 SE in July (Table A3.1).

There was large annual variation in TFA content in the midsections with a peak period in July with over 100 mg g^{-1} dw. The tips had relatively stable TFA contents between 73.0 mg g^{-1} dw ± 1.9 SE in September and 79.7 mg g^{-1} dw ± 4.6 SE in November 2011 and were also the section with the highest TFA content except for the period from June to July when the midsections were richer in TFA.

3.3.3. Fatty acid composition

There was a clear seasonal variation in specific fatty acid composition expressed as changes in the proportions of PUFA, MUFA and SFA (Figure 3.4a). At the start of the growth period in June, fatty acids of young plants consisted of 44 % PUFA, 21 % MUFA and 34 % SFA. During the initial growth phase from June to August, PUFA content declined to 41 % of TFA and further declined to 37 % of TFA toward September/ October when plants had reached their biomass peak. At the end of the life cycle in November, PUFA content further declined to 31 % of TFA. This general decline in PUFA is characterised by a 45 % decline of PUFA(n-3), in particular C18:4(n-3) which declined by over 60 % from June to November (Table 3.2). This decline in PUFA is generally mirrored by increasing saturation from 33 % in June to 42 % of TFA content in November.

Similar to TFA content, there was no clear relationship between plant size and fatty acid composition analysed for SFA ($r = 0.035$, $p = 0.712$, $n = 112$), MUFA ($r = 0.073$, $p = 0.442$, $n = 112$) and PUFA ($r = -0.118$, $p = 0.214$, $n = 112$) (Figure A3.2). Dominant SFA were C16:0 (24.64 % of TFA ± 0.93 SE) and C14:0 (7.83 % of TFA ± 0.10 SE) while the most abundant MUFA was C18:1(n-9) (17.57 % of TFA ± 0.49 SE) (Table 3.2). PUFA(n-3) were more abundant than PUFA(n-6) with an n-6/n-3 ratio of 0.74. Of the PUFA(n-3), the most abundant fatty acids were C18:4(n-3) (8.44 % of TFA ± 0.74 SE), C18:3(n-3) (5.10 % of TFA ± 0.15 SE) and C20:4(n-3) (3.86 % of TFA ± 0.20 SE). While the content of SFA was not correlated with water temperature ($r = 0.125$, $p =$

0.768, n = 8; Figure 3.3b), there was a strong negative correlation between water temperature and PUFA (r = -0.80, p = 0.018, n = 8; Figure 3.3c) and PUFA(n-3) (r = -0.81, p = 0.015, n = 8; Figure 3.3d).

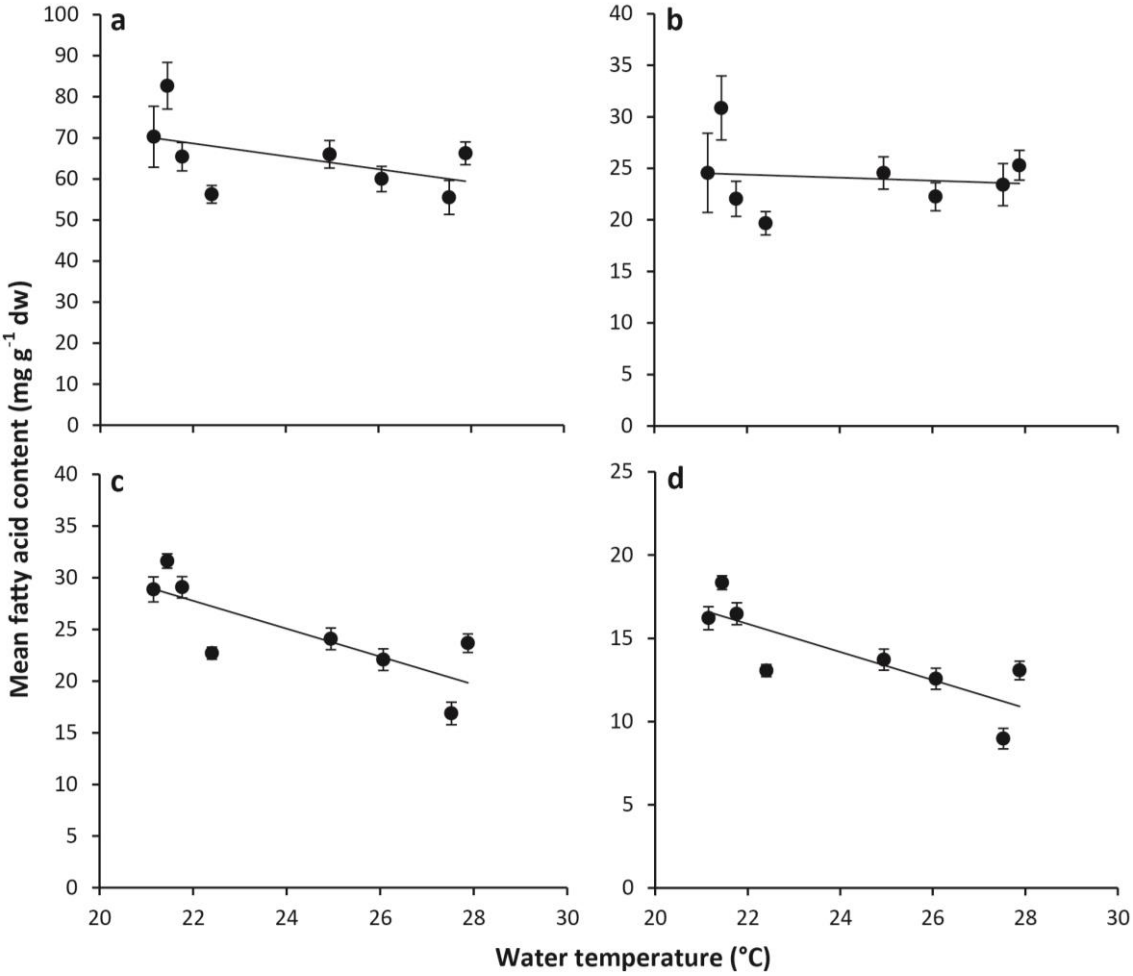

Figure 3.3. Correlation of mean (mg g^{-1} dw ± SE) **a** total fatty acids (TFA) (r = -0.501, p = 0.206, n = 8), **b** saturated fatty acids (SFA) (r = 0.125, p = 0.768, n = 8), **c** polyunsaturated fatty acids (PUFA) (r = -0.796, p = 0.018, n = 8) and **d** PUFA(n-3) (r = -0.811, p = 0.015, n = 8) with water temperature (°C).

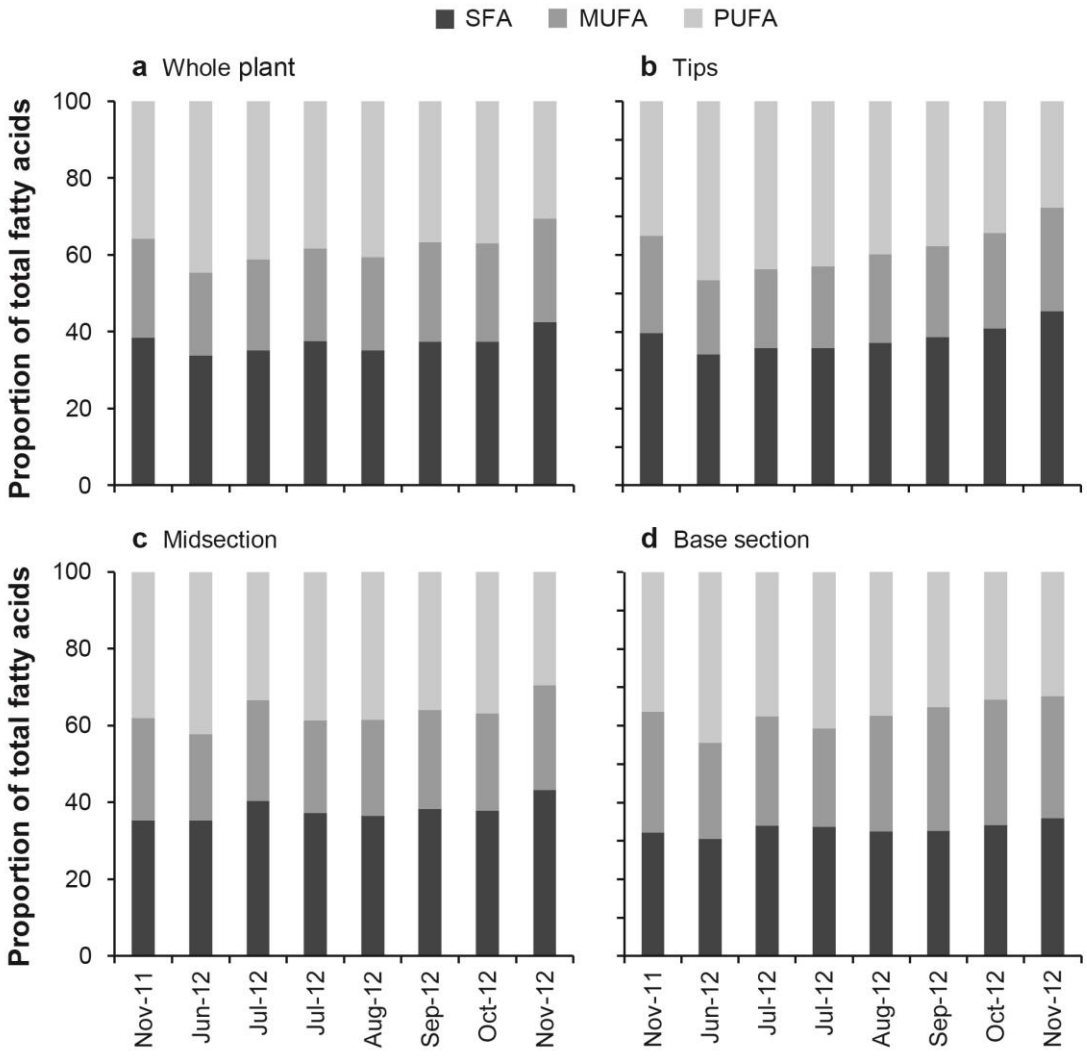

Figure 3.4. Proportion of SFA, MUFA and PUFA of TFA content of **a** whole plants and plant sections (**b** tips, **c** midsections, **d** base sections) of *S. macrodontum* sampled over one year. 15 plants per sampling period were used except November 2011 (n = 14) and 'early July' (n = 9). Specific sampling dates are provided in Table 3.2.

Fatty acid composition also varied distinctly within plants with the base section having a less saturated fatty acid profile (33 % SFA) than the midsections (38 % SFA) and tips (38 % SFA) (Figure 3.4bcd, Table A3.1). This low saturation of the base section is mainly a result of a relatively low content of C16:0 (21% of TFA) compared to the midsections (26 % of TFA) and the tips (26 % of TFA). While the PUFA content was similar across plant sections (37 – 38 % of TFA), the base section had a relatively high content of MUFA (29 % of TFA) compared to the tips (23 % of TFA) and midsections (25 % of TFA). Similar

to whole plants, PUFA content declined in all plant sections towards the end of the growth cycle with the largest decline from 46 % to 27 % of TFA in the tips and the smallest decline from 44 % to 32 % of TFA in the base sections.

3.3.4. Proximate and ultimate analysis

Ash content of the tips (7.9 % of dw ± 1.3 SE) and midsections (5.8 % of dw ± 1.2 SE) was similar while the base section had more than three times this ash content (23.2 % of dw ± 8.5 SE) (Table 3.1; ANOVA: $F_{3,14} = 5.350$, $p = 0.012$). Although statistically not significant, nitrogen content tended to be higher in the tips (2.5 % of dw ± 0.2 SE), followed by the midsections (2.4 % of dw ± 0.3 SE) and base sections (1.7 % of dw ± 0.3 SE) (Table 3.1; ANOVA: $F_{3,14} = 2.617$, $p = 0.092$). Carbon content was similar in the tips (42.4 % of dw ± 0.6 SE) and midsections (43.1 % of dw ± 1.5 SE) and significantly lower in the base sections (37.2 % of dw ± 1.4 SE) (Table 3.1; ANOVA: $F_{3,14} = 7.548$, $p = 0.003$). A similar pattern of carbon and nitrogen between the different plant sections was found when these values were expressed as proportions of ash free dry weight (afdw) (Table 3.1).

Table 3.1. Elemental analysis of different plant sections and whole plants collected in September 2012 as means (wt % ± SE, n = 3) based on dry weight (dw) and ash free dry weight (afdw). Significant and homogenous subsets (Tukey's HSD) are indicated by identical letters.

	% dw		% afdw			
	Carbon	Nitrogen	Carbon	Nitrogen	Ash	C:N ratio
Whole plant	41.9 ± 0.5 [a]	2.3 ± 0.1 [a]	44.6 ± 0.3 [a]	2.4 ± 0.1 [a]	9.4 ± 0.9 [a]	18.2 : 1 [a]
Tips	42.4 ± 0.6 [a]	2.5 ± 0.2 [a]	45.3 ± 0.9 [a]	2.7 ± 0.1 [a]	7.9 ± 1.3 [a]	16.8 : 1 [a]
Midsection	43.1 ± 1.5 [a]	2.4 ± 0.3 [a]	45.9 ± 1.0 [a]	2.5 ± 0.3 [a]	5.8 ± 1.2 [a]	18.1 : 1 [a]
Base section	37.2 ± 1.4 [b]	1.7 ± 0.3 [a]	40.8 ± 0.3 [b]	1.8 ± 0.3 [a]	23.2 ± 8.5 [b]	22.2 : 1 [a]

Significant and homogenous subsets (Tukey's HSD) are indicated by identical letters

Table 3.2. Average content of fatty acids (% of TFA ± SE) of *S. macrodontum* (whole plants) collected over one year and average of seasonal fatty acid content of *S. macrodontum* (% of TFA ± SE) compared with typical fatty acid profiles of traditional oil feedstock plant species (% of TFA) (reviewed in Dubois et al. 2007). Total fatty acid (TFA) content is presented as means (mg g^{-1} dw ± SE) and significant and homogenous subsets (Tukey's HSD) are indicated by identical letters. 15 plants per sampling period were used except November 2011 (n = 14) and 'early July' (n = 9). No samples found from January to May 2012.

	Fatty acids (% of TFA) of *Spatoglossum macrodontum*									Fatty acids (% of TFA) of oil crops			
	20/11/2011	4/6/2012	3/7/2012	30/7/2012	29/8/2012	26/9/2012	27/10/2012	26/11/2012	Annual average	Rapeseed	Palm oil	Soybean	Sunflower
C14:0	7.43 ± 0.21	8.13 ± 0.16	8.11 ± 0.38	8.22 ± 0.26	7.77 ± 0.22	7.80 ± 0.23	7.58 ± 0.21	7.63 ± 0.23	7.83 ± 0.10	0.1	1.1	0.1	0.1
C14:1	0.43 ± 0.03	0.48 ± 0.03	0.53 ± 0.04	0.35 ± 0.03	0.43 ± 0.02	0.45 ± 0.02	0.45 ± 0.04	0.43 ± 0.06	0.44 ± 0.02				
C15:0	0.49 ± 0.04	0.47 ± 0.02	0.66 ± 0.03	0.59 ± 0.02	0.77 ± 0.03	0.62 ± 0.02	0.65 ± 0.03	0.75 ± 0.03	0.63 ± 0.04				
C16:0	26.93 ± 0.80	21.94 ± 0.77	21.48 ± 1.58	24.74 ± 1.21	22.57 ± 0.89	25.10 ± 0.78	25.14 ± 0.58	29.20 ± 0.78	24.64 ± 0.93	5.1	43.8	10.8	6.4
C16:1(n-9)	0.37 ± 0.02	0.37 ± 0.02	0.65 ± 0.05	0.46 ± 0.03	0.62 ± 0.03	0.59 ± 0.03	0.60 ± 0.03	0.58 ± 0.05	0.53 ± 0.04				
C16:1(n-7)	3.58 ± 0.07	3.19 ± 0.13	3.41 ± 0.28	3.28 ± 0.11	3.56 ± 0.06	3.70 ± 0.12	3.84 ± 0.14	3.78 ± 0.14	3.54 ± 0.08	0.2	0.2	0.2	0.1
C16:1	0.61 ± 0.03	0.60 ± 0.05	0.69 ± 0.05	0.49 ± 0.04	0.63 ± 0.03	0.69 ± 0.03	0.77 ± 0.03	0.93 ± 0.05	0.68 ± 0.05				
C16:2	0.23 ± 0.04	0.33 ± 0.03	0.41 ± 0.09	0.25 ± 0.05	0.46 ± 0.02	0.47 ± 0.02	0.42 ± 0.06	0.18 ± 0.07	0.34 ± 0.04				
C17:0	0.38 ± 0.04	0.32 ± 0.04	0.59 ± 0.03	0.39 ± 0.03	0.50 ± 0.01	0.49 ± 0.04	0.40 ± 0.07	0.47 ± 0.08	0.44 ± 0.03				
C16:3(n-4)	0.41 ± 0.02	0.35 ± 0.03	0.64 ± 0.05	0.45 ± 0.03	0.56 ± 0.02	0.57 ± 0.02	0.62 ± 0.03	0.69 ± 0.03	0.54 ± 0.04				
C16:3(n-3)	0.46 ± 0.02	0.47 ± 0.03	0.62 ± 0.05	0.49 ± 0.03	0.57 ± 0.02	0.57 ± 0.03	0.62 ± 0.03	0.57 ± 0.05	0.55 ± 0.02				
C16:4(n-3)	0.12 ± 0.05	0.38 ± 0.04	0.48 ± 0.08	0.31 ± 0.05	0.37 ± 0.05	0.29 ± 0.06	0.15 ± 0.06	0.12 ± 0.06	0.28 ± 0.05				
C18:0	1.40 ± 0.05	1.17 ± 0.05	1.30 ± 0.07	1.21 ± 0.03	1.22 ± 0.05	1.37 ± 0.04	1.43 ± 0.03	1.78 ± 0.06	1.36 ± 0.07	1.7	4.4	3.9	4.5
C18:1(n-9)	19.20 ± 0.53	15.60 ± 0.54	15.79 ± 1.02	17.49 ± 0.73	17.03 ± 0.48	18.33 ± 0.39	17.83 ± 0.34	19.31 ± 0.33	17.57 ± 0.49	60.1	39.1	23.9	22.1
C18:1	0.83 ± 0.05	0.58 ± 0.06	1.04 ± 0.03	0.90 ± 0.03	1.00 ± 0.03	0.94 ± 0.05	1.10 ± 0.05	1.23 ± 0.06	0.95 ± 0.07				
C18:1(n-5)	0.42 ± 0.04	0.47 ± 0.03	0.60 ± 0.05	0.42 ± 0.03	0.53 ± 0.02	0.53 ± 0.02	0.57 ± 0.03	0.61 ± 0.05	0.52 ± 0.03				
C18:2(n-6)	2.94 ± 0.05	3.03 ± 0.06	2.67 ± 0.08	2.73 ± 0.05	2.54 ± 0.05	2.48 ± 0.04	2.75 ± 0.07	2.78 ± 0.06	2.74 ± 0.06	21.5	10.2	52.1	65.6
C18:3(n-6)	1.28 ± 0.03	1.55 ± 0.06	1.12 ± 0.04	0.99 ± 0.04	0.90 ± 0.02	0.92 ± 0.02	1.07 ± 0.04	0.98 ± 0.03	1.10 ± 0.08				
C18:3(n-3)	5.16 ± 0.16	4.79 ± 0.13	4.95 ± 0.24	4.71 ± 0.20	5.76 ± 0.15	5.34 ± 0.17	5.55 ± 0.19	4.51 ± 0.17	5.10 ± 0.15	9.9	0.3	7.8	0.5
C18:4(n-3)	7.77 ± 0.46	11.89 ± 0.47	10.04 ± 0.59	9.44 ± 0.59	8.87 ± 0.45	7.18 ± 0.43	7.19 ± 0.29	5.10 ± 0.29	8.44 ± 0.74				
C20:0	0.74 ± 0.03	0.69 ± 0.04	0.92 ± 0.07	0.72 ± 0.04	0.90 ± 0.04	0.89 ± 0.03	0.92 ± 0.03	1.00 ± 0.04	0.85 ± 0.04	0.6	0.3	0.3	0.3
C20:1(n-9)	0.19 ± 0.06	0.02 ± 0.02	0.11 ± 0.11	0.06 ± 0.03	0.00 ± 0.00	0.32 ± 0.07	0.15 ± 0.09	0.00 ± 0.00	0.11 ± 0.04	1.4	0.1	0.1	0.2
C20:2(n-6)	0.54 ± 0.03	0.55 ± 0.04	0.64 ± 0.07	0.48 ± 0.03	0.63 ± 0.03	0.64 ± 0.03	0.79 ± 0.05	0.57 ± 0.08	0.61 ± 0.03	0.1			
C20:3(n-6)	2.64 ± 0.08	4.46 ± 0.10	4.39 ± 0.21	4.34 ± 0.17	3.50 ± 0.07	3.08 ± 0.11	2.74 ± 0.10	2.16 ± 0.03	3.41 ± 0.32				
C20:4(n-6)	8.12 ± 0.32	9.33 ± 0.38	8.83 ± 0.54	7.44 ± 0.40	8.82 ± 0.33	7.68 ± 0.24	7.61 ± 0.20	7.09 ± 0.26	8.11 ± 0.28				
C20:3(n-3)	0.09 ± 0.05	0.00 ± 0.00	0.19 ± 0.10	0.45 ± 0.03	0.47 ± 0.04	0.38 ± 0.06	0.43 ± 0.08	0.04 ± 0.04	0.26 ± 0.07				
C20:4(n-3)	3.07 ± 0.11	4.14 ± 0.11	4.36 ± 0.27	4.13 ± 0.13	4.38 ± 0.08	4.10 ± 0.13	3.83 ± 0.13	2.87 ± 0.11	3.86 ± 0.20				
C20:5(n-3)	3.24 ± 0.11	3.80 ± 0.08	3.38 ± 0.14	3.51 ± 0.07	3.30 ± 0.12	3.11 ± 0.06	3.26 ± 0.06	3.14 ± 0.09	3.34 ± 0.08				
C22:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.13 ± 0.09	0.09 ± 0.06	0.00 ± 0.00	0.06 ± 0.06	0.04 ± 0.02	0.3	0.1	0.2	0.8
C22:1(n-9)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.18 ± 0.08	0.22 ± 0.07	0.25 ± 0.09	0.00 ± 0.00	0.08 ± 0.04	0.4			0.1
C24:0	0.53 ± 0.03	0.48 ± 0.04	0.84 ± 0.15	0.50 ± 0.04	0.61 ± 0.05	0.57 ± 0.07	0.77 ± 0.05	0.89 ± 0.05	0.65 ± 0.06	0.2	0.1	0.3	0.2

Table 3.2. (Continued)

	Fatty acids (% of TFA) of *Spatoglossum macrodontum*									Fatty acids (% of TFA) of oil crops			
	20/11/2011	4/6/2012	3/7/2012	30/7/2012	29/8/2012	26/9/2012	27/10/2012	26/11/2012	Annual average	Rapeseed	Palm oil	Soybean	Sunflower
Other FA	0.39 ± 0.02	0.398 ± 0.02	0.54 ± 0.04	0.441 ± 0.02	0.42 ± 0.04	0.482 ± 0.02	0.523 ± 0.03	0.579 ± 0.03	0.472 ± 0.025	0.3	0.6		0.5
Total SFA	37.90 ± 0.71	33.20 ± 0.78	33.89 ± 1.37	36.39 ± 0.99	34.47 ± 0.80	36.93 ± 0.65	36.89 ± 0.50	41.76 ± 0.67	36.43 ± 0.96	8	50.4	15.7	12.8
Total MUFA	25.63 ± 0.52	21.32 ± 0.42	22.83 ± 0.86	23.47 ± 0.66	23.96 ± 0.36	25.79 ± 0.37	25.57 ± 0.41	26.87 ± 0.30	24.43 ± 0.65	62.4	39.4	24.2	22.4
Total PUFA	36.08 ± 1.11	45.08 ± 1.14	42.74 ± 2.15	39.70 ± 1.63	41.15 ± 1.10	36.80 ± 0.93	37.02 ± 0.73	30.78 ± 0.88	38.67 ± 1.58	31.5	10.5	59.8	66
PUFA (n-3)	19.91 ± 0.72	25.47 ± 0.67	24.03 ± 1.28	23.04 ± 0.97	23.73 ± 0.68	20.96 ± 0.59	21.02 ± 0.42	16.34 ± 0.55	21.81 ± 1.02	9.9	0.3	7.8	0.5
PUFA (n-6)	15.52 ± 0.43	18.93 ± 0.50	17.66 ± 0.81	15.96 ± 0.64	16.40 ± 0.41	14.80 ± 0.34	14.96 ± 0.38	13.57 ± 0.33	15.98 ± 0.60	21.6	10.2	52.1	65.6
n-6/n-3	0.78 ± 0.01	0.74 ± 0.01	0.74 ± 0.01	0.70 ± 0.01	0.69 ± 0.01	0.71 ± 0.01	0.71 ± 0.02	0.84 ± 0.02	0.74 ± 0.02	2.18	34.00	6.68	131.20
TFA (mg g^{-1} dw)	66.22 ± 2.78 ab	65.42 ± 3.47 ab	70.28 ± 7.44 ab	82.69 ± 5.66 b	56.22 ± 2.32 a	65.99 ± 3.34 ab	59.99 ± 3.08 a	55.50 ± 4.15 a	65.29 ± 3.09				

FA fatty acids, *TFA* total fatty acids, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *dw* dry weight

3.4. Discussion

This study identified the brown seaweed *S. macrodontum* as an annual species which is apparent on the reef flat on Magnetic Island (Queensland, Australia) from June to November with a growth period from June to September. Its high TFA content and fatty acid composition make it a species of interest for the production of oil-based products, in particular nutraceuticals and chemicals. This study also clearly identified seasonal variation in TFA content and composition that are linked to morphology and broad seasonal changes in water temperature. However, across all seasons there was clear and consistent within-plant variation in TFA content and fatty acid composition, with the plant tips having higher overall TFA and a higher percentage of SFA, while the base-section was richest in MUFA.

3.4.1. Plant sizes and biomass seasonality

S. macrodontum is an annual seaweed with macrothalli present from at least June to November at the sample site. Although sampling was conducted from January on a monthly basis, no macrothalli were found until June. This is potentially because of their small size and their cryptic location within dense stands of *Sargassum* species. Although many tropical brown seaweed have no macrothalli present during the warmer summer, some *Dictyota* species survive as cryptic microthalli and start to grow once environmental conditions become favourable (Ateweberhan et al. 2005). In this Chapter, it could not be determined whether individual *S. macrodontum* plants survived the summer as the dormant stage, however, it seems likely that populations were completely renewed every year from propagules as there was no evidence of microthalli. In addition, it is possible that plants survived the summer in deeper water as the reported vertical distribution of this species ranges from the intertidal to 18 m depth (Kraft 2009).

The growth pattern of this species was characterised by a stage of rapid biomass increase from June to September followed by a stagnant phase and a subsequent biomass decline toward the end of the life cycle. During the 'growth phase', average SGR of *S. macrodontum* (2.49 % day^{-1} ± 0.99 SE) was similar to other benthic brown seaweeds such as *Desmarestia viridis* (2.9 % day^{-1}) (Blain and Gagnon 2013) or *Sargassum hemiphyllum* (0.62 – 1.65 % day^{-1}) (Yu et al. 2013) but lower than common commercial species such as *Kappaphycus alvarezii* (3.72 – 7.17 % day^{-1}) (Hurtado-Ponce 1992) or various species of *Gracilaria* (2.5 – 7.8 % day^{-1}) (Skriptsova and Nabivailo 2009). Biomass increase in *S. macrodontum* occurred when both water temperature and incidental light were at their annual minimum (AIMS 2014; BOM 2014). Changes in water temperature and light govern the seasonal growth patterns of a range of seaweed species, in particular brown seaweeds (Ateweberhan et al. 2005; Lüning 1990). For example, tropical *Spatoglossum asperum* (Ngan and Price 1980), temperate *Spatoglossum crassum* (Hwang et al. 2004), and other members of the Dictyotales (Ateweberhan et al. 2005) have a pattern of seasonal growth over the cooler winter. After the biomass peak in September, growth of *S. macrodontum* ceased, potentially triggered by increasing water temperatures and/ or a reallocation of resources from growth towards reproduction as reported for other benthic brown seaweeds (Ateweberhan et al. 2005; Blain and Gagnon 2013; Díaz-Villa et al. 2005). Without any further growth, degenerative processes such as grazing, physical fragmentation and general tissue decomposition could explain the observed decline of biomass toward the end of the life cycle. A similar annual growth pattern of *S. macrodontum* with a peak biomass in late autumn and biomass decay towards summer has been reported from Lord Howe Island (Kraft 2009), suggesting that the annual growth pattern described in this chapter is typical for this species.

3.4.2. Fatty acids

This study confirms the high TFA content of *S. macrodontum* which ranged between 55.5 and 82.7 mg g^{-1} dw depending on the time of collection. These levels are within the range

of fatty acid contents reported for this species sampled at the same location in September 2010 (57.40 mg g^{-1} dw ± 0.87 SE) (Chapter 2; Gosch et al. 2012). The TFA content of *S. macrodontum* is approximately twice as high as that of the related species *Dictyota bartayresii* (35.15 mg g^{-1} dw ± 3.89 SE) (Chapter 2; Gosch et al. 2012) and considerably higher than that of the common commercial seaweeds *Saccharina japonica* (15.91 – 30.63 mg g^{-1} dw) (Honya et al. 1994) and *Undaria pinnatifida* (total lipid: 24 mg g^{-1} dw) (Herbreteau et al. 1997). This makes *S. macrodontum* an interesting target to investigate the production of oil-based products for the nutraceutical industry but also for targeted pre-cursors for bio-polymers in the chemical industry.

S. macrodontum contains a diverse range of PUFA relevant to the nutraceutical market and is particularly rich in PUFA(n-3) with an n-6/n-3 ratio of 0.7, which is considerably lower than in most vegetable oils and similar to other seaweeds (Table 3.2; Dubois et al. 2007; Kumari et al. 2013a; Schmid et al. 2014). A typical western diet with a high n-6/n-3 ratio has been linked to health problems including cancer and cardiovascular diseases (Russo 2009). The most abundant PUFA(n-3) in *S. macrodontum* is C18:4(n-3) which is an omega-3 fatty acid that is important for cardiovascular health (Guil-Guerrero 2007) and is a precursor for the nutritionally essential EPA C20:5(n-3) (Guil-Guerrero 2007). Notably, C18:4(n-3) is absent or present in only minute quantities in traditional terrestrial oil crops (Dubois et al. 2007) and alternative sources of this fatty acid are fish oils and genetically manipulated terrestrial crops, which are problematic because of increasingly depleted fish stocks (Pauly et al. 2005) and low consumer acceptance of genetically manipulated foods (Bredahl 2001). Other abundant and nutritionally important PUFA(n-3) in *S. macrodontum* are C20:4(n-3), C20:5(n-3) and in particular C18:3(n-3) which has been found to decrease blood pressure, improve heart and liver function and also redistribute body fat in animal trials (Poudyal et al. 2012; Poudyal et al. 2013).

S. macrodontum has a fatty acid profile with 33 % to 42 % saturation, with the most abundant SFA being C16:0, followed by C14:0. Saturation in this species is within the range of several common seaweed species used for human consumption such as *Caulerpa lentillifera* and *C. racemosa* (40.8 – 43.3 % SFA; Paul et al. 2014) and *Saccharina japonica* (26 – 48 % SFA; Honya et al. 1994), but higher than that of *Undaria pinnatifida* (20.4 % SFA; Sánchez-Machado et al. 2004). The fatty acid profile is also more saturated than that of common industrial oil crops such as rapeseed, soybean or sunflower but much less saturated than, for example, palm oil or coconut oils (Dubois et al. 2007; Table 3.2). A high degree of saturation in a diet is generally associated with negative health effects as SFA, in particular C14:0 and C16:0, increase plasma cholesterol levels which contributes to cardiovascular diseases (Hunter 2001; Kris-Etherton and Yu 1997). However, the adverse effects of these SFA are generally associated with large quantities that are not usually consumed in the form of algal oils (Hayes and Khosla 1992). It is therefore likely that the effects of the PUFA content and the very low n-6/n-3 ratio of *S. macrodontum* will be beneficial.

S. macrodontum also has a high content of C18:1(n-9) (~ 18 % of TFA) which for example has demand in the chemical industry for the production of polyols and polyurethanes (Biermann et al. 2006; Lligadas et al. 2010). The relative and total content of this fatty acid is higher than reported in most seaweed species (Gosch et al. 2012; Kumari et al. 2010; Schmid et al. 2014) highlighting the potential of *S. macrodontum* as a renewable feedstock for the manufacture and synthesis of chemical products. Although terrestrial crops such as palm oil have a more favourable fatty acid profile for such chemical processes (Table 3.2), environmental concerns and competition with food crops remain controversial (Tan et al. 2009).

This study also provides clear evidence of seasonal variation in fatty acids in *S. macrodontum* with a higher TFA content during the colder winter months (June and July)

and a general trend of increasing saturation and concomitant decrease of PUFA towards the end of the growth period. Seasonal variation in TFA content and fatty acid composition can be a response to changes in the key environmental parameters of temperature and light. For example, lower TFA during winter have been reported for various seaweeds (Honya et al. 1994; Nelson et al. 2002). Although there is limited data on the direct effect of temperature on the TFA in macroalgae (e.g. Al-Hasan et al. 1991), various seaweed from cold water environments have a higher degree of unsaturation compared to seaweed from warm water environments (Pettitt et al. 1989; Nelson et al. 2002). This is possibly an environmental acclimatisation as PUFA have a lower melting point than SFA and therefore provide a physiological advantage in cold water environments by increased membrane lipid fluidity (Los et al. 2013; Thompson et al. 1992). This is supported by this Chapter as PUFA, and in particular PUFA(n-3), were negatively correlated with water temperature. In a similar manner, low light conditions not only lead to a higher TFA content in a range of seaweeds (Hotimchenko 2002) but also a higher degree of unsaturation (Klyachko-Gurvich et al. 1999). Previous work on marine microalgae suggests that this is a physiological response to improve the effectiveness of photosynthesis by increasing the fluidity of thylakoid membranes and thereby electron flow in the chloroplast (Mock and Kroon 2002).

Although environmental variation provides an explanation for the observed fatty acid patterns, it is possible that saturation also increases with age or growth stage as proposed for the brown seaweeds *Costaria costata* (Gerasimenko et al. 2010) and *Saccharina japonica* (Honya et al. 1994). This potentially relates to an increasing rate of PUFA oxidation in plants towards the end of their life cycle. Such an age/growth stage effect on fatty acids is unlikely in this Chapter because plant size did not significantly explain fatty acid variability in *S. macrodontum*. However, it remains unclear whether plant size is an accurate measure of age and growth stage in this species due to potential grazing or

fragmentation through physical disturbances, which is demonstrated by two distinct growth curves for length and weight at the study site.

In addition to this variation in fatty acids between whole plants, there was also internal within-plant variation in fatty acids with the tips and mid-sections having a higher TFA content and slightly more PUFA(n-3) while the base-section was proportionally less saturated and richer in MUFA. This within-plant pattern is similar to those in the brown seaweeds *Sargassum miyabei* and *Saccharina japonica* (Khotimchenko and Kulikova 2000; Kulikova and Khotimchenko 2000) and is likely related to the morphological, functional and physiological differentiation of plant parts required for growth, photosynthesis and energy storage (Gómez and Wiencke 1998; Lawrence and McClintock 1988; Stengel and Dring 1998). Generally it can be expected that the thin and branched tips of a seaweed such as *S. macrodontum* contain more photosynthetic tissue per unit biomass because of the higher surface area to volume ratio compared to the more compact holdfast region whose main function is structural support and attachment to the substratum (Arnold and Manley 1985; Littler and Littler 1985).

Functional differentiation between photosynthetic tips and structural base-sections may also provide an explanation for the within-plant variation in TFA content of *S. macrodontum*. The lower measured TFA content of the base-section can be a direct result of proportionally more carbohydrates than fatty acids in this region, reflecting it's structural and energy storage function, while the photosynthetically active tips are richer in lipids that support photosynthetic activity. The major lipid class of brown seaweeds are the glycolipids which are directly associated with the light harvesting complex and are found in the photosynthetic membranes of algae and plants (Dembitsky et al. 1991; Dörmann and Hölzl 2010; Sanina et al. 2004) and dominate the upper sections of a range of seaweeds (Khotimchenko and Kulikova 2000; Kulikova and Khotimchenko 2000). Glycolipids of several seaweeds have a high concentration of PUFA(n-3) and in

particular C18:4(n-3) and C20:5(n-3) (Khotimchenko 2003; Miyashita et al. 2013; Sanina et al. 2004). In *S. macrodontum*, however, there was no general dominance of PUFA(n-3) in the tips but a strong dependence on sampling time as PUFA(n-3) of the tips were generally more abundant from June to October, while there were no differences between tips and base-sections during the 'decline phase' in both November 2011 and 2012. It is possible that during the 'decline phase' there was a breakdown of tissue, oxidation of PUFA(n-3) and general physiological inactivity which together lead to a reduced PUFA(n-3) content in the tips. Although neither carbohydrates nor photosynthetic activity were measured *per se* in *S. macrodontum*, indirect evidence of the biochemical composition is provided in the form of a distinct within-plant variation in carbon and nitrogen content. The C:N ratio ranges from 16.8:1 in the tips to 22.2:1 in the base-section (Table 3.1). Such C:N ratios are typical for tropical seaweeds (Atkinson and Smith 1983) and indicate nitrogen limitation. Within-plant variation in carbon and nitrogen content is documented for a range of seaweeds such as *Macrocystis integrifolia* and *Nereocystis luetkeana* where the blades were richer in nitrogen than the stipes or bulbs across all seasons (Rosell and Srivastava 1985), or for *Saccharina japonica* were the C:N ratio increased from the tips (7.87:1) to the basal parts (11.02:1) of the thallus (Wang et al. 2013). The proportionally higher nitrogen content in specific thallus regions is likely a consequence of a high concentration of photosynthetic pigments and associated proteins (Bird et al. 1982) as measured for *Saccharina japonica* (Wang et al. 2013) or discussed by Rosell and Srivastava (1985). It is therefore likely, that the proportionally higher nitrogen content in the tips of *S. macrodontum* is also related to the higher concentration of photosynthetic pigments in this plant section. Conversely, the higher proportion of carbon in the base is likely due to a high concentration of carbohydrates as means of energy storage (Percival 1979) but also due to the function of the hold-fast in providing support, flexibility and firm attachment to the substratum rather than energy fixation (Arnold and Manley 1985; Littler and Littler 1985).

3.4.3. Conclusion

This study emphasizes the suitability of *S. macrodontum* as a novel species for the production of oil-based products because of its high TFA content and a fatty acid composition that is suitable for nutraceuticals, or chemicals. The high content of C18:1(n-9) make it a potential feedstock for the chemical industry. However, most importantly, the high and diverse content of PUFA(n-3) and its abundance of C18:4(n-3), which are absent in most traditional terrestrial oil crops, make it a suitable feedstock for the production of high value health products as nutraceuticals and a potential substitute for fish oil and terrestrial oil crops. Although this species is currently not cultured on a commercial scale, and only limited experimental data has demonstrated its growth in tank-based cultures (Israel and Hophy 2002), its commercial potential is likely found in the South Pacific where it is abundant on islands such as Vanuatu or Samoa (Skelton et al. 2007). Extensive seaweed culture is an established industry in nearby Asia, providing the knowledge and infrastructure required for the aquaculture of brown seaweeds (Paul et al. 2012). Considering its morphology and benthic growth form, a culture method similar to other brown seaweeds such as kelps or *Sargassum* (Yu et al. 2013) might be successfully applied.

CHAPTER 4

Spatial, seasonal and within-plant variation in total fatty acid content and composition in the brown seaweeds *Dictyota bartayresii* and *Dictyopteris australis* (Dictyotales, Phaeophyceae) [3]

4.1. Introduction

There is increasing awareness of the health benefits of a diet rich in PUFA(n-3) which includes improved cardiovascular health (Russo 2009), reduced severity of certain mental health disorders (Su et al. 2003) and the redistribution of body fat (Poudyal et al. 2012, 2013). However, the typical western diet is deficient in these PUFA(n-3) with an unfavorably high n-6/n-3 ratio in most animal oils (Simopoulos 2008) and vegetable oils (Dubois et al. 2007). Furthermore, there is substantial demand for PUFA(n-3) to improve animal health and growth and also product quality in meat (Díaz-Chirón et al. 2013) and aquaculture production (Dantagnan et al. 2009; Zhao et al. 2015; Naylor et al. 2009). Traditionally these PUFA(n-3) are derived from marine fish oils, and marketed in the form of functional foods, nutraceuticals and formulated animal feeds with a projected market of $34.7 billion in 2016 (Adarme-Vega et al. 2014). However, fish oils as a source for PUFA(n-3) are problematic as fish stocks are increasingly depleted (Pauly et al. 2005) and current demand for fish oil already exceeds supply (Globefish 2014). Furthermore, there are quality concerns with high loads of mercury in some fish species (Kris-Etherton

[3] Adapted from: Gosch BJ, Paul NA, de Nys R, Magnusson M (2015a) Spatial, seasonal and within-plant variation in total fatty acid content and composition in the brown seaweeds *Dictyota bartayresii* and *Dictyopteris australis* (Dictyotales, Phaeophyceae). Journal of Applied Phycology 27:1607-1622

et al. 2002), 'fishy' taste and increasing customer preference for vegetarian dietary products (Leitzmann 2014). Although some terrestrial crops such as chia seed and certain nuts have high PUFA(n-3) contents, they generally lack essential omega-3 fatty acids such as EPA or DHA (Adarme-Vega et al. 2014; Dubois et al. 2007; Simopoulos 2002), have low productivities of oil, and also compete with traditional food crops for arable land (Foley et al. 2011).

An alternative source of PUFA(n-3) is seaweed biomass which can be produced on non-arable land or in offshore cultivation and therefore does not compete with food crops. Despite high productivities in culture (Bolton et al. 2009; Capo et al. 1999; Magnusson et al. 2014; Mata et al. 2015) and an annual production of approximately 20 million tonnes per year (FAO 2014), seaweed as a bioresource for valuable oils has been largely overlooked, mainly because of its presumably low content of fatty acids. However, in an expanding field of research a range of oil rich species (> 10 % dw) with high total fatty acid (TFA) contents and high proportions of PUFA(n-3) have been identified and investigated in detail for their suitability as feedstock for oil-based bioproducts (Chapter 2; Gosch et al. 2012; Kumari et al. 2013a; Schmid et al. 2014). Recent examples include the brown seaweed *Spatoglossum macrodontum* with a TFA content of up to 8 % dw of which 20 % are in the form of valuable PUFA(n-3) (Chapter 3; Gosch et al. 2014) and the green seaweed *Derbesia tenuissima* with a TFA content of 5 % dw of which 40 % are PUFA(n-3) and a fatty acid productivity of 1.4 g dw m^2 day^{-1} (Magnusson et al. 2014).

However, the content and the composition of TFA of seaweed can vary considerably over space and time for individuals of the same species (Chapter 3; Gosch et al. 2014; Hernández-Carmona et al. 2009; Nelson et al. 2002). Although it is possible that some of the spatial variability of fatty acids in a species is caused by genotypic differentiation (Robinson et al. 2013), variation is generally linked to the environment, including changes in light (Hotimchenko 2002), salinity (Kumar et al. 2010), and in particular

nutrient availability (Gómez and Wiencke 1998; Gordillo et al. 2001) and water temperature (Al-Hasan et al. 1991; Floreto et al. 1993). The degree and direction of seasonal variation in the content and composition of fatty acids is species-specific (Schmid et al. 2014) and may also be related to plant size and the life-history stages of individuals (Gerasimenko et al. 2010; Honya et al. 1994). In addition, the content and composition of fatty acids can also vary between different parts of the thallus within an individual (Chapter 3; Gosch et al. 2014; Khotimchenko and Kulikova 2000; Kulikova and Khotimchenko 2000) and is likely related to the morphological and functional differentiation of the thallus (Lawrence and McClintock 1988; Stengel and Dring 1998).

This inherent variability of fatty acids constitutes a challenge for the commercial production of PUFA(n-3) from seaweed biomass as a stable supply as consistent quality is preferable (Gellenbeck 2012; Hafting et al. 2012). However, there is also an opportunity to exploit this natural variability and develop culture and harvest strategies that result in optimized yields of desired target fatty acids. A critical first step in the domestication of new species of seaweed for renewable oil products is therefore the quantification of the natural variation in fatty acid content and composition, and the drivers thereof. Subsequently, culture conditions and harvest strategies for this new target species can be designed to precisely and predictably optimize the fatty acid profile for the production of specific bioproducts.

Therefore, the overall objective of this study was to quantify variability in the content and composition of fatty acids in *Dictyota bartayresii* and *Dictyopteris australis* (Dictyotales, Phaeophyceae), which are broadly distributed in Northern Queensland, Australia, with emphasis on PUFA(n-3) and their potential for oil-based bioproducts. These species were selected because of their high fatty acid content with a high proportion of PUFA(n-3) (Chapter 2; Gosch et al. 2012) and a wide distribution in the Pacific region. The first aim was to quantify the seasonal variability in the content and composition of the fatty acids

of both species and also the spatial variation in *D. bartayresii*. The second aim was to quantify monthly variation in the fatty acids in both species at a single location. The variation in fatty acids was analyzed for whole plants and also for different parts of the thallus.

4.2. Materials and Methods

4.2.1. Study organism and field sites

The brown seaweeds *D. bartayresii* and *D. australis* were selected because they have been identified as a rich source of fatty acids with a high proportion of PUFA(n-3) and are commonly found along the North Queensland coast (Chapter 2; Gosch et al. 2012). *D. bartayresii* has a circumtropical distribution while *D. australis* is common in the Pacific and Indian Ocean including Chile, Hawaii, Australia and India (Guiry and Guiry 2014). *D. bartayresii* was collected from three locations (Nelly Bay, Orpheus Island and Kissing Point) in North Queensland, Australia, while *D. australis* was collected from Nelly Bay and was not present at Orpheus Island or Kissing Point. The field sites were selected based on the availability of the target species and include intertidal rocky shore to coral reef habitats. The sampling locations are described in detail in Chapter 2 (Gosch et al. 2012).

4.2.2. Biomass sampling and preparation

Sampling at each field site was conducted during particular months (permitting weather conditions, tides and logistical constraints) during summer and winter and 15 individual plants of each species were collected during each sampling month. The sampling months were defined as summer and winter months based on seawater temperature and light availability (Table 4.1). *D. bartayresii* was found at all sampling locations. At Nelly Bay, samples were collected during three summer (November 2011, February 2012, November 2012) and three winter months (May 2012, June 2012, August 2012). At Orpheus Island, samples were collected in two summer months (November 2011, November 2012) and

one winter month (August 2012). At Kissing Point, plants were collected during two winter months (May 2012, August 2012) while plants found in summer at this location were generally in a poor state and consisted mainly of fragments and were therefore not included in this Chapter. *D. australis* was only found at Nelly Bay and was collected in three summer (November 2011, February 2012, November 2012) and three winter months (June 2012, July 2012, August 2012).

Table 4.1. Climate data for the sampling locations (Kissing Point, Nelly Bay, Orpheus Island). Data as means from the Australian Institute of Marine Science (AIMS) and the Australian Bureau of Meteorology (BOM).

		Townsville - Kissing Point 146.77°E, 19.25°S			Magnetic Island - Nelly Bay 146.85°E, 19.15°S		Orpheus Island - Pioneer Bay 146.59°E, 18.71°S	
		Water temperature (°C)	Solar exposure (MJ m^{-2})	Rainfall (mm)	Water temperature (°C)	Solar exposure (MJ m^{-2})	Water temperature (°C)	Solar exposure (MJ m^{-2})
Summer	November	28.6	25.8	50.7	28.0	26.5	27.2	23.6
	December	29.5	24.9	151.5	29.8	25.7	28.4	23.7
	January	31.2	22.4	313.3	29.6	23.1	29.0	20.1
	February	30.5	22.1	406.4	29.6	22.5	29.0	18.8
	March	28.8	21.7	139.4	28.7	22.3	28.4	20.3
	April	26.9	19.4	48.8	27.1	19.9	27.1	19.1
Winter	May	25.4	16.7	11.5	25.0	17.2	25.2	16.9
	June	22.1	15.4	22.0	22.9	15.6	23.3	14.7
	July	21.8	16.8	14.3	21.9	17.1	22.2	16.1
	August	22.7	19.6	12.3	22.6	20.0	22.1	18.5
	September	24.2	23.0	13.2	24.9	23.6	23.7	21.5
	October	26.7	25.4	17.4	27.0	25.9	25.6	23.6
	Summer	29.7	23.4	212.3	29.1	24.0	28.4	21.3
	Winter	23.2	18.3	14.7	23.4	18.7	23.3	17.5

Water temperature: Townsville (Port of Townsville) (1961 - 1971, Kenny 1997); Nelly Bay (2002 - 2011, Australian Institute of Marine Science (AIMS): 2.4m NELFL1 Reef Flat Site 1); Orpheus Island (2002 - 2011, AIMS: 1.9m Orpheus Island Platform)
Solar exposure: Townsville and Nelly Bay (2007 - 2011, Australian Government Bureau of Meteorology (BOM)); Orpheus island (2007 - 2011 data from Palm Island, BOM)
Rainfall: Tonwnsville only (2002 - 2011, BOM)

A plant was considered as an individual if it had a separate holdfast, was not connected to another plant and was clearly spatially separate (> 1 m). Collected plants were transported on ice to James Cook University, Townsville where they were cleaned in freshwater to remove debris, epiphytes and animals, and the total length and fresh weight of each individual plant was measured to assess any relationship between TFA content or composition with plant size. To estimate the content and composition of TFA in 'whole plants' of *D. australis*, a representative portion, which included tips, midsection and base section (Figure A4.1) in similar proportion to whole plants, was removed from each

individual plant. Furthermore, to obtain a detailed understanding of the variation of fatty acids within plants, three cuttings, approximately 1 cm^2 in dimension, respectively from the tips, midsection and base section of each plant of *D. australis* were taken and averaged for analysis. Because plants of *D. bartayresii* were generally too small to remove cuttings for detailed fatty acid analysis, plants were cut in half (upper section, lower section) (Figure A4.2) to analyse the variation of fatty acids within plants and the TFA content and composition for the 'whole plant' was estimated from the biomass based proportional results from both the upper and lower section of each plant. All biomass samples were frozen to -20°C, freeze-dried and ground to a fine powder. The seaweed powder was sealed in airtight jars and stored at -20°C until fatty acid analysis.

4.2.3. Fatty acid analysis

Fatty acids were analysed for each plant section and each representative portion. A direct trans-esterification method was used to simultaneously extract and esterify the fatty acids to fatty acid methyl esters (FAMEs) from 0.0200 g dw subsamples for analysis by gas chromatography mass spectrometry (GC-MS; 7890 GC, 5975c MS, DB-23 capillary column with 15 µm cyanopropyl stationary phase, 60 m length and 0.25 mm inner diameter (Agilent Technologies Australia Pty Ltd.)), as described in detail in Chapter 2 (Gosch et al. 2012). TFA content was determined as the sum of all FAMEs with fatty acids being designated as CX:Y(n-z), where X is the total number of carbon, Y is the number of double bonds, and z is the position of the ultimate double bond from the terminal methyl group.

4.2.4. Statistical analysis

The relationships between plant size as measured in thallus length (cm) and fresh weight (g), and TFA content and fatty acid composition (SFA, MUFA, PUFA) were analysed by correlations for all individual plants (Correlation, IBM SPSS version 21). The seasonal (both species), spatial (*D. bartayresii* only), and within-plant (both species) variation in

fatty acid composition (% of TFA) was analysed with a non-metric multidimensional scaling (MDS, Primer 6) using the average monthly fatty acid proportion of 15 individual plants as the sample. Samples from distinct locations and sampling times (sampling months) are visualized as distinct dots and seasonal clusters with similar fatty acid profiles are visualized as circles. A vector loading bi-plot (Pearson's product correlations) shows the relative load of individual fatty acids for the samples with the lengths and directions of the vectors representing the strength and direction of correlations.

4.3. Results

4.3.1. Plant sizes

Plants of both species were generally present year round, but sampling was only conducted during summer and winter. There were distinct differences in both the length and fresh weight of *D. bartayresii* at different locations as plants from Nelly Bay were larger in both length (14.4 cm ± 0.7 SE) and fresh weight (6.0 g ± 1.0 SE) than plants from Orpheus Island, which had the smallest thallus length (6.6 cm ± 0.8 SE), and plants from Kissing Point, with the lowest fresh weight (2.6 g ± 0.1 SE) (Figure 4.1a). There was no clear seasonal pattern in plant size in *D. bartayresii* across locations. The average fresh weight of the thalli collected at Nelly Bay was 6.0 g ± 1.0 SE and ranged from 2.3 g ± 0.3 SE in November 2011 to 8.6 g ± 0.8 SE in August 2012. The average annual thallus length was 14.4 cm ± 0.7 SE with small monthly variation from 11.2 cm ± 0.7 SE in February 2012 to 15.8 cm ± 0.5 SE in November 2012 (Figure 4.1b). Plants of *D. australis* had considerably higher mean annual fresh weight (23.6 g ± 3.7 SE) than *Dictyota* and greater variation from 9.6 g ± 1.1 SE in June 2012 to 33.6 g ± 3.3 SE in November 2011, but with a similar and relatively uniform plant length throughout the year (15.6 cm – 19.8 cm) (Figure 4.1c).

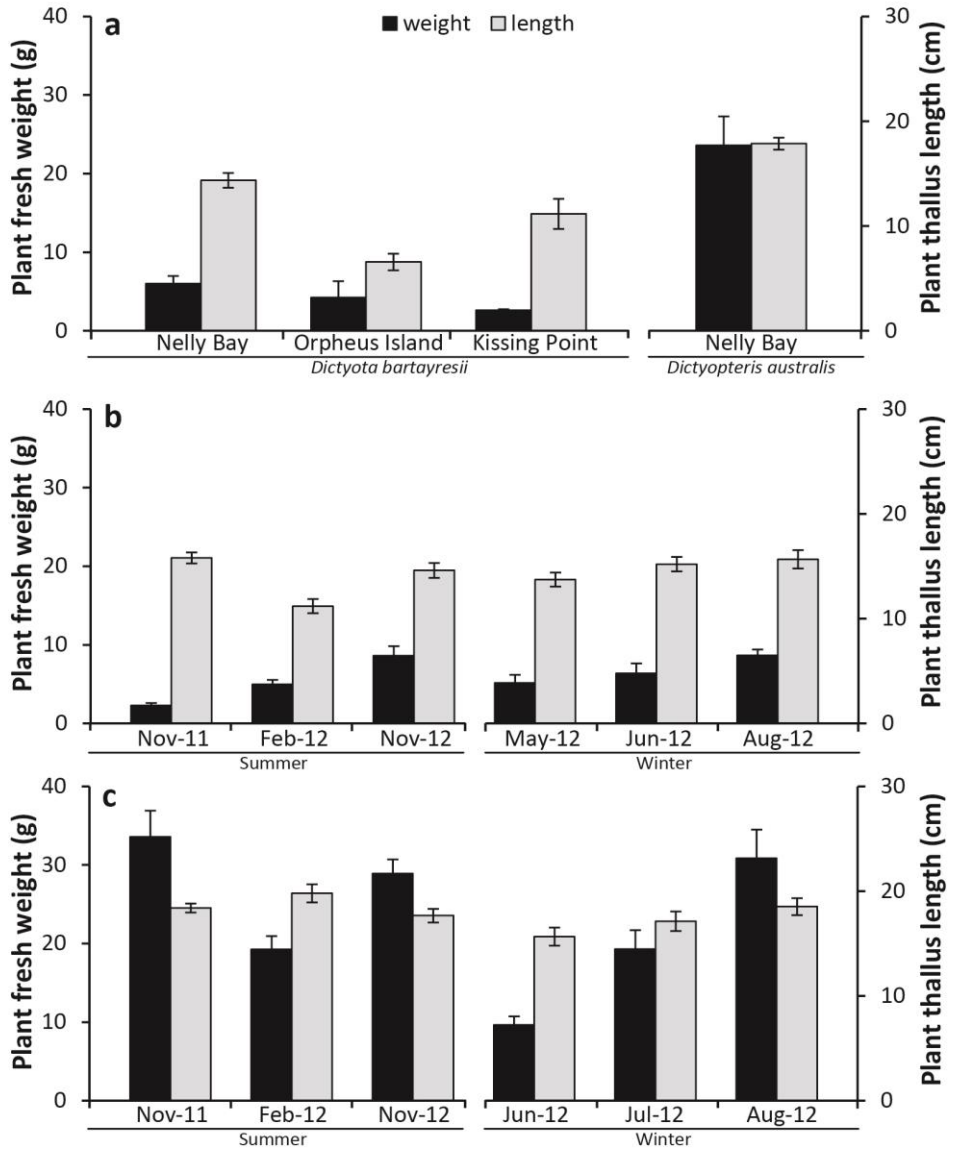

Figure 4.1. Average fresh weight (g ± SE) and plant thallus length (cm ± SE) as **a** annual averages (based on monthly averages with n months for each location and with each month being the average of 15 individual plants) of *Dictyota bartayresii* from different locations (Nelly Bay (n = 6), Orpheus Island (n = 3), Kissing Point (n = 2)) and *Dictyopteris australis* from Nelly Bay (n = 6), **b** monthly averages (n = 15 plants for each month) of *Dictyota bartayresii* from Nelly Bay and **c** monthly averages (n = 15 plants for each month) of *Dictyopteris australis* from Nelly Bay.

4.3.2. Total fatty acid content - plant sizes

TFA content was not significantly correlated to the fresh weight (g) of individual plants in *D. bartayresii* ($r = -0.067$, $p = 0.398$, $n = 161$), however, plants with longer thalli (cm) had a significantly higher content of TFA across all locations ($r = 0.722$, $p < 0.001$, $n =$

161). This relationship was also strong if analyzed separately for Nelly Bay (r = 0.535, p < 0.001, n = 90), Kissing Point (r = 0.529, p < 0.001, n = 27) and Orpheus Island (r = 0.480, p < 0.001, n = 44) (Figure 4.2ab). In *D. australis*, there was no relationship between the TFA content and either fresh weight (r = 0.145, p = 0.206, n = 78) or thallus length (r = 0.050, p < 0.657, n = 81) (Figure 4.2cd).

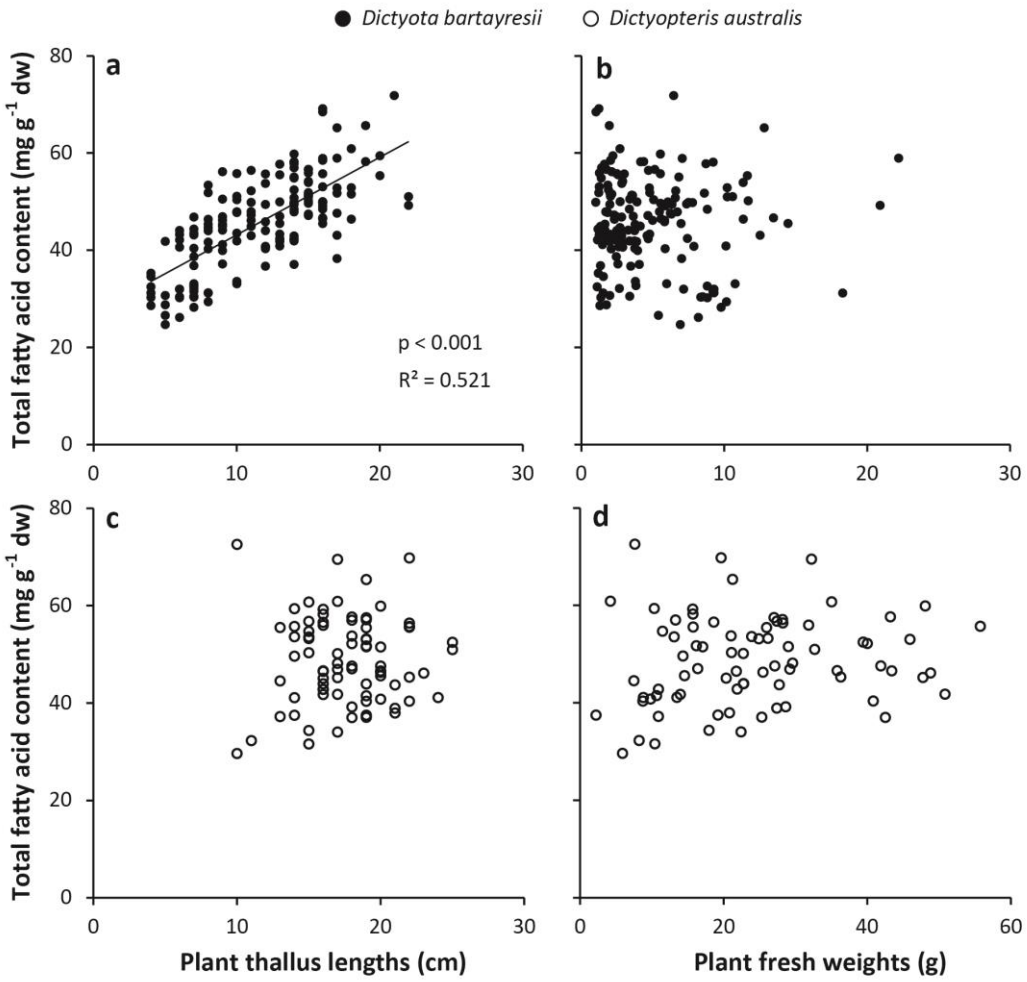

Figure 4.2. Relationship between TFA content (mg g^{-1} dw) with individual plant thallus lengths (cm) and plant fresh weights (g) in all individual plants of **a b** *Dictyota bartayresii* (n = 161) and **c d** *Dictyopteris australis* (n = 80).

4.3.3. Total fatty acid content – spatial and seasonal variation

The TFA content in *D. bartayresii* differed substantially between all locations with plants from Kissing Point (54.2 mg g^{-1} dw ± 1.9 SE) having the highest TFA content, followed by plants from Nelly Bay (49.6 mg g^{-1} dw ± 1.9 SE) and Orpheus Island (35.8 mg g^{-1} dw

± 3.6 SE) (Figure 4.3a). The TFA content was higher during summer at both Nelly Bay (51.8 mg g^{-1} dw ± 3.5 SE) and Orpheus Island (38.7 mg g^{-1} dw ± 3.9 SE) compared to winter (47.4 mg g^{-1} dw ± 1.3 SE, 30.1 mg g^{-1} dw) (Table 4.2). At Nelly Bay, TFA also varied on a monthly basis with a 20 % difference in TFA between the months of the highest TFA content in November 2011 (55.4 mg g^{-1} dw ± 1.5 SE) and 2012 (55.3 mg g^{-1} dw ± 1.8 SE) and the months with the lowest TFA content in February (44.8 mg g^{-1} dw ± 1.7 SE) and May (44.8 mg g^{-1} dw ± 1.7 SE) (Figure 4.3b).

Across species, the TFA content in *D. australis* (48.6 mg g^{-1} dw ± 3.1 SE) was similar to *D. bartayresii* from Nelly Bay (49.6 mg g^{-1} dw ± 1.9 SE) but differed from those *Dictyota* plants from either Kissing Point (54.2 mg g^{-1} dw ± 1.9 SE) or Orpheus Island (35.8 mg g^{-1} dw ± 3.6 SE) (Figure 4.3a). In *D. australis*, the broad seasonal pattern was the opposite to that of *Dictyota* with TFA being higher in winter (51.0 mg g^{-1} dw ± 6.3 SE) compared to summer (46.2 mg g^{-1} dw ± 1.6 SE) (Table 4.2). In a similar manner to *Dictyota*, there was monthly variation in the TFA content. While the TFA content was relatively stable during summer (43.3 – 48.6 mg g^{-1} dw), there was large variation during winter with a rapid increase in TFA from June (38.8 mg g^{-1} dw ± 1.8 SE) to July (60.0 mg g^{-1} dw ± 1.7 SE), followed by a slight decrease toward August (54.3 mg g^{-1} dw ± 1.3 SE) (Figure 4.3c).

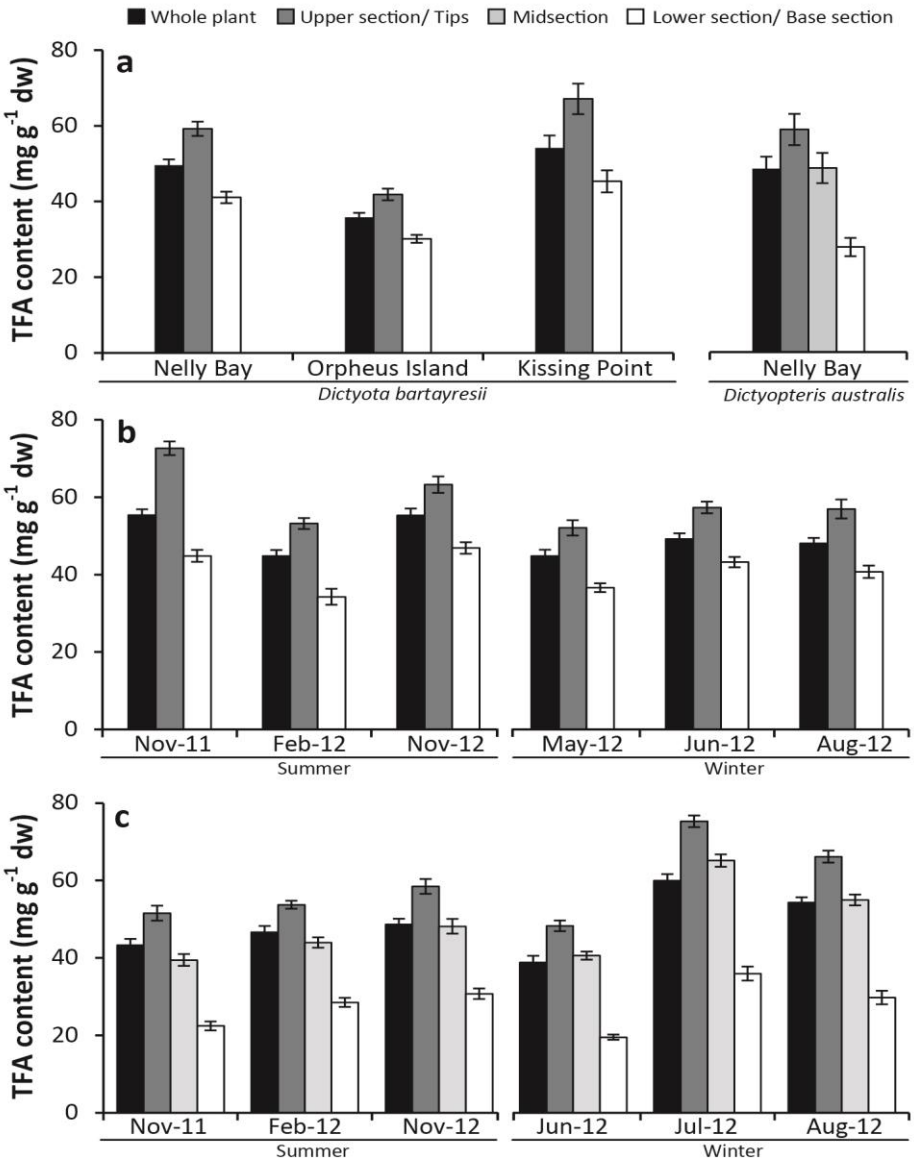

Figure 4.3. Average content of TFA (mg g^{-1} dw ± SE) in 'whole plants' and plant sections (upper section, lower section) in *Dictyota bartayresii* and 'whole plants' and plant sections (tips, midsection, base section) in *Dictyopteris australis* as **a** annual averages (based on monthly averages with n months for each location and with each month being the average of 15 individual plants) of *Dictyota bartayresii* from different locations (Nelly Bay (n = 6), Orpheus Island (n = 3), Kissing Point (n = 2)) and *Dictyopteris australis* from Nelly Bay (n = 6), **b** monthly averages (n = 15 plants for each month) of *Dictyota bartayresii* from Nelly Bay and **c** monthly averages (n = 15 plants for each month) of *Dictyopteris australis* from Nelly Bay.

4.3.4. Total fatty acid content – within-plant variation

The content of TFA also varied within plants of *D. bartayresii* with the upper sections always having a higher TFA content than the lower sections, but with different compositions depending on location and time of the year. The differences between the

upper and lower sections were largest in plants from Kissing Point (39 %), followed by Nelly Bay (36 %) and Orpheus Island (30 %) (Figure 4.3a, Table 4.2). At Nelly Bay, the TFA content in the upper section ranged from 52.1 mg g^{-1} dw ± 2.1 SE in May to 72.6 mg g^{-1} dw ± 1.8 SE in November 2011 while TFA in the lower section ranged from 34.3 mg g^{-1} dw ± 2.1 SE in February to 46.9 mg g^{-1} dw ± 1.6 SE in November 2012. The largest differences between upper and lower sections were in November 2011 (47 %) and the lowest differences in June 2012 (28 %) (Figure 4.3b). In *D. australis*, the TFA content was always highest in the tips, followed by the midsection and the base section, with distinct seasonal variability (Figure 4.3a, Table 4.2). The TFA content in the tips ranged from 48.3 mg g^{-1} dw ± 1.4 SE in June to 75.3 mg g g^{-1} dw ± 1.5 in July, while the midsection had 76 to 81 % of the TFA of the tips, and the base section only 43 to 53 % (Figure 4.3c).

4.3.5. Fatty acid composition - plant sizes

There was significant within-species variation in the composition of fatty acids in both *D. bartayresii* and *D. australis* that was related to individual plant sizes (Figure 4.4). Larger plants of *D. bartayresii*, as measured in fresh weight (g), generally had a higher proportion of PUFA(n-3) ($r = 0.207$, $p = 0.008$, $n = 162$) (Figure 4.4a) and a lower proportion of PUFA(n-6) ($r = -0.331$, $p < 0.01$, $n = 162$) resulting in a lower n-6/n-3 ratio. Other groups of fatty acids (SFA, MUFA) were unrelated to the fresh weight of plants. Furthermore, plants with longer thalli (cm) had less saturated fatty acids ($r = -0.178$, $p = 0.024$, $n = 162$) with a higher proportion of PUFA(n-3) ($r = 0.172$, $p = 0.029$, $n = 162$) (Figure 4.4b) and a higher proportion of PUFA(n-6) ($r = 0.157$, $p = 0.047$, $n = 162$). In *D. australis*, the broad fatty acid groups SFA, MUFA, PUFA(n-3) were unrelated to the fresh weight (g) of plants, however, larger plants tended to have a higher proportion of PUFA(n-6) ($r = 0.290$, $p = 0.01$, $n = 78$) (Figure 4.4cd). Thallus length had no effect on the fatty acid composition in this species.

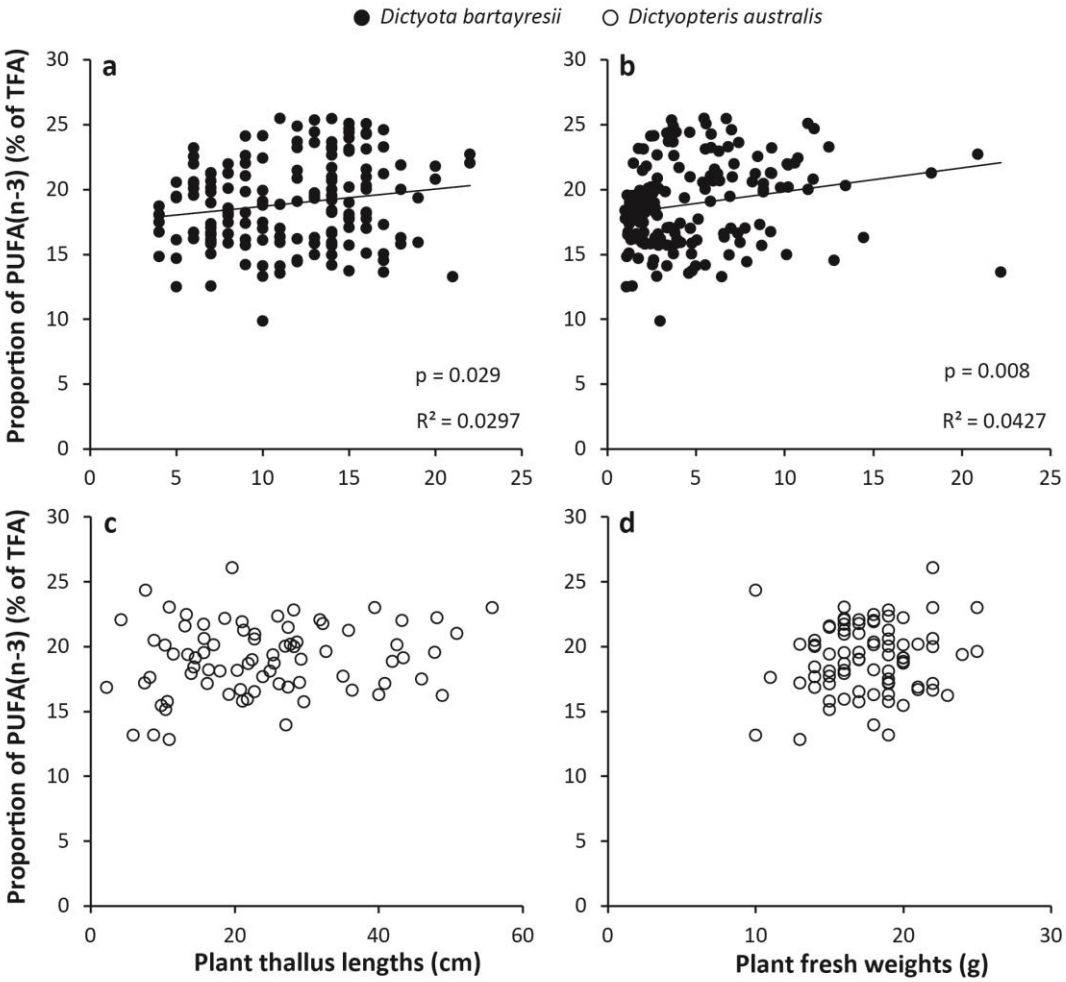

Figure 4.4. Relationship between the proportion of PUFA(n-3) with plant thallus length (cm) and plant fresh weight (g) in all individual plants of **a b** *Dictyota bartayresii* (n = 154) and **c d** *Dictyopteris australis* (n = 80).

4.3.6. Fatty acid composition – spatial and seasonal variation

In addition to the variation in the content of TFA, both species also had a distinct seasonal composition of fatty acids and in the case of *D. bartayresii* there was also a small variation in the composition of fatty acids between locations. Plants of *Dictyota* from Nelly Bay had a lower proportion of SFA (33.6 % of TFA ± 0.8 SE) and a higher proportion of PUFA(n-3) (19.6 % of TFA ± 1.5 SE) than plants from Orpheus Island (35.0 % of TFA ± 0.6 SE, 18.3 % of TFA ± 1.5 SE) or Kissing Point (35.2 % ± 0.2 SE, 17.9 % of TFA ± 0.6 SE). However, the proportion of MUFA was similar across locations (Figure 4.5a). Comparing *Dictyota* and *Dictyopteris* from Nelly Bay (Figure

4.5a), both species had a similar degree of saturation with the most abundant saturated fatty acids being C16:0 and C14:0 (Table 4.2). The proportion of MUFA was higher in *D. bartayresii* (28.4 % of TFA ± 0.8 SE) than in *Dictyopteris* (23.5 % of TFA ± 0.8 SE) but with a lower proportion of C18:1(n-9). Although *D. australis* had a higher proportion of total PUFA (n-3 and n-6), this was due to a higher proportion of PUFA(n-6), with the proportion of PUFA(n-3) being similar between species. However, specific PUFA(n-3) differed between species with *Dictyota* having a higher proportion of C20:5(n-3) and *Dictyopteris* a higher proportion of C18:3(n-3) (Figure 4.6, Table 4.2).

There was also temporal variation in the composition of fatty acids in both species. For *D. bartayresii* collected at Nelly Bay, the general groupings of fatty acids (SFA, MUFA, PUFA(n-3)) differed between summer and winter (Figure 4.5b, Table 4.2). The proportions of SFA and MUFA were higher in summer (35.2 % of TFA ± 0.3 SE, 29.5 % of TFA ± 1.2 SE) than in winter (32.1 % of TFA ± 0.9 SE, 27.3 % of TFA ± 0.4 SE), while the proportion of PUFA(n-3) was higher in winter (22.9 % of TFA ± 0.5 SE) than in summer (16.2 % of TFA ± 0.7 SE). In particular, C18:3(n-3), C18:4(n-3) and C20:5(n-3) were higher in winter, while C20:4(n-3) was higher in summer (Table 4.2). There was also a small difference in the proportion of PUFA(n-6) between summer (16.7 % of TFA ± 1.2 SE) and winter (15.1 % of TFA ± 0.7 SE). The seasonal variation in the broad fatty acid groups at Orpheus Island was similar to that at Nelly Bay with more PUFA(n-3) during winter (21.2 % of TFA) than summer (16.9 % of TFA ± 0.6 SE) (Table 4.2). At Nelly Bay the detailed monthly variation in the broad fatty acid groups was relatively low. The proportion of SFA ranged from 30.1 % of TFA in June to 35.6 % of TFA in February and the monthly variation in PUFA(n-3) was low during both summer (22.1 – 23.6 % of TFA) and winter (15.5 – 17.6 % of TFA) (Figure 4.5b).

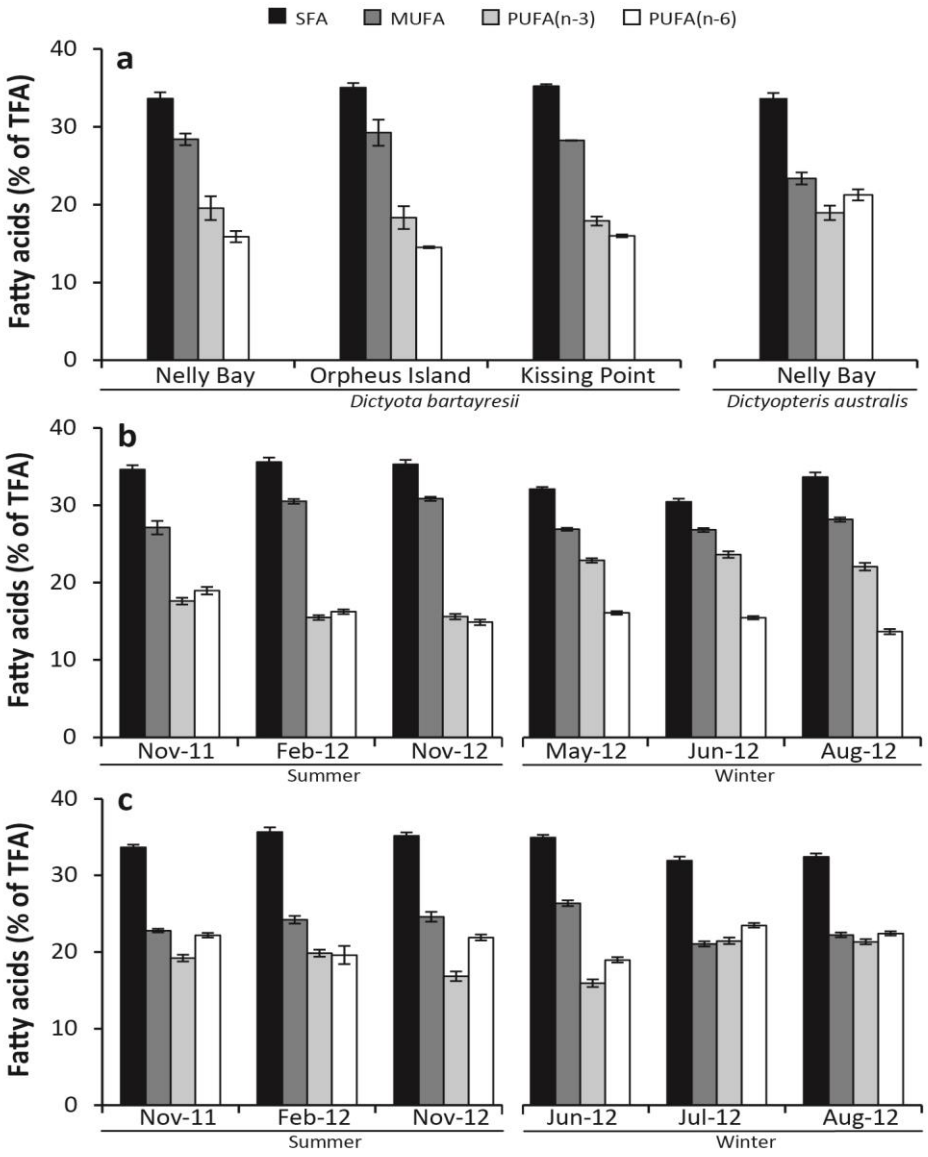

Figure 4.5. Average proportion of fatty acids groups (SFA, MUFA, PUFA(n-3), PUFA(n-6)) (% of TFA ± SE) as **a** annual averages (based on monthly averages with n months for each location and with each month being the average of 15 individual plants) of *Dictyota bartayresii* from different locations (Nelly Bay (n = 6), Orpheus Island (n = 3), Kissing Point (n = 2)) and *Dictyopteris australis* from Nelly Bay (n = 6), **b** monthly averages (n = 15 plants for each month) of *Dictyota bartayresii* from Nelly Bay and **c** monthly averages (n = 15 plants for each month) of *Dictyopteris australis* from Nelly Bay.

In *D. australis* there was no consistent seasonal pattern (summer *vs*. winter) in the general groupings of fatty acids (SFA, MUFA, PUFA(n-3), PUFA(n-6)) and the differences between summer and winter were generally marginal (Table 4.2). However, there was considerable monthly variation in the fatty acid groups (Figure 4.5c). The proportion of

SFA ranged from 31.9 % of TFA in July to 35.7 % of TFA in February. The proportion of MUFA was highest in June (26.4 % of TFA) and sharply decreased to 21.1 % of TFA in July. Both PUFA(n-3) (15.9 % of TFA) and PUFA(n-6) (18.9 % of TFA) were lowest in June and increased to a maximum in July of 21.5 % and 23.5 % of TFA, respectively.

4.3.7. Fatty acid composition - within-plant variation

There was significant variation in the composition of fatty acids between parts of the thallus in *D. australis* with seasonal variation in fatty acids within plants reflecting that of whole plants. The tips of plants of *D. australis* had a higher proportion of SFA and PUFA(n-3), while the base had a higher proportion of MUFA (Table 4.2). There was no significant variation of fatty acids between the upper and lower section of the thallus in *D. bartayresii*, and seasonal variation of the sections again reflected that of whole plants.

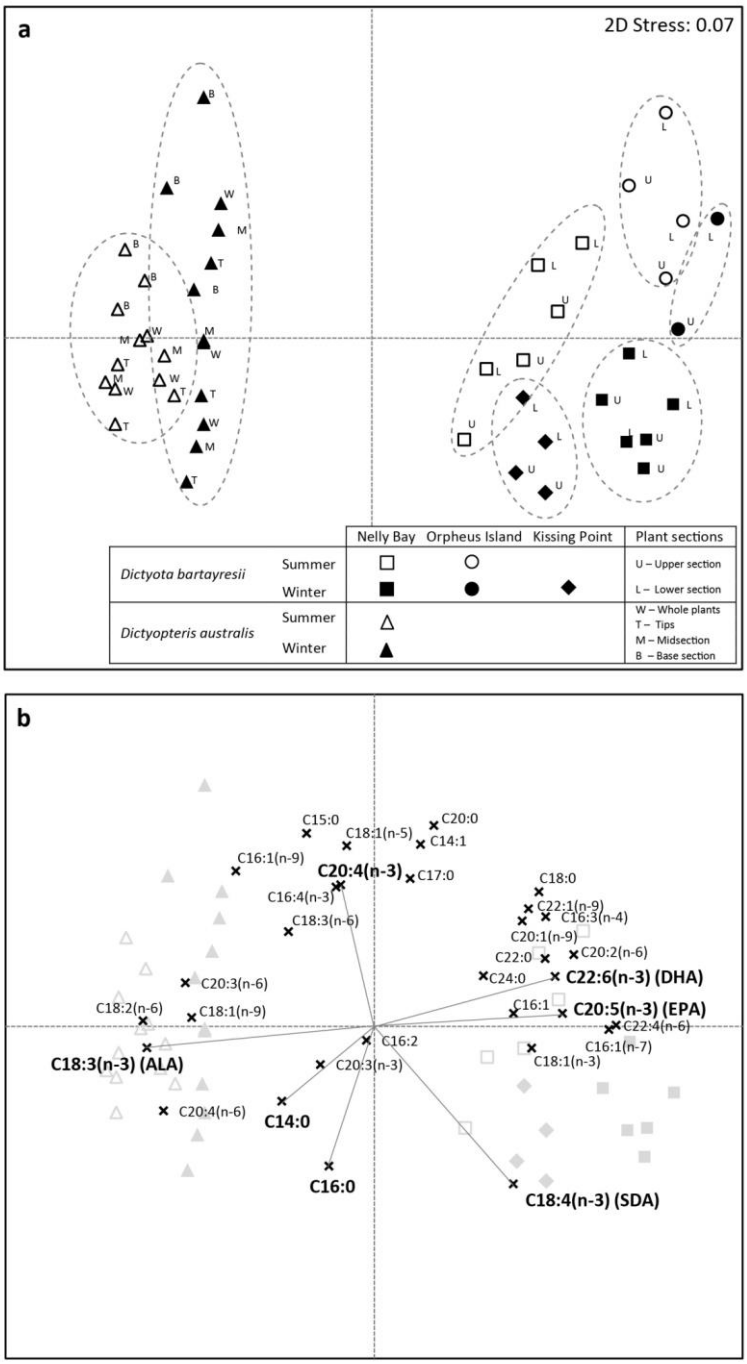

Figure 4.6. Multidimensional Scaling (MDS) showing the differences in average fatty acid composition (% of TFA) (based on monthly averages with n months for each location and with each month being the average of 15 individual plants) in whole plants and plant sections (*Dictyota*: upper section, lower section; *Dictyopteris*: tips, midsection, base section) across locations (*Dictyota*: Nelly Bay (n = 6), Orpheus Island (n = 3), Kissing Point (n = 2); *Dictyopteris*: Nelly Bay (n = 6)) between summer (Nov-11, Feb-12, Nov-12) and winter (*Dictyota*: May-12, Jun-12, Aug-12; *Dictyopteris*: Jun-12, Jul-12, Aug-12). For the particular sampling months at a location and season refer to the materials and methods section. **a** MDS ordinates and **b** MDS ordinates with overlayed vector loadings of individual fatty acids. Vectors only shown for the fatty acids of particular interest and discussed in this Chapter.

Table 4.2. Average TFA content (mg g^{-1} dw ± SE), the average proportion of the broad fatty acid groups (SFA, MUFA, PUFA(n-3), PUFA(n-6)) and selected fatty acids of particular interest (% of TFA ± SE) across locations (*Dictyota*: Nelly Bay (n = 6), Orpheus Island (n = 3), Kissing Point (n = 2); *Dictyopteris*: Nelly Bay (n = 6)) between summer (Nov-11, Feb-12, Nov-12) and winter (*Dictyota*: May-12, Jun-12, Aug-12; *Dictyopteris*: Jun-12, Jul-12, Aug-12). No plants were found at Kissing Point during summer. Averages based on monthly averages with n months for each location and with each month being the average of 15 individual plants. For the particular sampling months at a location and season refer to the materials and methods section. All data: Table A4.1.

	Dictyota bartayresii									***Dictyopteris australis***			
	Nelly Bay - Summer (n = 3)			Kissing Point - Summer			Orpheus Island - Summer (n = 2)			Nelly Bay - Summer (n = 3)			
	Whole plant	Upper section	Lower section	Whole plant	Upper section	Lower section	Whole plant	Upper section	Lower section	Whole plant	Tips	Midsection	Base section
C14:0	6.8 ± 0.0	7.2 ± 0.1	6.0 ± 0.1				6.6 ± 0.1	7.1 ± 0.1	5.8 ± 0.2	8.0 ± 0.6	9.0 ± 0.6	7.6 ± 0.7	6.8 ± 0.7
C16:0	22.0 ± 0.7	22.7 ± 0.8	21.1 ± 0.8				20.2 ± 0.3	20.9 ± 0.1	19.0 ± 0.7	22.7 ± 0.1	23.0 ± 0.1	22.6 ± 0.2	21.1 ± 0.8
C18:3(n-3), ALA	2.3 ± 0.2	2.3 ± 0.1	2.4 ± 0.2				1.7 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	8.2 ± 0.6	7.3 ± 0.3	8.3 ± 0.5	7.0 ± 0.5
C18:4(n-3), SDA	8.7 ± 0.7	9.1 ± 0.7	8.0 ± 0.8				6.6 ± 0.7	6.9 ± 0.7	6.2 ± 0.6	6.1 ± 0.3	6.7 ± 0.4	5.6 ± 0.2	4.0 ± 0.2
C20:5(n-3), EPA	2.8 ± 0.2	2.7 ± 0.1	3.0 ± 0.1	No plants analysed			4.4 ± 0.1	3.9 ± 0.0	4.9 ± 0.2	2.3 ± 0.1	1.9 ± 0.1	2.5 ± 0.1	2.6 ± 0.1
C22:6(n-3), DHA	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0				1.1 ± 0.3	0.8 ± 0.4	1.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
Total SFA	35.2 ± 0.3	36.0 ± 0.1	34.0 ± 0.3				35.1 ± 1.0	36.1 ± 0.9	33.8 ± 1.4	34.8 ± 0.6	35.7 ± 0.6	34.5 ± 0.8	33.5 ± 1.4
Total MUFA	29.5 ± 1.2	29.1 ± 1.2	30.0 ± 1.2				30.8 ± 1.1	30.7 ± 0.9	30.9 ± 1.4	23.9 ± 0.5	22.7 ± 0.3	24.5 ± 0.3	27.3 ± 0.4
Total PUFA	34.8 ± 1.7	34.3 ± 1.6	35.4 ± 1.8				33.6 ± 0.6	32.6 ± 0.6	34.9 ± 0.4	40.4 ± 1.0	40.7 ± 0.7	40.1 ± 0.9	38.2 ± 0.6
Total PUFA(n-3)	16.2 ± 0.7	16.3 ± 0.6	16.0 ± 0.8				16.9 ± 0.6	16.4 ± 0.5	17.5 ± 0.6	18.6 ± 0.9	17.8 ± 0.7	18.7 ± 0.5	15.9 ± 0.7
Total PUFA(n-6)	16.7 ± 1.2	16.4 ± 1.1	17.1 ± 1.2				14.5 ± 0.2	14.2 ± 0.2	15.1 ± 0.2	21.2 ± 0.8	22.4 ± 0.3	20.7 ± 0.6	21.6 ± 1.2
n-6/n-3	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.1				0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.4 ± 0.1
TFA (mg g^{-1} dw)	51.8 ± 3.5	63.0 ± 5.6	42.0 ± 3.9				38.7 ± 3.9	44.6 ± 3.9	32.4 ± 3.6	46.2 ± 1.6	54.6 ± 2.0	43.9 ± 2.5	27.2 ± 2.5
	Nelly Bay - Winter (n = 3)			Kissing Point - Winter (n = 2)			Orpheus Island - Winter (n = 1)			Nelly Bay - Winter (n = 3)			
	Whole plant	Upper section	Lower section	Whole plant	Upper section	Lower section	Whole plant	Upper section	Lower section	Whole plant	Tips	Midsection	Base section
C14:0	7.1 ± 0.1	7.4 ± 0.1	6.6 ± 0.2	7.7 ± 0.0	8.1 ± 0.0	7.3 ± 0.0	6.8	7.1	5.6	7.4 ± 0.5	8.2 ± 0.5	7.2 ± 0.5	6.4 ± 0.5
C16:0	19.9 ± 0.8	20.3 ± 0.8	19.6 ± 0.8	23.4 ± 0.1	24.0 ± 0.3	22.6 ± 0.5	19.7	21.1	19.7	20.1 ± 0.6	21.3 ± 0.6	20.3 ± 0.3	17.7 ± 0.9
C18:3(n-3), ALA	3.3 ± 0.2	3.3 ± 0.3	3.3 ± 0.2	2.8 ± 0.1	2.8 ± 0.2	2.8 ± 0.0	2.0	1.4	1.5	6.5 ± 0.3	6.0 ± 0.4	7.0 ± 0.3	5.7 ± 0.4
C18:4(n-3), SDA	12.5 ± 1.1	12.8 ± 1.1	12.1 ± 1.0	10.8 ± 0.5	11.2 ± 0.8	10.3 ± 0.1	8.6	7.5	6.8	7.9 ± 1.6	8.5 ± 1.4	8.2 ± 1.8	5.5 ± 1.1
C20:5(n-3), EPA	4.8 ± 0.2	4.8 ± 0.1	4.9 ± 0.3	3.3 ± 0.3	3.2 ± 0.4	3.4 ± 0.1	7.7	3.9	5.1	2.5 ± 0.2	2.2 ± 0.2	2.6 ± 0.3	2.8 ± 0.3
C22:6(n-3), DHA	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	1.4	1.2	1.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total SFA	32.1 ± 0.9	32.5 ± 1.0	31.5 ± 0.9	35.2 ± 0.2	36.0 ± 0.6	34.3 ± 0.1	34.9	35.2	34.5	33.1 ± 0.9	34.1 ± 1.0	32.6 ± 1.4	31.6 ± 0.7
Total MUFA	27.3 ± 0.4	26.8 ± 0.5	27.9 ± 0.3	28.2 ± 0.0	27.9 ± 0.5	28.6 ± 0.5	26.1	25.8	26.5	23.2 ± 1.6	21.7 ± 1.5	23.1 ± 1.8	27.0 ± 1.0
Total PUFA	39.1 ± 0.9	39.0 ± 1.1	39.1 ± 0.7	35.0 ± 0.4	34.7 ± 1.3	35.4 ± 0.4	36.9	36.9	37.0	42.1 ± 2.9	42.7 ± 2.5	42.8 ± 3.4	40.2 ± 1.8
Total PUFA(n-3)	22.9 ± 0.5	23.0 ± 0.6	22.7 ± 0.4	17.9 ± 0.6	18.2 ± 1.3	17.7 ± 0.2	21.2	21.7	20.7	19.6 ± 1.8	18.9 ± 1.1	20.5 ± 2.1	17.0 ± 2.2
TOTAL PUFA(n-6)	15.1 ± 0.7	15.0 ± 0.7	15.1 ± 0.7	16.0 ± 0.2	15.6 ± 0.2	16.4 ± 0.2	14.5	14.1	14.8	21.6 ± 1.4	22.9 ± 1.7	21.4 ± 1.6	22.6 ± 0.2
n-6/n-3	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.0	0.7	0.7	0.7	1.1 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	1.4 ± 0.2
TFA (mg g^{-1} dw)	47.4 ± 1.3	55.4 ± 1.7	40.2 ± 1.9	54.2 ± 1.9	67.1 ± 4.4	45.3 ± 0.8	30.1	36.4	25.5	51.0 ± 6.3	63.2 ± 7.9	53.6 ± 7.1	28.4 ± 4.8

TFA total fatty acids, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *ALA* α-linolenic acid, *SDA* stearidonic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *dw* dry

4.4. Discussion

Both *Dictyota* and *Dictyopteris* were present at the sampling sites throughout the year with relatively uniform thallus lengths but seasonal variation in the biomass of individuals. Overall, both species had an annualized TFA content of 5 % dw, of which approximately 20 % was in the form of PUFA(n-3). Spatial variation in the fatty acids of *Dictyota* between locations was mainly restricted to differences in TFA (~ 40 %), while the fatty acid composition was similar, and is possibly related to distinct populations of *Dictyota* or spatial variation in the broad environmental conditions at the three locations. Seasonal variation in the TFA content (~ 10 %) in both species was small and possibly related to the seasonal variation of water temperature and light or plant sizes, but with no general pattern emerging. *Dictyota* had a slightly higher TFA content in summer with a higher proportion of PUFA(n-3) in winter while *Dictyopteris* had a higher TFA content in winter and a higher proportion of PUFA(n-3) in summer, highlighting the significance of species-specific seasonal harvest strategies. There was also within-plant variation in fatty acids with a higher TFA content in the tips decreasing toward the base while fatty acid composition was relatively uniform within plants. Overall, it is unlikely that there is a single environmental or biotic driver that shapes the fatty acid patterns in seaweeds as the effects were species-specific in strength and direction.

4.4.1. Distribution and plant sizes

D. bartayresii was present year round at all sampling sites aside from Kissing Point which only had floating fragments present during summer. This is in agreement with previous records from Kissing Point where no *D. bartayresii* plants were found during summer (Dec, Jan, Feb) (Ngan and Price 1980). The absence of *Dictyota* from Kissing Point is possibly related to the warmer summer temperature at this location (summer 1961 – 1971: 28.6 – 31.2°C, Kenny 1974) compared to Nelly Bay (summer 2002 – 2011: 28.0 – 29.8°C, AIMS 2014) and Orpheus Island (summer 2002 – 2011: 27.2 – 29.0°C, AIMS 2014). Many species of *Dictyota* have a narrow range of thermal tolerance that

restricts their distribution (Tronholm et al. 2012) and they appear as summer annuals at locations close to their lower thermal limit (Peckol 1982) and as winter annuals when close to their upper thermal limit (Ateweberhan et al. 2005). *Dictyopteris* was restricted to the fringing reef habitat on Magnetic Island with no plants found on Orpheus Island or at Kissing Point and I therefore suggest this species has specific requirements for substrate and other physical environmental conditions.

Both *Dictyota* and *Dictyopteris* had relatively uniform monthly thallus lengths of 14 cm and 18 cm respectively. The average fresh weight, however, varied between months but with no seasonal pattern. This is in contrast to annual species with well-defined seasonal growth patterns such as *S. macrodontum* (Chapter 3; Gosch et al. 2014) or *Sargassum baccularia* (Schaffelke and Klumpp 1997). Thallus length can be closely related to the stage of development as demonstrated for *Dictyota cervicornis* (Ateweberhan et al. 2005) and *Dictyota dichotoma* (Hwang et al. 2005) where reproduction was observed in the largest plants, and also to different life cycle stages as sporophytes of *D. dichotoma* were significantly larger than non-fertile specimens (Tronholm et al. 2008). While monthly variation in average thallus length in *D. bartayresii* was small, there was a large range of individual plant sizes each month suggesting that plants of different age and life cycle stage coexist year round with continuous population renewal. Such non-synchronous size structure has been described for *Dictyota cervicornis* (Ateweberhahn et al. 2005) and a detailed analysis of the life cycle in *D. dichotoma* showed that plants of different life cycle stages coexist in successive and overlapping generations (Tronholm et al. 2008).

4.4.2. Total fatty acid content and composition

The annualized mean TFA content in *Dictyota* and *Dictyopteris* was approximately 5 % dw which is towards the upper range of TFA measured in seaweeds. Only a few other species, such as *Derbesia tenuissima* (5 % dw) (Magnusson et al. 2014) and *S. macrodontum* (5 – 8 % dw) (Chapter 3; Gosch et al. 2014), have a similar or higher TFA

content while the majority of seaweed species have a TFA content below 3 % dw (Chapter 2; Gosch et al. 2012; Schmid et al. 2014). Both *Dictyota* and *Dictyopteris* had a fatty acid composition of 16 – 23 % PUFA(n-3) which is higher than most terrestrial oil crops (Dubois et al. 2007) and comparable to some of the commercially utilized seaweeds such as the kelp *Laminaria ochroleuca* (25 % of TFA) (Sánchez-Machado et al. 2004) and *Chondrus crispus* (22 % of TFA) (van Ginneken et al. 2011) but lower than others such as *Undaria pinnatifida* (45 % of TFA) (Sánchez-Machado et al. 2004) and *Palmaria palmata* (63 % of TFA) (van Ginneken et al. 2011). However, all of these seaweeds have considerably lower TFA contents than *D. bartayresii* and *D. australis* (*L. ochroleuca*: 0.92 % dw (total lipid), *C. crispus*: 0.13 % dw, *U. pinnatifida*: 0.92 % dw, *P. palmata*: 0.72 % dw; Fleurence et al. 1994; Sánchez-Machado et al. 2004) and accordingly also have a lower PUFA(n-3) content. This highlights the importance of considering both the TFA content and the fatty acid composition when selecting species for the production of oil-based bioproducts.

The composition of PUFA(n-3) was diverse with a high proportion of C18:4(n-3) (stearidonic acid, SDA) in both *Dictyota* (6.6 – 12.5 % of TFA) and *Dictyopteris* (6.1 – 8.1 % of TFA). SDA has an important role in cardiovascular health (Guil-Guerrero 2007) and is a characteristic fatty acid of brown seaweeds (Chapter 2; Gosch et al. 2012). The essential PUFA C18:3(n-3) (ALA) was particularly abundant in *Dictyopteris* (6.5 – 8.1 % of TFA) and is considered beneficial for the function of the cardiovascular system and liver, and is an effective agent in the redistribution of body fat as demonstrated in animal trials (Poudyal et al. 2012, 2013). While ALA is also abundant in certain higher plants (Dubois et al. 2007), and the intake of ALA through the consumption of nuts, seeds and vegetable oils is considered adequate in the western diet (Harris 2005), the long-chained PUFA(n-3) C20:5(n-3) (EPA) and C22:6(n-3) (DHA) are deficient and supplementation is recommended (Burdge 2004). EPA and DHA, in particular, are highly beneficial with anti-inflammatory properties, improved fetal development and a positive impact on the

cognition in infants and children (Dunstan et al. 2007; Krauss-Etschmann et al. 2008). Unlike higher plants, algae – which includes seaweed – convert ALA to EPA and DHA through elongation and desaturation (Bell and Tocher 2009; Pereira et al. 2003) and are therefore sources for these long-chained PUFA(n-3). In this Chapter, *Dictyota* had a relatively high proportion of EPA (2.8 – 7.7 % of TFA) compared to *Dictyopteris* (2.3 – 2.5 % of TFA), but like other brown seaweeds (Chapter 2; Gosch et al. 2012; Kumari et al. 2010) the proportion of DHA (< 1.4 % of TFA) was low. Of importance for the oil quality is also the degree of saturation, as saturated fats are generally considered detrimental from a health perspective (Kris-Etherton and Yu 1997) and are associated with increased plasma cholesterol levels (Hunter 2001). The saturation in both *Dictyota* and *Dictyopteris* was 32 – 35 % of TFA which is at the lower range of seaweed species (Chapter 2; Gosch et al. 2012; Kumari et al. 2013a) with the most abundant SFA being C16:0 followed by C14:0. Overall, both species had a high quantity of the "high quality" fatty acids, defined by a high proportion of PUFA(n-3) and a low degree of saturation compared to other seaweeds. These results highlight their suitability for the production of oil-based health and food products.

4.4.3. Seasonal and spatial variability in fatty acids

Although the oil quantity and quality are the paramount selection criteria for the commercial utilization of a bioresource feedstock for oil-based products, a consistent quantity and quality of the oils is also preferable (Gellenbeck 2012; Hafting et al. 2012). In *Dictyota*, location had the largest impact on the variability of the TFA content (~ 40 %) with the highest TFA content found in plants from Kissing Point and the lowest TFA content in plants from Orpheus Island. The broad seasonal variation in TFA contents in *Dictyota*, and also in *Dictyopteris*, was very low differing 10 % between summer and winter. It is possible that the spatial variability in TFA content is caused by genotypic variation between different populations of *Dictyota* which is also reflected in different plant sizes. Genetic diversity in populations of *D. dichotoma* has been linked to long-term

adaption to specific environmental conditions and geographic isolation (Hwang et al. 2005). While variation in fatty acids based on genotype and subsequent strain selection for improved fatty acid contents and composition is well researched and practiced in microalgae (Grima et al. 1995; Rodolfi et al. 2009), little information on the heritability of fatty acid properties is available in seaweed (Robinson et al. 2013). However, the heritability of other biochemical compounds such as furanones (Wright et al. 2004) and phlorotannins (Honkanen and Jormalainen 2005) has been demonstrated in seaweeds and might also exist for fatty acids.

Furthermore, the environmental conditions at the three locations were distinct, which is reflected in larger monthly temperature fluctuations in shallow intertidal habitats like Kissing Point (Port of Townsville (1961 – 1971): 21.8 – 31.2°C, Kenny 1974) and Nelly Bay (2002 – 2011: 21.9 – 29.8°C, AIMS 2014) as opposed to the more offshore location at Orpheus Island (2002 – 2011: 22.1 – 29.0°C, AIMS 2014). It is therefore possible that these factors (genotype, environment), or an interaction thereof, shaped the fatty acid content and composition in this seaweed. The direct effect of water temperature (Al-Hasan et al. 1991) and light (Floreto et al. 1993; Hotimchenko 2002) on the TFA content (or lipid content) has been demonstrated experimentally, and seasonal field studies support a pattern where higher content of TFA occur during the winter when light and water temperature are at their annual minimum (Chapter 3; Gosch et al. 2014; Nelson et al. 2002). In this Chapter I did not detect a general seasonal pattern in the TFA content as *Dictyota* had its highest TFA content in summer when water temperature and light availability were at their annual maximum while *Dictyopteris* had a higher TFA content in winter at the same sampling location when water temperature and light availability were at their annual minimum. Although the degree and direction of seasonal TFA contents can be species specific (Schmid et al. 2014), and environmental conditions have potentially opposing effects on individual species, it appears unlikely that water temperature and light are the main drivers for the observed seasonal variability as I also

observed erratic monthly fluctuations in the TFA content of up to 40 % within a timeframe of relatively stable light intensity and water temperature (Table 4.1; AIMS 2014).

It is therefore likely that biotic factors such a plant size and life stages of individual plants had an impact on the TFA content. In *Dictyota*, plants with longer thalli had consistently higher TFA contents and monthly TFA contents generally followed monthly thallus length. The relationship between plant size and the TFA content has been demonstrated for the brown seaweed *Costaria costata* where larger plants had higher TFA contents (Gerasimenko et al. 2010) and this might also be related to the reproductive stage of individual plants (Honya et al. 1994). Although I did not determine the age or life history stages of individual plants, it is likely that these parameters are directly related to plant sizes as demonstrated for other species of *Dictyota* (Ateweberhahn et al. 2005; Tronholm et al. 2008). In *Dictyopteris* I did not detect any size-dependent TFA relationships that would explain seasonal variability in TFA. It is therefore possible that plant size and development stage are not directly related as in *Dictyota.*

In a similar manner to TFA content, there was also seasonal variation in the composition of fatty acids in both species. *Dictyota* from both Nelly Bay and Orpheus Island had a consistently higher proportion of PUFA(n-3) and a less saturated fatty acid composition during winter than summer. The elevated PUFA(n-3) content in winter can be a physiological response to colder water temperature and lower light availability. PUFA have a lower melting point than saturated fatty acids and therefore provide a physiological advantage in cold water environments as membrane fluidity increases concomitantly with PUFA concentrations (Los et al. 2013; Thomson et al. 1992). Furthermore, some algae respond to low light conditions with increased production of chloroplasts and associated membrane lipids (Sharma et al. 2012) which have high proportions of PUFA(n-3) (Sanina et al. 2004). A high proportion of PUFA(n-3) can

increase the fluidity of the thylakoid membranes and so increase the electron flow in the chloroplast (Mock and Kroon 2002) and provide a physiological advantage at low light conditions. The positive effects of colder water temperatures (Al-Hasan et al. 1991; Floreto et al. 1993) and lower light availability (Hotimchenko 2002; Khotimchenko and Yakovleva 2005) on the concentration of PUFA in seaweed has been demonstrated and is largely supported by seasonal field studies (Nelson et al. 2002; Nomura et al. 2013). However, this pattern cannot be generalized as some species, including *Dictyopteris* from the current study, have higher proportions of PUFA(n-3) during the warmer summer months (Honya et al. 1994; Schmid et al. 2014) suggesting that the effect of water temperature and light availability is small and other factors have a more profound effect on the fatty acid composition in these species.

The effect of plant sizes on the composition of fatty acids was fairly weak in this Chapter with larger *Dictyota* plants (length, weight) having slightly more PUFA(n-3) and larger plants (length) of *Dictyopteris* having a higher proportion of PUFA(n-6). Size-dependent variation in fatty acid composition has also been demonstrated for species of *Caulerpa* where larger plants had a lower proportion of EPA and the harvest of smaller plants therefore provides a biomass feedstock of higher quality (Paul et al. 2014). Size-dependent variation in fatty acid composition can be related to developmental stage and degree of maturation as demonstrated for *Saccharina japonica* where PUFA decreased as plants matured (Honya et al. 1994). However, from a practical perspective, the small measured variability in fatty acid composition in relation to plant sizes in both *Dictyota* and *Dictyopteris* is negligible, and the broad seasonal changes have a more profound impact on the nutritionally important proportion of PUFA(n-3).

4.4.4. Within-plant fatty acid variation

Although location and to a lesser degree the time of harvest appear to be the most important sources of variation in fatty acids in whole plants, I also found within-plant

variation in the TFA content and composition. The TFA content decreased in both *Dictyota* and *Dictyopteris* from the tips (upper section) toward the base (lower section) consistent with previous analysis of fatty acids within plants of *S. macrodontum* (Chapter 3; Gosch et al. 2014). Such within-plant variation in TFA content (or total lipid content) can be caused by heterogenic microhabitats experienced by different parts of the thallus where the upper sections are exposed to high light conditions while the lower sections are shaded. The effect of microhabitats on the within-plant variation of pigments and other biochemical compounds has been demonstrated (Stengel and Dring 1998) and is likely also present for fatty acids. It has been shown for seaweed that total lipid content decreased in the shade (Hotimchenko 2002) and increased with increasing light intensity (Floreto et al. 1993) which indicates a self-shading effect on the within-plant variation of fatty acids in this Chapter. Different sections of the thallus are also affected by biotic factors such as grazing and fouling with accordingly heterogeneous within-plant production and distribution of chemical defence compounds (de Nys et al. 1996). Antimicrobial activity has also been attributed to unsaturated fatty acids in a range of seaweeds (Alamsjah et al. 2008) with a non-uniform level of activity within the thallus (Rosell and Srivastava 1987) supporting corresponding variation in the content of unsaturated fatty acids.

Within-plant variation in fatty acids may also be related to morphological and functional differentiation in the thallus, with a structural base for attachment to the substratum and bioactive midsections and tips which have various functions including photosynthesis and reproduction (Lawrence and McClintock 1988). In particular the membranes of the light harvesting complex are associated with a high content of glycolipids which contain proportionally more PUFA(n-3) fatty acids, in particular C18:3(n-3) and C20:5(n-3) (Sanina et al. 2004), which would explain the higher proportion of these fatty acids found in the tips of *Dictyopteris*. This is supported by analysis of lipid classes within plants of *Sargassum miyabei* and *Saccharina japonica* where the upper sections not only had a

higher proportion of glycolipids but also a higher proportion of PUFA(n-3) (Khotimchenko and Kulikova 2005; Kulikova and Khotimchenko 2000).

4.4.5. Biomass application

Overall, both *Dictyota* and *Dictyopteris* are suitable feedstock species for applications in health and nutrition as their TFA content exceeds 5 % dw and is among the highest measured for any seaweed, with 20 % PUFA(n-3) (Gill and Valivety 1997). Although the content of TFA varied considerably between locations and also within plants, the variation in fatty acid composition was generally low over space and time. Only in *Dictyota*, was there a distinct seasonal variation in PUFA(n-3) which could be attributed to changes in water temperature. Such variation in fatty acids is interesting from a biological perspective but can be challenging commercially as a stable supply of fatty acids with consistent content and quality is preferable (Gellenbeck 2012; Hafting et al. 2012). However, it also provides the opportunity to exploit this natural variability and develop culture and harvest strategies that result in optimized yields of desired target fatty acids.

CHAPTER 5

Environmental effects on growth and fatty acids in three isolates of *Derbesia tenuissima* (Bryopsidales, Chlorophyta) [4]

5.1. Introduction

Derbesia tenuissima (Bryopsidales, Chlorophyta) is a species of seaweed recently identified as a biomass resource rich in polyunsaturated omega-3 fatty acids (PUFA(n-3)) with applications in functional foods and nutraceuticals. *D. tenuissima* is a species rich in lipids (> 12 % dw) with a content of TFA greater than 5 % dw of which 40 % are in the form of valuable PUFA(n-3) (Chapter 2, Gosch et al. 2012; Magnusson et al. 2014). It also has a high biomass (15 – 25 g dw m^{-2} day^{-1}), and consequently, fatty acid productivity (0.8 –1.4 g dw m^{-2} day^{-1}) (Magnusson et al. 2014; Mata et al. 2015). Furthermore, the productivity of biomass and fatty acids in *D. tenuissima* is stable over time and consistency in the production of biomass and fatty acids are key criteria for the successful commercialization of new species for the nutraceutical market (Gellenbeck 2012; Hafting et al. 2012). Although the general suitability for cultivation and biomass applications have been established in *D. tenuissima*, biomass and fatty acid productivities are likely not yet fully exploited and may be improved through refined culture strategies of environmental manipulation and the selection of strains with advantageous attributes. The basis for these improvements is the identification and quantification of natural variability in growth and the content or composition of fatty acids, and subsequent quantification of the drivers thereof.

[4] Adapted from: Gosch BJ, Lawton RJ, Paul NA, de Nys R, Magnusson M (2015b) Environmental effects on growth and fatty acids in three isolates of *Derbesia tenuissima* (Bryopsidales, Chlorophyta). Algal Research 9:82-93

The environmental parameters of temperature, light and nutrients are key factors for growth in large, foliose seaweeds (Lobban and Harrison 1997) and it is predicted that they are also important drivers for growth and the content and composition of fatty acids in filamentous, clonal seaweeds such as *Derbesia*. While there is extensive literature on environmental effects on the content and composition of fatty acids in microalgae (Cohen et al. 1988; Solovchenko et al. 2008), research on seaweeds is largely restricted to field studies based on broad environmental correlations with fatty acids (Gosch et al. 2014; Gosch et al. 2015a; Nelson et al. 2002; Schmid et al. 2014) and only a few studies in a limited number of species have experimentally quantified the effects of temperature (Al-Hasan et al. 1991; Floreto et al. 1993) and light (Hotimchenko 2002; Khotimchenko and Yakovleva 2005) on fatty acids. Additionally, while the effect of nitrogen starvation can lead to substantial increases in content and composition of PUFA(n-3) in microalgae (Grima et al. 1995), the quantification of the effects of nitrogen availability on the content and composition of fatty acids in seaweeds is restricted to a few species of *Ulva* (Floreto et al. 1996; Gordillo et al. 2001; Pinchetti et al. 1998) and *Gracilaria* (Dawes et al. 1993). Importantly, the direction and degree of these effects for microalgae and macroalgae are species specific (Gordillo et al. 2001; Dawes et al. 1993; Sharma et al. 2012) and environmental conditions often have opposing effects on growth and the content and composition of fatty acids (Gordillo et al. 2001; Sharma et al. 2012), and so may result in highly specific net changes in fatty acid productivity.

Furthermore, while the content and composition of fatty acids vary considerably between the broad taxonomic groups of red, green and brown seaweeds and at the taxonomic level of families, orders and within a genus (Gosch et al. 2012; Kumari et al. 2013a), there is no evidence for genotypic variation of the content and composition of fatty acids within species of seaweed (Robinson et al. 2013). While strain selection in species and subsequent crop improvements are common in terrestrial plants (Downey and Craig 1964; Khush 2001), and more recently in microalgae with substantial improvements in

growth and the quantity and quality of fatty acids (Grima et al. 1995; Sharma et al. 2012; Rodolfi et al. 2009), this process is still in its infancy in seaweeds (Robinson et al. 2013). Strain selection and subsequent improvements in growth, temperature tolerance and the yield of iodine in the Chinese kelp industries have progressed since the 1960s (Chaoyuan and Guangheng 1987), and there is strong support for a genetic component of basic morphological features (Shao-jun et al. 1997) and growth rates (Lawton et al. 2013b) in seaweeds. A limited number of studies have also confirmed the genotypic variation and heritability of the natural products furanones (Wright et al. 2004) and phlorotannins (Honkanen and Jormalainen 2005) and the selective breeding of the brown seaweed *Macrocystis* has resulted in increases in total lipids and protein compared to natural populations (Westermeier et al. 2012). Importantly however, genotypic variation in the content and composition of fatty acids, in particular the nutritionally important PUFA(n-3), within seaweeds has not been demonstrated (Robinson et al. 2013). Although field-based studies have quantified variability in the content and composition of fatty acids between individual conspecific individuals from within and between spatially isolated populations of seaweeds (Gosch et al. 2012; Gosch et al. 2015a), the distinctions between the effects of local environmental conditions and a genetic component remain speculative.

The quantification and portioning of genetic and environmental components in the variability of growth and the content and composition of fatty acids is the first step to fully exploit the potential of *D. tenuissima* and other target seaweeds. Therefore, I quantified the effects of temperature, light and nitrogen availability as the key environmental factors on the growth and content and composition of fatty acids in three isolates of *D. tenuissima* in controlled growth trials.

5.2. Materials and Methods

5.2.1. Study organism and biomass collection

D. tenuissima is a filamentous marine macroalga found in temperate to tropical waters including the North Atlantic, Mediterranean and South Pacific (van den Hoek and Womersley 1984; Kobara and Chihara 1981; Sears and Wilce 1970). It was selected as an oil rich species (> 12 % dw) with a high content of TFA and a high proportion of PUFA(n-3) (Chapter 2, Gosch et al. 2012). Furthermore, it has high biomass productivities in culture which makes it an interesting species for the production of oil-based bioproducts (Magnusson et al. 2014; Mata et al. 2015). Biomass isolates of this species were collected from an intertidal flat at Rowes Bay, Townsville, Australia (19.14°S, 146.48°E) (isolate 1) and a local pet shop (Pet HQ) in Townsville where it was found as fouling organism in a fish tank (isolate 2). A third biomass sample was collected from the Marine & Aquaculture Research Facility (MARFU) at James Cook University, Townsville were it was identified as a fouling species in cultures of other seaweeds (isolate 3). Biomass of the three isolates was transported to James Cook University, Townsville where cultures were established and scaled up and kept separate as stock cultures under controlled conditions (12h:12h, light:dark cycle, 24°C) in aerated 2 L culture vessels with weekly changes of autoclaved seawater enriched with F/2 medium (Algaboost F/2 (1000×) silicate free, AusAqua) (Guillard and Ryther 1962) (~12 mg N L^{-1}).

5.2.2. Species identification

Strains were identified by comparing the morphology with taxonomic keys (van den Hoek and Womersley 1984) and literature (Kobara and Chihara 1981; Sears and Wilce 1970), and by DNA barcoding. This approach compares short DNA sequences from a standardised region of the genome - the 'barcode' - to a library of reference sequences derived from individuals of known identity (Hebert et al. 2003). Genomic DNA was isolated from fresh tissue samples of each isolate using a Qiagen DNEasy Plant Mini Kit

following the manufacturer's instructions and amplified at the DNA barcoding marker rbcL3' using the primers GrbcLFi (Saunders and Kucera 2010) and 1385R [40]. Polymerase chain reaction (PCR) amplifications were performed in a 25 µl reaction mixture containing 1.5 U of MyTaq HS DNA polymerase (Bioline), 5 x MyTaq reaction buffer, 0.4 µm each primer, and 1 µl of genomic DNA (25 – 30 ng). Amplifications were performed on a BioRad C1000 Thermal Cycler with a touchdown PCR cycling profile (cycling parameters: 5 min at 94°C, 30 cycles of 30 s denaturing at 95°C, 45 s annealing at 56°C with the annealing temperature decreasing by 0.5°C each cycle, 60 s extension at 72°C, and a final extension at 72°C for 5 min). PCR products were column purified using Sephadex G-25 resin and sequenced in both directions by the Australian Genome Research Facility (Brisbane, Australia). If sequences were unreadable or contaminated a second PCR attempt was made and sequenced. If these sequences were also unreadable or contaminated, then DNA was re-extracted from a fresh sample and further PCRs and sequencing attempts were made. All attempts to generate readable sequences were unsuccessful for isolate 3. Attempts were also made to amplify and sequence all isolates at the alternative barcoding marker *tufA* using a range of primers and PCR conditions, however we were unable to obtain readable sequences for any isolate at this marker.

Sequences were edited using Bioedit (Hall 1999) and submitted to GenBank under the accession numbers KM998970 for isolate 1 and KM998971 for isolate 2. Sequence similarity searches using a nucleotide BLAST search in GenBank (http://www.ncbi.nlm.nih.gov/blast/) failed to find an exact match. Therefore we identified our isolates by constructing phylogenetic trees using sequences downloaded from Genbank. All publically available *Derbesia*, *Bryopsis* and *Pedobesia* rbcL sequences were downloaded. Duplicate sequences were removed from each dataset and then all remaining sequences were aligned with ours and trimmed to a standard length in MEGA 5.0 (Tamura et al. 2011). Maximum likelihood (ML) phylogenetic trees were constructed in MEGA using a *Caulerpa filiformis* sequence (Genbank accession:

AY004763) as an outgroup. We used the simple Kimura two-parameter model to estimate genetic distance (Kimura 1980) as this is the standard model of molecular evolution used in barcoding studies (Hebert et al. 2003). The reliability of tree topologies was estimated using bootstrapping (9,999 replicates).

5.2.3. Experimental design

We tested the effects (high *vs.* low) of water temperature (high: 29°C, low: 21°C), nitrogen concentration of the culture medium (high: 12 mg N L^{-1}, low: 3 mg N L^{-1}) and light intensity (high: 100 µmol photons m^{-2} s^{-1}, low: 24 µmol photons m^{-2} s^{-1}) on the specific growth rate (SGR % day^{-1}), internal concentrations of nitrogen and carbon, and the content and composition of fatty acids in three isolates of *D. tenuissima*. As the concentration of phosphorous (P) was kept constant (N:P ratio of 5:1 in initial F/2 media), the N:P ratio was accordingly increased at the high nitrogen treatments. The selected water temperatures represent typical summer and winter seawater conditions at Rowes Bay (Australian Institute of Marine Science) and were controlled in culture chambers (New Brunswick Biological Shakers Innova 44/44R, Eppendorf). The tested nitrogen concentrations were controlled by adding F/8 (3 mg N L^{-1}) to both treatments and an additional 9 mg N L^{-1} in the form of sodium nitrate ($NaNO_3$) to the 'high nitrogen' treatment culture medium. These nitrogen concentrations provided 0.96 mg nitrogen per 80 mL jar in the 'high nitrogen' treatment cultures and therefore provided an excess of nitrogen at an estimated harvest biomass of 25 mg dw^{-1} and an estimated internal nitrogen concentration of 3 % of dw biomass, while the 'low nitrogen' treatment cultures had only 0.24 mg N per jar and therefore nitrogen was clearly limiting at the same estimated biomass growth and internal biomass nitrogen concentration. The culture chambers were equipped with photosynthetic lamps (New Brunswick photosynthetic growth lamp, Eppendorf) and culture light intensity was controlled by using clear 200 mL culture jars with semi-transparent lids for the 'high light' treatments (100 µmol photons m^{-2} s^{-1}) and paper wrapped culture jars with light-blocking lids for the 'low light' treatments (24 µmol

photons m^{-2} s^{-1}). The effects of temperature, light, nitrogen and isolate were tested with a factorial design where each culture treatment in each growth trial was duplicated to account for methodical variability and also to obtain enough biomass for subsequent biochemical analyses. A sample was then considered the average of the duplicate treatments and was replicated 3 times in successive and identical growth trials.

5.2.4. Acclimation and growth trials

Biomass was acclimated in culture chambers in 200 mL plastic jars filled with 80 mL autoclaved seawater and added F/4 medium (~ 6 mg N L^{-1}) seven days prior to a growth trial. A total of 10 jars for each isolate were stocked with approximately 250 mg fresh weight (fw) biomass per jar at conditions that were similar to the experimental growth trials with a 12h:12h light:dark photoperiod and activated shaking function of the culture chamber (100 rpm) to provide gas exchange (water movement) and prevent biomass from attaching to the culture jars. However, water temperature (24°C) and nitrogen concentration (F/4 ~ 6 mg N L^{-1}) were set intermediate of the specified treatment conditions of the growth trials to account for possible stress responses and therefore bias toward either of the tested treatment conditions. After the acclimation period, the biomass of each isolate was harvested separately and spin-dried for 3 minutes and considered as 'fresh weight' (fw) biomass. Three random 150 mg fw biomass samples from the fresh weight biomass of each isolate were directly frozen to -80°C and freeze-dried to calculate the average fresh weight to dry weight ratio (fw:dw) of the biomass in each growth trial. For each growth trial, 200 mL plastic jars filled with 80 mL autoclaved seawater and treatment specific nitrogen addition were inoculated with 20 mg ± 1 mg fresh weight biomass and randomly placed in the incubator chambers. Positions of jars were randomly changed every day. After a 7 day culture period, samples were harvested, and stored at -80°C. The growth trial was replicated 3 times with new and acclimated biomass from the stock cultures.

5.2.5. Carbon, nitrogen and fatty acid analysis

Frozen samples were freeze-dried and ground to a fine powder of which 10 mg were sent to OEAlabs (www.oealabs.com) for analysis of internal carbon and nitrogen content while another 20 mg were used for the analysis of fatty acids. A direct transesterification method was used to simultaneously extract and esterify the fatty acids to fatty acid methyl esters (FAMEs) for analysis by gas chromatography mass spectrometry (GC-MS; 7890A GC, 5975C MS, DB-23 capillary column with 15 µm cyanopropyl stationary phase, 60 m length and 0.25 mm inner diameter (Agilent Technologies Australia Pty Ltd.)), as described in detail in Chapter 2 (Gosch et al. 2012). The content of TFA was determined as the sum of all FAMEs with fatty acids being designated as CX:Y(n-z), where X is the total number of carbon, Y is the number of double bonds, and z is the position of the ultimate double bond from the terminal methyl group.

5.2.6. Growth rates

Growth of biomass was determined as specific growth rate (SGR) with the following equation SGR (% day^{-1}) = 100 x [$\ln(W_f/W_i)$] / t; with W_f and W_i being the final and initial dry weight biomass of the samples and t the culture period (7 days). Although the initial stocking biomass was measured out as fresh weight biomass, it was converted to dry weight on the basis of the fresh weight to dry weight conversion factor estimated for three replicates for each growth trial and isolate.

5.2.7. Statistical analysis

Four-way factorial ANOVA and Tukey's honest significant difference (HSD) post-hoc tests (IBM SPSS Statistics version 21) were used to test for the effects of temperature, light, nitrogen and isolate (all fixed factors) on the SGR, C:N ratio, TFA and fatty acid composition (SFA, MUFA, PUFA(n-3) and PUFA(n-6)) in *D. tenuissima* with 3 replicate samples for each treatment combination. Eta-squared (%) $\eta^2 = SS_{factor} / SS_{total} \times 100$; with SS_{factor} and SS_{total} being the sum of squares of a particular factor and the total sum of

squares respectively was calculated as a measure of effect size describing the proportion of variation (%) of the total variation of the independent variable explained by a particular factor or factor interaction (Richardson 2011). The relationships between the SGR and the C:N ratio with the content of TFA and fatty acid composition (SFA, MUFA, PUFA(n-3), PUFA(n-6)) were analysed by correlations (Correlation, IBM SPSS version 21). The variation in average composition of fatty acids (% of TFA) between the three isolates (iso1, iso2, iso3) and the different culture treatment conditions of temperature, nitrogen and light was analysed using non-metric multidimensional scaling (MDS, Primer 6). Groups of samples forming distinct groups based on their composition of fatty acids were visualized as shaded circles. A vector loading bi-plot (Pearson's product correlations) was used to visualize the relative load of individual fatty acids for the samples, with the lengths and directions of the vectors representing the strength and direction of correlations.

5.3. Results

5.3.1. Species and isolate identification

The sequences of isolates 1 and 2 formed a distinct, well supported (99 % bootstrap support) clade that did not contain any other Genbank samples (Figure 5.1). These sequences fell within a larger, well supported (86 % bootstrap support) clade that was distinct from all other sections of the phylogenetic tree and contained *Derbesia marina* and a sequence identified as *Derbesia* sp. 1GWS. These results demonstrated that isolates 1 and 2 are from the genus *Derbesia* and not *Pedobesia*, and more specifically are not the species *D. marina* (Figure 5.1). There were no *D. tenuissima* sequences available in Genbank for the region of the rbcL gene that was sequenced. However, samples matched morphological descriptions of *D. tenuissima* (van den Hoek and Womersley 1984; Kobara and Chihara 1981; Sears and Wilce 1970) (Figure 5.2, Table 5.1) including the general characteristics of cylindrical siphonous filaments without septa, infrequent

branching, and elongated chloroplast (5 – 10 µm) with a single pyrenoid (Figure 5.2, Table 5.1).

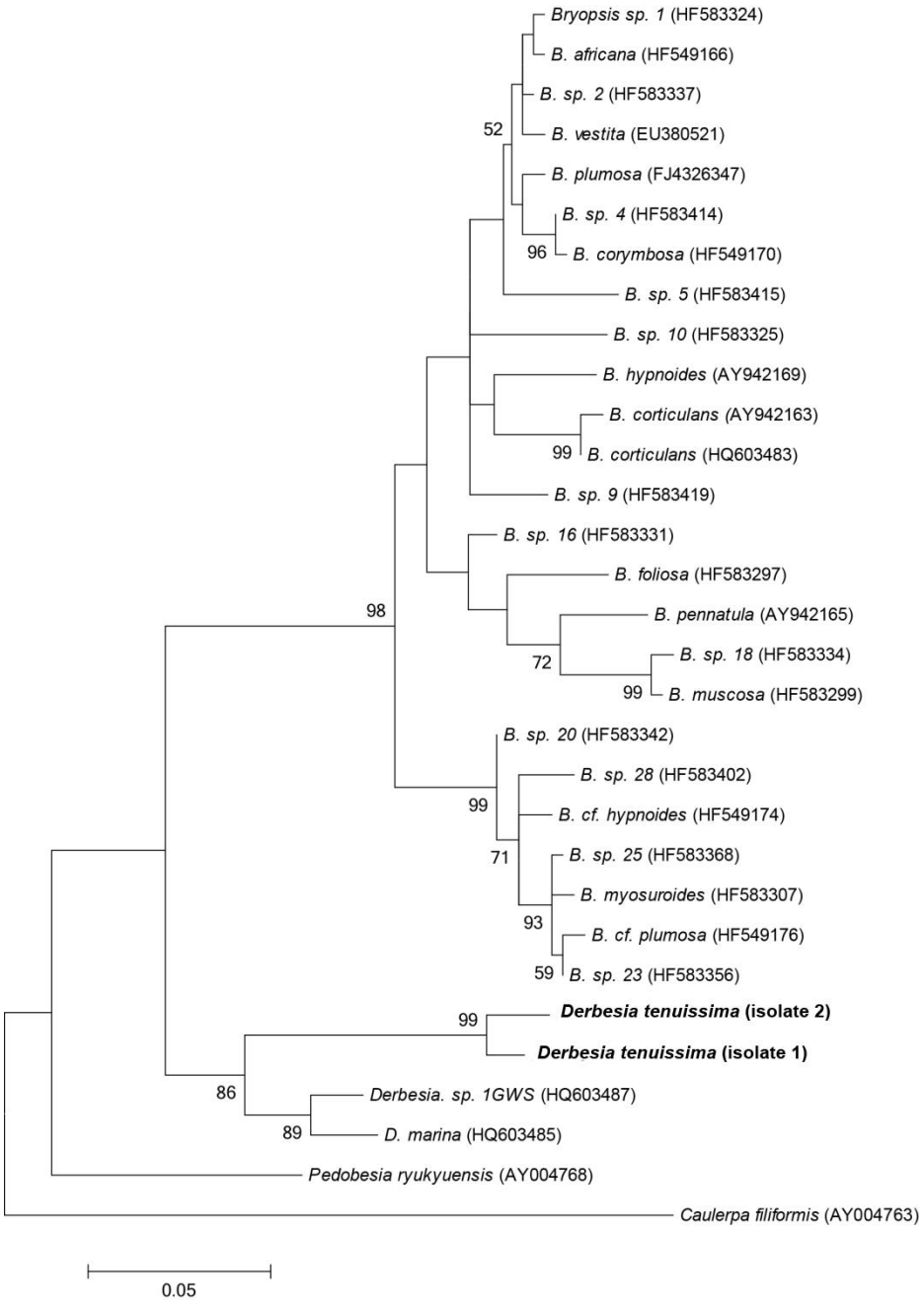

Figure 5.1. Maximum likelihood tree of *Derbesia* and *Bryopsis* rbcL sequence data (scale at bottom). Numbers accompanying the species names are GenBank accession numbers for the sequences used in the analysis. Numbers near each node refer to bootstrap support values, nodes with <50 % bootstrap support are not labelled. Isolates from the current study shown in bold, and after morphological analysis are identified as *Derbesia tenuissima*.

It was not possible to obtain readable sequences for isolate 3 and there were differences in filament diameter with isolates 1 and 2 (14 – 20 µm) having smaller filament diameter than isolate 3 (20 – 30 µm) (Figure 5.2, Table 5.1). However, all remaining morphological characteristics of isolate 3 were the same as isolates 1 and 2, therefore I have also identified it as *D. tenuissima* (Figure 5.2, Table 5.1).

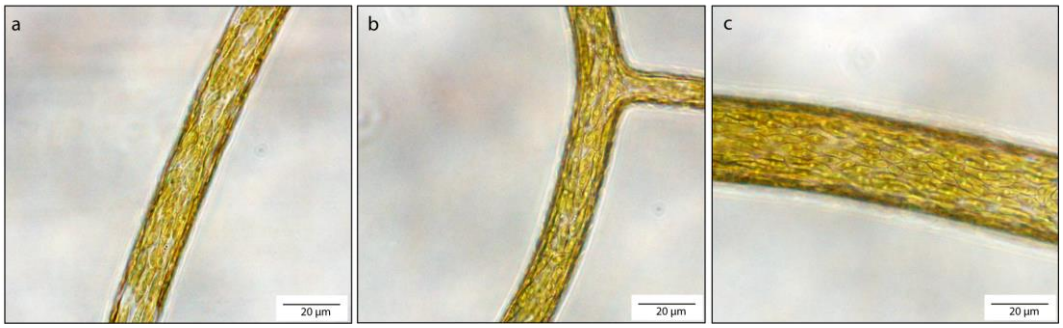

Figure 5.2. Histological photographs (40 × magnification) of three isolates (**a** isolate 1, **b** isolate 2, **c** isolate 3) of *Derbesia tenuissima*.

Table 5.1. Key morphological features of three isolates of *Derbesia tenuissima* (based on 10 individual filaments of each isolate).

	Isolate 1	Isolate 2	Isolate 3
Filament shape	Cylindrical and siphonous; no visible septa	Cylindrical and siphonous; no visible septa	Cylindrical and siphonous; no visible septa
Filament diameter	14 – 20 µm	14 – 20 µm	20 – 30 µm
Branching pattern	Irregular and infrequent; filament with small diameter can branch from filament with larger diameter	Irregular and infrequent; filament with small diameter can branch from filament with larger diameter	Irregular and infrequent; filament with small diameter can branch from filament with larger diameter
Chloroplasts	5 – 10 µm long; single pyrenoid	5 – 10 µm long; single pyrenoid	5 – 10 µm long; single pyrenoid
Sporangia	Not found in specimen	Not found in specimen	Not found in specimen

Table 5.2. Output of 4-way factorial ANOVA testing the effects of temperature (T) (29°C, 21°C), light intensity (L) (100 µmol photons m^{-2} s^{-1}, 24 µmol photons m^{-2} s^{-1}) and nitrogen concentrations (12 mg N L^{-2}, 3 mg N L^{-1}) in three isolates of *Derbesia tenuissima* (I) (isolate 1, isolate 2, isolate 3) on the specific growth rate (SGR, % day^{-1}), carbon to nitrogen ratio (C:N ratio), total fatty acids (TFA, mg g^{-1} dw), saturated fatty acids (SFA, % of TFA), monounsaturated fatty acids (MUFA, % of TFA), polyunsaturated fatty acids (PUFA, % of TFA), PUFA(n-3) (% of TFA) and PUFA(n-6) (% of TFA). Reported is the F-statistic (F), the significance level at 0.05 (p) with significant effects in bold and the eta-squared value (η^2, % of variance (Var)) as a measure of effect size.

		SGR			C:N ratio			TFA			SFA			MUFA			PUFA(n-3)			PUFA(n-6)		
Source of variation	df	F	p	η^2	F	p	η^2	F	p	η^2	F	p	η^2	F	p	η^2	F	p	η^2	F	p	η^2
T	1	3.239	0.078	1.0	3.129	0.083	0.5	0.872	0.355	0.2	89.046	**<0.001**	34.1	23.903	**<0.001**	22.8	117.998	**<0.001**	42.1	643.502	**<0.001**	57.8
L	1	154.596	**< 0.001**	47.9	151.175	**<0.001**	22.7	86.096	**<0.001**	18.2	71.311	**<0.001**	27.3	2.767	0.103	2.6	33.757	**<0.001**	12.0	24.559	**<0.001**	2.2
N	1	0.434	0.513	0.1	167.120	**<0.001**	25.1	47.332	**<0.001**	10.0	14.119	**<0.001**	5.4	1.827	0.183	1.7	12.340	**0.001**	4.4	0.989	0.325	0.1
I	2	43.787	**<0.001**	27.1	20.979	**<0.001**	6.3	115.253	**<0.001**	48.7	0.419	0.660	0.3	8.400	**0.001**	16.0	2.842	0.068	2.0	163.534	**<0.001**	29.4
T x L	1	12.995	**<0.001**	4.0	4.454	**0.040**	0.7	0.345	0.560	0.1	3.598	0.064	1.4	4.156	**0.047**	4.0	7.589	**0.008**	2.7	2.136	0.150	0.2
T x N	1	0.103	0.750	0.0	0.189	0.665	0.0	0.239	0.627	0.1	0.955	0.333	0.4	1.312	0.258	1.3	0.090	0.765	0.0	0.074	0.786	0.0
T x I	2	0.956	0.391	0.6	2.927	0.063	0.9	0.391	0.678	0.2	11.411	**<0.001**	8.8	0.505	0.607	1.0	20.801	**<0.001**	14.8	21.836	**<0.001**	3.9
L x N	1	0.892	0.350	0.3	106.608	**<0.001**	16.0	19.712	**<0.001**	4.2	1.749	0.192	0.7	0.323	0.573	0.3	4.703	**0.035**	1.7	2.707	0.106	0.2
L x I	2	2.219	0.120	1.4	13.074	**<0.001**	3.9	8.879	**0.001**	3.8	0.108	0.898	0.1	0.267	0.767	0.5	0.047	0.954	0.0	2.646	0.081	0.5
N x I	2	0.225	0.799	0.1	39.451	**<0.001**	11.8	5.732	**0.006**	2.4	0.289	0.750	0.2	0.074	0.928	0.1	0.952	0.393	0.7	2.632	0.082	0.5
T x L x N	1	3.016	0.089	0.9	6.894	**0.012**	1.0	2.389	0.129	0.5	0.560	0.458	0.2	0.174	0.679	0.2	0.332	0.567	0.1	0.470	0.496	0.0
T x L x I	2	1.535	0.226	1.0	0.628	0.538	0.2	0.846	0.436	0.4	0.668	0.518	0.5	0.797	0.456	1.5	1.693	0.195	1.2	0.682	0.510	0.1
T x N x I	2	0.123	0.885	0.1	0.613	0.546	0.2	0.658	0.523	0.3	1.979	0.149	1.5	0.322	0.726	0.6	0.067	0.935	0.0	2.509	0.092	0.5
L x N x I	2	0.157	0.855	0.1	11.172	**<0.001**	3.4	2.245	0.117	0.9	0.758	0.474	0.6	0.280	0.757	0.5	0.976	0.384	0.7	1.019	0.369	0.2
T x L x N x I	2	0.668	0.517	0.4	0.780	0.464	0.2	0.100	0.905	0.0	0.089	0.915	0.1	0.475	0.625	0.9	0.333	0.718	0.2	0.235	0.792	0.0
Residuals	48			14.9			7.2			10.1			18.4			45.8			17.1			4.3

SGR specific growth rate; *C:N ratio* carbon to nitrogen ratio; *TFA* total fatty acids; *SFA* saturated fatty acids; *MUFA* monounsaturated fatty acids; *PUFA* polyunsaturated fatty acids

5.3.2. Specific growth rate

The SGR ranged from 12.8 to 33.4 % day^{-1} across all treatments and isolates, and 27 % of the total variation in SGR can be explained by the type of isolate with isolate 1 and isolate 2 having similar SGR (~ 27 % day^{-1}) while the SGR of isolate 3 was significantly lower at 21 % day^{-1} (ANOVA: *Isolate* (η^2 = 27.1 %), Tukey's HSD: $p < 0.001$, Table 5.2, Figure 5.3). The most important factor, however, that explained nearly 50 % (η^2 = 47.9 %) of the variation in SGR was light intensity. Low light intensity significantly reduced the SGR between 27 and 50 % in all three isolates (ANOVA: *Light* (η^2 = 47.9 %), Table 5.1, Figure 5.3). There was a significant interaction effect between temperature and light intensity (ANOVA: *Temperature* × *Light* (η^2 = 4.0 %), Table 5.2), however, this interaction was relatively weak and the SGR was only slightly higher at the treatment combination of high light intensity and high temperature compared to the treatment combination of high light intensity and low temperature. There was no such temperature effect on the SGR at low light intensity (Table 5.2, Figure 5.3). The addition of nitrogen had no significant effect on growth in any of the three isolates (Table 5.2, Figure 5.3).

5.3.3. Carbon to nitrogen ratio

The C:N ratio ranged from 6.3:1 to 13.1:1 across all treatments and isolates and the most important factors explaining the variation were nitrogen concentration (η^2 = 25.1 %, Table 5.2) and light (η^2 = 22.7 %, Table 5.2) with a strong interaction between the two factors (ANOVA: *Light* × *Nitrogen* (η^2 = 16.0 %), Table 5.2, Figure 5.4) that also differed significantly between isolates (ANOVA: *Light* × *Nitrogen* × *Isolate* (η^2 = 3.4 %), Table 5.2). In isolate 1 and isolate 2, there was a significant and strong interaction between light and nitrogen with a higher C:N ratio (11.1:1 – 13.1:1) at the low nitrogen treatment when grown at high light conditions while at low light conditions, this nitrogen effect was not evident (Figure 5.4). This interaction between light and nitrogen on the C:N ratio did not occur in isolate 3 where the C:N ratio was relatively uniform across treatments with a range from 6.6:1 to 8.3:1 (Figure 5.4).

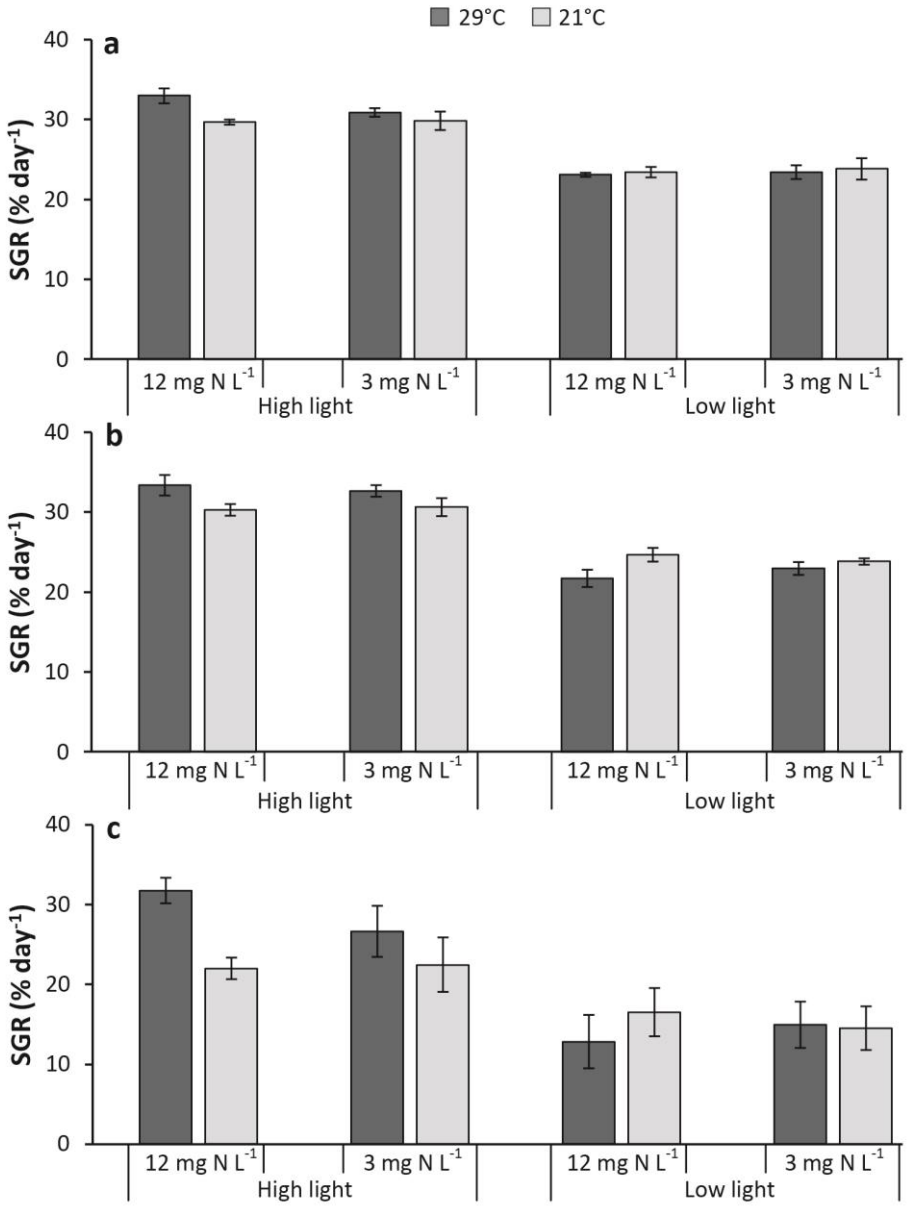

Figure 5.3. Mean specific growth rate (SGR) (% day^{-1} ± SE, n = 3) in three isolates of *Derbesia tenuissima* (**a** isolate 1, **b** isolate 2, **c** isolate 3) grown at different temperatures (29°C, 21°C), different nitrogen concentrations (12 mg N L^{-1}, 3 mg N L^{-1}) and different light conditions (100 µmol photons m^{-2} s^{-1}, 24 µmol photons m^{-2} s^{-1}).

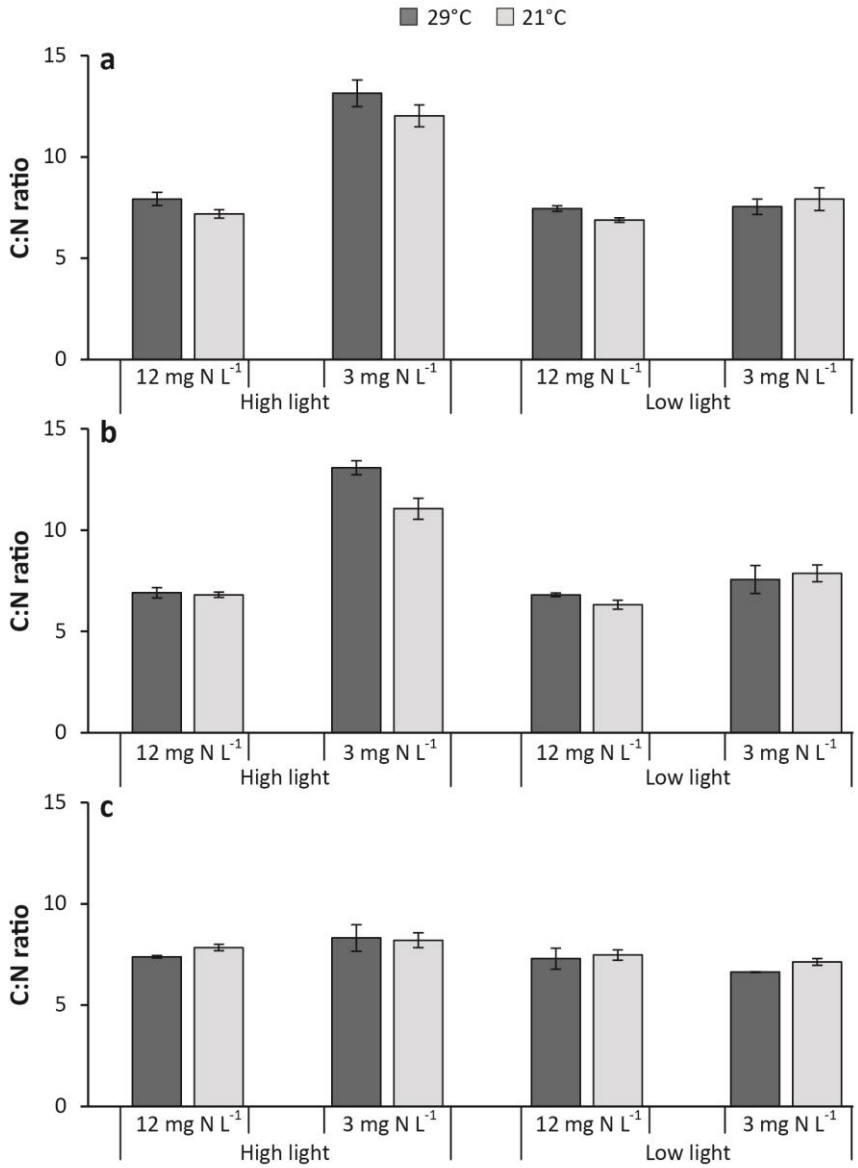

Figure 5.4. Mean of the carbon to nitrogen ratio (C:N ratio) (ratio ± SE, n = 3) in three isolates of *Derbesia tenuissima* (**a** isolate 1, **b** isolate 2, **c** isolate 3) grown at different temperatures (29°C, 21°C), different nitrogen concentrations (12 mg N L^{-1}, 3 mg N L^{-1}) and different light conditions (100 µmol photons m^{-2} s^{-1}, 24 µmol photons m^{-2} s^{-1}).

5.3.4. Total fatty acid content

The content of TFA ranged from 34.8 to 54.1 mg g^{-1} dw across all treatments and isolates and the most important factor explaining the variability in the content of TFA was isolate (η^2 = 48.7 %, Table 5.2) with isolate 1 (47.2 mg g^{-1} dw) and isolate 2 (48.3 mg g^{-1} dw) having similar contents of TFA, while isolate 3 had a significantly lower content of TFA (38.2 mg g^{-1} dw) (ANOVA: *Isolate*, Tukey's HSD: $p < 0.001$, Table 5.2, Figure 5.5). The parameter with the second strongest effect was light intensity (ANOVA: *Light* (η^2 = 18.2 %), Table 5.2) followed by nitrogen (ANOVA: *Nitrogen*, η^2 = 10.0 %, Table 5.2, Figure 5.5) with a weak interaction between the two factors (ANOVA: *Light* × *Nitrogen* (η^2 = 4.2 %), Table 5.2). The content of TFA was reduced by up to 20 % at the treatment combination of high light and low nitrogen while at low light there was no reduction in the content of TFA irrespective of the nitrogen concentration (Table 5.2, Figure 5.5). Further, the effects of light and nitrogen were only present in isolate 1 and isolate 2 while isolate 3 had a relatively uniform content of TFA (ANOVA: *Light* × *Isolate* (η^2 = 3.8 %); ANOVA: *Nitrogen* × *Isolate* (η^2 = 2.4 %); Table 5.2, Figure 5.5). Temperature had no significant effect on the content of TFA in all isolates and at all treatment combinations (Table 5.2, Figure 5.5). The content of TFA was not correlated with the SGR ($r = 0.071$, $p = 0.554$, $n = 72$; Figure 5.7a) but was negatively correlated with the C:N ratio ($r = -0.401$, $p < 0.001$, $n = 72$; Figure 5.7b).

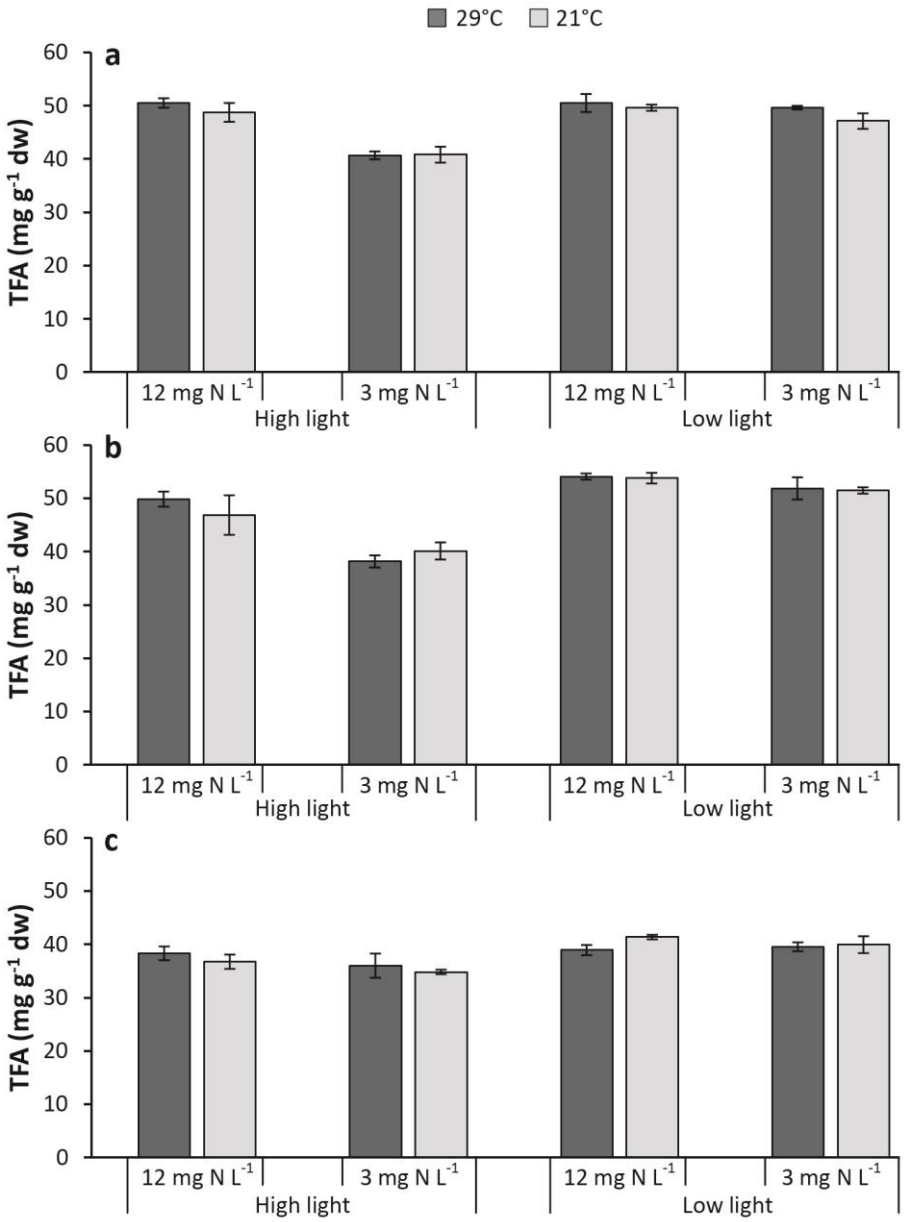

Figure 5.5. Mean total fatty acid content (TFA) (mg g^{-1} dw ± SE, n = 3) in three isolates of *Derbesia tenuissima* (**a** isolate 1, **b** isolate 2, **c** isolate 3) grown at different temperatures (29°C, 21°C), different nitrogen concentrations (12 mg N L^{-1}, 3 mg N L^{-1}) and different light conditions (100 µmol photons m^{-2} s^{-1}, 24 µmol photons m^{-2} s^{-1}).

5.3.5. Fatty acid composition

The average proportion of SFA was 32.2 % of TFA ± 0.6 SE across all treatments and isolates, and ranged from 27.7 to 37.9 % of TFA. The most abundant SFA was C16:0 which ranged from 21.5 to 29.3 % of TFA. Although temperature alone explained 34.1% (η^2, Table 5.2) of the variation in SFA, the effect of temperature was largely restricted to

isolate 1 and isolate 2 where the proportion of SFA was approximately 15 % lower in the low temperature treatments, while in isolate 3, temperature had no significant effect on the proportion of SFA (ANOVA: *Temperature* × *Isolate* (η^2 = 8.8 %), Table 5.2, Figure 5.6). The second largest effect on the proportion of SFA was light in all three isolates and, irrespective of the temperature, the proportion of SFA was significantly higher (~ 10%) at the high light treatments (ANOVA: *Light* (η^2 = 27.3 %), Table 5.2, Figure 5.6). In addition there was a small, but significant, positive effect of nitrogen on the proportion of SFA (ANOVA: *Nitrogen* (η^2 = 5.4 %), Table 5.2, Figure 5.6). The proportion of SFA was weakly correlated with the SGR (r = 0.332, p = 0.004, n = 72; Figure 5.7c) and more strongly correlated with the C:N ratio (r = 0.517, p < 0.001, n = 72; Figure 5.7d).

The proportion of MUFA was 11.0 % of TFA ± 0.3 SE across all treatments and isolates and ranged from 8.6 to 14.1 % of TFA. The most abundant MUFA was C18:1(n-9) (3.6 % – 7.2 % of TFA). Overall, temperature had a strong effect (ANOVA: *Temperature* (η^2 = 22.8 %), Table 5.2, Figure 5.6) on the variability of the proportion of MUFA and the increases in the proportion of MUFA at the low temperature treatments were larger at high light (23 %) compared to low light (10 %) (ANOVA: *Temperature* × *Light* (η^2 = 4.0 %), Table 5.2, Figure 5.6). The second most important source of variation was the type of isolate (ANOVA: *Isolate* (η^2 = 16.0 %), Table 5.2) with isolate 1 (11.3 % of TFA) and isolate 3 (11.7 % of TFA) having similar proportions of MUFA while the proportion of MUFA in isolate 2 (10.0 % of TFA) was significantly lower. The proportion of MUFA was only weakly correlated with the SGR (r = -0.237, p = 0.045, n = 72) and there was no correlation with the C:N ratio (r = 0.071, p = 0.556, n = 72).

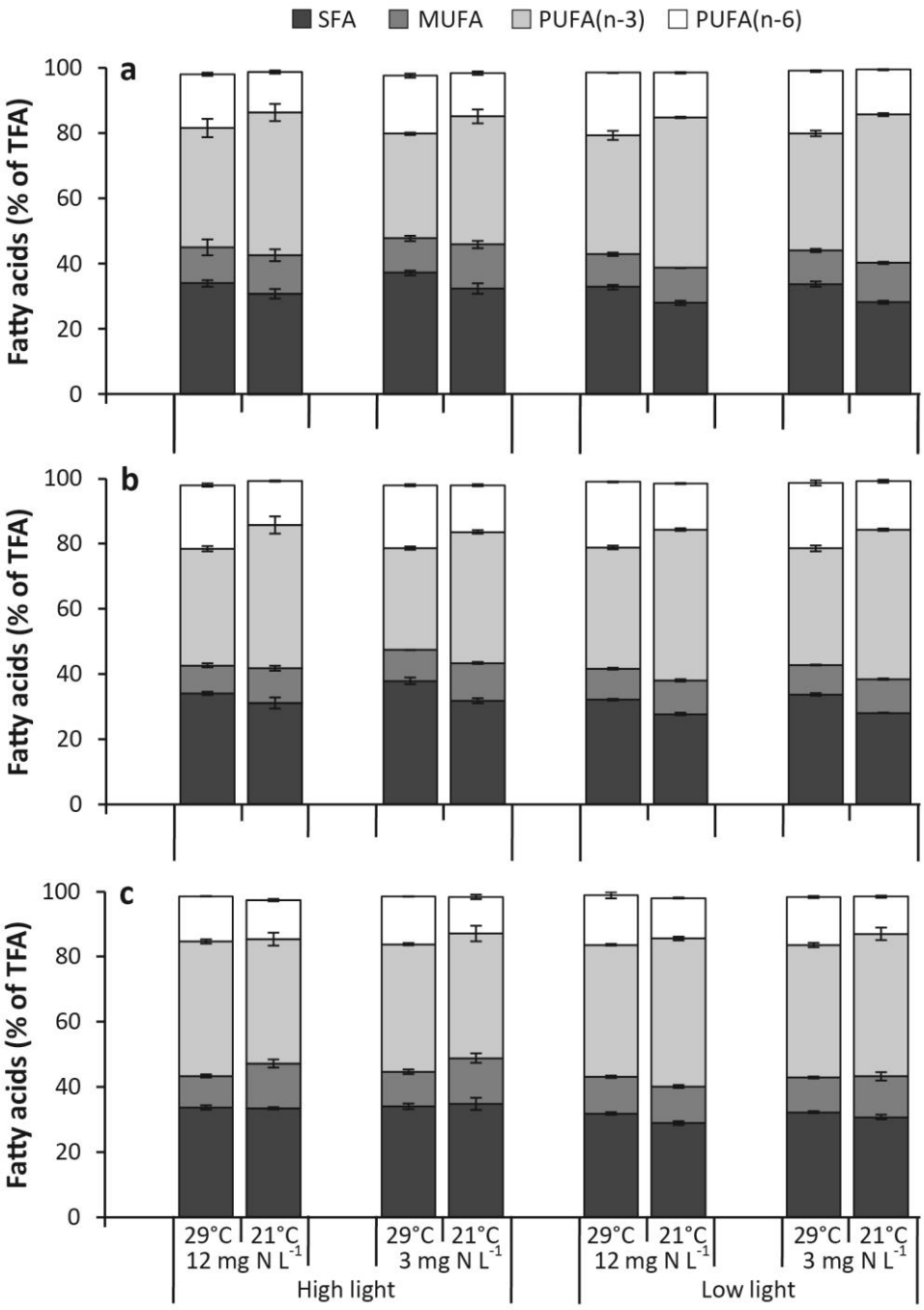

Figure 5.6. Mean composition (% of TFA ± SE, n = 3) of the broad fatty acid groups (*SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA(n-3) / (n-6)* polyunsaturated omega-3/ omega-6 fatty acids) in three isolates of *Derbesia tenuissima* (**a** isolate 1, **b** isolate 2, **c** isolate 3) grown at different temperatures (29°C, 21°C), different nitrogen concentrations (12 mg N L^{-1}, 3 mg N L^{-1}) and different light conditions (100 µmol photons m^{-2} s^{-1}, 24 µmol photons m^{-2} s^{-1}).

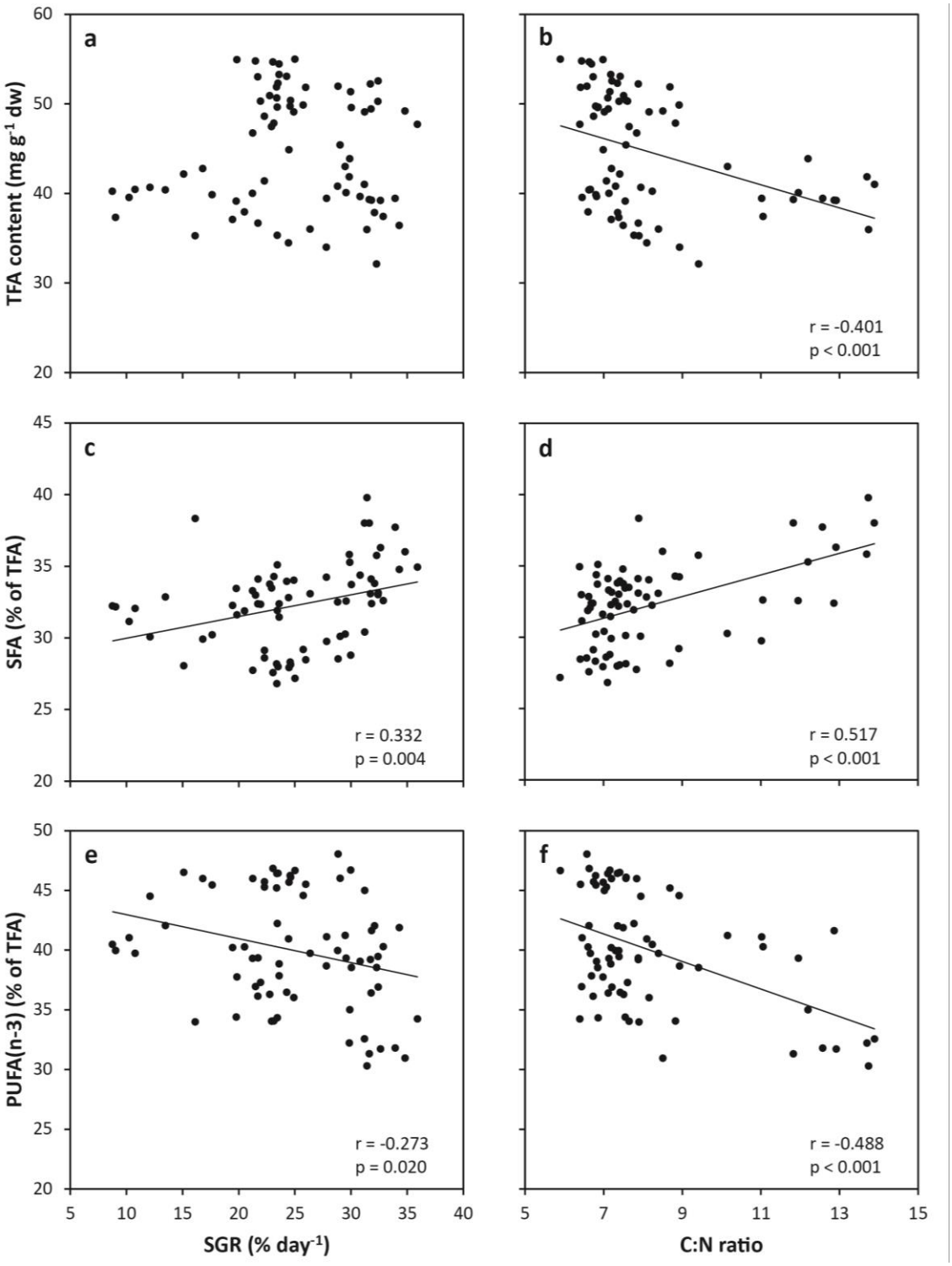

Figure 5.7. Correlations (n = 72) of **a b** the total fatty acid content (TFA) (mg g^{-1} dw), **c d** saturated fatty acids (SFA) (% of TFA) and **e f** polyunsaturated omega-3 fatty acids (PUFA(n-3)) (% of TFA) with the specific growth rate (SGR) (% day^{-1}) and the carbon to nitrogen ratio (C:N ratio).

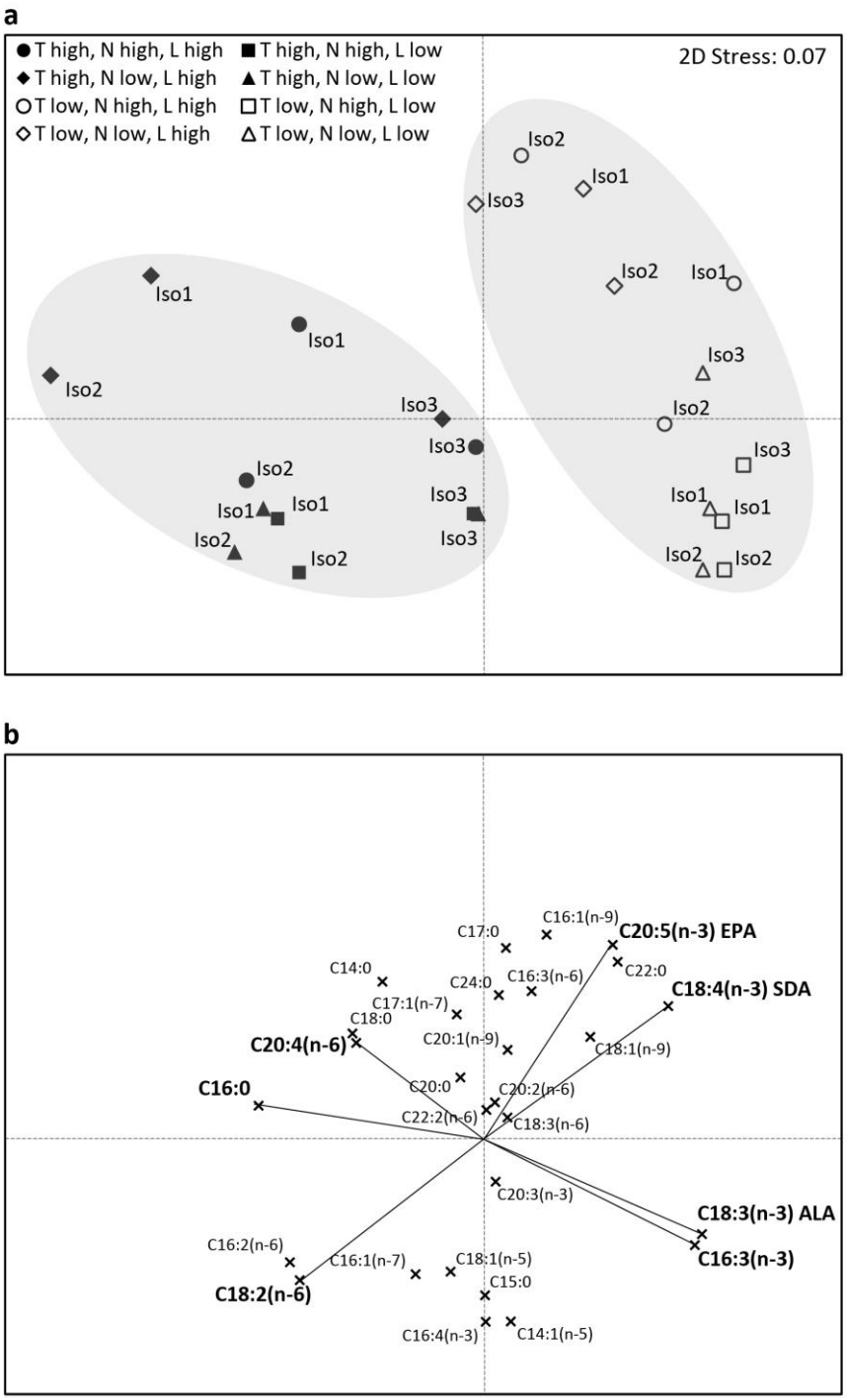

Figure 5.8. Multidimensional Scaling (MDS) showing the differences in average fatty acid composition (% of TFA) of three isolates (iso1, iso2, iso3) of *Derbesia tenuissima* grown at different levels (high, low) of temperature (T), nitrogen (N) and light (L) with **a** MDS ordinates and **b** overlayed vector loadings of individual fatty acids. Vectors only shown for the most abundant fatty acids and fatty acids of particular interest and discussed in this Chapter.

The proportion of PUFA(n-3) was high with 40.0 % of TFA ± 0.9 SE across all treatments and isolates and ranged from 31.3 % to 46.3 % of TFA. The most abundant PUFA(n-3) was C18:3(n-3) (17.3 – 25.6 % of TFA) followed by C16:3(n-3) (9.9 % – 16.1 % of TFA). Although temperature had a strong overall effect (η^2 = 42.1 %) on the variability in the proportion of PUFA(n-3), this effect was restricted to isolate 1 and isolate 2 where the proportion of PUFA(n-3) was over 20 % higher at the low temperature treatments, while in isolate 3 temperature had no significant effect on the proportion of PUFA(n-3) (ANOVA: *Temperature* × *Isolate* (η^2 = 14.8 %), Table 5.2, Figure 5.6). This effect of temperature on the most abundant individual PUFA(n-3) (C16:3(n-3), C18:3(n-3)) was also restricted to isolate 1 and isolate 2 while the proportion of C20:5(n-3) increased in all three isolates at low temperature as demonstrated by MDS (Figure 5.8). There was also a significant but weak interaction between the effects of temperature and light (ANOVA: *Temperature* × *Light* (η^2 = 2.7 %), Table 5.2) and the lowest proportion of PUFA(n-3) was found for the high temperature and high light treatment combination (Figure 5.6). The proportion of PUFA(n-3) was only weakly negatively correlated with the SGR ($r = -0.273$, $p = 0.020$, $n = 72$; Figure 5.7e) but there was a stronger negative correlation with the C:N ratio ($r = -0.488$, $p < 0.001$, $n = 72$; Figure 5.7f).

The average proportion of PUFA(n-6) was 15.3 % of TFA ± 0.6 SE across all treatments and isolates, and ranged from 11.2 % to 20.1 % of TFA. Similar to PUFA(n-3), the overall variability in the proportion of PUFA(n-6) was mainly affected by the culture temperature (η^2 = 57.8 %, Table 5.2), however, in an opposing direction with a higher proportion of PUFA(n-6) at the high temperature treatments. The variability in the proportion of PUFA(n-6) was also affected by the type of isolates (η^2 = 29.4 %) with isolate 2 (17.0 % of TFA) having the highest proportion of PUFA(n-6) followed by isolate 1 (15.7 % of TFA) and isolate 3 (13.2 % of TFA). Furthermore, the effect of temperature on the proportion of PUFA(n-6) was strongest in isolates 1 and 2 with a 32 –

33 % difference between the temperature treatments, while in isolate 3 there was only a 23 % difference in the proportion of PUFA(n-6) (ANOVA: *Temperature* × *I*solate (η^2 = 3.9 %), Table 5.2). This impact of temperature in the isolates was also evident on individual PUFA(n-6) as demonstrated by MDS (Figure 5.8). The proportion of PUFA(n-6) was only weakly correlated with the SGR (r = 0.240, p = 0.042, n = 72) and there was no correlation with the C:N ratio (r = 0.150, p = 0.207, n = 72).

5.4. Discussion

This study confirms the suitability of the green seaweed *D. tenuissima* as a biomass feedstock for the production of functional foods and nutraceuticals because of its high growth rate and high proportion of PUFA(n-3). While its cultivation has been demonstrated at a large scale, the full growth potential has not been exploited. Here I demonstrate that the growth and the proportion of PUFA(n-3) can be further increased through environmental manipulations and also careful strain selection. The growth and the proportion of PUFA(n-3) were different between isolates and in two isolates substantial improvements in the proportion of PUFA(n-3) were achieved at low water temperature treatments. Importantly, these increases in the proportion of PUFA(n-3) were achieved in the presence of high contents of TFA and high growth rates leading to net increases in the productivity of PUFA(n-3).

5.4.1. Species identification and morphology

Although analysis of DNA sequences was inconclusive, the morphological features of the filaments, including the size and shape of the chloroplasts and the presence of a single pyrenoid in each chloroplast, strongly suggest that all three isolates are *D. tenuissima* (van den Hoek and Womersley 1984; Kobara and Chihara 1981). However, unlike in the literature, where filament diameter commonly ranges from 25 to 80 µm (van den Hoek and Womersley 1984; Kobara and Chihara 1981; Sears and Wilce 1970), the filaments in

this Chapter measured only between 14 µm (isolate 1, isolate 2) and 30 µm (isolate 3) and are therefore the smallest filament sizes reported for *D. tenuissima*. Many of the larger filament sizes previously reported are field-collected samples from the North Atlantic and Southern Australia with filament diameter of up to 80 µm (van den Hoek and Womersley 1984) while Mediterranean isolates had smaller filament diameter (30 – 50 µm) (Kobara and Chihara 1981) that were even smaller in culture (30 µm) (Sears and Wilce 1970). Variation in morphology within species has been reported for many seaweeds including *Asparagopsis taxiformis* (Zanolla et al. 2014), *Caulerpa taxifolia* (Wright 2005) and *Dictyota dichotoma* (Hwang et al. 2005) and was attributed to both environmental plasticity and differences between genotypes. Therefore, I propose that the observed variation in the diameter of filaments in *D. tenuissima* reported in this Chapter and the disparity with the previously reported diameter of filaments is within the natural variation of this species. Further, it is likely that the variation in the diameter of filaments between the isolates from this Chapter are a genotypic characteristic rather than a phenotypic expression as the filament size differences between the isolates persisted under identical culture conditions over several months.

5.4.2. Growth and internal nitrogen

Overall, the SGR (referred to as growth hereafter) of *D. tenuissima* ranged from 13 to 33 % day^{-1} and approximately 27 % of that variation was due to differences in growth between isolates. While isolate 1 and isolate 2 had similar average growth rates of approximately 27 % day^{-1}, isolate 3 only had an average SGR of 21 % day^{-1}. Since the isolates were previously kept under identical culture conditions, it is likely that the observed variation in growth is due to genotypic differences between the isolates. Similar variation in growth between different isolates has been reported for the green seaweed *Ulva ohnoi* and the possibility of genotypic variation as the driver for this variation in growth was suggested for strains from different locations (Lawton et al. 2013b). Further, natural selection for high growth and its heritability has been experimentally

demonstrated in the brown seaweed *Fucus vesiculosus* (Jormalainen and Honkanen 2004). High growth rates are characteristic for opportunistic and invasive species of plants where faster growth rates are favoured over other traits such as herbivory defence (Herms and Mattson 1992). Growth rates in seaweed are also typically influenced by the cell size or filament morphology, where a larger surface area to volume ratio is associated with higher growth rates through more efficient photosynthesis and increased nutrient uptake as demonstrated in many species of seaweed (Raven and Taylor 2003). Therefore, the variation in growth rates found for isolates of *D. tenuissima* in this Chapter can be a direct result of differences in the size of filaments that in itself can be a genotypic trait of a broader survival strategy.

In addition to the variation between isolates, growth was also strongly affected by the environmental treatments and in particular light intensity which explained approximately 50 % of the total variation in growth. The strong effect of light on the growth in this species has been demonstrated previously in large-scale outdoor cultures under non-limiting conditions, where light intensity was either controlled by stocking density (Magnusson et al. 2014) or varied seasonally (Mata et al. 2015). Notably, the addition of extra nitrogen had no effect on the growth in any of the three isolates which suggests that nitrogen was in no case a limiting factor for growth. The C:N ratio, as an indicator for nitrogen limitation for growth (Atkinson and Smith 1983), was uniformly low across most treatments with an approximate ratio of 7:1 and was similar to outdoor cultures of *D. tenuissima* under non-limiting conditions (Magnusson et al. 2014; Mata et al. 2015). However, at the high light and low nitrogen treatment combinations a higher C:N ratio of over 13:1 was measured in isolates 1 and 2, which indicates that the available nitrogen supported the high growth but at the cost of internal nitrogen depletion. This internal depletion of nitrogen was not observed in isolate 3 which suggests that the full growth potential of this isolate was not realized possibly due to suboptimal conditions of light and temperature or the limitation of another factor such as phosphorous. Although the

N:P ratio was not measured in this Chapter, it is possible that phosphorous became a limiting factor at the high nitrogen treatment conditions and therefore not all available nitrogen was utilised for growth.

5.4.3. Total fatty acid content and fatty acid composition

The content of TFA in *D. tenuissima* ranged between 35 and 54 mg g^{-1} dw with an average fatty acid composition of 32 % SFA, 11 % MUFA and 40 % PUFA(n-3) which is within the range of earlier reports for this species (Gosch et al. 2012; Magnusson et al. 2014). Approximately 17 – 26 % of the PUFA(n-3) were in the form of C18:3(n-3) (α-linolenic acid, ALA) which is beneficial for cardiovascular health and has potential applications in the redistribution of body fat (Poudyal et al. 2012; Poudyal et al. 2013) and therefore is an important fatty acid for applications in health and nutrition. However, there was substantial variation in the content and composition of fatty acids across treatments and isolates. The content of TFA was approximately 20 % lower in isolate 3 compared to the other isolates and this difference between isolates explained nearly 50 % of the total variation in the content of TFA. In a similar manner, the proportion of the fatty acid groups (SFA, MUFA, PUFA) also differed between isolates but to a smaller degree than the content of TFA, and the environmental conditions had a larger impact on the composition of fatty acids. Variation in the content and composition of fatty acids has been demonstrated for genotypes of terrestrial oil crops such as rapeseed (Downey and Craig 1964) and soybean (Byrum et al. 1997) and also for various species of microalgae (Grima et al. 1995; Sharma et al. 2012; Rodolfi et al. 2009), with significant increases in the content and also composition of fatty acids through selective breeding and strain selection (Vollmann and Rajcan 2010). In seaweed, however, this branch of research is still in its infancy but first improvements of the total lipid content by 20 % in the commercially important kelp *Macrocystis* through selective breeding have been reported (Westermeier et al. 2012) and mark a first step in the domestication of seaweeds for oil-based bioproducts. Of particular importance, however, is the quality of the oil and

specifically a high proportion of PUFA(n-3) which have applications in nutraceuticals, functional foods and as a fish oil replacement (Adarme-Vega et al. 2014). The genotypic variation of PUFA(n-3) has previously not been demonstrated for seaweeds (Robinson et al. 2013). Here I provide the first strong support that part of the variability of fatty acids in seaweed is genotypic, as evidenced by the portioning of the source of the variation in TFA and fatty acid composition between the isolates and the environmental treatment conditions. This has important implications for future strain selection and subsequent selective breeding for improvements in fatty acid quality and in particular increases in the content and composition of PUFA(n-3).

As well as the opportunity for strain selection, improvements in the content and composition of fatty acids can be achieved by exploiting the natural variability driven by environmental factors such as temperature, light and nutrient availability through modified culture and harvest strategies. For the content of TFA, the effect of the environment was smaller than that of the genotype but nevertheless remained substantial. There was a strong interaction of the factors light and nitrogen, resulting in a reduction of up to 20 % in the content of TFA at the high light and low nitrogen treatment combination. The effect of light on the fatty acids in algae has been investigated previously, and in many microalgae the content of TFA (or total lipids) increases as a result of high light intensity (Solovchenko et al. 2008), and is generally attributed to increased conversion of photoassimilates into triacylglycerol (TAG) as a means of energy storage (Sharma et al. 2012). However, for the *Derbesia* strains investigated here, high light intensity does not lead to accumulation of lipids which is supported by a previous study on *D. tenuissima* (Magnusson et al. 2014), and in a similar manner several other green seaweeds have higher total lipid contents at low light conditions (Hotimchenko 2002; Khotimchenko and Yakovleva 2005). While high light resulted in a slight general decrease in the content of TFA, a particularly strong reduction only occurred in combination with low nitrogen availability and specifically in the cases where the internal

nitrogen was depleted as measured by a high C:N ratio. This is in contrast to many species of microalgae (Sharma et al. 2012; Adarme-Vega et al. 2014) where the depletion of nitrogen leads to increases in the content of TFA (or total lipids) through increased production of TAG. However, there appears to be no general trend as the content of TFA decreases with nitrogen depletion in some species of microalgae (Sharma et al. 2012) and lipid increases induced by nitrogen depletion have only rarely been observed in seaweeds (Gordillo et al. 2001). This inconsistency between species and studies can be related to interactions with other environmental factors such as light (Solovchenko et al. 2008) or carbon availability (Gordillo et al. 2001) and associated heterogeneous responses to nitrogen depletion. Furthermore, nitrogen starvation also results in a reduction of chloroplasts (Pinchetti et al. 1998; Siaut et al. 2011) with a subsequent reduction in the associated membrane lipids (Siaut et al. 2011) and therefore possible net reductions of total lipids. In green algae specifically, the production of starch, as the major energy storage product, also competes with TAG for the carbon precursor (Kumari et al. 2013b; Li et al. 2010) and the production of starch can be favoured over TAG under nitrogen depletion (Siaut et al. 2011). These factors can therefore result in overall net decreases in the content of TFA under nitrogen depletion as occurred in *D. tenuissima*. Temperature had no effect on the content of TFA in this Chapter. Although there are examples where a reduced culture temperature resulted in increased total lipid contents in both microalgae (Sharma et al. 2012) and seaweeds (Floreto et al. 19993), this seems to be a reflection of a narrow range of tested culture temperatures as studies that included multiple temperature treatments often show an optimal temperature that leads to increased total lipid content while temperatures above and below that optimum support lower lipid contents (Renaud et al. 1995; Renaud et al. 2002).

While temperature had no effect on the content of TFA, this parameter had the strongest effect on the composition of fatty acids either as a main effect or in various interactions with light and isolate. In isolates 1 and 2, the proportion of PUFA(n-3) in TFA increased

by 20 % to 45 % at the low temperature treatment with a concomitant decrease in PUFA(n-6) and saturation. Importantly, temperature did not substantially affect the growth or the content of TFA and therefore the overall quality of the biomass as defined by high PUFA(n-3) productivity was improved at low temperature. This temperature effect on the proportion of PUFA(n-3) was not present in isolate 3 where the proportion of PUFA(n-3) remained stable across temperatures at approximately 40 % of TFA, emphasizing the significance of appropriate strain selection for different climates to maximise the production of PUFA(n-3). The effect of water temperature on the composition of fatty acids is generally understood as a physiological response to improve membrane fluidity at lower temperatures, as unsaturated fatty acids have a lower melting point than saturated fatty acids (Los et al. 2013; Thompson et al. 1992). However, the increased unsaturation with decreased temperature occurs in many (Sharma et al. 2012; Zhu et al. 1997), but not all species of microalgae (Renaud et al. 2002) investigated to date. This temperature effect on the composition of fatty acids has been investigated experimentally for only a few species of seaweed (Al-Hasan et al. 1991; Floreto et al. 1993), but similar to microalgae there seems to be no general trend as many field-based studies also show increased PUFA(n-3) contents during the warmer summer months (Gosch et al. 2015a; Schmid et al. 2014) and species-specific interactions between light and growth rates therefore seem likely. Nevertheless, I provide here the first evidence for the temperature dependence of PUFA(n-3) content in *D. tenuissima,* and the observed variation of this temperature effect below the species level has implications for strain selection for different climates.

The composition of fatty acids was also affected by light intensity and there was an increased proportion of SFA at the high light treatment in all three isolates with the lowest overall proportion of PUFA(n-3) at the high light and high temperature treatment combination. Similar effects of light on the fatty acid composition occurred for species of seaweed where the fatty acid profile was more saturated at high light conditions with a

lower proportion of PUFA (Hotimchenko 2002; Khotimchenko and Yakovleva 2005). Light intensity can provoke a range of physiological responses including increased production of chloroplasts and associated membrane lipids at low light conditions (Sharma et al. 2012). Such membrane lipids generally have a more unsaturated composition of fatty acids (Sanina et al. 2004) which increase the fluidity of thylakoid membranes and thereby electron-flow in the chloroplasts (Mock and Kroon 2002). Furthermore, increased rates of oxidation at high light intensity can lead to a breakdown of double bonds and thereby increase the degree of saturation (Sharma et al. 2012). In addition to the direct influence of light on the composition of fatty acids, it must be emphasized that the composition of fatty acids can also change depending on growth rates. I observed weak correlations between high growth rates and reduced PUFA(n-3) and a higher proportion of SFA, and these highest growth rates were achieved at the high light treatments. There is evidence that growth rate can affect the proportion of fatty acids through proportional changes of lipid classes with a higher production of the structural and more unsaturated glycolipids during periods of high growth (Cohen et al. 1988), reflecting the increased production of organelles and in particular chloroplasts. Many species of microalgae therefore show increased levels of PUFA(n-3) during the exponential growth phase (Zhu et al. 1997). Growth rate and light intensity therefore have opposing effects on the proportion of PUFA(n-3), which can explain the discrepancies between studies and reported species with either a decrease (Hotimchenko 2002; Khotimchenko and Yakovleva 2005) or an increase (Hotimchenko 2002) with light intensity.

5.4.4. Conclusion

This study confirms the high growth rates, and the high content and proportion of the nutritionally important PUFA(n-3) in *D. tenuissima*. Furthermore, the results of this study clearly demonstrate that there is potential to enhance the biomass growth and the content and composition of fatty acids in *D. tenuissima* through environmental manipulations and

also careful strain selection. There were substantial differences between the isolates and the selection of a particular isolate of *D. tenuissima* will have important implications for choosing culture strategies because of strong genotype by environment interactions. Isolate 1 and isolate 2 had both higher growth rates and higher contents of TFA, and the proportion of PUFA(n-3) was elevated to nearly 45% of TFA in the low water temperature treatment. Importantly, these increases in the proportion of PUFA(n-3) were achieved in the presence of a stable content of TFA and high growth rates, resulting in a net increase in PUFA(n-3) productivity. Isolate 3, however, had a lower growth rate and a lower content of TFA, but the proportion of the nutritionally important PUFA(n-3) remained stable at around 40 % of TFA across treatments. This stability in the composition of fatty acids is beneficial for commercial cultivation for health and food applications where a stable supply of PUFA(n-3) is preferred (Gellenbeck 2012; Hafting et al. 2012). While it remains to be confirmed that these results are transferable to scaled-up, long-term outdoor cultivation systems, this study provides the theoretical framework for the implementation of strain selection and culture management to improve productivity of selected bioproducts in seaweed at scale.

Chapter 6

Synthesis and discussion

6.1. General statement

While there is a global production of over 20 million tonnes of seaweed annually (FAO 2014), the majority of this biomass production is targeted at dried foods and the production of phycocolloids. I have now demonstrated in this thesis that there are a range of potential extensions to the current product portfolio for seaweeds which are oil-based bioproducts and in particular functional foods, nutraceuticals, feeds for livestock and aquatic animals, and biodiesel and biomaterials. However, while terrestrial plants and increasingly microalgae are utilised for these oil-based products, seaweeds remain a largely untapped bioresource. Some seaweeds have high biomass productivities (Bolton et al. 2009; Capo et al. 1999; Mata et al. 2010b) and there is industrial scale production of feedstock to deliver scalable biomass (FAO 2014). However, this biomass has not been considered as a suitable feedstock for oil-based bioproducts because of its low oil content. This thesis investigated the potential of seaweed biomass for oil-based bioproducts through the identification of novel high lipid species and the subsequent identification and quantification of natural environmental and genetic variability between plants (inter-plant and population variation) within these species, and also within plants of these species (intra-plant variation), as the basis for cultivation and selection strategies to improve biomass and lipid productivity.

The major findings of this thesis were:

1. The identification of key target species of seaweed with high total lipid and high total fatty acid contents with a composition of fatty acids suitable for oil-based

bioproducts. Two of these species are new seaweed benchmarks in terms of the content of PUFA(n-3);

2. The identification and quantification of the natural variability in fatty acids within species;
3. The identification and quantification of the environmental drivers (temperature, light, nitrogen) of variability in fatty acids within species;
4. The identification and quantification of the biotic drivers (plant size, life history stage, growth rate) of variability in fatty acids within species;
5. The identification and quantification of a genotypic driver of variability in fatty acids within species;
6. The identification and quantification of the variability in fatty acids within the thallus of plants.

6.2. Species selection and profiling

Seaweeds have not traditionally been perceived as a suitable feedstock for oil-based bioproducts because of their low content of lipids and fatty acids. In contrast to this perception three species of seaweed were identified through this work with the potential to be considered as a feedstock for oil-based bioproducts based on three selection criteria which are the total lipid content, total fatty acid content and fatty acid composition.

6.2.1. Total content of lipids and fatty acids

The first selection criterion was the total lipid content. Notably, the majority of examined species were unsuitable for oil-based bioproducts because of their low total lipid content with a designated selection point of a minimum of 10 % dw. However, there were species with a high total lipid content above 10 % dw within orders of green (Bryopsidales, Cladophorales) and brown seaweeds (Dictyotales). The species with the highest total lipid content were *Dictyota bartayresii* (12 %) and *Spatoglossum*

macrodontum (12 %) from the Dictyotales and *Caulerpa sertularioides* (13 %) and *Derbesia tenuissima* (12 %) from the Bryopsidales.

The second selection criterion was the content of TFA. This is the critical parameter in determining the yield and suitability of seaweed biomass as feedstock for oil-based bioproducts. The TFA fraction is present in the form of esterified fatty acids as part of the triacylglycerols (TAG) and other complex lipids (Christie 2003; Kumari et al. 2013b). Based on their content of TFA, the most suitable species for oil-based bioproducts were *S. macrodontum* (6 – 8 % dw), *D. bartayresii* (4 – 5 % dw) and *D. tenuissima* (4 – 5 % dw).

These key species of seaweed have total lipid contents at the upper range of previous reports for seaweeds (Herbreteau et al. 1997; Kumari et al. 2010; Montgomery and Gerking, 1980; McDermid and Stuercke 2003) and *S. macrodontum* has the highest content of TFA reported for seaweeds to date (Honya et al. 1994; Schmid et al. 2014; van Ginneken et al. 2011). This challenges the assumption that seaweed biomass is unsuitable for the production of oil-based bioproducts because of their presumably low content of oils. While these total lipid contents are among the highest for seaweeds, they are at the lower range of common terrestrial oil crops (20 – 50 % dw; Issariyakul and Dalai 2014) and eukaryote microalgae (10 – 50 % dw; Griffiths and Harrison 2009; Huerlimann et al. 2010) but at the upper range of cultured cyanobacteria including *Spirulina* spp. (5 – 13 % dw; Griffiths and Harrison 2009). Microalgae in particular, are considered as the benchmark for oil production from algal biomass. For example, a lipid productivity of 5 g oil m^{-2} day^{-1} has been reported as the critical economical benchmark for the production of biodiesel from microalgae (Stephens et al. 2010a). To meet this benchmark, a species with a high biomass productivity (20 g dw m^{-2} day^{-1}) would require a total lipid content of 25 % dw which is not achieved by any of the species of seaweed identified in this thesis. However, while the total lipid content is the critical

benchmark for low value products such as biodiesel, the critical parameter for high value products such as functional foods, nutraceuticals and animal feed ingredients, based on omega-3 fatty acids, is the quality of the oils.

6.2.2. Fatty acid composition

The third selection criterion was therefore the composition of fatty acids as this determines the suitability of species for specific oil-based bioproducts. The fatty acid compositions of the identified key species were largely unknown and this thesis therefore provides an important contribution to this field of research. Overall, the biomass of the key species of brown seaweed had a fatty acid composition of 20 % PUFA(n-3) dominated by ALA, SDA and EPA, while *D. tenuissima* had over 40 % PUFA(n-3) dominated by C16:3(n-3) and ALA. A high proportion of PUFA(n-3), and in particular a high proportion of the essential omega-3 fatty acids of ALA, EPA and DHA, are considered key criteria for the inclusion in functional foods and nutraceuticals (Adarme-Vega et al. 2014; Gill and Valivety 1997), and also animal feeds (Bahar et al. 2012; Muller-Feuga 2000; Tocher et al. 2010). The essential PUFA C18:3(n-3) (ALA) is beneficial for the function of the cardiovascular system and liver and is an effective agent in the redistribution of body fat as demonstrated in animal trials (Poudyal et al. 2012, 2013). While the intake of ALA through the consumption of nuts, seeds and vegetable oils is considered adequate in the western diet (Harris 2005), the long-chained PUFA(n-3) C20:5(n-3) (EPA) and C22:6(n-3) (DHA) are deficient and supplementation is recommended (Burdge 2004). EPA and DHA, in particular, are highly beneficial with anti-inflammatory properties, improved fetal development and a positive impact on the cognition in infants and children (Dunstan et al. 2007; Krauss-Etschmann et al. 2008) and are considered important for larval development in aquaculture (Muller-Feuga 2000; Tocher 2010; Tocher 2015).

The benchmark for commercial production of functional foods and nutraceuticals from novel biomass sources is the fatty acid compositions of fish oils, terrestrial crops, and microalgae as these are currently the only commercial sources for omega-3 fatty acids. Compared to the majority of terrestrial crops, the key species of seaweed identified in this thesis had a higher proportion and diversity of PUFA(n-3). Terrestrial oil crops typically have low proportions of PUFA(n-3) which are less than 1 % of TFA in palm oil, sunflower oil and olive oil, and between 5 and 10 % of TFA in soybean and rapeseed (Dubois et al. 2007). Although some terrestrial crops such as linseed (50 % of TFA) and chia seed (60 % of TFA) have higher proportions of PUFA(n-3) (Dubois et al. 2007), this is in the form of ALA only and they lack EPA and DHA (Dubois et al. 2007). Unlike terrestrial plants, algae (micro- and macroalgae) convert ALA to EPA and DHA through elongation and desaturation (Bell and Tocher 2009; Christie 2003; Pereira et al. 2003) and are therefore the natural sources of these long-chained PUFA(n-3). Microalgae have been considered a suitable substitute for fish oils (Brown et al. 1997; Muller-Feuga 2000) and many species of microalgae have a high proportion of PUFA(n-3) and also high proportions of the essential EPA and DHA which can exceed 50 % of TFA in some species (Griffiths and Harrison 2009; Huerlimann et al. 2010; Spolaore et al. 2006). EPA and DHA are also major fatty acids in many fish oils (30 – 40 % EPA and DHA of TFA, Ackman et al. 1988; Bandarra et al. 1997; Fernandes et al. 2014) which are the traditional and most common sources of PUFA(n-3) for functional foods, nutraceuticals and aquaculture feeds (Naylor et al. 2009; Tacon and Metian 2008). The proportion of DHA was marginal (< 1 % of TFA) in the three key species of seaweed, which makes them an unsuitable replacement for fish oils for applications such as specialized aquaculture larval feeds (Muller-Feuga 2000; Tocher 2010; Tocher 2015) and omega-3 infant formulas (Forsyth et al. 2003; Ratledge 2013) where a high proportion of DHA is preferable. Overall, the quality of the oil in the key species of seaweed identified in this thesis is higher than that of terrestrial oil crops but lower than

that of many microalgae and fish oils in terms of long-chain (≥ 20 carbon) n-3 fatty acids.

Comparatively, amongst the seaweeds, the key species of seaweed identified in this thesis have a higher proportion of PUFA(n-3) (as a % of TFA) than the brown seaweed *Ascophyllum nodosum* (8 % of TFA) (van Ginneken et al. 2011) which is the main seaweed utilized for livestock feeds (Evans and Critchley 2014). The key brown seaweeds identified in this thesis, *D. bartayresii* and *S. macrodontum*, have proportions of PUFA(n-3) comparable to commercial species directed at human consumption including the red seaweed *Chondrus crispus* (22 % of TFA) (van Ginneken et al. 2011) and the brown seaweed *Saccharina ochroleuca* (25 % of TFA) (Sánchez-Machado et al. 2004). *D. tenuissima* was comparable to the kelp *Undaria pinnatifida* (45 % of TFA) (Sánchez-Machado et al. 2004) and the red seaweed *Palmaria palmata* (30 – 60 % of TFA) (Mæhre et al. 2014; van Ginneken et al. 2011). However, *P. palmata* is the benchmark for EPA (34 % of TFA) in seaweeds (Mæhre et al. 2014) but at a considerably lower level of TFA (Table 6.1). Based on the current knowledge of reported species, DHA is rare in seaweeds, but some species such as the brown seaweed *Sargassum natans* (13 % of TFA) (van Ginneken et al. 2011) and the green seaweed *Ulva fasciata* (6 % of TFA) (Kumari et al. 2010) have high proportions of DHA. However, they also have a very low content of TFA (or total lipids) (Table 6.1). This highlights the importance of considering both the TFA content and the fatty acid composition when selecting species for the production of oil-based bioproducts. The first major outcome of this thesis is the provision of two new benchmark species of seaweed based on their high content of total faty acids (% of dw), high proportion of PUFA(n-3) (% of TFA) and therefore their high total content of PUFA(n-3) (% of dw) (Table 6.1):

1) *Spatoglossum macrodontum* at 1.4 % PUFA(n-3) of dw;

2) *Derbesia tenuissima* at 1.8 % PUFA(n-3) of dw.

However, these are only seaweed benchmarks and these species of seaweed have lower oil quality than many species of microalgae and fish oils for applications in health and nutrition, and animal feeds.

Table 6.1. A comparative profile of the contents of total lipids and fatty acids of selected seaweeds, microalgae and terrestrial oil crops. The two in this thesis identified benchmark species of seaweed are highlighted.

		% dw			% of TFA				
Species	Type	Total lipid	TFA	PUFA(n-3)	PUFA(n-3)	ALA	EPA	DHA	Reference
Spatoglossum macrodontum	S	12	7	1.4	20	5	3	nd	Gosch et al. 2012; Gosch et al. 2014
Dictyota bartayresii	S	12	4.5	0.9	20	2.5	5	1	Gosch et al. 2012; Gosch et al. 2015a
Derbesia tenuissima	S	12	4.5	1.8	40	18	4	nd	Gosch et al. 2012; Gosch et al. 2015b
Ascophyllum nodosum	S		4.5	0.4	8	2	4	nd	van Ginneken et al. 2011
Chondrus crispus	S		1.5	0.3	22	2	8	nd	van Ginneken et al. 2011
Saccharina japonica	S	3.4	2.3			5	7	nd	Honya et al. 1994
Laminaria ochroleuca	S	0.9			25	5.2	8.6	nd	Sánchez-Machado et al. 2004
Palmaria palmata	S		1.5	0.9	63	2	59	nd	van Ginneken et al. 2011
Sargassum natans	S		0.7	0.1	20	2	5	13	van Ginneken et al. 2011
Ulva fasciata	S	1.8			10	1.3	2.4	6	Kumari et al. 2010
Undaria pinnatifida	S		1.8	0.6	35	7	16	nd	van Ginneken et al. 2011
Tetraselmis sp.	M	13			50	20	4	<1	Renaud et al. 1999
Isochrysis sp.	M	23			47	6	1	10	Renaud et al. 1999
Schizochytrium sp.	M		24		34	<1	1	33	Jiang et al. 2004
Spirulina sp.	M	9			7	1	2	3	Griffiths and Harrison 2009; Tokuşoglu and Ünal 2003
Rapeseed	T	35			10	10	nd	nd	Dubois et al. 2007; Issariyakul and Dalai 2014
Palm oil	T	40			< 1	< 1	nd	nd	Dubois et al. 2007; Issariyakul and Dalai 2014
Soybean	T	20			8	8	nd	nd	Dubois et al. 2007; Issariyakul and Dalai 2014
Sunflower	T	45			< 1	< 1	nd	nd	Dubois et al. 2017; Issariyakul and Dalai 2014

S seaweeds, *M* microalgae, *T* terrestrial plants, *nd* not detected

For biodiesel, a feedstock with a more saturated fatty acid profile, with a low proportion of PUFA and a high proportion of C18:1(n-9), is preferred (Hu et al. 2008; Knothe 2008). The MUFA C18:1(n-9) also has demand in the chemical industry for the synthesis of chemical compounds and in particular renewable, non-toxic and biodegradable bio-polymers (Biermann et al. 2006; Biermann and Metzger 2008; Gandini 2008; Lligadas et al. 2010). From the species examined in detail in this thesis, *S. macrodontum* had the most suitable fatty acid profile for the production of biodiesel and bio-materials, as it has the most saturated fatty acid profile (~36 % SFA of TFA) and the highest proportion of C18:1(n-9) (~18 % of TFA). However, its high proportion of PUFA(n-3) would be problematic for the production of biodiesel as this reduces oxidative stability and therefore provides a lower quality fuel (Knothe 2006). In contrast, many terrestrial oil crops such as palm oil and rapeseed have low proportions

of PUFA(n-3) and more saturated fatty acid profiles with higher proportions of C18:1(n-9) than seaweeds (e.g. palm oil: 50 % SFA of TFA, 40 % C18:1(n-9) of TFA, Dubois et al. 2007) which makes their oils better suited for biodiesel production. Therefore, from an oil quality perspective, seaweed biomass is a potential feedstock for biodiesel and biomaterial production; however, compared to the currently utilized terrestrial oil crops their fatty acid quality parameters make them a secondary feedstock choice. Furthermore, the production of biodiesel is clearly uneconomic at this stage and this is discussed and supported in detail in section 6.4.2.

6.3. Fatty acid variability within species

Although the species examined in detail in this thesis have lower oil contents than required for economical biodiesel production, and their contents of EPA and DHA are lower than in many species of microalgae and fish oils, which limits their potential for specific health and aquaculture feeds, there is significant scale for improvement of the yield and quality of fatty acids through refined culture methods and strain selection. The basis for these improvements is the identification and quantification of natural variability in growth, and in the content and composition of fatty acids. While many species of terrestrial crops (Byrum et al. 1997; Downey and Craig 1964; Khush 2001) and microalgae (Grima et al. 1995; Rodolfi et al. 2009; Sharma et al. 2012; Wijffels and Barbosa 2010) have undergone a process of domestication with substantial improvements in their content and composition of fatty acids through advanced culture methods and strain selection, this process is still in its infancy for seaweeds (Robinson et al. 2013) and similar improvements are predicted.

The second major outcome of this thesis was therefore the identification and quantification of natural variability in fatty acids within species on a seasonal and spatial scale, and also within individual plants. For *S. macrodontum*, the content of TFA (55 – 83 mg g^{-1} dw) and the proportion of PUFA(n-3) (16 – 25 % of TFA) varied substantially

(~ 50 %) on a temporal scale (monthly). For *D. bartayresii*, the content of TFA (45 – 55 mg g^{-1} dw) varied slightly (~ 20%) and the proportion of PUFA(n-3) (16 – 24 % of TFA) varied substantially (~ 50 %) on a temporal scale (monthly). There was also spatial variation for *D. bartayresii* which was 50 % for the content of TFA (36 – 54 mg g^{-1} dw) but less than 10 % for the proportion of PUFA(n-3) (18 – 20 % of TFA). This within species variability in fatty acids was attributed to the environmental parameters temperature and light, but also to biotic factors such as plant sizes, life history events and a genotypic component. In addition, there was also substantial within-plant variability in the content of TFA and to a lesser degree in the composition of fatty acids in *S. macrodontum* (TFA: 21 – 106 mg g^{-1} dw) and *D. bartayresii* (TFA: 40 – 57 mg g^{-1} dw). The possible drivers identified for this variability were the morphological and functional differentiation of the thallus, exposure to environmental microhabitats, heterogenic herbivory and microbial defence. The drivers of variability and implications for culture are discussed in detail in sections 6.3.1 to 6.3.4.

6.3.1. Variability – Environmental drivers

The natural variability in the content and composition of fatty acids within a species can be a response to changes in the environmental conditions of water temperature, light availability and nitrogen. While there is extensive literature regarding environmental effects on the content and composition of fatty acids in microalgae (Cohen et al. 1988; Sharma et al. 2012; Solovchenko et al. 2008), research on seaweeds is largely restricted to a small number of field studies based on broad environmental correlations with fatty acids (Nelson et al. 2002; Schmid et al. 2014) and only a few studies in a limited number of species have experimentally quantified the effects of temperature (Al-Hasan et al. 1991; Floreto et al. 1993) and light (Hotimchenko 2002; Khotimchenko and Yakovleva 2005) on fatty acids. Additionally, while the effect of nitrogen starvation can lead to substantial changes in the content and composition of PUFA(n-3) in microalgae (Grima et al. 1995), the quantification of the effects of nitrogen availability on the

content and composition of fatty acids in seaweeds is restricted to a few species of *Ulva* (Floreto et al. 1996; Gordillo et al. 2001; Pinchetti et al. 1998) and *Gracilaria* (Dawes et al. 1993). Overall, the effects of environmental parameters on the fatty acids in seaweeds are species specific and no generalizations can be drawn from information in the available literature.

The third major outcome of this thesis was therefore the demonstration of the effect of environmental parameters on the fatty acids in four species of seaweed and therefore the provision of a basis for future optimization of the production of fatty acids in seaweed culture. The first line of evidence was from seasonal field-based studies on *S. macrodontum* and *D. bartayresii* which showed a higher proportion of PUFA(n-3) in winter when water temperature and light availability were at their annual minimum. This suggests that water temperature and light are potential drivers for fatty acid variation in seaweeds. While these field studies show a correlation between fatty acids and the environmental parameters of water temperature and light, it was not possible to distinguish between the possible effects of the two environmental drivers, as other factors such as plant sizes are also potential drivers. In a second line of evidence based on controlled growth trials of *D. tenuissima*, it was possible to not only quantify the overall variation in the content and composition of fatty acids but also identify and quantify the individual drivers of this variation. These experimental results support the field-based studies, and colder water temperature was identified as the major driver to improve the proportion of PUFA(n-3) in biomass. In a similar manner, high light intensity reduced the quality of the biomass by increasing saturation.

These results are in congruence with the current physiological understanding of the effects of water temperature and light on the composition of fatty acids in algae. A higher proportion of PUFA(n-3) at colder water temperatures is a physiological response to improve the membrane fluidity in the thylakoid membranes as PUFA(n-3) have a

lower melting point than saturated fatty acids (Los et al. 2013; Thompson et al. 1992). In a similar manner, a higher proportion of PUFA(n-3) at low light intensity can be caused by increased production of chloroplasts and associated membrane lipids (Sharma et al. 2012) which are primary sources of PUFA(n-3) (Sanina et al. 2004). The increased fluidity of the thylakoid membranes imparted by higher amounts of PUFA(n-3) also increase electron-flow in the chloroplasts providing a physiological advantage at low light intensity (Mock and Kroon 2002). Furthermore, increased rates of oxidation at high light intensity can lead to a breakdown of double bonds and thereby increase the degree of saturation (Sharma et al. 2012). While an increase in the amount and/or proportions of PUFA(n-3) at low water temperature and low light intensity were identified for the three key species of seaweeds identified in this thesis, this is not a general pattern for seaweeds, as the opposite was found for *Dictyopteris australis* which had a higher proportion of PUFA(n-3) during summer when water temperature and light intensity were at their annual maximum. Similar species-specific changes in seasonal oil contents and quality have been reported previously (Schmid et al. 2014). Furthermore, in one isolate of *D. tenuissima*, the proportion of PUFA(n-3) remained stable across water temperature treatments (Chapter 5), highlighting that environmental effects on fatty acids can be very specific between species and also within-species. From a practical perspective, these responses to environmental factors in selected species, or genotypes within a species, can be exploited through seasonal culture and harvest practices to obtain a biomass feedstock of higher quality defined by a higher oil productivity and more specifically a higher PUFA(n-3) productivity. While the seasonal variation in the content and quality of oils provides the opportunity to optimize the content and quality of feedstock oils, there are trade-offs. For example, many species of seaweed have distinct seasonal growth patterns where the largest biomass density and growth rates do not coincide with the period of highest biomass quality. The effect of biotic factors on the content and quality of oils is discussed in detail in section 6.3.2.

6.3.2. Variability – biotic drivers

The natural variability in growth, and the content and composition of fatty acids, within a species can be due to biotic factors. The relationship between biotic factors such as plant size or reproductive stage and content or quality of fatty acids has been demonstrated in only a few species of seaweed. For example, larger plants of the brown seaweed *Costaria costata* had a higher content of TFA (Gerasimenko et al. 2010), while larger plants of species of *Caulerpa* had a lower proportion of EPA and therefore the harvest of smaller plants provides a biomass feedstock of higher quality (Paul et al. 2014). Although the reason for this size dependence of specific fatty acids in some species remains unclear, it is potentially driven by changes in the stage of development and reproduction which can also be linked to size as demonstrated for species of *Dictyota* (Ateweberhahn et al. 2005; Tronholm et al. 2008).

The fourth major outcome of this thesis was therefore the identification of a relationship between plant size and life cycle events with the content and composition of fatty acids in key species of seaweed for oil-based bioproducts. In *D. bartayresii*, plants with a larger thallus length had significantly higher contents of TFA and slightly higher proportions of PUFA(n-3). From a commercial perspective, the biomass of larger plants is therefore more suitable for the production of oil-based bioproducts. In this thesis, it was also found that the fatty acid composition of *S. macrodontum* was not correlated to size, but that older plants in their "decline phase" had a more saturated fatty acid profile than younger plants in their "growth phase". The harvest of younger biomass therefore provides a feedstock with a higher proportion of PUFA(n-3) and is therefore more suitable for bioproducts in health and nutrition. Therefore, the careful species-specific optimization of harvest timing in terms of biomass size and biomass oil quality is a critical component of an optimal culture and harvest strategy.

6.3.3. Variability – genotypic drivers

In addition to the environmental and biotic factors that can drive the natural variability in growth and the content and composition of fatty acids within a species, this variability can also be due to genotypic traits for individuals and populations of a species. While the genotypic variability of fatty acids has been examined in terrestrial crops such as soybean (Byrum et al. 1997) and rapeseed (Downey and Craig 1964) to improve the content and composition of fatty acids for particular oil-based bioproducts, and specifically for the production of biodiesel, this branch of research is still in its infancy in seaweeds. However, the first improvements of the lipid content through selective breeding have been made in the commercially important kelp *Macrocystis* with a 20 % increase in total lipids (Westermeier et al. 2012). This marks the first step in the domestication of seaweeds for oil-based bioproducts. There has to date been no evidence of the genotypic variation of fatty acids in species of seaweeds (Robinson et al. 2013).

The fifth major outcome of this thesis was therefore the provision of two lines of evidence for the genotypic variability of fatty acids in seaweeds. First, there was substantial spatial variability (~ 40 – 60 %) in the content of fatty acids between the sampling locations for *Dictyota dichotoma* (Chapter 2) and in particular *D. bartayresii* (Chapters 2 and 4). Because neither environmental differences between the locations, nor the differences in plant sizes adequately explained this large variation, it was suggested that this variability was due to genotypic differences between populations of *Dictyota*. However, due to the nature of field-based studies, it was not possible to quantify the environmental, biotic and possible genotypic component of this variation. For correct quantification and portioning of the environmental and the genotypic component of variability in fatty acids, the effects of the environmental parameters temperature, light and nitrogen were tested for three isolates of *D. tenuissima* in a controlled manipulative experiment (Chapter 5). This provided a second line of strong

evidence for the genotypic variation of fatty acids. Nearly 50 % of the total variation in the content of TFA was explained by differences between isolates and in a similar manner, the proportion of the fatty acid groups (SFA, MUFA, PUFA) also differed between isolates. Environmental conditions also had an impact on the composition of fatty acids and consequently there were strong "genotype × environment" interactions. Notably, the environmental parameters of temperature and light only had an effect in two of the three isolates. This thesis therefore provides the first strong support that part of the variability of the content and composition of fatty acids in seaweeds is genotypic, as evidenced by the portioning of the source of the variation in the content of TFA and composition of fatty acids between the isolates and environmental treatments.

6.3.4. Variability within the thallus

There was also strong natural variability in the content and composition of fatty acids within the thallus of individual plants of *D. bartayresii*, *D. australis* and *S. macrodontum*. This heterogeneous distribution of oils and fatty acids is typical for terrestrial oil crops where the oils are concentrated in seeds and fruits while the remaining plant material is of low economic value for oil production (del Río-Celestino et al. 2008). Seaweed biomass, however, is generally harvested as whole biomass and little attention is given to possible within-plant variation of fatty acids. A limited number of studies have analysed the within-plant variation of fatty acids and only in a few selected species of seaweed (Khotimchenko and Kulikova 2000; Kulikova and Khotimchenko 2000; Schmid and Stengel 2014). The sixth major outcome of this thesis was therefore the provision of a substantial contribution to this field of research and the provision of the first data on the within-plant variation of fatty acids in key species of seaweed suitable for the production of oil-based bioproducts. The content of TFA differed between tips and base by 160 % in *S. macrodontum* and by 35 % in *D. bartayresii*. Overall, the upper sections of these brown seaweeds were the most suitable plant sections for oil-based bioproducts because of a higher content of TFA and a higher

proportion of PUFA(n-3). This pattern is similar to a previous study (Schmid and Stengel 2014) and therefore is likely to be a typical pattern for many species of seaweed, in particular brown seaweeds.

This thesis identified several possible drivers for this within-plant variation in fatty acids. The thallus of some brown seaweeds has a high degree of morphological and functional differentiation with a structural base for attachment to the substratum and bioactive midsections and tips for photosynthesis and reproduction (Lawrence and McClintock 1988). Photosynthetic membranes have been associated with a higher proportion of glycolipids and therefore a higher proportion of PUFA(n-3) (Sanina et al. 2004). Further, fatty acids can have functions in microbial defence (Alamsjah et al. 2008; Rosell and Srivastava 1987) and the heterogenic distribution of microbial defence and antifouling compounds has been demonstrated previously in seaweeds (Alamsjah et al. 2008; de Nys et al. 1996). In addition, environmental parameters such as light can differ on a small scale through the self-shading of plants and therefore provide heterogeneous microhabitats that affect fatty acids in plant sections differently. This within-plant variation of fatty acids can be of practical importance as it allows for specialised partial harvesting techniques, analogous to the selective harvest of terrestrial oil crop seeds, to improve the oil yield of seaweed production. Furthermore, future selective breeding for plant morphologies that maximize plant sections with inherently higher levels of oil can be an important step towards domestication and oil yield optimization in seaweeds. In addition, there is the possibility to selectively increase the oil content in plant sections through the over-expression of specific genes, as demonstrated for terrestrial plants (Vanhercke et al. 2014), and similar approaches may be applicable to seaweeds.

6.4. Suitability for oil-based applications

6.4.1. Cultivation methods

A critical parameter for the identification of a novel bioresource is its suitability for cultivation and biomass productivity at scale. However, there are no tested methods for the cultivation of the tropical species of brown seaweed identified in this thesis. Notably culture strategies will differ significantly between species based on their growth habit, thallus size and life history.

S. macrodontum is currently not utilised commercially and data on its potential for cultivation is limited to a single study that included experimental growth trials in tank-based systems (Israel and Hophy 2002) and no data on its biomass productivity in culture has been published. As part of this thesis I had attempted to culture *S. macrodontum* on a number of occassions and this was not successful using fragmentation approaches common for land-based cultivation. Based on the seasonal changes of biomass in the field (Chapter 3), an average SGR of 2.5 % day^{-1} has been calculated which is lower than for the extensive culture of major commercial seaweeds such as *Kappaphycus* (3.72 – 7.17 % day^{-1}; Hurtado-Ponce 1992) or *Gracilaria* (2.5 – 7.8 % day^{-1}; Skriptsova and Nabivailo 2009). The commercial potential for the cultivation of *S. macrodontum* if it were to be pursued would occur in the South Pacific where it is naturally abundant on islands such as Vanuatu and Samoa (Skelton et al. 2007). In a similar manner, *D. bartayresii* is largely untapped as a renewable bioresource and there is only limited culinary use of *Dictyota* in parts of Asia (Indonesia, Malaysia, Thailand) and Hawaii where wild-collected plants are used for traditional dishes (De Clerck et al. 2001). *Dictyota* is a potential species for cultivation as it has a circumtropical distribution with locally high population densities in excess of 700 to 800 g fw m^{-2} (Beach and Walters 2000) and rapid population recovery through asexual reproduction (fragmentation) has been reported (Herren et al. 2006), highlighting its potential for cultivation.

For *S. macrodontum* and *D. bartayresii*, a culture method similar to other brown seaweeds such as kelps or *Sargassum* might be applied. Kelps and *Sargassum* are typically cultured on floating long lines with juvenile plants being attached to the ropes for grow out (Tseng 2003; Yu et al. 2013). The quantification of natural seasonal variation in the content and composition of fatty acids and the seasonal variation in biomass provide the framework on which to develop culture and harvest strategies to maximise the net recovery of target fatty acids. For example, for *S. macrodontum* and *D. bartayresii*, a harvest during the colder winter months would provide a feedstock with a higher proportion of PUFA(n-3) which is the target for functional foods and nutraceuticals (Adarme-Vega et al. 2014; Gill and Valivety 1997). Likewise a harvest during summer would provide a feedstock with a more saturated fatty acid profile which makes it more suitable for biodiesel production (Meher et al. 2006; Ramos et al. 2009; Ramírez-Verduzco et al. 2012). Furthermore, there is potential for partial harvest to utilise the within-plant variation of fatty acids. Partial harvest of the tips and midsection can provide a biomass feedstock with a higher content of TFA and higher proportion of PUFA(n-3) compared to whole plants.

In contrast to the foliose brown seaweeds where little is known about their cultivation, the suitability of *D. tenuissima* for large-scale tank-based cultivation has been confirmed, where it has a high biomass (15 – 25 g dw m^{-2} day^{-1}), and consequently, fatty acid productivity (0.8 – 1.4 g dw m^{-2} day^{-1}) (Magnusson et al. 2014; Mata et al. 2015). This biomass productivity is within the range of many microalgae where biomass productivities between 10 and 50 g dw m^{-2} day^{-1} are common (Huerlimann et al. 2010; Mata et al. 2010a). Although the general suitability for cultivation and biomass applications has been established in *D. tenuissima*, biomass and fatty acid productivities are likely not yet fully exploited and may be improved through refined culture strategies of environmental manipulation and the selection of strains with advantageous attributes.

This thesis provided a detailed framework for potential culture management strategies for *D. tenuissima*. There were substantial differences in the content and composition of fatty acids between isolates and the selection of a particular isolate of *D. tenuissima* will have important implications for choosing culture strategies because of strong genotype by environment interactions. Some genotypes of this species had higher growth rates and higher contents of TFA, and the proportion of PUFA(n-3) was elevated to nearly 45 % of TFA in the low water temperature treatment. Importantly, these increases in the proportion of PUFA(n-3) were achieved in the presence of a stable content of TFA and high growth rates, resulting in a net increase in PUFA(n-3) productivity. However, in another genotype of this species, water temperature had no effect on the content and composition of fatty acids. This stability in the composition of fatty acids is beneficial for commercial cultivation for health and food applications where a stable supply of PUFA(n-3) is preferred (Gellenbeck 2012; Hafting et al. 2012).

6.4.2. Economic potential

Biomass of certain species of seaweeds contains high proportions of omega-3 fatty acids with clear therapeutic value which justifies their inclusion as an ingredient in functional foods, nutraceuticals and animal feeds. The simplest way to include seaweeds as an ingredient is through the addition of dried seaweed biomass to foods and feeds to enhance the nutritional value by improving the proportion of essential omega-3 fatty acids. In a similar manner, dried seaweed biomass powder can be processed to tablets or encapsulated to be marketed as a nutraceutical. A current example of this approach is the dried powder of the filamentous cyanobacteria *Spirulina* spp. which is marketed in the form of tablets and capsules as a novel ‘superfood’ (Borowitzka 2013; Carvalho and Pereira 2015).

Alternatively, there is the option to extract the fatty acids for concentrated omega-3 products, in a similar manner to plant and microalgal oils. A particularly important

consideration is the choice of the extraction method from economic, extraction efficiency and oil quality standpoints. Oils from dried biomass are traditionally extracted by Soxhlet solvent (hexane) extractions (Wang and Weller 2006) which have high extraction efficiencies but this method is time consuming and the high quantity of toxic solvents used is concerning (Wang and Weller 2006). An alternative extraction method is supercritical carbon dioxide fluid extraction (Chan and Ismail 2009; Crampon et al. 2011; Herrero and Ibáñez 2015) which uses less solvent and therefore is more suited for oils in the health and nutrition sector. However, a major drawback of this extraction method is that, similar to Soxhlet extraction, dried biomass is required as feedstock. Novel extraction methods for wet biomass such as supercritical methanol extraction (Patil et al. 2011) or liquid dimethyl ether extraction (Kanda et al. 2012) can also be used. However, these methods have only been tested on microalgae and their suitability for seaweed biomass needs to be confirmed.

The total market size for the omega-3 EPA and DHA oils has been estimated at 135,000 – 190,000 tonnes in 2015 (Frost and Sullivan 2010). Considering a market price of US$ 140 kg^{-1} for algal derived EPA and DHA oils (Borowitzka 2013), there is substantial economic potential. However, a major drawback is the low proportion of DHA in seaweed oils. In addition to the traditional source of DHA from fish oils, DHA is currently sourced from microalgae and commercialized under the brand DHA-SCO™ at an annual production of approximately 3000 tonnes in 2013 (Ratledge 2013). Nevertheless, the total market for omega-3 based functional foods and nutraceuticals has been estimated at over US$ 34 billion (Adarme-Vega et al. 2014) and the demand for omega-3 rich ingredients in the fast growing aquaculture sector is enormous, providing a substantial market for omega-3 oils rich in ALA and EPA as derived from seaweed biomass. Previous concerns about the low scalability of high value products because of presumably rapid market saturation (Stephens et al. 2010a) are unjustified in light of the size and growth of the omega-3 market.

Although the biomass of the key species identified in this thesis, and in particular *S. macrodontum*, is technically suitable for the extraction of oils with subsequent transesterification of fatty acids to methyl esters for biodiesel, the utilization of seaweed oils for biodiesel is clearly not economic. The benchmark lipid productivity for economical biodiesel production from microalgae has been estimated at 5 g lipid m^{-2} day^{-1} to provide an internal rate of return of 15 % (Stephens et al. 2010a). To achieve this benchmark with seaweeds, a species with a 10 % total lipid content would need to be cultured at a biomass productivity of over 50 g dw m^{-2} day^{-1}. While *S. macrodontum* had the most suitable fatty acid profile for biodiesel, its highly seasonal growth with an average specific growth rate of 2.5 % day^{-1} make it an unrealistic biomass feedstock for such applications. Although *D. tenuissima* has much higher growth rates (12 – 33 % day^{-1}, Chapter 5) and therefore higher biomass productivities (~ 20 g dw m^{-2} day^{-1}, Magnusson et al. 2014; Mata et al. 2015), its lipid productivity would be 2 g lipid m^{-2} day^{-1} and therefore below the benchmark for economical biodiesel production. Furthermore, these economic analyses were based on a crude oil price of US$ 100 bbl^{-1} (Stephens et al. 2010a) which has decreased to a current value of approximately US$ 50 bbl^{-1} (IndexMundi 2015). Biofuels are therefore clearly low value products which are not justified with the current high production costs of algal biomass. Even when considering the lower cost of production of seaweed biomass as opposed to microalgae (Paul et al. 2012) and the potential of improvements in the content and composition of fatty acids through refined culture strategies and selective breeding, the differential in value between algal derived omega-3 oils (US$ 140 kg^{-1}, Borowitzka 2013) and crude oil for the fuel market (US$ 1.13 kg^{-1}, Borowitzka 2013) is two orders of magnitude and therefore utilising oils derived from seaweed in the fuel market, as opposed to the omega-3 market, is clearly uneconomic. The only potential options for biofuels from seaweed biomass are therefore bio-ethanol (John and Anisha 2011; Kraan 2013) and liquid bio-oils where biomass is thermo-chemically processed by hydrothermal liquefaction (Anastasakis and Ross 2011; Neveux et al. 2014a) or pyrolysis (Budarian et

al. 2011; Ross et al. 2008; Rowbotham et al. 2012) after the extraction of high value by-products such as omega-3 oils or proteins (Neveux et al. 2014b; Stephens et al. 2013a).

6.5. Future research directions

This thesis identified three key species of seaweed with high oil contents and fatty acid profiles suitable for oil-based bioproducts. The high diversity of seaweeds with an estimated 8000 species (Lüning 1991) provides further opportunity for the identification of novel high lipid species. The phylogenetic pattern of lipids and fatty acids found in this thesis allows for efficient identification of further high lipid species through phylogeny-guided screening. Therefore, species of the Dictyotales (244 species, Guiry and Guiry 2014) and Bryopsidales (542 species, Guiry and Guiry 2014) should be the prime targets for future profiling. Of particular interest are species with a high content of the essential omega-3 polyunsaturated fatty acids (ALA, EPA, DHA) that are targeted for applications in the health and animal feed sectors that have the highest economic potential. However, the low content of DHA in seaweeds, including the species identified in this thesis, has been identified as a point-of-concern for seaweed oils for these applications. Identification of species with a high total content of fatty acids and a high content of DHA should be the focus of future bioprospecting.

In addition, there is the possibility to improve oil quality in seaweeds through genetic engineering. There have been recent advances in producing transgenic terrestrial oil crops to produce high levels of DHA (> 12 % of TFA) through the introduction of transgenic desaturase pathways derived from microalgae and yeasts (Petrie et al. 2014). Similar genetic engineering of novel and also traditional commercial seaweeds for EPA and DHA production is therefore a clear future direction for research. While fish oils are the traditional source of EPA and DHA, there have been recent concerns about the oxidative stability of these fatty acids in nutraceutical products with lower proportions of both EPA and DHA in products than advertised (Albert et al. 2015). In contrast to

fish oils, seaweeds are rich in natural antioxidants (Jiménez-Escrig et al. 2012; Kindleysides et al. 2012; Magnusson et al. *accepted*; Sabeena and Jacobsen 2013) which have been successfully used to reduce lipid oxidation in canned fish samples (Ortiz et al. 2014). The potential of the natural antioxidants to preserve PUFA(n-3) in commercial seaweed based omega-3 products can be a substantial advantage of seaweed oils compared to fish oils, which remains to be confirmed.

Most of the oil rich species identified in this thesis have not been considered for cultivation and cultivation methods have only been established for *D. tenuissima* (Magnusson et al. 2014; Mata et al. 2015). However, the suitability for cultivation, and the corresponding productivity of biomass and oils and target fatty acids, are key measures for selecting and developing oil-based bioproducts from seaweeds and therefore need to be addressed for *D. bartayresii* and *S. macrodontum*. Although there is a potential for these brown seaweed to be cultured commercially, the labor intensive cultivation methods (as described in section 6.4.1), the seasonal growth patterns and the slow growth compared to other seaweeds will likely restrict their commercial utilization to few localities and niche markets. Overall, *D. tenuissima* is the most suitable species for the commercial production of omega-3 products because of its suitability for intensive land-based culture where it has high biomass productivities (Magnusson et al. 2014; Mata et al. 2015). Furthermore, its proportion of PUFA(n-3) is among the highest for seaweeds and this thesis provided clear evidence for the potential of further improvements in growth and the content and composition of fatty acids through culture strategies but in particular provides the basis for selective breeding of this species. While the small-scale experiment for *D. tenuissima* successfully quantified and portioned the drivers for biomass and fatty acid variability into an environmental and a genotypic component, it remains to be confirmed that these results are transferable to scaled-up, long-term outdoor cultivation systems.

6.6. Conclusion

Overall, this thesis provides the basic framework on which to develop strategies for the domestication of seaweeds for the production of oil-based bioproducts in a similar manner to the past improvements in the oil yield of terrestrial oil crops and also microalgae. The most important consideration for this domestication process is the selection of a species which has high biomass productivities in culture with a high yield of target fatty acids (omega-3). The next step will then be the improvement of the biomass productivity and the yield of the target fatty acids. While there is potential for improvements through culture strategies by exploiting natural variability caused by environmental and biotic factors, the major avenue for improvements is the selective breeding of a species to create new strains with permanently improved attributes in growth and yield of target fatty acids. Based on the findings of this thesis and the literature, *D. tenuissima* is the most suitable species for this approach because of its high biomass productivity in culture and its high content and high proportion of PUFA(n-3). Based on this species, this thesis provides for the first time evidence for the genotypic variability of PUFA(n-3) and therefore the potential for selective breeding of seaweed in the context of oil production for health and nutrition.

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APPENDIX

Supplementary Tables

Table A2.1. Total lipid content (mg g^{-1} dw ± STDEV) of seaweeds.

Species	Type	Total lipids	STDEV
Boodlea composita	Chlorophyta	24.7	3.1
Bryopsis sp	Chlorophyta	71.3	8.1
Caulerpa lentillifera	Chlorophyta	54.6	7.5
Caulerpa racemosa clavifera	Chlorophyta	44.1	5.7
Caulerpa racemosa laetevirens	Chlorophyta	22.7	3.0
Caulerpa racemosa occidentalis	Chlorophyta	47.3	4.9
Caulerpa serrulata	Chlorophyta	72.8	0.9
Caulerpa sertularioides	Chlorophyta	130.4	14.6
Caulerpa taxifolia	Chlorophyta	65.4	7.3
Chaetomorpha linum	Chlorophyta	44.1	10.7
Chlorodesmis fastigiata	Chlorophyta	95.8	9.3
Cladophora coelothrix	Chlorophyta	54.8	5.9
Cladophora patentiramea	Chlorophyta	98.7	4.3
Cladophora regulosa	Chlorophyta	24.2	2.3
Cladophora rugulosa	Chlorophyta	65.8	6.8
Derbesia tenuissima	Chlorophyta	121.4	5.9
Enteromorpha clathrata	Chlorophyta	17.9	1.0
Udotea argentea	Chlorophyta	31.7	5.1
Ulva fleximosa	Chlorophyta	65.1	7.7
Ulva rigida	Chlorophyta	32.4	1.3
Ulva rigida	Chlorophyta	24.8	4.5
Colpomenia sinuosa	Phaeophyceae	29.0	7.3
Cystoseira trinodis	Phaeophyceae	44.6	2.7
Dictyopteris australis	Phaeophyceae	84.7	2.9
Dictyopteris delicatula	Phaeophyceae	58.5	5.7
Dictyota bartayresii	Phaeophyceae	119.1	20.0
Dictyota dichotoma	Phaeophyceae	107.9	9.8
Lobophora variegata	Phaeophyceae	29.9	3.7
Padina australis	Phaeophyceae	41.1	2.8
Rosenvingea intricata	Phaeophyceae	17.9	1.8
Sargassum flavicans	Phaeophyceae	21.9	4.8
Sargassum neurophorum	Phaeophyceae	26.7	3.1
Sargassum spinifex	Phaeophyceae	24.2	2.6
Spatoglossum macrodontum	Phaeophyceae	117.3	4.9
Turbinaria ornata	Phaeophyceae	63.8	5.0
Acanthophora spicifera	Rhodophyta	20.2	0.6
Asparagopsis taxiformis	Rhodophyta	14.9	11.3
Ceramium	Rhodophyta	56.9	2.0
Champia parvula	Rhodophyta	37.2	1.3
Gracilaria sp	Rhodophyta	52.0	5.8
Halymenia sp	Rhodophyta	14.8	2.0
Hypnea cervicornis	Rhodophyta	17.5	4.3
Hypnea pannosa	Rhodophyta	22.4	3.8
Jania sp	Rhodophyta	18.4	4.5
Laurencia majuscula	Rhodophyta	41.5	3.4
Laurencia sp	Rhodophyta	51.2	2.0
Plocamium sp	Rhodophyta	37.9	0.3

Table A2.2. Fatty acid compostion of analysed brown seaweeds (mg g^{-1} dw ± STDEV).

	Brown seaweeds													
	Colpomenia sinuosa	*Cystoseira trinodis*	*Dictyopteris australis*	*Dictyopteris delicatula*	*Dictyota bartayresii*	*Dictyota dichotoma*	*Lobophora variegata*	*Padina australis*	*Rosenvingea intricata*	*Sargassum flavicans*	*Sargassum neurophorum*	*Sargassum spinifex*	*Spatoglossum macrodontum*	*Turbinaria ornata*
C14:0	1.27	1.13	2.88	2.49	2.54 ±0.70	2.28 ±0.65	1.91	1.43 ±0.19	1.00	0.67	0.85	0.72	4.95 ±0.30	0.64 ±0.04
C14:1	0.21	0.21	0.21	0.24	0.38 ±0.12	0.35 ±0.12	0.20	0.22 ±0.00	0.23	0.19	0.20	0.20	0.21 ±0.00	
C15:0	0.24	0.26	0.26	0.25	0.34 ±0.07	0.34 ±0.09	0.25	0.30 ±0.00	0.23	0.23	0.23	0.23	0.32 ±0.03	0.23 ±0.00
C16:0	3.77	7.83	6.61	5.24	7.17 ±2.14	6.85 ±1.49	5.79	7.36 ±0.07	4.87	4.13	5.16	4.89	14.30 ±1.42	4.78 ±0.40
C16:1(n-9)	0.19	0.21	0.20	0.20	0.28 ±0.02	0.24 ±0.05	0.24	0.21 ±0.01	0.21	0.20	0.20	0.21	0.24 ±0.00	0.20 ±0.01
C16:1(n-7)	0.50	1.19	0.82	0.83	0.62 ±0.09	0.82 ±0.37	0.47	1.06 ±0.20	0.46	0.80	0.80	1.09	1.89 ±0.16	0.62 ±0.08
C16:1	0.24	0.21	0.25	0.25	2.21 ±0.65	2.09 ±1.50		1.10 ±0.09	1.02	0.20		0.21	0.38 ±0.00	
C16:2(n-6)	0.23	0.22			0.35 ±0.03	0.29 ±0.11		0.24 ±0.02					0.26 ±0.01	0.25
C16:2(n-4)					0.23 ±0.02	0.27 ±0.08				0.21	0.20		0.22 ±0.01	0.25 ±0.03
C17:0	0.19	0.22	0.20	0.20	0.22 ±0.01	0.34 ±0.20		0.21 ±0.00	0.21	0.21	0.21	0.21	0.22 ±0.01	0.21 ±0.00
C16:3(n-6)	0.20	0.21	0.22	0.23	0.42 ±0.10	0.34 ±0.09	0.20	0.22 ±0.01		0.21	0.20	0.21	0.23 ±0.01	0.20 ±0.00
C16:3(n-3)			0.20		0.26 ±0.04	0.43 ±0.18					0.22		0.29 ±0.02	0.22 ±0.01
C16:4(n-3)	0.22	0.24	0.34	0.25	0.35 ±0.09	0.41 ±0.26	0.25	0.24	0.21	0.21	0.24	0.23	0.28 ±0.00	0.22 ±0.01
C18:0	0.30	0.41	0.40	0.50	0.78 ±0.16	0.60 ±0.11	0.32	0.64 ±0.36	0.33	0.35	0.29	0.33	0.74 ±0.05	0.30 ±0.02
C18:1(n-9)	2.39	3.19	5.19	3.80	4.65 ±1.38	4.43 ±0.51	3.33	3.84 ±0.61	2.02	1.53	2.10	1.78	10.31 ±0.91	2.13 ±0.17
C18:2(n-6)trans			0.30	0.24	0.42 ±0.29	0.35 ±0.13	0.29	0.27					0.26 ±0.00	
C18:2(n-6)cis	0.42	1.15	1.60	0.73	0.92 ±0.33	0.71 ±0.08	0.33	1.29 ±0.38	0.56	0.72	0.96	0.58	1.54 ±0.04	1.17 ±0.12
C18:3(n-6)	0.27	0.27	0.42	0.32	0.49 ±0.17	0.49 ±0.13	0.27	0.55 ±0.15	0.29	0.31	0.32	0.23	0.65 ±0.04	0.31 ±0.03
C18:3(n-3)	0.36	1.31	2.64	0.83	1.31 ±0.57	0.97 ±0.38	0.27	1.37 ±0.02	0.41	0.71	1.36	0.44	2.62 ±0.06	1.48 ±0.24
C18:4(n-3)	0.52	0.94	2.03	1.06	3.42 ±1.21	2.51 ±0.81	0.43	3.10 ±0.00	0.40	0.64	1.32	0.34	5.21 ±0.52	1.18 ±0.34
C20:0	0.28	0.26	0.30	0.34	0.45 ±0.09	0.37 ±0.07	0.27	0.31 ±0.09	0.25	0.23	0.23	0.24	0.45 ±0.00	0.23 ±0.02
C21:0		0.23	0.42	0.32	0.84 ±0.23	0.64 ±0.37	0.48	0.20			0.26		0.34 ±0.00	0.28 ±0.01
C20:3(n-6)	0.23	0.43	0.74	0.46	0.54 ±0.31	0.47 ±0.23	0.32	0.60 ±0.01	0.25	0.32	0.35	0.29	2.15 ±0.09	0.40 ±0.05
C20:4(n-6)	0.70	2.75	3.07	2.33	2.57 ±1.58	2.76 ±1.75	0.81	2.28 ±0.45	0.95	1.75	2.46	1.37	4.47 ±0.37	2.06 ±0.25
C20:4(n-3)	0.23	0.32	0.66	0.40	0.77 ±0.18	0.63 ±0.24	0.24	0.48 ±0.02	0.24	0.28	0.31	0.24	2.21 ±0.01	0.38 ±0.05
C22:0	0.60	1.34	0.97	1.80	1.22 ±0.47	1.16 ±0.68	0.72	0.63 ±0.07	0.56	0.87	1.28	0.60	1.96 ±0.18	1.15 ±0.38
C20:5(n-3)	0.61	1.24	0.75	1.61	1.42 ±0.32	1.40 ±0.67	0.68	0.61 ±0.08	0.62	0.80	1.36	0.58	2.03 ±0.08	1.21 ±0.39
C24:0	0.27	0.31		0.25	0.20	0.22	0.24		0.27	0.26	0.27		0.30 ±0.01	0.28 ±0.03
C22:6(n-3)					0.27 ±0.02	0.27 ±0.01								
Other FAs	0.00	0.00	0.47	0.26	1.18 ±0.47	1.21 ±0.38	0.26	0.24 ±0.01	0.15	0.09	0.00	0.57	0.29 ±0.16	0.00
Total FAs	13.25	24.27	31.22	23.79	35.15 ±8.69	32.19 ±7.76	17.37	27.81 ±0.73	15.07	14.22	19.73	14.95	57.40 ±1.23	18.21 ±2.24
Saturated FAs	5.98	10.37	10.83	9.66	12.20 ±3.03	11.17 ±2.41	8.91	9.96 ±0.02	6.82	5.68	7.25	6.30	21.62 ±1.28	6.37 ±0.59
Total MUFA	3.40	4.73	6.61	5.25	8.14 ±1.88	7.85 ±1.61	4.10	6.44 ±0.52	3.94	2.59	3.03	3.42	12.95 ±1.17	2.93 ±0.26
Total PUFA	3.56	8.67	12.86	8.25	13.23 ±4.61	11.58 ±4.41	3.85	10.70 ±1.19	3.87	5.49	9.01	4.28	21.99 ±1.09	8.52 ±1.61
PUFA (n-3)	1.72	3.89	6.50	4.02	7.57 ±2.33	6.22 ±2.28	1.78	5.63 ±0.16	1.82	2.40	4.66	1.75	12.41 ±0.61	4.35 ±1.14
PUFA (n-6)	1.85	4.79	6.36	4.23	5.62 ±2.52	5.27 ±2.08	2.07	5.07 ±1.03	2.05	3.02	4.29	2.53	9.47 ±0.42	4.11 ±0.46

Table A2.2. (Continued) Fatty acid compostion of analysed green seaweeds (part 1) (mg g^{-1} dw ± STDEV).

Green seaweeds - part 1									
	Boodlea composita	*Bryopsis sp*	*Caulerpa lentillifera*	*Caulerpa racemosa clavifera*	*Caulerpa racemosa laetevirens*	*Caulerpa racemosa occidentalis*	*Caulerpa serrulata*	*Caulerpa sertularioides*	*Caulerpa taxifolia*
C14:0	1.36	0.50	0.44	0.43	0.51	0.63 ±0.20	0.72 ±0.09	1.32 ±0.27	0.60
C14:1	0.21	0.21	0.21			0.20	0.19		0.24
C15:0	0.21	0.20	0.21	0.20	0.19	0.21 ±0.00	0.20 ±0.00	0.21 ±0.00	0.21
C16:0	5.64	5.94	3.80	5.44	3.53	5.45 ±0.67	6.31 ±0.33	9.39 ±1.56	7.09
C16:1(n-9)	0.28	0.34	0.26	0.25	0.23	0.26 ±0.05	0.29 ±0.02	0.37 ±0.06	0.25
C16:1(n-7)	0.53	0.95	0.70	0.71	0.48	0.69 ±0.04	0.89 ±0.02	1.26 ±0.28	1.32
C16:1	0.22		0.20		0.20	0.21 ±0.02	0.20 ±0.00	0.21 ±0.02	0.27
C16:2(n-6)	0.26	0.80	0.34	0.40	0.24	0.35 ±0.01	0.46 ±0.06	0.61 ±0.20	0.51
C16:2(n-4)	0.35	0.21							
C17:0	0.20	0.23	0.21	0.19	0.20	0.20 ±0.01	0.20 ±0.00	0.23 ±0.02	0.29
C16:3(n-6)	0.20			0.19	0.20	0.21 ±0.00	0.20	0.21	
C16:3(n-3)		1.56	0.62	0.48	0.32	0.74 ±0.22	0.90 ±0.11	1.75 ±0.35	1.37
C16:4(n-3)	0.41	0.36	0.22	0.22	0.20	0.22 ±0.01	0.25 ±0.04	0.25 ±0.02	0.25
C18:0	0.43	0.32	0.27	0.34	0.29	0.37 ±0.02	0.33 ±0.01	0.45 ±0.06	0.31
C18:1(n-9)	2.52	1.53	0.97	0.99	0.78	1.05 ±0.03	1.19 ±0.03	1.28 ±0.05	1.18
C18:2(n-6)trans									
C18:2(n-6)cis	1.06	1.92	0.66	0.91	0.48	0.87 ±0.09	1.15 ±0.15	1.97 ±0.58	1.47
C18:3(n-6)	1.14	0.37	0.25	0.26	0.22	0.28 ±0.07	0.35 ±0.04	0.39 ±0.05	0.55
C18:3(n-3)	0.41	2.35	0.80	1.12	0.53	1.54 ±0.22	1.74 ±0.15	2.62 ±0.41	3.20
C18:4(n-3)	0.27	0.45	0.24	0.28		0.28 ±0.02	0.42 ±0.03	0.52 ±0.12	0.88
C20:0									
C21:0						0.27 ±0.04	0.27 ±0.02	0.32 ±0.04	
C20:3(n-6)	0.35	0.32	0.23			0.29	0.24 ±0.01	0.32 ±0.04	
C20:4(n-6)	0.92	0.93	0.37	0.31	0.32	0.60 ±0.27	0.54 ±0.07	0.41 ±0.06	0.84
C20:4(n-3)	0.22	0.22				0.25	0.22	0.36 ±0.05	
C22:0	0.34	0.50	0.41	0.57	0.26	0.80 ±0.08	0.74 ±0.12	1.34 ±0.24	1.40
C20:5(n-3)	0.37	0.57	0.38	0.51	0.30	0.71 ±0.01	0.89 ±0.11	1.28 ±0.33	1.38
C24:0		0.50	0.67	0.75	0.48	0.74 ±0.11	0.83 ±0.05	0.98 ±0.16	0.84
C22:6(n-3)	0.34			0.20		0.22 ±0.00	0.26 ±0.01	0.26 ±0.03	0.33
Other FAs	0.14	0.00	0.33	0.56	0.59	0.84 ±0.06	0.87 ±0.05	0.81 ±0.19	0.94
Total FAs	17.51	20.59	12.02	14.36	9.93	16.88 ±2.06	19.62 ±1.44	27.61 ±4.85	24.58
Saturated FAs	7.54	7.43	5.32	7.15	4.90	7.64 ±1.00	8.71 ±0.55	12.84 ±2.09	9.30
Total MUFA	3.69	2.97	2.21	1.95	1.55	2.21 ±0.07	2.54 ±0.04	2.98 ±0.40	3.26
Total PUFA	5.86	9.76	3.89	4.51	2.63	5.88 ±1.01	7.30 ±0.81	10.65 ±2.11	10.71
PUFA (n-3)	1.76	5.36	2.03	2.57	1.29	3.58 ±0.40	4.58 ±0.47	7.05 ±1.31	7.34
PUFA (n-6)	3.86	4.34	1.85	1.94	1.34	2.30 ±0.61	2.73 ±0.34	3.60 ±0.80	3.37

Table A2.2. (Continued) Fatty acid compostion of analysed green seaweeds (part 2) (mg g^{-1} dw ± STDEV).

Green seaweeds - part 2										
	Chaetomorpha linum	*Chlorodesmis fastigiata*	*Cladophora coelothrix*	*Cladophora patentiramea*	*Cladophora regulosa*	*Derbesia tenuissima*	*Udotea argentea*	*Enteromorpha clathrata*	*Ulva fleximosa*	*Ulva rigida*
C14:0	1.45	0.60	1.32	1.53	0.96 ±0.52	1.34 ±0.17	0.29 ±0.01	0.29	1.58	0.35 ±0.04
C14:1	0.25		0.29	0.23	0.21 ±0.01	0.30 ±0.03		0.29	0.27	0.21
C15:0	0.23	0.21	0.23	0.23	0.20 ±0.00	0.24 ±0.01		0.23	0.24	0.22 ±0.00
C16:0	5.21	9.26	8.44	9.84	4.63 ±3.02	11.96 ±0.54	3.35 ±0.15	3.59	7.28	6.20 ±0.51
C16:1(n-9)	0.21	0.33	0.51	0.48	0.53 ±0.25	0.39 ±0.05	0.24 ±0.01	0.20	0.32	0.22 ±0.01
C16:1(n-7)	1.20	1.26	1.15	1.05	0.59 ±0.22	1.91 ±0.37	0.57 ±0.11	0.35	1.44	1.16 ±0.12
C16:1	0.21	0.20	0.22	0.20	0.21 ±0.00	0.24 ±0.02	0.20		0.23	0.20
C16:2(n-6)	0.28	0.80	0.65	0.39	0.30 ±0.08	0.88 ±0.27	0.83 ±0.33	0.20	0.46	0.26 ±0.06
C16:2(n-4)	1.21		0.33	3.39	1.37 ±1.37	0.21 ±0.05			0.72	0.26 ±0.05
C17:0	0.32	0.23	0.23			0.24 ±0.02			0.24	0.21 ±0.01
C16:3(n-6)	0.20	0.23	0.29	0.22	0.20 ±0.00	0.20 ±0.01			0.24	0.21 ±0.00
C16:3(n-3)	0.24	0.88	0.33	0.22		3.34 ±1.71	0.69	0.23	0.77	0.33 ±0.09
C16:4(n-3)	0.85	0.27	0.68	0.74	0.65 ±0.54	0.43 ±0.03	0.23	0.49	1.50	2.00 ±1.38
C18:0	0.33	0.45	0.35	0.35	0.28 ±0.04	0.50 ±0.09	0.26 ±0.03	0.25	0.53	0.30 ±0.03
C18:1(n-9)	1.65	1.72	2.34	4.48	2.90 ±1.79	2.41 ±0.32	1.58 ±0.25	1.03	2.93	1.81 ±0.27
C18:2(n-6)trans						0.20				
C18:2(n-6)cis	2.99	4.19	2.21	3.03	2.95 ±3.08	2.75 ±0.39	0.94 ±0.12	0.89	3.73	0.79 ±0.25
C18:3(n-6)	0.28	0.40	0.46	3.31	0.47 ±0.30	0.77 ±0.19	0.30 ±0.01	0.30	0.41	0.29 ±0.06
C18:3(n-3)	1.36	1.66	2.27	0.52	0.31	6.14 ±2.42	1.10 ±0.01	0.81	2.09	2.26 ±1.02
C18:4(n-3)	0.52	0.31	0.97	0.45	0.33	0.64 ±0.13	0.30 ±0.01	0.45	0.48	2.53 ±1.43
C20:0			0.20			0.22 ±0.02		0.28	0.23	
C21:0	0.21	0.55							0.22	
C20:3(n-6)	0.23	0.33	0.25	0.23	0.22	0.31 ±0.04	0.29 ±0.02	0.30	0.25	
C20:4(n-6)	0.49	0.59	1.11	2.77	1.09 ±0.99	1.17 ±0.23	0.41 ±0.02	0.39	0.96	0.27 ±0.07
C20:4(n-3)	0.20	0.26	0.23		0.21	0.24 ±0.04	0.25 ±0.01	0.25	0.22	0.26 ±0.04
C22:0	0.30	1.01	1.46	0.47	0.47 ±0.27	1.56 ±0.41	0.97 ±0.62	0.44	0.83	0.65 ±0.05
C20:5(n-3)	0.36	1.63	1.49	0.60	0.46 ±0.17	1.51 ±0.33	0.70 ±0.25	0.45	0.97	0.62 ±0.07
C24:0	0.29	0.87	0.29	0.24		0.90 ±0.17	0.41 ±0.06		0.41	0.23
C22:6(n-3)	0.22	0.34	0.35	0.26	0.41		0.29 ±0.06	0.40	0.39	0.62 ±0.15
Other FAs	0.52	1.44	0.26	0.26	0.00	0.28 ±0.24	0.77 ±0.24	0.00	0.39	0.03 ±0.05
Total FAs	21.26	28.98	27.46	34.63	18.65 ±13.57	39.58 ±5.09	12.85 ±0.28	11.53	29.31	21.39 ±6.11
Saturated FAs	7.44	12.07	10.95	11.96	6.03 ±3.67	15.31 ±0.54	4.58 ±0.10	4.56	10.29	7.10 ±0.64
Total MUFA	3.53	3.45	4.42	6.37	4.38 ±2.36	5.19 ±0.61	2.39 ±0.25	1.80	5.11	3.33 ±0.60
Total PUFA	9.22	11.58	11.44	15.82	8.04 ±7.54	18.35 ±4.25	5.11 ±0.31	4.94	13.03	10.50 ±4.82
PUFA (n-3)	3.61	5.19	6.25	2.57	1.67 ±1.50	12.23 ±4.61	2.64 ±0.58	3.00	6.34	8.54 ±4.18
PUFA (n-6)	4.40	6.39	4.96	9.86	5.01 ±4.67	5.98 ±0.33	2.46 ±0.27	1.95	5.97	1.70 ±0.59

Table A2.2. (Continued) Fatty acid compostion of analysed red seaweeds (mg g^{-1} dw ± STDEV).

	Red seaweeds											
	Acanthophora spicifera	*Asparagopsis taxiformis*	*Ceramium*	*Champia parvula*	*Gracilaria* sp	*Halymenia* sp	*Hypnea cervicornis*	*Hypnea pannosa*	*Jania* sp	*Laurencia majuscula*	*Laurencia* sp	*Plocamium* sp
C14:0	0.49	0.60	0.54	1.29	1.52	0.34 ±0.07	1.45	0.88	0.21	1.25 ±0.24	1.59 ±0.36	0.84 ±0.37
C14:1	0.27		0.21	0.22	0.24	0.71	0.26	0.24		0.36 ±0.03	0.29 ±0.09	0.27 ±0.05
C15:0	0.22	0.19	0.24	0.21	0.24	0.28 ±0.08	0.23	0.25		0.22 ±0.00	0.25 ±0.06	0.23 ±0.01
C16:0	2.33	2.35	7.69	6.61	7.07	8.36 ±1.13	5.72	4.16	1.64	4.91 ±1.45	5.48 ±1.04	7.02 ±1.13
C16:1(n-9)	0.20	0.39	0.21	0.19	0.24					0.19 ±0.00	0.20 ±0.01	0.23 ±0.05
C16:1(n-7)	0.30	0.33	0.62	0.46	0.58	0.42 ±0.09	1.23	0.64		0.35 ±0.06	0.48 ±0.15	0.35 ±0.08
C16:1	0.22	0.20	0.19	0.19				0.27		0.21 ±0.00	0.24 ±0.05	0.20 ±0.01
C16:2(n-6)			0.24	0.19	0.22	0.29					0.23 ±0.03	0.25
C16:2(n-4)			0.22									0.23
C17:0			0.24	0.20	0.29		0.23			0.19	0.20	0.42
C16:3(n-6)			0.20	0.19		0.21		0.23		0.20	0.20	0.21 ±0.02
C16:3(n-3)			0.30			0.28				0.25	0.20 ±0.02	
C16:4(n-3)			0.42	0.26	0.24	0.22 ±0.02	0.20	0.24		0.20 ±0.00	0.24 ±0.01	0.21 ±0.01
C18:0	0.25	0.26	0.30	0.29	0.41	0.30 ±0.06	0.31	0.27		0.28 ±0.04	0.33 ±0.03	0.37 ±0.03
C18:1(n-9)	0.92	0.77	1.31	1.38	1.90	1.99 ±0.68	1.38	1.11	0.56	1.40 ±0.38	1.51 ±0.24	1.86 ±0.60
C18:2(n-6)trans											0.20 ±0.01	
C18:2(n-6)cis	0.27	0.23	0.71	0.45	0.35	0.36 ±0.17	0.42	0.28	0.21	0.32 ±0.03	0.38 ±0.05	0.25 ±0.01
C18:3(n-6)	0.26		0.30	0.33	0.24	0.27 ±0.06	0.32			0.21 ±0.02	0.24 ±0.02	0.22 ±0.01
C18:3(n-3)	0.26		0.95	0.66	0.20		0.22			0.26 ±0.05	0.30 ±0.08	0.20 ±0.01
C18:4(n-3)			0.68	0.53		0.29	0.25			0.21 ±0.02	0.39 ±0.19	0.21
C20:0											0.21	
C21:0											0.81	
C20:3(n-6)			0.29	0.47	0.25	0.28	0.27		0.26	0.21 ±0.01	0.28 ±0.07	0.30 ±0.08
C20:4(n-6)	0.40	0.35	5.83	3.59	2.25	1.73 ±1.45	0.57	0.39	0.22	0.70 ±0.19	0.95 ±0.34	0.34 ±0.05
C20:4(n-3)											0.30 ±0.10	0.22
C22:0	0.48	0.50	2.15	3.17	1.66	2.41 ±2.57	1.26	0.47	0.33	1.10 ±0.02	0.78 ±0.47	0.93 ±0.25
C20:5(n-3)	0.53	0.48	1.62	3.30	1.64	2.29 ±2.51	1.19	0.46	0.36	1.09 ±0.22	1.43 ±0.72	0.72 ±0.09
C24:0					0.24		0.23	0.32				0.23
C22:6(n-3)												
Other FAs	0.00	0.00	0.00	0.00	0.10	0.49 ±0.13	0.00	0.00	0.00	1.11 ±0.83	0.89 ±0.68	0.00
Total FAs	6.50	5.69	23.78	21.39	18.23	18.58 ±6.07	14.71	9.80	3.10	13.55 ±0.77	15.71 ±2.21	13.33 ±1.89
Saturated FAs	3.24	3.38	9.24	9.24	9.63	9.81 ±1.98	8.05	5.77	1.95	6.81 ±1.73	7.86 ±1.08	8.45 ±1.23
Total MUFA	1.76	1.19	2.42	2.26	2.96	2.77 ±0.08	2.87	2.26	0.56	2.41 ±0.52	2.49 ±0.56	2.71 ±0.73
Total PUFA	1.29	1.06	11.63	9.62	5.02	5.24 ±3.95	3.32	1.52	0.59	2.96 ±0.69	4.17 ±1.29	1.79 ±0.45
PUFA (n-3)	0.52	0.48	3.98	4.66	1.95	2.63 ±2.12	1.73	0.70	0.36	1.64 ±0.49	2.29 ±0.68	0.80 ±0.43
PUFA (n-6)	0.76	0.58	7.50	4.96	3.07	2.61 ±1.83	1.59	0.82	0.23	1.33 ±0.20	1.88 ±0.61	0.94 ±0.10

Table A2.3. Fatty acid composition (mg g^{-1} dw) of all investigated *Dictyota bartayresii* plants (every replicate from all samples of this species).

	Dictyota bartayresii														
Sample:	South Nelly Bay 8/9/2010			North Nelly Bay 8/9/2010			North Nelly Bay 21/9/2010			Kissing Point 6/10/2010			Orpheus Island 3/11/2010		
Replicates:	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
C14:0	2.96	3.34	3.94	2.17	2.39	2.56	2.63	2.85	2.59	2.80	2.57	2.87	1.34	1.77	1.32
C14:1	0.39	0.33	0.37	0.32	0.31	0.32	0.37	0.31	0.28	0.57	0.59	0.62	0.31	0.30	0.27
C15:0	0.41	0.32	0.35	0.34	0.34	0.30	0.33	0.31	0.30	0.48	0.46	0.43	0.27	0.22	0.27
C16:0	8.53	8.91	10.68	5.91	6.79	6.59	6.78	6.76	6.22	9.55	8.82	9.18	4.01	4.83	3.92
C16:1(n-9)	0.31	0.31	0.29	0.31	0.28	0.28	0.28	0.23	0.25	0.31	0.27	0.31	0.26	0.27	0.25
C16:1(n-7)	0.69	0.60	0.63	0.57	0.52	0.54	0.63	0.64	0.55	0.76	0.76	0.74	0.50	0.54	0.59
C16:1	2.51	3.06	3.15	2.06	1.84	2.30	2.85	2.83	2.88	1.48	1.39	1.59	1.62	2.20	1.38
C16:2(n-6)		0.38	0.39	0.36	0.33	0.36		0.33	0.33	0.30	0.28	0.36		0.38	0.31
C16:2(n-4)	0.25		0.25						0.21						
C17:0							0.23			0.21	0.21	0.21			
C16:3(n-6)	0.49	0.52	0.52	0.45	0.43	0.45	0.42	0.42	0.41	0.24	0.24	0.25	0.50	0.50	0.46
C16:3(n-3)	0.24	0.28	0.29		0.21			0.26		0.28	0.28		0.38	0.23	
C16:4(n-3)	0.52	0.42	0.48		0.27	0.27	0.57	0.30	0.30	0.34	0.30	0.34		0.30	0.26
C18:0	0.94	0.98	1.17	0.72	0.83	0.88	0.68	0.67	0.70	0.65	0.65	0.56	0.81	0.67	0.77
C18:1(n-9)	5.87	5.87	8.06	4.10	5.29	4.71	4.56	4.71	4.17	5.04	4.62	4.60	2.67	3.09	2.38
C18:2(n-6)trans	0.30	0.32	0.31	0.27	0.26	0.29	0.33	0.32	0.30	1.32	0.28	1.22	0.23	0.27	0.23
C18:2(n-6)cis	1.06	1.07	1.28	0.75	0.85	0.84	1.09	1.08	0.93	1.21	1.16	1.27	0.36	0.43	0.42
C18:3(n-6)	0.42	0.49	0.50	0.36	0.40	0.40	0.61	0.57	0.62	0.60	0.66	0.84	0.25	0.32	0.27
C18:3(n-3)	2.00	1.68	1.89	1.23	1.12	1.16	1.78	1.36	0.97	1.74	1.69	1.83	0.37	0.44	0.45
C18:4(n-3)	3.85	4.29	4.45	3.42	2.88	3.55	3.93	3.69	3.76	4.35	4.23	4.73	1.43	1.71	1.07
C20:0	0.62	0.53	0.58	0.48	0.44	0.46		0.46	0.48	0.36	0.36	0.36		0.44	0.34
C21:0	0.90	1.03	1.08	0.87	0.66	0.87	1.11	1.13	1.18	0.67	0.61		0.64	0.70	0.52
C20:3(n-6)	0.50	0.52	0.50	0.38	0.36	0.45	0.45	0.53	0.41	1.05	1.08	1.07		0.26	0.25
C20:4(n-6)	2.12	2.43	2.43	1.77	1.68	2.02	2.27	2.57	2.15	5.16	4.93	5.67	1.08	1.34	0.99
C20:4(n-3)	0.92	0.90	0.90	0.76	0.67	0.77	0.77	0.74	0.71	1.01	0.91	0.95		0.58	0.43
C22:0	1.57	0.22		1.31			1.20			1.96			1.23		0.24
C20:5(n-3)	1.57	1.47	1.60	1.31	1.27	1.34	1.20	1.24	1.17	1.96	1.84	1.94	1.23	1.21	0.98
C24:0															0.20
C22:6(n-3)	0.34	0.26	0.29	0.27	0.27	0.27	0.24				0.28		0.28	0.25	0.25
Other FAs	1.47	1.46	1.49	1.01	0.94	1.08	1.16	1.13	0.70	0.41	0.64	0.82	2.18	1.55	1.72
Total FAs	41.77	42.02	47.86	31.48	31.61	33.03	36.48	35.43	32.56	44.81	40.10	42.76	21.96	24.82	20.60
Saturated FAs	15.94	15.32	17.80	11.78	11.45	11.66	12.96	12.17	11.47	16.67	13.67	13.60	8.30	8.64	7.60
Total MUFA	9.77	10.18	12.51	7.36	8.24	8.14	8.69	8.72	8.12	8.16	7.63	7.87	5.37	6.39	4.88
Total PUFA	14.59	15.06	16.06	11.32	10.98	12.15	13.68	13.40	12.28	19.56	18.16	20.47	6.11	8.24	6.39
PUFA (n-3)	9.45	9.32	9.88	6.99	6.67	7.35	8.51	7.57	6.90	9.68	9.53	9.78	3.69	4.72	3.45
PUFA (n-6)	4.89	5.74	5.93	4.34	4.31	4.80	5.17	5.83	5.16	9.88	8.63	10.69	2.42	3.52	2.94

Table A2.4. Fatty acid profile (mg g^{-1} dw) of all investigated *Dictyota dichotoma* plants (every replicate from all samples of this species).

Dictyota dichotoma									
Sample:	North Nelly Bay 8/9/2010			North Nelly Bay 27/10/2010			Kissing Point 7/9/2010		
Replicates:	1	2	3	1	2	3	1	2	3
C14:0	2.70	3.13	3.24	2.00	1.82	1.71	1.61	2.56	1.77
C14:1	0.38	0.36	0.34	0.23	0.71		0.22	0.24	0.23
C15:0	0.28	0.30	0.29	0.44			0.26	0.32	0.29
C16:0	6.97	7.70	7.63	7.95	8.23	7.70	4.34	6.41	4.73
C16:1(n-9)	0.28	0.32	0.29		0.21	0.20	0.21	0.21	0.20
C16:1(n-7)	0.62	0.60	0.61	1.29	1.50	0.93	0.58	0.66	0.58
C16:1	3.28	4.14	3.99	1.53	1.56	1.33	0.82	1.24	0.91
C16:2(n-6)	0.36	0.47	0.44		0.22	0.21		0.24	
C16:2(n-4)				0.27		0.38	0.21		
C17:0		0.57				0.26	0.20		0.20
C16:3(n-6)	0.41	0.41	0.52	0.22	0.31	0.29	0.27	0.32	0.29
C16:3(n-3)	0.30	0.30		0.89	0.57	0.20			
C16:4(n-3)		0.28	0.29	0.55	0.59	0.96	0.24	0.24	0.24
C18:0	0.48	0.59	0.60	0.75	0.80	0.61	0.46	0.61	0.49
C18:1(n-9)	3.57	4.52	4.52	4.41	6.60	4.03	3.31	5.16	3.75
C18:2(n-6)trans	0.32	0.30	0.30	0.52	0.45		0.21	0.27	0.26
C18:2(n-6)cis	0.59	0.61	0.65	0.79	0.70	0.70	0.73	0.90	0.71
C18:3(n-6)	0.47	0.46	0.46	0.68	0.53	0.67	0.34	0.38	0.39
C18:3(n-3)	0.70	0.62	0.70	1.36	1.50	1.32	0.68	1.04	0.80
C18:4(n-3)	2.93	2.69	2.95	3.18	3.16	2.89	1.18	2.04	1.53
C20:0	0.43	0.45	0.44		0.37	0.35	0.25	0.33	0.30
C21:0	0.92	1.14	1.13	0.49	0.55	0.34	0.33	0.47	
C20:3(n-6)	0.36	0.30	0.34	0.77	0.71	0.72	0.31	0.37	0.31
C20:4(n-6)	2.27	1.96	2.07	4.67	5.12	4.44	1.18	1.76	1.36
C20:4(n-3)	0.63	0.66	0.68		0.89	0.83	0.33	0.44	0.40
C22:0	1.60		0.33	1.93			0.60		
C20:5(n-3)	1.60	1.50	1.61	1.93	2.04	1.94	0.60	0.72	0.64
C24:0							0.22		
C22:6(n-3)		0.25	0.31		0.30	0.23	0.26	0.28	
Other FAs	1.15	1.97	1.63	0.45	1.18	2.05	0.63	1.08	0.75
Total FAs	33.62	36.60	36.33	37.29	40.64	35.29	20.56	28.28	21.12
Saturated FAs	13.38	13.88	13.65	13.54	11.77	10.97	8.27	10.70	7.77
Total MUFA	8.13	9.94	9.74	7.46	10.58	6.49	5.14	7.51	5.67
Total PUFA	10.96	10.81	11.31	15.84	17.11	15.78	6.53	8.99	6.93
PUFA (n-3)	6.17	6.29	6.54	7.91	9.06	8.36	3.28	4.76	3.62
PUFA (n-6)	4.80	4.51	4.77	7.65	8.05	7.03	3.04	4.23	3.31
Total FAs	33.62	36.60	36.33	37.29	40.64	35.29	20.56	28.28	21.12

Table A3.1. Average content of fatty acids (% of TFA ± SE) of *Spatoglossum macrodontum* plant sections (Tips, Midesctions, Base sections) collected over one year. Total fatty acid (TFA) content presented as means (mg g^{-1} dw ± SE). 15 plants per sampling period were used except November 2011 (n = 14) and 'early July' (n = 9). No samples found from January to May 2012.

	20/11/2011			4/06/2012			3/07/2012			30/07/2012		
	Tips	Midsection	Base section	Tips	Midsection	Base section	Tips	Midsection	Base section	Tips	Midsection	Base section
C14:0	7.68 ± 0.23	7.04 ± 0.23	6.60 ± 0.22	9.05 ± 0.19	8.18 ± 0.24	6.65 ± 0.17	9.55 ± 0.26	7.91 ± 0.23	6.76 ± 0.17	8.97 ± 0.27	7.86 ± 0.26	7.17 ± 0.15
C14:1	0.33 ± 0.05	0.41 ± 0.08	0.33 ± 0.07	0.44 ± 0.02	0.44 ± 0.03	0.67 ± 0.03	0.44 ± 0.01	0.38 ± 0.04	0.08 ± 0.05	0.32 ± 0.03	0.31 ± 0.02	0.33 ± 0.05
C15:0	0.55 ± 0.06	0.67 ± 0.04	0.91 ± 0.07	0.43 ± 0.01	0.45 ± 0.02	0.68 ± 0.02	0.57 ± 0.01	0.53 ± 0.03	1.15 ± 0.15	0.59 ± 0.02	0.64 ± 0.03	0.86 ± 0.03
C16:0	27.52 ± 0.95	23.96 ± 0.82	20.90 ± 0.36	21.59 ± 0.67	23.11 ± 1.08	20.09 ± 0.28	21.81 ± 0.72	27.21 ± 1.86	19.30 ± 0.25	22.86 ± 0.72	24.85 ± 1.21	20.90 ± 1.14
C16:1(n-9)	0.34 ± 0.02	0.51 ± 0.05	0.51 ± 0.10	0.34 ± 0.02	0.35 ± 0.02	0.59 ± 0.01	0.57 ± 0.02	0.47 ± 0.04	0.91 ± 0.18	0.48 ± 0.02	0.47 ± 0.03	0.57 ± 0.05
C16:1(n-7)	3.28 ± 0.07	4.03 ± 0.12	4.67 ± 0.11	2.35 ± 0.06	3.18 ± 0.18	4.21 ± 0.09	2.28 ± 0.07	2.92 ± 0.09	5.06 ± 0.14	2.62 ± 0.15	3.41 ± 0.13	4.16 ± 0.13
C16:1	0.74 ± 0.06	0.72 ± 0.04	0.98 ± 0.04	0.62 ± 0.08	0.65 ± 0.14	0.82 ± 0.02	0.64 ± 0.02	0.50 ± 0.05	1.56 ± 0.08	0.49 ± 0.03	0.47 ± 0.05	0.71 ± 0.06
C16:2	0.21 ± 0.03	0.16 ± 0.04	0.00 ± 0.00	0.32 ± 0.03	0.26 ± 0.04	0.13 ± 0.03	0.43 ± 0.03	0.36 ± 0.04	0.13 ± 0.09	0.36 ± 0.01	0.33 ± 0.03	0.26 ± 0.05
C17:0	0.31 ± 0.03	0.26 ± 0.05	0.04 ± 0.02	0.25 ± 0.03	0.30 ± 0.03	0.17 ± 0.07	0.44 ± 0.03	0.45 ± 0.03	0.77 ± 0.18	0.39 ± 0.01	0.44 ± 0.02	0.46 ± 0.04
C16:3(n-4)	0.36 ± 0.04	0.59 ± 0.03	0.67 ± 0.07	0.27 ± 0.03	0.33 ± 0.03	0.62 ± 0.04	0.49 ± 0.02	0.45 ± 0.03	1.30 ± 0.09	0.36 ± 0.04	0.45 ± 0.03	0.69 ± 0.05
C16:3(n-3)	0.39 ± 0.04	0.64 ± 0.05	0.50 ± 0.06	0.43 ± 0.02	0.44 ± 0.03	0.67 ± 0.02	0.48 ± 0.02	0.43 ± 0.04	0.71 ± 0.16	0.46 ± 0.02	0.47 ± 0.03	0.71 ± 0.05
C16:4(n-3)	0.12 ± 0.04	0.05 ± 0.02	0.00 ± 0.00	0.31 ± 0.02	0.35 ± 0.04	0.37 ± 0.06	0.33 ± 0.05	0.34 ± 0.04	0.14 ± 0.10	0.32 ± 0.03	0.34 ± 0.04	0.43 ± 0.05
C18:0	1.56 ± 0.05	1.27 ± 0.04	1.35 ± 0.05	1.27 ± 0.04	1.13 ± 0.05	1.12 ± 0.02	1.48 ± 0.08	1.48 ± 0.11	1.82 ± 0.07	1.28 ± 0.02	1.21 ± 0.03	1.16 ± 0.04
C18:1(n-9)	18.61 ± 0.54	18.56 ± 0.39	21.38 ± 0.47	14.52 ± 0.51	16.41 ± 0.78	16.88 ± 0.25	15.36 ± 0.60	19.60 ± 1.35	18.19 ± 0.37	15.84 ± 0.50	17.53 ± 0.72	17.49 ± 0.50
C18:1	0.86 ± 0.12	1.18 ± 0.07	1.84 ± 0.10	0.39 ± 0.05	0.47 ± 0.06	0.99 ± 0.08	0.61 ± 0.10	0.93 ± 0.02	1.60 ± 0.22	0.85 ± 0.03	0.89 ± 0.04	1.23 ± 0.06
C18:1(n-5)	0.33 ± 0.04	0.55 ± 0.06	0.91 ± 0.05	0.32 ± 0.02	0.42 ± 0.03	0.76 ± 0.03	0.40 ± 0.06	0.40 ± 0.04	1.13 ± 0.16	0.33 ± 0.03	0.39 ± 0.04	0.66 ± 0.06
C18:2(n-6)	3.13 ± 0.07	2.90 ± 0.07	2.60 ± 0.14	3.47 ± 0.06	2.96 ± 0.09	2.54 ± 0.07	3.33 ± 0.07	2.82 ± 0.06	2.79 ± 0.08	3.07 ± 0.05	2.72 ± 0.05	2.33 ± 0.07
C18:3(n-6)	1.49 ± 0.09	1.42 ± 0.05	1.61 ± 0.09	1.62 ± 0.06	1.47 ± 0.04	1.76 ± 0.05	1.08 ± 0.03	0.94 ± 0.04	1.49 ± 0.03	1.01 ± 0.03	0.97 ± 0.03	1.02 ± 0.04
C18:3(n-3)	4.88 ± 0.30	6.55 ± 0.28	5.70 ± 0.20	4.60 ± 0.10	4.63 ± 0.15	5.33 ± 0.15	4.65 ± 0.12	4.02 ± 0.28	3.92 ± 0.14	4.72 ± 0.13	4.94 ± 0.25	5.62 ± 0.23
C18:4(n-3)	8.11 ± 0.40	7.30 ± 0.39	4.64 ± 0.35	12.21 ± 0.37	11.57 ± 0.50	9.90 ± 0.26	9.61 ± 0.33	7.87 ± 0.68	4.89 ± 0.30	10.11 ± 0.32	9.41 ± 0.53	8.84 ± 0.45
C20:0	0.85 ± 0.06	0.90 ± 0.04	1.21 ± 0.07	0.71 ± 0.03	0.64 ± 0.03	0.96 ± 0.02	0.90 ± 0.03	0.69 ± 0.06	1.66 ± 0.07	0.75 ± 0.02	0.68 ± 0.04	0.93 ± 0.06
C20:1(n-9)	0.30 ± 0.10	0.31 ± 0.08	0.56 ± 0.11	0.04 ± 0.02	0.01 ± 0.01	0.03 ± 0.03	0.00 ± 0.00	0.03 ± 0.02	0.00 ± 0.00	0.07 ± 0.03	0.08 ± 0.02	0.07 ± 0.03
C20:2(n-6)	0.47 ± 0.08	0.71 ± 0.05	1.06 ± 0.09	0.48 ± 0.02	0.59 ± 0.06	0.85 ± 0.02	0.50 ± 0.03	0.44 ± 0.05	0.45 ± 0.20	0.45 ± 0.03	0.50 ± 0.05	0.87 ± 0.08
C20:3(n-6)	3.01 ± 0.11	2.21 ± 0.07	2.15 ± 0.06	5.16 ± 0.15	4.19 ± 0.15	3.97 ± 0.11	5.10 ± 0.11	3.82 ± 0.27	4.47 ± 0.15	5.05 ± 0.15	4.30 ± 0.16	4.10 ± 0.14
C20:4(n-6)	7.62 ± 0.29	8.40 ± 0.22	9.62 ± 0.15	9.24 ± 0.33	8.74 ± 0.48	10.50 ± 0.15	9.03 ± 0.32	6.74 ± 0.72	10.58 ± 0.22	8.38 ± 0.24	7.33 ± 0.36	8.67 ± 0.36
C20:3(n-3)	0.06 ± 0.03	0.07 ± 0.04	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.07	0.38 ± 0.07	0.00 ± 0.00	0.43 ± 0.03	0.45 ± 0.03	0.34 ± 0.06
C20:4(n-3)	2.87 ± 0.17	3.97 ± 0.16	3.98 ± 0.13	4.26 ± 0.14	4.08 ± 0.18	4.20 ± 0.10	4.56 ± 0.17	3.61 ± 0.33	3.52 ± 0.14	4.55 ± 0.12	4.16 ± 0.14	4.84 ± 0.22
C20:5(n-3)	3.11 ± 0.12	3.45 ± 0.12	3.40 ± 0.14	4.42 ± 0.08	3.79 ± 0.10	3.25 ± 0.07	3.89 ± 0.10	3.17 ± 0.18	2.94 ± 0.08	3.98 ± 0.08	3.55 ± 0.06	3.34 ± 0.11
C22:0	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.07	0.05 ± 0.05	0.33 ± 0.23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C22:1(n-9)	0.22 ± 0.06	0.21 ± 0.06	0.11 ± 0.06	0.03 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.02 ± 0.02
C24:0	0.37 ± 0.05	0.63 ± 0.06	0.84 ± 0.09	0.50 ± 0.02	0.44 ± 0.04	0.71 ± 0.07	0.67 ± 0.07	0.66 ± 0.11	2.06 ± 0.21	0.50 ± 0.02	0.44 ± 0.04	0.68 ± 0.05
Other FA	0.36 ± 0.04	0.36 ± 0.04	0.88 ± 0.10	0.36 ± 0.01	0.41 ± 0.04	0.56 ± 0.03	0.43 ± 0.01	0.39 ± 0.04	0.30 ± 0.15	0.38 ± 0.02	0.41 ± 0.02	0.54 ± 0.04
Total SFA	38.85 ± 0.94	34.74 ± 0.82	31.88 ± 0.35	33.80 ± 0.60	34.27 ± 0.95	30.38 ± 0.31	35.49 ± 0.60	38.98 ± 1.60	33.85 ± 0.43	35.35 ± 0.56	36.13 ± 1.00	32.16 ± 1.03
Total MUFA	24.99 ± 0.44	26.47 ± 0.43	31.29 ± 0.67	19.06 ± 0.44	21.94 ± 0.62	24.96 ± 0.33	20.30 ± 0.51	25.24 ± 1.15	28.53 ± 0.51	21.03 ± 0.52	23.55 ± 0.60	25.24 ± 0.56
Total PUFA	35.80 ± 1.20	38.43 ± 0.91	35.95 ± 0.55	46.79 ± 1.02	43.38 ± 1.55	44.09 ± 0.54	43.78 ± 1.07	35.39 ± 2.70	37.33 ± 0.58	43.24 ± 0.99	39.92 ± 1.56	42.06 ± 1.38
Total PUFA (n-3)	19.53 ± 0.82	22.03 ± 0.64	18.24 ± 0.48	26.22 ± 0.54	24.85 ± 0.86	23.73 ± 0.38	23.82 ± 0.68	19.82 ± 1.57	16.12 ± 0.58	24.56 ± 0.58	23.32 ± 0.94	24.13 ± 0.93
Total PUFA (n-6)	15.71 ± 0.44	15.64 ± 0.30	17.03 ± 0.20	19.98 ± 0.53	17.95 ± 0.69	19.62 ± 0.28	19.04 ± 0.42	14.76 ± 1.08	19.78 ± 0.39	17.95 ± 0.44	15.82 ± 0.60	16.98 ± 0.54
n-6/n-3	0.81 ± 0.02	0.71 ± 0.01	0.94 ± 0.03	0.76 ± 0.01	0.72 ± 0.01	0.83 ± 0.01	0.80 0.01	0.75 ± 0.02	1.24 ± 0.06	0.73 ± 0.01	0.68 ± 0.01	0.71 ± 0.02
TFA (mg g^{-1} dw)	79.65 ± 4.60	52.87 ± 2.71	28.78 ± 0.88	76.62 ± 2.87	74.91 ± 5.05	39.97 ± 1.14	77.58 3.01	105.80 ± 10.32	28.20 ± 1.52	77.25 ± 3.13	82.22 ± 5.72	49.29 ± 4.63

Table A3.1. (Continued)

	29/08/2012			26/09/2012			27/10/2012			26/11/2012		
	Tips	Midsection	Base section	Tips	Midsection	Base section	Tips	Midsection	Base section	Tips	Midsection	Base section
C14:0	8.41 ± 0.23	7.54 ± 0.21	6.74 ± 0.13	8.91 ± 0.20	7.84 ± 0.22	6.96 ± 0.13	8.19 ± 0.20	7.30 ± 0.16	7.36 ± 0.12	8.02 ± 0.28	7.52 ± 0.21	7.24 ± 0.13
C14:1	0.38 ± 0.01	0.43 ± 0.01	0.12 ± 0.05	0.41 ± 0.01	0.42 ± 0.02	0.10 ± 0.07	0.38 ± 0.01	0.47 ± 0.03	0.06 ± 0.04	0.41 ± 0.02	0.48 ± 0.02	0.05 ± 0.03
C15:0	0.63 ± 0.02	0.72 ± 0.03	1.29 ± 0.04	0.56 ± 0.01	0.59 ± 0.02	1.18 ± 0.04	0.55 ± 0.02	0.67 ± 0.02	1.38 ± 0.04	0.61 ± 0.02	0.72 ± 0.02	1.54 ± 0.06
C16:0	24.39 ± 0.92	24.23 ± 0.82	19.39 ± 0.21	25.33 ± 0.49	26.17 ± 0.58	19.51 ± 0.25	28.38 ± 0.64	25.85 ± 0.62	19.82 ± 0.18	32.19 ± 0.77	30.36 ± 0.57	21.47 ± 0.30
C16:1(n-9)	0.54 ± 0.02	0.59 ± 0.02	0.90 ± 0.08	0.56 ± 0.02	0.57 ± 0.02	0.97 ± 0.09	0.48 ± 0.02	0.62 ± 0.02	0.60 ± 0.13	0.46 ± 0.02	0.55 ± 0.03	0.24 ± 0.09
C16:1(n-7)	2.89 ± 0.06	3.72 ± 0.07	4.96 ± 0.10	2.81 ± 0.08	3.59 ± 0.13	5.20 ± 0.12	3.08 ± 0.09	3.92 ± 0.10	5.11 ± 0.14	3.28 ± 0.09	3.78 ± 0.11	5.03 ± 0.22
C16:1	0.59 ± 0.03	0.58 ± 0.02	1.23 ± 0.05	0.73 ± 0.02	0.66 ± 0.03	1.27 ± 0.04	0.65 ± 0.02	0.77 ± 0.02	1.54 ± 0.05	0.85 ± 0.06	0.86 ± 0.03	1.64 ± 0.08
C16:2	0.40 ± 0.01	0.44 ± 0.01	0.19 ± 0.08	0.44 ± 0.01	0.44 ± 0.02	0.19 ± 0.07	0.38 ± 0.01	0.38 ± 0.05	0.00 ± 0.00	0.21 ± 0.05	0.19 ± 0.05	0.00 ± 0.00
C17:0	0.45 ± 0.01	0.50 ± 0.01	0.54 ± 0.12	0.47 ± 0.01	0.50 ± 0.02	0.14 ± 0.06	0.48 ± 0.02	0.47 ± 0.05	0.04 ± 0.04	0.53 ± 0.02	0.59 ± 0.03	0.17 ± 0.09
C16:3(n-4)	0.44 ± 0.02	0.54 ± 0.01	1.10 ± 0.06	0.48 ± 0.01	0.53 ± 0.02	1.14 ± 0.04	0.49 ± 0.02	0.67 ± 0.03	1.30 ± 0.07	0.54 ± 0.02	0.66 ± 0.02	1.43 ± 0.07
C16:3(n-3)	0.47 ± 0.02	0.54 ± 0.02	0.86 ± 0.10	0.49 ± 0.01	0.54 ± 0.02	0.99 ± 0.06	0.47 ± 0.02	0.61 ± 0.03	0.44 ± 0.11	0.38 ± 0.03	0.52 ± 0.04	0.36 ± 0.11
C16:4(n-3)	0.37 ± 0.02	0.36 ± 0.03	0.06 ± 0.04	0.28 ± 0.04	0.28 ± 0.03	0.02 ± 0.02	0.29 ± 0.02	0.28 ± 0.05	0.00 ± 0.00	0.04 ± 0.02	0.04 ± 0.03	0.08 ± 0.08
C18:0	1.33 ± 0.04	1.23 ± 0.03	1.70 ± 0.06	1.43 ± 0.03	1.39 ± 0.02	1.64 ± 0.04	1.60 ± 0.04	1.47 ± 0.06	1.92 ± 0.05	1.86 ± 0.04	1.75 ± 0.05	2.20 ± 0.07
C18:1(n-9)	16.96 ± 0.54	17.67 ± 0.41	19.54 ± 0.31	17.44 ± 0.27	18.49 ± 0.33	20.88 ± 0.25	18.41 ± 0.40	17.50 ± 0.29	21.59 ± 0.28	20.18 ± 0.40	19.65 ± 0.25	21.28 ± 0.41
C18:1	0.89 ± 0.03	0.99 ± 0.03	1.61 ± 0.07	0.81 ± 0.02	0.92 ± 0.03	1.55 ± 0.06	1.04 ± 0.03	1.13 ± 0.04	1.85 ± 0.05	1.11 ± 0.03	1.28 ± 0.05	2.23 ± 0.08
C18:1(n-5)	0.44 ± 0.02	0.50 ± 0.01	1.09 ± 0.06	0.47 ± 0.01	0.50 ± 0.02	0.95 ± 0.07	0.39 ± 0.02	0.52 ± 0.03	1.09 ± 0.13	0.47 ± 0.02	0.60 ± 0.02	1.24 ± 0.13
C18:2(n-6)	2.94 ± 0.05	2.43 ± 0.06	2.43 ± 0.05	2.89 ± 0.04	2.53 ± 0.04	2.21 ± 0.05	3.12 ± 0.03	2.84 ± 0.06	2.67 ± 0.05	2.75 ± 0.04	2.69 ± 0.04	2.85 ± 0.09
C18:3(n-6)	0.96 ± 0.03	0.85 ± 0.01	1.19 ± 0.05	0.96 ± 0.02	0.90 ± 0.02	0.95 ± 0.09	0.99 ± 0.03	1.02 ± 0.02	1.03 ± 0.13	0.87 ± 0.02	0.93 ± 0.02	0.88 ± 0.16
C18:3(n-3)	5.22 ± 0.16	5.80 ± 0.18	4.57 ± 0.18	4.72 ± 0.16	5.19 ± 0.17	4.11 ± 0.10	4.40 ± 0.10	5.91 ± 0.21	3.91 ± 0.13	3.81 ± 0.17	4.50 ± 0.15	4.28 ± 0.17
C18:4(n-3)	8.90 ± 0.43	7.96 ± 0.36	4.63 ± 0.21	7.71 ± 0.22	7.13 ± 0.40	3.61 ± 0.21	6.93 ± 0.42	6.77 ± 0.36	3.02 ± 0.15	4.69 ± 0.20	4.78 ± 0.19	3.01 ± 0.20
C20:0	0.83 ± 0.03	0.86 ± 0.02	1.50 ± 0.06	0.91 ± 0.03	0.86 ± 0.03	1.54 ± 0.04	0.83 ± 0.02	0.95 ± 0.03	1.80 ± 0.04	0.89 ± 0.03	0.96 ± 0.03	1.84 ± 0.07
C20:1(n-9)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.21 ± 0.05	0.29 ± 0.07	0.64 ± 0.15	0.02 ± 0.02	0.10 ± 0.06	0.30 ± 0.10	0.06 ± 0.03	0.02 ± 0.02	0.03 ± 0.03
C20:2(n-6)	0.45 ± 0.02	0.58 ± 0.03	1.09 ± 0.17	0.49 ± 0.02	0.58 ± 0.03	1.48 ± 0.08	0.52 ± 0.02	0.78 ± 0.04	1.54 ± 0.12	0.39 ± 0.04	0.46 ± 0.07	1.32 ± 0.20
C20:3(n-6)	4.00 ± 0.12	3.26 ± 0.07	3.72 ± 0.11	3.81 ± 0.12	3.08 ± 0.12	3.32 ± 0.08	3.28 ± 0.09	2.64 ± 0.05	3.13 ± 0.09	2.46 ± 0.07	2.14 ± 0.04	2.40 ± 0.08
C20:4(n-6)	8.77 ± 0.42	7.99 ± 0.31	10.00 ± 0.14	8.28 ± 0.18	7.46 ± 0.19	9.72 ± 0.14	6.81 ± 0.24	7.23 ± 0.20	9.92 ± 0.13	6.46 ± 0.30	6.64 ± 0.19	9.16 ± 0.17
C20:3(n-3)	0.37 ± 0.03	0.47 ± 0.02	0.03 ± 0.03	0.25 ± 0.04	0.35 ± 0.04	0.04 ± 0.04	0.27 ± 0.03	0.36 ± 0.06	0.04 ± 0.04	0.04 ± 0.02	0.03 ± 0.02	0.00 ± 0.00
C20:4(n-3)	3.70 ± 0.09	4.51 ± 0.12	4.08 ± 0.13	3.64 ± 0.06	3.80 ± 0.08	4.19 ± 0.14	3.01 ± 0.09	3.69 ± 0.19	3.26 ± 0.09	2.53 ± 0.08	2.87 ± 0.07	3.02 ± 0.11
C20:5(n-3)	3.24 ± 0.05	3.37 ± 0.07	3.27 ± 0.15	3.15 ± 0.09	3.07 ± 0.06	2.95 ± 0.05	3.28 ± 0.05	3.52 ± 0.08	2.83 ± 0.08	2.79 ± 0.07	3.07 ± 0.07	3.32 ± 0.10
C22:0	0.04 ± 0.02	0.13 ± 0.07	0.30 ± 0.17	0.07 ± 0.03	0.01 ± 0.01	0.26 ± 0.13	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C22:1(n-9)	0.09 ± 0.05	0.15 ± 0.06	0.43 ± 0.18	0.26 ± 0.05	0.28 ± 0.06	0.60 ± 0.16	0.27 ± 0.07	0.20 ± 0.07	0.59 ± 0.20	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
C24:0	0.54 ± 0.03	0.60 ± 0.03	1.10 ± 0.11	0.60 ± 0.03	0.60 ± 0.03	1.35 ± 0.06	0.60 ± 0.03	0.84 ± 0.05	1.78 ± 0.12	0.66 ± 0.05	0.85 ± 0.04	1.64 ± 0.18
Other FA	0.39 ± 0.01	0.45 ± 0.01	0.34 ± 0.09	0.42 ± 0.01	0.46 ± 0.01	0.32 ± 0.09	0.41 ± 0.02	0.50 ± 0.02	0.08 ± 0.04	0.43 ± 0.02	0.50 ± 0.01	0.07 ± 0.05
Total SFA	36.62 ± 0.75	35.81 ± 0.68	32.58 ± 0.38	38.29 ± 0.33	37.96 ± 0.46	32.58 ± 0.27	40.63 ± 0.55	37.57 ± 0.62	34.10 ± 0.28	44.75 ± 0.56	42.74 ± 0.52	36.09 ± 0.34
Total MUFA	22.78 ± 0.51	24.63 ± 0.40	29.87 ± 0.49	23.70 ± 0.27	25.71 ± 0.33	32.16 ± 0.43	24.72 ± 0.41	25.23 ± 0.29	32.72 ± 0.41	26.85 ± 0.31	27.22 ± 0.24	31.75 ± 0.58
Total PUFA	40.22 ± 1.25	39.11 ± 1.03	37.22 ± 0.57	37.60 ± 0.56	35.88 ± 0.72	34.93 ± 0.38	34.25 ± 0.93	36.70 ± 0.82	33.11 ± 0.39	27.96 ± 0.84	29.53 ± 0.62	32.09 ± 0.58
Total PUFA (n-3)	22.27 ± 0.66	23.01 ± 0.68	17.49 ± 0.63	20.25 ± 0.33	20.35 ± 0.45	15.91 ± 0.38	18.65 ± 0.60	21.14 ± 0.61	13.52 ± 0.42	14.29 ± 0.45	15.81 ± 0.36	14.06 ± 0.61
Total PUFA (n-6)	17.12 ± 0.59	15.11 ± 0.38	18.44 ± 0.24	16.43 ± 0.31	14.56 ± 0.33	17.68 ± 0.22	14.73 ± 0.35	14.51 ± 0.25	18.29 ± 0.25	12.92 ± 0.38	12.87 ± 0.24	16.61 ± 0.24
n-6/n-3	0.77 ± 0.01	0.66 ± 0.01	1.08 ± 0.05	0.81 ± 0.01	0.72 ± 0.01	1.12 ± 0.04	0.79 ± 0.01	0.69 ± 0.02	1.37 ± 0.05	0.91 ± 0.01	0.82 ± 0.01	1.21 ± 0.05
TFA (mg g^{-1} dw)	73.69 ± 3.87	61.25 ± 2.66	24.57 ± 1.10	73.01 ± 1.86	71.45 ± 3.37	26.36 ± 1.07	79.39 ± 3.32	57.44 ± 2.51	21.66 ± 0.70	76.89 ± 4.03	59.40 ± 2.72	20.98 ± 1.33

Table A4.1. Average TFA content (mg g-1 dw ± SE), the average proportion of the broad fatty acid groups (SFA, MUFA, PUFA(n-3), PUFA(n-6)) and individual fatty acids (% of TFA ± SE) across locations (*Dictyota*: Nelly Bay (n = 6), Orpheus Island (n = 3), Kissing Point (n = 2); *Dictyopteris*: Nelly Bay (n = 6)) between summer (Nov-11, Feb-12, Nov-12) and winter (*Dictyota*: May-12, Jun-12, Aug-12; *Dictyopteris*: Jun-12, Jul-12, Aug-12). No plants were found at Kissing Point during summer. Averages based on monthly averages with n months for each location and with each month being the average of 15 individual plants.

	***Dictyota bartayresii* - Nelly Bay**						***Dictyota bartayresii* - Kissing Point**					
	Summer (Nov-11, Feb-12, Nov-12; n = 3)			Winter (May-12, Jun-12, Aug-12; n = 3)			Summer			Winter (May-12, Aug-12; n = 2)		
	Whole plant	Upper section	Lower section	Whole plant	Upper section	Lower section	Whole plant	Upper section	Lower section	Whole plant	Upper section	Lower section
C14:0	6.8 ± 0.0	7.2 ± 0.1	6.0 ± 0.1	7.1 ± 0.1	7.4 ± 0.1	6.6 ± 0.2				7.7 ± 0.0	8.1 ± 0.0	7.3 ± 0.0
C14:1	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1				0.4 ± 0.1	0.3 ± 0.0	0.5 ± 0.1
C15:0	0.8 ± 0.1	0.7 ± 0.1	1.0 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0				0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
C16:0	22.0 ± 0.7	22.7 ± 0.8	21.1 ± 0.8	19.9 ± 0.8	20.3 ± 0.8	19.6 ± 0.8				23.4 ± 0.1	24.0 ± 0.3	22.6 ± 0.5
C16:1(n-9)	2.5 ± 0.2	2.3 ± 0.2	2.8 ± 0.2	2.1 ± 0.2	1.8 ± 0.2	2.4 ± 0.3				2.1 ± 0.0	1.9 ± 0.2	2.3 ± 0.1
C16:1(n-7)	6.9 ± 0.4	7.1 ± 0.5	6.4 ± 0.3	7.4 ± 0.5	7.4 ± 0.5	7.4 ± 0.5				7.6 ± 0.5	7.7 ± 0.3	7.6 ± 0.7
C16:1	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1				0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
C16:2	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.2				0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
C17:0	1.6 ± 0.3	1.5 ± 0.3	1.8 ± 0.3	1.0 ± 0.1	1.0 ± 0.2	1.1 ± 0.1				0.8 ± 0.0	0.7 ± 0.1	0.9 ± 0.0
C16:3(n-4)	1.2 ± 0.2	1.1 ± 0.2	1.5 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.2				0.7 ± 0.2	0.6 ± 0.1	0.8 ± 0.3
C16:4(n-3)	0.6 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0				0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
C18:0	1.9 ± 0.2	2.0 ± 0.2	1.9 ± 0.2	1.7 ± 0.2	1.8 ± 0.2	1.7 ± 0.2				1.6 ± 0.2	1.6 ± 0.2	1.5 ± 0.2
C18:1(n-9)	15.7 ± 0.5	15.9 ± 0.3	15.5 ± 0.6	14.9 ± 0.8	15.0 ± 0.9	14.9 ± 0.7				16.2 ± 0.4	16.2 ± 0.9	16.1 ± 0.1
C18:1(n-3)	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.3				0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
C18:1(n-5)	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0				0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0
C18:2(n-6)	2.4 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1				2.4 ± 0.1	2.3 ± 0.1	2.5 ± 0.2
C18:3(n-6)	1.9 ± 0.2	1.6 ± 0.2	2.2 ± 0.2	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.2				1.5 ± 0.1	1.2 ± 0.0	1.7 ± 0.1
C18:3(n-3)	2.3 ± 0.2	2.3 ± 0.1	2.4 ± 0.2	3.3 ± 0.2	3.3 ± 0.3	3.3 ± 0.2				2.8 ± 0.1	2.8 ± 0.2	2.8 ± 0.0
C18:4(n-3)	8.7 ± 0.7	9.1 ± 0.7	8.0 ± 0.8	12.5 ± 1.1	12.8 ± 1.1	12.1 ± 1.0				10.8 ± 0.5	11.2 ± 0.8	10.3 ± 0.1
C20:0	1.1 ± 0.2	1.0 ± 0.2	1.2 ± 0.2	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0				0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
C20:1(n-9)	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1				0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
C20:2(n-6)	2.2 ± 0.2	2.0 ± 0.2	2.4 ± 0.2	2.7 ± 0.2	2.5 ± 0.2	2.8 ± 0.3				2.1 ± 0.2	1.9 ± 0.2	2.4 ± 0.3
C20:3(n-6)	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.8 ± 0.0				1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
C20:4(n-6)	7.4 ± 1.2	7.6 ± 1.2	7.1 ± 1.3	6.6 ± 0.5	6.9 ± 0.5	6.3 ± 0.5				7.4 ± 0.2	7.7 ± 0.2	7.2 ± 0.1
C20:3(n-3)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1				0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
C20:4(n-3)	1.5 ± 0.0	1.4 ± 0.0	1.7 ± 0.0	1.1 ± 0.5	1.1 ± 0.6	1.1 ± 0.5				0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.2
C20:5(n-3)	2.8 ± 0.2	2.7 ± 0.1	3.0 ± 0.1	4.8 ± 0.2	4.8 ± 0.1	4.9 ± 0.3				3.3 ± 0.3	3.2 ± 0.4	3.4 ± 0.1
C22:0	0.6 ± 0.2	0.5 ± 0.1	0.7 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1				0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C22:1(n-9)	0.5 ± 0.0	0.4 ± 0.0	0.7 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1				0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
22:4(n-6)	1.5 ± 0.2	1.4 ± 0.2	1.6 ± 0.2	2.2 ± 0.0	2.1 ± 0.1	2.3 ± 0.0				1.6 ± 0.0	1.5 ± 0.0	1.7 ± 0.0
C24:0	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1				0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
C22:6(n-3)	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.2				0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.1
Other FA	0.6 ± 0.3	0.6 ± 0.3	0.5 ± 0.3	1.6 ± 0.6	1.6 ± 0.6	1.6 ± 0.5				1.5 ± 0.2	1.4 ± 0.1	1.7 ± 0.2
Total SFA	35.2 ± 0.3	36.0 ± 0.1	34.0 ± 0.3	32.1 ± 0.9	32.5 ± 1.0	31.5 ± 0.9				35.2 ± 0.2	36.0 ± 0.6	34.3 ± 0.1
Total MUFA	29.5 ± 1.2	29.1 ± 1.2	30.0 ± 1.2	27.3 ± 0.4	26.8 ± 0.5	27.9 ± 0.3				28.2 ± 0.0	27.9 ± 0.5	28.6 ± 0.5
Total PUFA	34.8 ± 1.7	34.3 ± 1.6	35.4 ± 1.8	39.1 ± 0.9	39.0 ± 1.1	39.1 ± 0.7				35.0 ± 0.4	34.7 ± 1.3	35.4 ± 0.4
Pufa(n-3)	16.2 ± 0.7	16.3 ± 0.6	16.0 ± 0.8	22.9 ± 0.5	23.0 ± 0.6	22.7 ± 0.4				17.9 ± 0.6	18.2 ± 1.3	17.7 ± 0.2
PUFA(n-6)	16.7 ± 1.2	16.4 ± 1.1	17.1 ± 1.2	15.1 ± 0.7	15.0 ± 0.7	15.1 ± 0.7				16.0 ± 0.2	15.6 ± 0.2	16.4 ± 0.2
n-6/n-3	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0				0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.0
TFA (mg g^{-1} dw)	51.8 ± 3.5	63.0 ± 5.6	42.0 ± 3.9	47.4 ± 1.3	55.4 ± 1.7	40.2 ± 1.9				54.2 ± 1.9	67.1 ± 4.4	45.3 ± 0.8

No plants analysed

TFA total fatty acids, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *ALA* α-linolenic acid, *SDA* stearidonic acid, *ETA* eicosatetraenoic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *dw* dry weight

Table A4.1. (Continued)

	Dictyota bartayresii - Orpheus Island					
	Summer (Nov-11, Nov-12; n = 2)			Winter (Aug-12; n = 1)		
	Whole plant	Upper section	Lower section	Whole plant	Upper section	Lower section
C14:0	6.6 ± 0.1	7.1 ± 0.1	5.8 ± 0.2	6.8	7.1	5.6
C14:1	1.1 ± 0.2	1.0 ± 0.2	1.3 ± 0.2	0.9	0.8	1.0
C15:0	1.0 ± 0.1	0.9 ± 0.1	1.2 ± 0.2	0.9	0.8	1.0
C16:0	20.2 ± 0.3	20.9 ± 0.1	19.0 ± 0.7	19.7	21.1	19.7
C16:1(n-9)	3.2 ± 0.8	3.0 ± 0.8	3.5 ± 0.9	2.7	2.2	2.6
C16:1(n-7)	8.8 ± 0.1	9.4 ± 0.0	8.1 ± 0.4	5.9	9.4	7.7
C16:1	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.9	0.5	0.6
C16:2	0.5 ± 0.5	0.4 ± 0.4	0.5 ± 0.5	0.0	0.9	1.0
C17:0	2.3 ± 0.6	2.2 ± 0.6	2.4 ± 0.6	1.6	2.8	3.0
C16:3(n-4)	1.7 ± 0.7	1.6 ± 0.5	1.8 ± 0.9	1.3	1.0	0.9
C16:4(n-3)	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.3	0.5	0.8	1.0
C18:0	2.3 ± 0.0	2.2 ± 0.0	2.4 ± 0.1	2.9	2.3	2.5
C18:1(n-9)	13.4 ± 0.1	13.5 ± 0.0	13.3 ± 0.1	13.6	13.5	13.4
C18:1(n-3)	0.6 ± 0.6	0.6 ± 0.6	0.7 ± 0.7	0.0	1.2	1.4
C18:1(n-5)	1.3 ± 0.4	1.2 ± 0.3	1.4 ± 0.4	0.8	0.9	1.0
C18:2(n-6)	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.7	1.6	1.7
C18:3(n-6)	1.7 ± 0.0	1.6 ± 0.0	2.0 ± 0.1	1.1	1.6	2.1
C18:3(n-3)	1.7 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	2.0	1.4	1.5
C18:4(n-3)	6.6 ± 0.7	6.9 ± 0.7	6.2 ± 0.6	8.6	7.5	6.8
C20:0	1.6 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	1.4	1.4	1.5
C20:1(n-9)	0.9 ± 0.2	0.8 ± 0.2	1.1 ± 0.2	0.6	0.7	0.8
C20:2(n-6)	2.6 ± 0.0	2.6 ± 0.1	2.7 ± 0.0	2.5	2.7	2.7
C20:3(n-6)	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.0	1.0	0.9	1.0
C20:4(n-6)	5.3 ± 0.4	5.3 ± 0.4	5.2 ± 0.4	5.5	5.7	5.7
C20:3(n-3)	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.2	0.0	0.5	0.5
C20:4(n-3)	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.2	0.9	1.5	1.6
C20:5(n-3)	4.4 ± 0.1	3.9 ± 0.0	4.9 ± 0.2	7.7	3.9	5.1
C22:0	0.5 ± 0.3	0.5 ± 0.3	0.6 ± 0.3	0.6	0.8	0.9
C22:1(n-9)	1.1 ± 0.3	1.0 ± 0.3	1.3 ± 0.3	0.8	0.7	1.0
22:4(n-6)	2.1 ± 0.1	2.0 ± 0.1	2.3 ± 0.1	2.7	1.9	2.1
C24:0	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.3	0.9	0.8	0.9
C22:6(n-3)	1.1 ± 0.3	0.8 ± 0.4	1.4 ± 0.2	1.4	1.2	1.6
Other FA	0.5 ± 0.5	0.6 ± 0.6	0.4 ± 0.4	2.1	0.0	0.0
Total SFA	35.1 ± 1.0	36.1 ± 0.9	33.8 ± 1.4	34.9	37.0	35.1
Total MUFA	30.8 ± 1.1	30.7 ± 0.9	30.9 ± 1.4	26.1	29.9	29.6
Total PUFA	33.6 ± 0.6	32.6 ± 0.6	34.9 ± 0.4	36.9	33.2	35.3
Pufa(n-3)	16.9 ± 0.6	16.4 ± 0.5	17.5 ± 0.6	21.2	16.9	18.1
PUFA(n-6)	14.5 ± 0.2	14.2 ± 0.2	15.1 ± 0.2	14.5	14.3	15.3
n-6/n-3	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.7	0.9	0.9
TFA (mg g^{-1} dw)	38.7 ± 3.9	44.6 ± 3.9	32.4 ± 3.6	30.1	36.4	25.5

Table A4.1. (Continued)

	Dictyopteris australis - Nelly Bay							
	Summer (Nov-11, Feb-12, Nov-12; n = 3)				Winter (Jun-12, Jul-12, Aug-12; n = 3)			
	Whole plant	Tips	Midsection	Base section	Whole plant	Tips	Midsection	Base section
C14:0	8.0 ± 0.6	9.0 ± 0.6	7.6 ± 0.7	6.8 ± 0.7	7.4 ± 0.5	8.2 ± 0.5	7.2 ± 0.5	6.4 ± 0.5
C14:1	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.9 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	1.0 ± 0.1
C15:0	0.8 ± 0.1	0.7 ± 0.0	0.8 ± 0.0	1.1 ± 0.0	1.0 ± 0.2	0.8 ± 0.2	1.0 ± 0.2	1.8 ± 0.3
C16:0	22.7 ± 0.1	23.0 ± 0.1	22.6 ± 0.2	21.1 ± 0.8	20.1 ± 0.6	21.3 ± 0.6	20.3 ± 0.3	17.7 ± 0.9
C16:1(n-9)	3.3 ± 0.2	3.1 ± 0.3	3.4 ± 0.2	3.9 ± 0.4	4.0 ± 0.5	3.3 ± 0.5	3.8 ± 0.5	5.0 ± 0.2
C16:1(n-7)	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.2 ± 0.2	1.1 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	2.0 ± 0.4
C16:1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
C16:2	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.2 ± 0.2
C17:0	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.5 ± 0.1	1.8 ± 0.4	1.4 ± 0.3	1.6 ± 0.4	1.3 ± 0.2
C16:3(n-4)	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.4 ± 0.4
C16:4(n-3)	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.1	0.7 ± 0.0	0.8 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	1.2 ± 0.3
C18:0	1.1 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.5 ± 0.1	1.4 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	2.1 ± 0.4
C18:1(n-9)	18.0 ± 0.6	17.0 ± 0.6	18.2 ± 0.6	19.7 ± 0.1	15.9 ± 0.4	15.6 ± 0.3	16.0 ± 0.5	17.5 ± 0.6
C18:1(n-3)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C18:1(n-5)	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.0 ± 0.3	0.8 ± 0.2	0.9 ± 0.2	1.0 ± 0.0
C18:2(n-6)	5.8 ± 0.5	5.8 ± 0.3	5.7 ± 0.6	6.4 ± 0.5	5.9 ± 0.7	5.9 ± 0.6	5.9 ± 0.7	6.5 ± 0.2
C18:3(n-6)	1.6 ± 0.3	1.8 ± 0.3	1.6 ± 0.3	1.8 ± 0.4	1.8 ± 0.1	1.9 ± 0.2	1.7 ± 0.1	1.9 ± 0.1
C18:3(n-3)	8.2 ± 0.6	7.3 ± 0.3	8.3 ± 0.5	7.0 ± 0.5	6.5 ± 0.3	6.0 ± 0.4	7.0 ± 0.3	5.7 ± 0.4
C18:4(n-3)	6.1 ± 0.3	6.7 ± 0.4	5.6 ± 0.2	4.0 ± 0.2	7.9 ± 1.6	8.5 ± 1.4	8.2 ± 1.8	5.5 ± 1.1
C20:0	0.8 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	1.1 ± 0.1	1.1 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	1.8 ± 0.4
C20:1(n-9)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.3 ± 0.3	0.1 ± 0.1
C20:2(n-6)	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.6 ± 0.1	1.3 ± 0.2	1.0 ± 0.2	1.2 ± 0.2	2.3 ± 0.5
C20:3(n-6)	2.0 ± 0.4	2.4 ± 0.3	1.9 ± 0.3	1.8 ± 0.3	2.6 ± 0.2	3.2 ± 0.2	2.6 ± 0.2	2.5 ± 0.4
C20:4(n-6)	10.8 ± 0.1	11.6 ± 0.5	10.4 ± 0.3	10.0 ± 0.2	10.0 ± 1.2	10.9 ± 1.5	10.0 ± 1.4	9.5 ± 0.6
C20:3(n-3)	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.1 ± 0.1
C20:4(n-3)	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.5 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	1.7 ± 0.2
C20:5(n-3)	2.3 ± 0.1	1.9 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.5 ± 0.2	2.2 ± 0.2	2.6 ± 0.3	2.8 ± 0.3
C22:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
C22:1(n-9)	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
22:4(n-6)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C24:0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.2
C22:6(n-3)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Other FA	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.4	0.9 ± 0.4	1.6 ± 0.5	1.4 ± 0.2	1.4 ± 0.2	1.2 ± 0.4
Total SFA	34.8 ± 0.6	35.7 ± 0.6	34.5 ± 0.8	33.5 ± 1.4	33.1 ± 0.9	34.1 ± 1.0	32.6 ± 1.4	31.6 ± 0.7
Total MUFA	23.9 ± 0.5	22.7 ± 0.3	24.5 ± 0.3	27.3 ± 0.4	23.2 ± 1.6	21.7 ± 1.5	23.1 ± 1.8	27.0 ± 1.0
Total PUFA	40.4 ± 1.0	40.7 ± 0.7	40.1 ± 0.9	38.2 ± 0.6	42.1 ± 2.9	42.7 ± 2.5	42.8 ± 3.4	40.2 ± 1.8
Pufa(n-3)	18.6 ± 0.9	17.8 ± 0.7	18.7 ± 0.5	15.9 ± 0.7	19.6 ± 1.8	18.9 ± 1.1	20.5 ± 2.1	17.0 ± 2.2
PUFA(n-6)	21.2 ± 0.8	22.4 ± 0.3	20.7 ± 0.6	21.6 ± 1.2	21.6 ± 1.4	22.9 ± 1.7	21.4 ± 1.6	22.6 ± 0.2
n-6/n-3	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	1.1 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	1.4 ± 0.2
TFA (mg g^{-1} dw)	46.2 ± 1.6	54.6 ± 2.0	43.9 ± 2.5	27.2 ± 2.5	51.0 ± 6.3	63.2 ± 7.9	53.6 ± 7.1	28.4 ± 4.8

APPENDIX

Supplementary figures

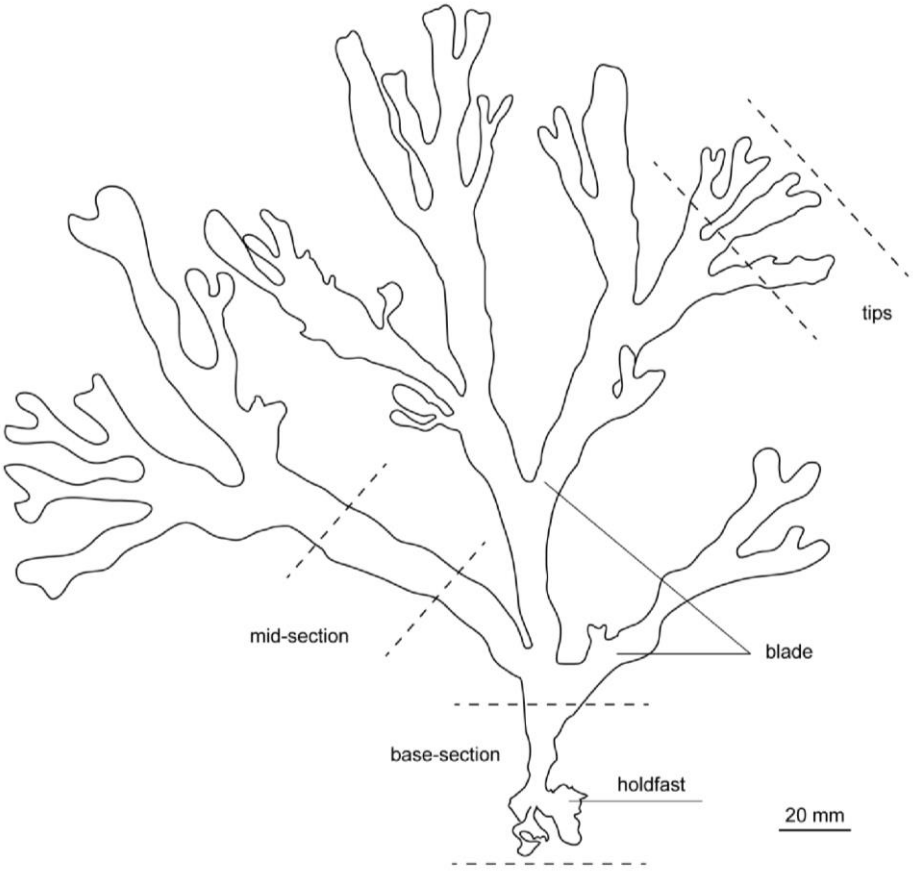

Figure A3.1. Shematic drawing of typical *Spatoglossum macrodontum* plant with plant sections used in this Chapter (tips, midsections, base sections).

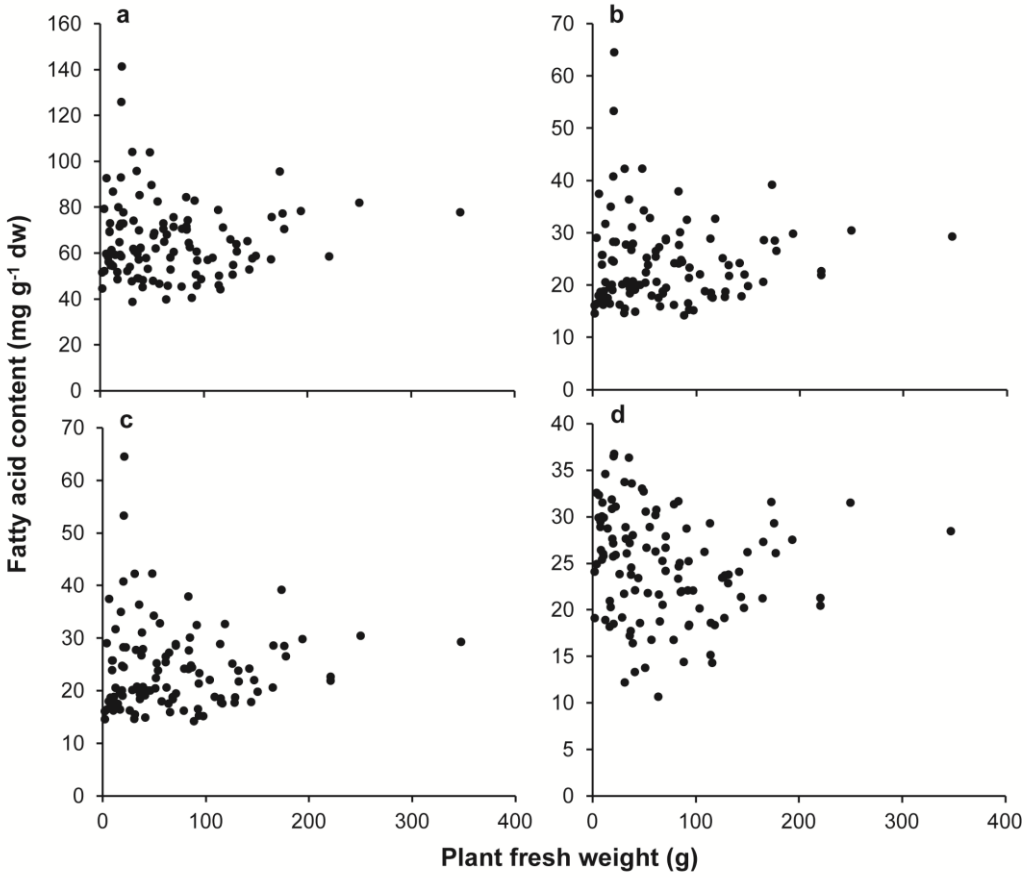

Figure A3.2. Correlation of plant fresh weight (g) and content (mg g^{-1} dw) of **a** total fatty acids (r = -0.001, p = 0.991, n =112), **b** monounsaturated fatty acids (MUFA) (r = 0.073, p = 0.442, n = 112), **c** saturated fatty acids (SFA) (r = 0.035, p = 0.712, n = 112) and d polyunsaturated fatty acids (PUFA) (r = -0.118, p = 0.214, n = 112) in *Spatoglossum macrodontum*.

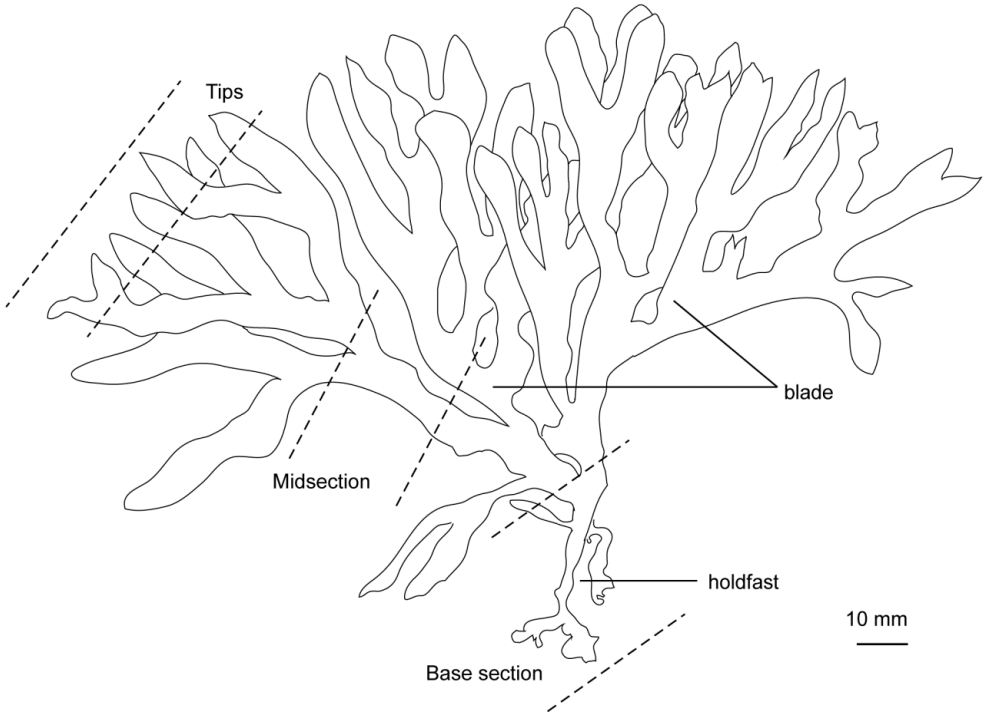

Figure A4.1. Shematic drawing of typical *Dictyopteris australis* plant with plant sections used in this Chapter (tips, midsection, base section).

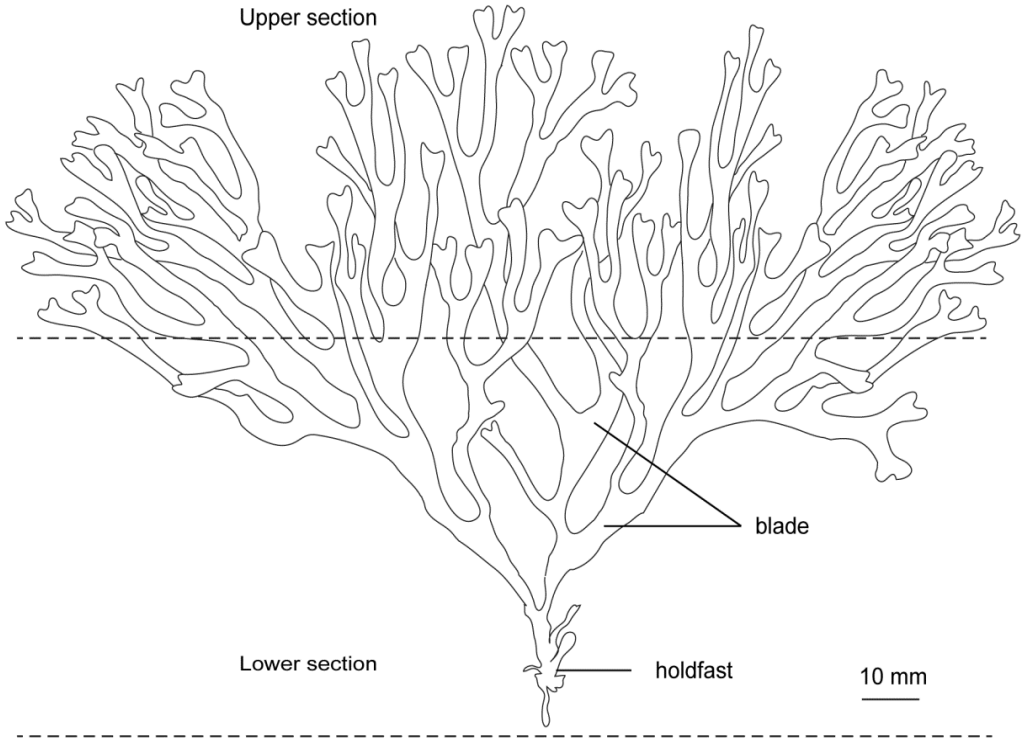

Figure A4.2. Shematic drawing of typical *Dictyota bartayresii* plant with plant sections used in this Chapter (upper sections, lower sections).