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

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Abstract We investigated the spatial, seasonal, and within-plant variation in total fatty acids (TFA) and fatty acid (FA) composition in the brown seaweeds *Dictyota bartayresii* sampled from Nelly Bay, Orpheus Island, and Kissing Point (Northern Queensland, Australia) and *Dictyopteris australis* sampled from Nelly Bay. Both species were present year round, but with no consistent seasonal pattern in plant size, and had an annualized mean content of TFA of 5 % dry weight (dw) that is among the highest recorded for seaweeds. For *Dictyota*, larger plants had a higher content of TFA and a higher proportion of polyunsaturated omega-3 FA (PUFA(n-3)) than smaller plants, while for *Dictyopteris*, the TFA content and the proportion of PUFA(n-3) were unrelated to plant size. TFA in *Dictyota* varied considerably between locations (~40 %) (3.5–5.5 % dw) and to a lesser degree between seasons (~10 %) with a lower content of TFA and higher proportion of PUFA(n-3) in winter. *Dictyopteris* had an ~10 % higher content of TFA in winter but with no seasonal pattern in the composition of FA. There was also within-plant variation in FA, as TFA decreased from the tips toward the base in both species, and in *Dictyopteris*, the tips had a higher proportion of saturated FA (SFA) and PUFA(n-3) than the base. The presence of site- and species-specific variation in the content and composition of fatty acids reinforces the need for detailed analyses of the biochemical profile of seaweed biomass, including their extracts or purified compounds, if the focus is nutritional applications based on lipids.

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Introduction

There is increasing awareness of the health benefits of a diet rich in polyunsaturated omega-3 fatty acids (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 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 eicosapentaenoic acid (EPA) or docosahexaenoic acid (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., The intensive land based production for the green seaweeds Derbesia tenuissima and Ulva ohnoi: biomass and bioproducts, in review) and an annual production of approximately 20 million tons 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 % dry weight (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 (Gosch et al. 2012; Kumari et al. 2013; 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) (Gosch et al. 2014) and the green seaweed Derbesia tenuissima with a TFA 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 (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 are 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 (Gosch et al. 2014; Khotimchenko and Kulikova 2000; Kulikova and Khotimchenko 2000) and are 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) (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.

Materials and methods

Study organism and field sites

The brown seaweeds Dictyota bartayresii Lamouroux and Dictyopteris australis (Sonder) Askenasy 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 (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 Gosch et al. (2012).

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 (Online Resource 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 study. *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).

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 were measured to assess any relationship between TFA content and 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 (Online Resource 2) 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) (Online Resource 3) to analyze the variation of fatty acids within plants, and the TFA content and composition for the "whole plant" were 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.

Fatty acid analysis

Fatty acids were analyzed 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)), as described in detail in 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.

Statistical analysis

The relationships between plant size as measured in thallus length (cm) and fresh weight (g) and TFA content and fatty acid composition (saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA)) were analyzed 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 analyzed 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.

Results

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 standard error (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) (Fig. 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 (Fig. 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 (15.6–19.8 cm) (Fig. 1c).

Fig. 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
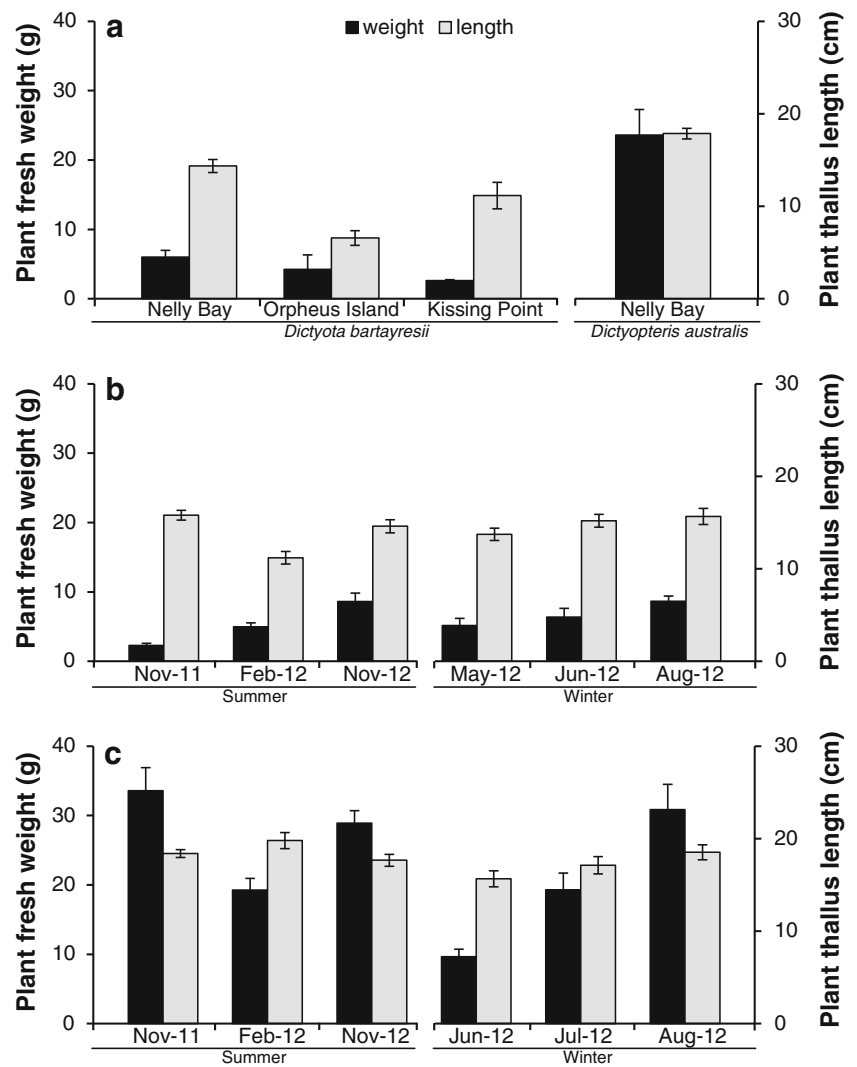
Total fatty acid content—plant sizes

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 ($r=0.480$, $p<0.001$, $n=44$) (Fig. 2a, b). 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 ($r=0.050$, $p<0.657$, $n=81$) (Fig. 2c, d).

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) (Fig. 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 1). 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) (Fig. 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) (Fig. 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}

Fig. 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* ($n=80$)
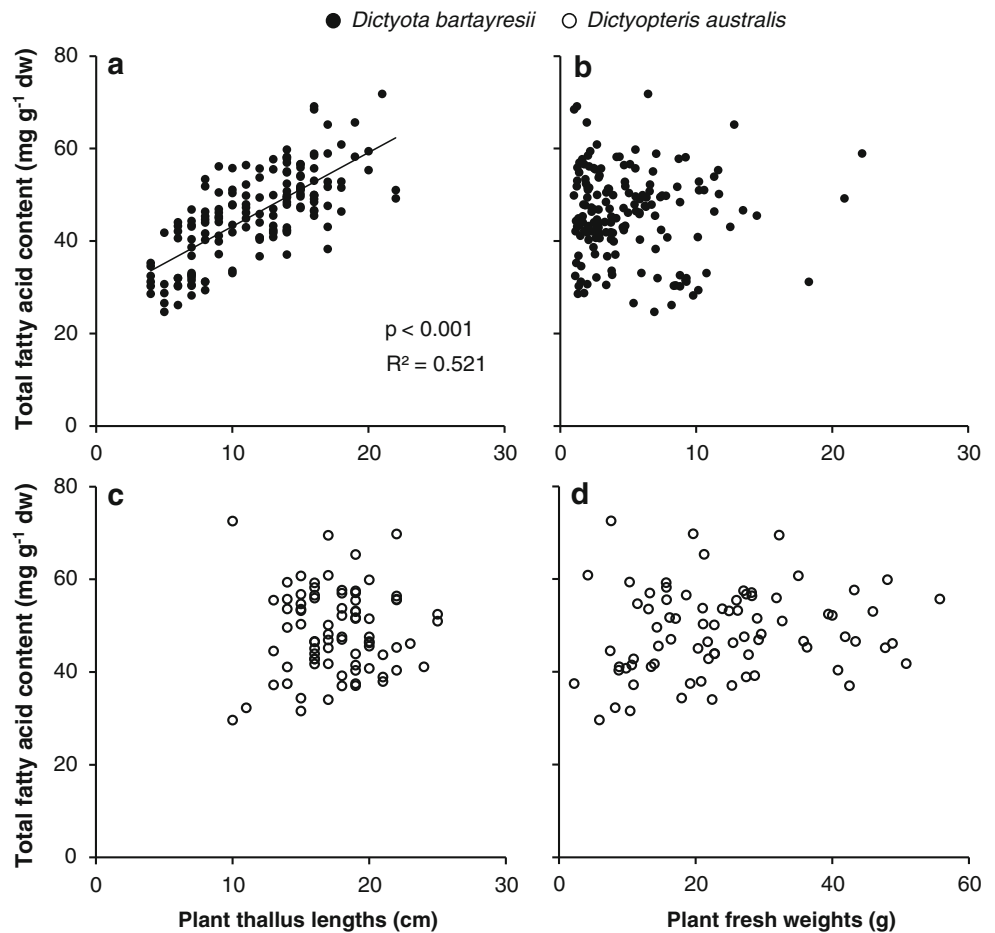
dw±6.3 SE) compared to summer (46.2 mg g^{-1} dw±1.6 SE) (Table 1). In a similar manner to *Dictyota*, there was monthly variation in the TFA content. While the TFA content was relatively stable during summer (43.3–48.6 mg g^{-1} dw), there was large variation during winter with a rapid increase in TFA 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) (Fig. 3c).

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 %) (Fig. 3a and Table 1). 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 %) (Fig. 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 (Fig. 3a and Table 1). 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 % (Fig. 3c).

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 (Fig. 4). Larger plants of *D. bartayresii*, as measured in fresh weight (g), generally had a higher proportion of PUFA(n-3) ($r=0.207$, $p=0.008$, $n=162$) (Fig. 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) ($r=0.172$, $p=0.029$, $n=162$) (Fig. 4b) and a higher proportion of

Fig. 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) and **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
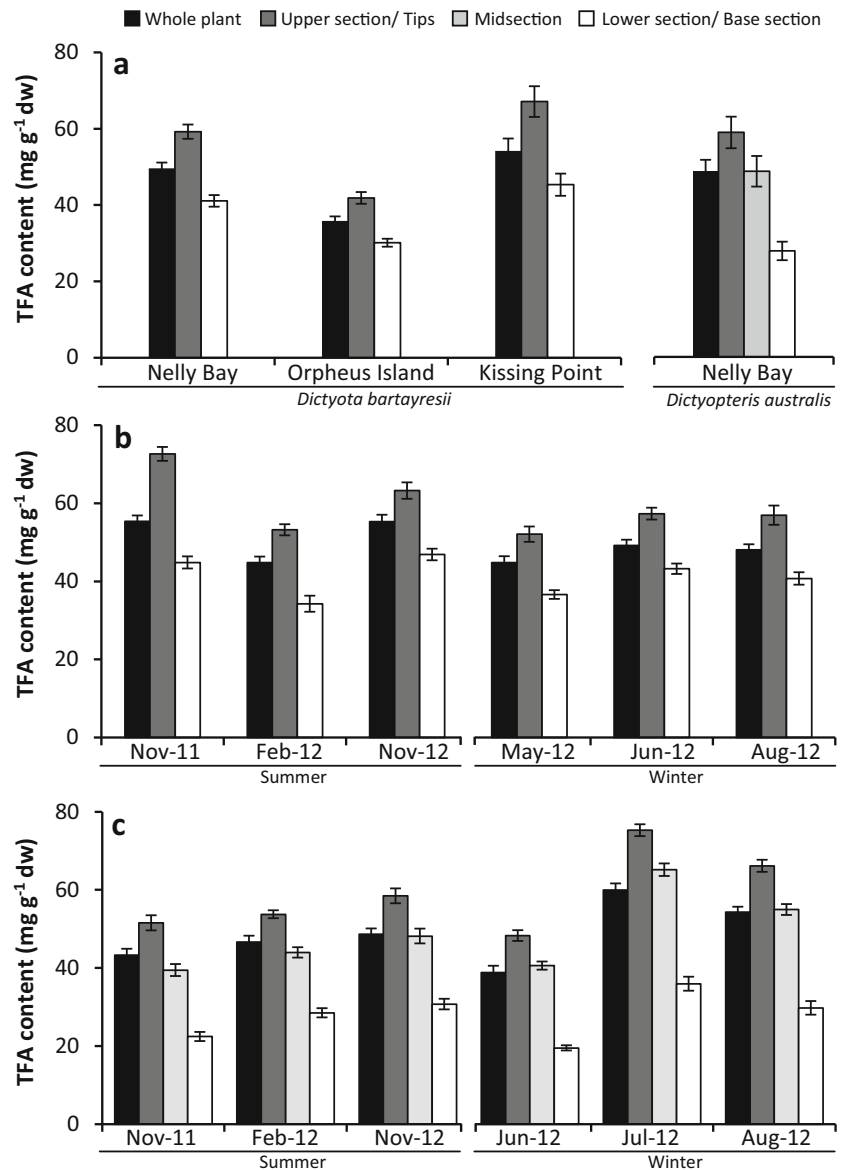
PUFA(n-6) (*r*=0.157, *p*=0.047, *n*=162). In *D. australis*, the broad fatty acid groups SFA, MUFA, and PUFA(n-3) were unrelated to the fresh weight (g) of plants; however, larger plants tended to have a higher proportion of PUFA(n-6) (*r*=0.290, *p*=0.01, *n*=78) (Fig. 4c, d). Thallus length had no effect on the fatty acid composition in this species.

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 (Fig. 5a). Comparing *Dictyota* and *Dictyopteris* from Nelly Bay (Fig. 5a), both species had a similar degree of saturation with the most abundant saturated fatty acids being C16:0 and C14:0 (Table 1). 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) (Fig. 6 and Table 1).

Table 1 Average TFA content (mg g^{-1} dw±SE), 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=6), Kissing Point (n=3); Dictyopteris: Nelly Bay (n=2)) between summer (November 2011, February 2012, November 2012) and winter (Dictyota: May 2012, June 2012, August 2012; Dictyopteris: June 2012, July 2012, August 2012). 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, and for a full fatty acid table, refer to Online Resource 4

Dictyota bartayresii

	Nelly Bay—summer (n=3)			Kissing Point—summer			Kissing Point—winter (n=2)			Nelly Bay—winter (n=3)			Orpheus Island—summer (n=2)			Orpheus Island—winter (n=1)		
	Whole plant	Upper section	Lower section	Whole plant	Upper section	Lower section	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				7.7±0.0	8.1±0.0	7.3±0.0	7.1±0.1	7.4±0.1	6.6±0.2	6.6±0.1	7.1±0.1	5.8±0.2	6.8	7.1	5.6
C16:0	22.0±0.7	22.7±0.8	21.1±0.8				23.4±0.1	24.0±0.3	22.6±0.5	19.9±0.8	20.3±0.8	19.6±0.8	20.2±0.3	20.9±0.1	19.0±0.7	19.7	21.1	19.7
C18:3(n-3), ALA	2.3±0.2	2.3±0.1	2.4±0.2				2.8±0.1	2.8±0.2	2.8±0.0	3.3±0.2	3.3±0.3	3.3±0.2	1.7±0.2	1.6±0.2	1.7±0.2	2.0	1.4	1.5
C18:4(n-3), SDA	8.7±0.7	9.1±0.7	8.0±0.8				10.8±0.5	11.2±0.8	10.3±0.1	12.5±1.1	12.8±1.1	12.1±1.0	6.6±0.7	6.9±0.7	6.2±0.6	8.6	7.5	6.8
C20:5(n-3), EPA	2.8±0.2	2.7±0.1	3.0±0.1				3.3±0.3	3.2±0.4	3.4±0.1	4.8±0.2	4.8±0.1	4.9±0.3	4.4±0.1	3.9±0.0	4.9±0.2	7.7	3.9	5.1
C22:6(n-3), DHA	0.1±0.0	0.0±0.0	0.1±0.0				0.2±0.1	0.2±0.0	0.3±0.1	0.6±0.1	0.6±0.1	0.7±0.2	1.1±0.3	0.8±0.4	1.4±0.2	1.4	1.2	1.6
Total SFA	35.2±0.3	36.0±0.1	34.0±0.3				35.2±0.2	36.0±0.6	34.3±0.1	32.1±0.9	32.5±1.0	31.5±0.9	35.1±1.0	36.1±10.9	33.8±11.4	34.9	35.2	34.5
Total MUFA	29.5±1.2	29.1±11.2	30.0±11.2				28.2±0.0	27.9±0.5	28.6±0.5	27.3±0.4	26.8±0.5	27.9±0.3	30.8±11.1	30.7±10.9	30.9±11.4	26.1	25.8	26.5
Total PUFA	34.8±1.7	34.3±11.6	35.4±11.8				35.0±0.4	34.7±1.3	35.4±0.4	39.1±0.9	39.0±1.1	39.1±0.7	33.6±10.6	32.6±10.6	34.9±10.4	36.9	36.9	37.0
Total PUFA(n-3)	16.2±0.7	16.3±0.6	16.0±0.8				17.9±0.6	18.2±1.3	17.7±0.2	22.9±0.5	23.0±0.6	22.7±0.4	16.9±0.6	16.4±0.5	17.5±0.6	21.2	21.7	20.7
Total PUFA(n-6)	16.7±1.2	16.4±11.1	17.1±11.2				16.0±0.2	15.6±0.2	16.4±0.2	15.1±0.7	15.0±0.7	15.1±0.7	14.5±10.2	14.2±10.2	15.1±10.2	14.5	14.1	14.8
n-6/n-3	1.0±0.0	1.0±10.0	1.1±10.1				0.9±0.0	0.9±0.1	0.9±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.9±10.0	0.9±10.0	0.9±10.0	0.7	0.7	0.7
TFA (mg g^{-1} dw)	51.8±3.5	63.0±15.6	42.0±13.9				54.2±1.9	67.1±4.4	45.3±0.8	47.4±1.3	55.4±1.7	40.2±1.9	38.7±13.9	44.6±13.9	32.4±13.6	30.1	36.4	25.5

(Kissing Point—summer: No plants analyzed)

Dictyopteris australis

	Nelly Bay—summer (n=3)				Nelly Bay—winter (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
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
C18:3(n-3), ALA	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), SDA	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
C20:5(n-3), EPA	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
C22:6(n-3), DHA	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
Total SFA	34.8±10.6	35.7±10.6	34.5±10.8	33.5±11.4	33.1±0.9	34.1±1.0	32.6±1.4	31.6±0.7
Total MUFA	23.9±10.5	22.7±10.3	24.5±10.3	27.3±10.4	23.2±1.6	21.7±1.5	23.1±1.8	27.0±1.0
Total PUFA	40.4±11.0	40.7±10.7	40.1±10.9	38.2±10.6	42.1±2.9	42.7±2.5	42.8±3.4	40.2±1.8
Total 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
Total PUFA(n-6)	21.2±10.8	22.4±10.3	20.7±10.6	21.6±11.2	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±10.1	1.4±10.1	1.1±0.0	1.2±0.0	1.1±0.0	1.4±0.2
TFA (mg g^{-1} dw)	46.2±11.6	54.6±12.0	43.9±2.5	27.2±2.5	51.0±6.3	63.2±7.9	53.6±7.1	28.4±4.8

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 weight

Fig. 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)
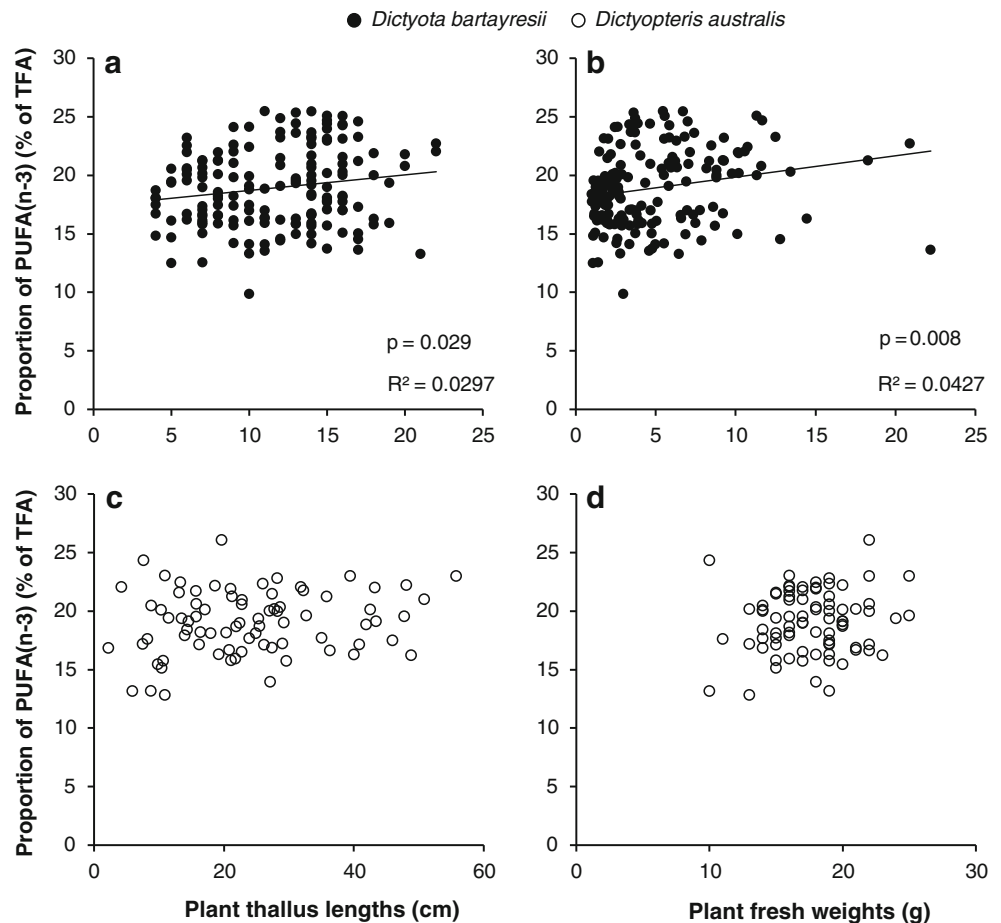
There was also temporal variation in the composition of fatty acids in both species. For *D. bartayresii* collected at Nelly Bay, the general groupings of fatty acids (SFA, MUFA, PUFA(n-3)) differed between summer and winter (Fig. 5b and Table 1). The proportions of SFA and MUFA were higher in summer (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 1). 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 1). 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) (Fig. 5b).

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 1). However, there was considerable monthly variation in the fatty acid groups (Fig. 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.

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 1). There was no significant variation of fatty acids between the upper and lower section

Fig. 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
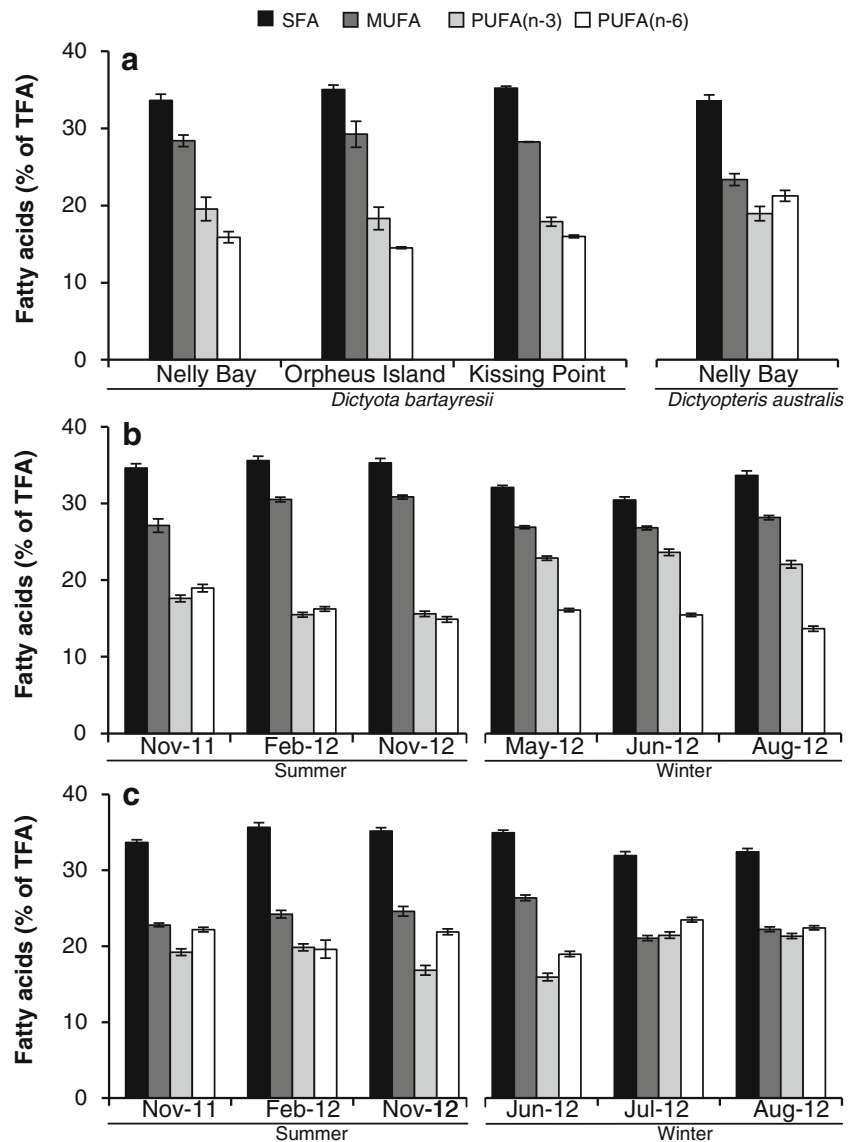
of the thallus in *D. bartayresii*, and seasonal variation of the sections again reflected that of whole plants.

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.

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 (December, January, February) (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 we 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 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* (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 *D. cervicornis* (Ateweberhan 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).

Total fatty acid content and composition

The annualized mean TFA content in *Dictyota* and *Dictyopteris* was approximately 5 % dw which is toward the upper range of TFA measured in seaweeds. Only a few other species, such as *D. tenuissima* (5 % dw) (Magnusson et al.

Fig. 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 (November 2011, February 2012, November 2012) and winter (*Dictyota*: May 2012, June 2012, August 2012; *Dictyopteris*: June 2012, July 2012, August 2012). 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 are only shown for the fatty acids of particular interest and discussed in this paper. "Whole plants" of *Dictyota* are not used in this MDS, as they are calculated from the proportional biomass of the upper and lower sections and therefore do not constitute unique biomass samples (see "Materials and methods" section)

2014) and *S. macrodontum* (5–8 % dw) (Gosch et al. 2014), have a similar or higher TFA content, while the majority of seaweed species have a TFA content below 3 % dw (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); *Chondrus 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 (Gosch et al. 2012). The essential PUFA C18:3(n-3) (α-linolenic acid, 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)
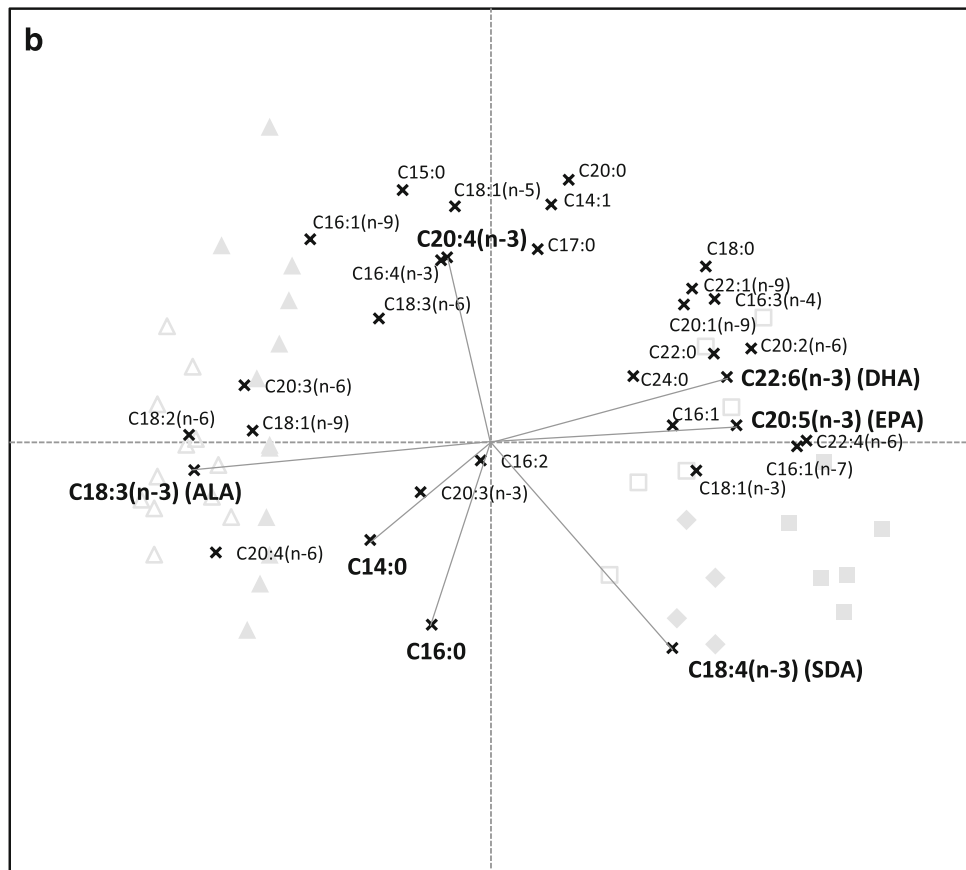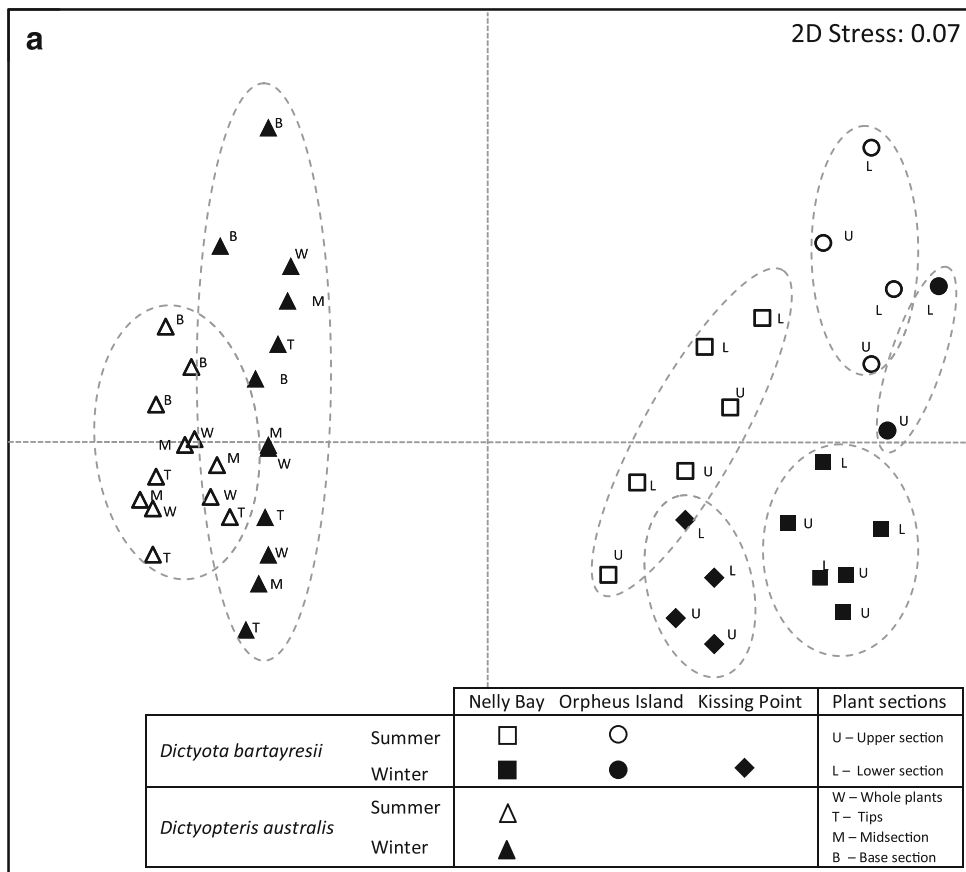
C20:5(n-3) (EPA) and C22:6(n-3) (DHA) are deficient and supplementation is recommended (Burdge 2004). EPA and DHA, in particular, are highly beneficial with anti-inflammatory properties, improved fetal development, and a positive impact on the cognition in infants and children (Dunstan et al. 2007; Krauss-Etschmann 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 study, *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 (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 (Gosch et al. 2012; Kumari et al. 2013) 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.

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 are 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 occurs during the winter when light and water temperature are at their annual minimum (Gosch et al. 2014; Nelson et al. 2002). In our study, we 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 we also observed erratic monthly fluctuations in the TFA content of up to 40 % within a timeframe of relatively stable light intensity and water temperature (Online Resource 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 we 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* (Ateweberhan et al. 2005; Tronholm et al. 2008). In *Dictyopteris*, we 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; Thompson 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 have been demonstrated and are 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 study 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* (*Laminaria*) *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).

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, we 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* (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 our study. 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 defense 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 *S. japonica* where the upper sections not only had a higher proportion of glycolipids but also a higher proportion of PUFA(n-3) (Khotimchenko and Yakovleva 2005; Kulikova and Khotimchenko 2000).

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.

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