# Seasonal Lipid Composition in Macroalgae of the Northeastern Pacific Ocean

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The lipid classes and fatty acid profiles of macrophytic algae from the Phaeophyta (*Egregia menziesii*), Rhodophyta (*Chondracanthus canaliculatus*) and Chlorophyta (*Ulva lobata*) were determined. *Ulva lobata* contained the highest amounts of lipids (20–29 mg g$^{-1}$ dry mass), followed by *Egregia menziesii* (9–16 mg) and *Chondracanthus canaliculatus* (2–3 mg), with increased lipids in winter and spring. This was reflected in total fatty acid content (14–24, 6–13 and 1–2 mg g$^{-1}$ dry mass, respectively). Major lipid classes were polar lipids (44–94 % of total lipids) and sterols (3–8 %). Higher levels of triacylglycerols occurred in *Egregia menziesii* during spring (22 %) and in *Ulva lobata* during summer (12 %). Free fatty acids were also variable (0–26 %). Triacylglycerols were not detected in *Chondracanthus canaliculatus*. Low levels of wax esters and diacylglyceryl ethers were detected in *Ulva lobata* (0.1 %). The major fatty acid common to all species was 16:0. *Egregia menziesii* and *Chondracanthus canaliculatus* contained 14:0, 18:1(n-9), 20:4(n-6) and 20:5(n-3) as major components. *Egregia menziesii* and *Ulva lobata* also contained 18:2(n-6), 18:3(n-3) and 18:4(n-3), and *Chondracanthus canaliculatus* and *Ulva lobata* contained 18:1(n-7). Large amounts of C$_{16}$ polyunsaturated fatty acids and the presence of 22:0 and C$_{22}$ polyunsaturated fatty acids were unique to *U. lobata*. Knowledge gained regarding macroalgal lipid composition may prove useful in raising mariculture species.

## Introduction

Variability in chemical components and growth of algae may be interspecific, intra-annual or inter-annual. A number of temporal variations are documented for macroalgae, including seasonal differences in the antimicrobial activity of phaeophytes (Hornsey and Hide 1974, Rao and Parekh 1981, Indiapadmakumar and Ayyakkannu 1997) and in growth rates of *Macrocystis pyrifera* (L.) C. Agardh (Hernández-Carmona *et al.* 2000). Also, temperature was shown to affect the growth rate of the rhodophyte *Palmaria palmata* (L.) O. Kuntze (Mishra *et al.* 1993). Studies on *Laminaria japonica* Areschoug (Honya *et al.* 1994), *Macrocystis pyrifera* (Rodríguez-Montesinos and Hernández-Carmona 1991, McKee *et al.* 1992, Castro-Gonzalez *et al.* 1994) and *Nereocystis luetkeana* (Mertens) Postels *et* Ruprecht (Rosell and Srivastava 1985) revealed seasonal variation in the chemical content. There is also evidence for temporal variability in algal lipid composition. Lipid levels in some algae increase in winter and decrease in summer (Rodríguez-Montesinos and Hernández-Carmona 1991, Mercer *et al.* 1993). In the three main macroalgal classes, the fatty acid profile (Johns *et al.* 1979) and sterol composition (Ackman 1981) varied seasonally.

Very few studies to date have simultaneously examined seasonal changes in lipid classes and fatty acids (FA) in algae (Mishra *et al.* 1993), and none have compared all seasons. This study examines lipid classes and fatty acid profiles of *Egregia menziesii* (Turner) Areschoug, *Chondracanthus canaliculatus* (Harvey) Guiry (formerly *Gigartina canaliculata* Harvey) and *Ulva lobata* (Kützing) Setchell *et* Gardner. Algae analyzed here were chosen based on their potential for use as a food source for the green abalone, *Haliotis fulgens* Philippi, an important mariculture species. *Egregia menziesii* was reported as highly preferred by *Haliotis fulgens* (Leighton 1966, Leighton and Peterson 1998, Nelson 1999), *Chondracanthus canaliculatus* is readily consumed by abalone (Leighton 1960), and the genus *Ulva* is a potential food source for wild abalone (Leighton 1971). Examination of interspecific and intra-annual differences in lipid profiles of these three macroalgae may also be useful for clarifying physiological requirements of grazers, especially with respect to mariculture species.

## Materials and Methods

### Seasonal samples

Algae of three classes, the phaeophyte *Egregia menziesii*, the rhodophyte *Chondracanthus canaliculatus* and the chlorophyte *Ulva lobata*, were collected during each season in southern California, during 1997–98 (December, March, July and October). The brown alga (freshly detached; blades excluding pneumoato-

cysts) was collected from the foot of Archer St., Tourmaline Beach, and the green alga from Pérez Cove and Dana Landing Marinas located off Ingraham St. in Mission Bay, San Diego, California. The red alga was collected from the west end of Shore Line Park, Santa Barbara, California, and shipped to San Diego overnight on ice. Specimens were carefully selected for high quality (live, healthy plants); freshness, full color, with neither obvious deterioration nor epiphytes. They were kept on ice or under refrigeration (–20 °C) for *ca* 1 h until prepared. After rinsing and agitation under flowing seawater for *ca* 10 min, the algae were dried on paper towels to remove surface moisture and then weighed. Duplicate samples (comprising material from several plants) were prepared for each alga per season for lipid analyses. Samples were frozen immediately at –70 °C and lyophilized. Lyophilization allowed determination of dry mass without sacrificing material. Samples were then transported frozen (dry ice) by air to CSIRO Marine Research, in Hobart, Tasmania, where they were maintained at –70 °C prior to analysis.

### Lipid extraction

Algae were homogenized with a mortar and pestle and rehydrated with millipore-filtered $H_2O$ for 1 h. Rehydration results in superior lipid extraction in comparison to non-rehydrated lyophilized samples (Dunstan *et al.* 1993). Samples were quantitatively extracted overnight using a modified Bligh and Dyer (1959) one-phase MeOH-CHCl$_3$-H$_2$O extraction (2:1:0.8). The phases were separated by the addition of CHCl$_3$-H$_2$O (final solvent ratio, 1:1:0.9 MeOH-CHCl$_3$-H$_2$O). The total solvent extract (TSE) was concentrated using rotary evaporation at 30 °C. All samples were made up to a known volume with CHCl$_3$ and stored at –20 °C.

### Lipid classes

An aliquot of the TSE was analyzed using a thin layer chromatograph-flame ionization detector (TLC-FID) analyzer to quantify individual lipid classes (Volkman and Nichols 1991). Samples were applied in duplicate to silica gel chromarods (5 μm particle size) using disposable micropipettes. The primary solvent system used for the lipid separation was hexane-Et$_2$O-HOAc (60:17:0.1), a mobile phase resolving non-polar compounds such as wax esters (WE), triacylglycerols (TAG), free fatty acids (FFA) and sterols (ST). A second non-polar solvent system of hexane-Et$_2$O (96:4) was also used to resolve hydrocarbons, WE, TAG and diacylglyceryl ethers (DAGE). The FID was calibrated for each compound class [phosphatidylcholine, cholesterol, cholesteryl oleate, oleic acid, squalene, TAG (derived from fish oil), WE (derived from orange roughy oil) and diacylglyceryl ether (DAGE) (derived from shark liver oil); 0.1–10 μg

range]. The TLC-FID results are generally reproducible to ± 5–10 % (Volkman and Nichols 1991).

### Fatty acids

An aliquot of the TSE was saponified in KOH-MeOH (5 % w:v) under N$_2$ for 3 h at 80 °C. Non-saponifiable neutral lipids were then extracted into hexane-CHCl$_3$ (4:1, 3 × 1.5 mL). Following acidification of the aqueous layer using HCl (pH=2), FA were extracted and methylated to produce their corresponding fatty acid methyl (Me) esters (FAME) using MeOH-CHCl$_3$-conc. HCl (10:1:1, 80 °C, 2 h). Products were then extracted into hexane-CHCl$_3$ (4:1, 3 × 1.5 mL) and stored at –20 °C. The non-saponifiable neutral lipid fractions were treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA 50 μL, 60 °C, overnight) to convert ST to their corresponding trimethylsilyl (TMSi) ethers.

Gas chromatograph analyses were performed with a cross-linked Me silicone fused silica capillary column (50 m × 0.32 mm) with H$_2$ as the carrier gas, an FID, a split/splitless injector, and an auto sampler. Following addition of a Me tricosanoate internal standard, samples were injected in splitless mode at an oven temperature of 50 °C. After 1 min, the oven temperature was raised to 150 °C at 30 °C min$^{-1}$, then to 250 °C at 2 °C min$^{-1}$ and finally to 300 °C at 5 °C min$^{-1}$. Individual components were identified using gas chromatograph-mass spectrometer data and by comparing R$_t$ data with those obtained for authentic and laboratory standards. Gas chromatograph results are subject to an error of ± 5 %.

### Determination of double bond configuration in fatty acids

Dimethyl disulphide (DMDS) adducts of monounsaturated FA were formed by treating the total FA fractions with DMDS (Dunkelblum *et al.* 1985, Nichols *et al.* 1986). Adducts were then extracted using hexane-CHCl$_3$ (4:1) and treated with BSTFA to form TMSi derivatives prior to gas chromatograph-mass spectrometry (GC-MS) analysis. Analyses were performed utilizing an on-column injector. The GC was fitted with a capillary column similar to that described above.

## Results and Discussion

### Lipid classes

In all algal samples, polar lipids (PL) were the dominant lipid class (44–94 % of total lipids, Table I), an indication that most lipids are structurally bound in membranes. ST ranged from 3 to 8 % and exhibited a decreasing trend toward autumn in *Ulva lobata*. The content of FFA was generally low (≤ 5 %) as expected with expedient sampling and processing of fresh algae,

Table I. Percentage lipid class composition of macroalgae.

| Algae Season | Wax esters | DAGE | TAG | Free fatty acids | Sterols | Polar lipids | Lipid: mg g⁻¹ dry mass |
|---|---|---|---|---|---|---|---|
| *Egregia menziesii* | | | | | | | |
| Winter | – | – | $0.9 \pm 0.0$ | $18.2 \pm 9.9$ | $7.4 \pm 1.4$ | $73.6 \pm 8.4$ | $15.5 \pm 2.6$ |
| Spring | – | – | $22.0 \pm 0.5$ | $26.3 \pm 0.6$ | $7.4 \pm 1.1$ | $44.3 \pm 1.0$ | $16.3 \pm 0.9$ |
| Summer | – | – | $8.6 \pm 2.1$ | $0.7 \pm 0.1$ | $6.7 \pm 1.7$ | $83.9 \pm 3.9$ | $8.8 \pm 0.2$ |
| Autumn | – | – | $2.2 \pm 0.7$ | $3.5 \pm 0.8$ | $6.3 \pm 1.1$ | $87.9 \pm 2.6$ | $11.5 \pm 0.7$ |
| *Chondracanthus caniculatus* | | | | | | | |
| Winter | – | – | – | $4.8 \pm 1.2$ | $5.1 \pm 0.1$ | $90.1 \pm 1.1$ | $3.1 \pm 0.1$ |
| Spring | – | – | – | $5.1 \pm 0.6$ | $4.7 \pm 0.9$ | $90.2 \pm 1.5$ | $2.8 \pm 0.3$ |
| Summer | – | – | – | $2.9 \pm 0.3$ | $7.2 \pm 1.2$ | $89.9 \pm 1.4$ | $1.7 \pm 0.3$ |
| Autumn | – | – | – | $2.5 \pm 1.1$ | $3.8 \pm 1.2$ | $93.7 \pm 2.3$ | $2.6 \pm 0.4$ |
| *Ulva lobata* | | | | | | | |
| Winter | $0.1 \pm 0.0$ | $0.1 \pm 0.0$ | $4.4 \pm 1.3$ | $0.1 \pm 0.0$ | $6.8 \pm 0.6$ | $88.5 \pm 0.7$ | $25.1 \pm 0.1$ |
| Spring | – | – | $3.2 \pm 0.2$ | $0.2 \pm 0.0$ | $7.8 \pm 0.4$ | $88.8 \pm 0.3$ | $29.1 \pm 2.4$ |
| Summer | $0.1 \pm 0.1$ | – | $11.9 \pm 1.0$ | – | $3.8 \pm 0.5$ | $84.2 \pm 1.5$ | $20.1 \pm 0.9$ |
| Autumn | $0.2 \pm 0.0$ | – | $3.7 \pm 0.9$ | – | $2.7 \pm 0.5$ | $93.3 \pm 0.4$ | $21.4 \pm 0.5$ |

Presented as mean ± sd, n = 2; (–), not detected; DAGE, diacylglyceryl ethers; TAG, triacylglycerols.

except in *Egregia menziesii* in winter (18 %) and spring (26 %). The elevated FFA concentration in *E. menziesii* in spring and concurrent increase in TAG from 1 to 22 % correlate with the fact that spring samples contained young recruiting plants. The cause for the high FFA is unknown. *Ulva lobata* exhibited a similar increase in TAG (from 3 to 12 %) in the summer. No TAG were detected in *Chondracanthus canaliculatus*, which also had low lipid content (1.7–3.1 dry mass), only 10–20 % of other algae. *Ulva lobata* contained the most lipids (20.1–29.1 mg g⁻¹); *Egregia menziesii* contained 8.8–16.3 mg g⁻¹. Lipid mass was elevated in winter and spring in all algal species. Low levels of wax esters (WE) were only detected in *Ulva lobata* (0.1–0.2 %). Surprisingly, 0.1 % of diacylglyceryl ethers (DAGE) was detected in *Ulva lobata* from winter. This species is commonly a host to epiphytic organisms, although we attempted to eliminate them (selection of epiphyte-free algae and rinsing with seawater). If epiphytes were the source of DAGE, one would expect to see high values in spring and summer due to increased epiphyte activity and growth. The presence of WE and DAGE in these macroalgae and the abalone that consume them (*Haliotis fulgens*; Nelson *et al.* 1999) suggests a dietary source for DAGE in the abalone.

## Fatty acids

To our knowledge there are no studies which have previously published FA and ST spectra on the algal species analyzed here. Results for algal FA composition of *Egregia menziesii* (Table II) agree with antecedent studies on other related species. Major components found in brown algae included 14:0, 16:0,

18:1(n-9), 18:2(n-6), 18:3(n-3), 18:4(n-3), 20:4(n-6) and 20:5(n-3) (Jamieson and Reid 1972, Ackman and McLachlan 1977, Johns *et al.* 1979, Ackman 1981, Rosell and Srivastava 1987, Stefanov *et al.* 1988, Dembitsky *et al.* 1990b, Banaimoon 1992, Fleurence *et al.* 1994, Honya *et al.* 1994, Virtue and Nichols 1994, Khotimchenko 1995, Mai *et al.* 1996, Vaskovsky *et al.* 1996). Major components found in *Chondracanthus canaliculatus* included 14:0, 16:0, 18:1(n-9), 18:1(n-7), 20:4(n-6) and 20:5(n-3) (Table II), which correlates with the FA composition for many other red algae (Jamieson and Reid 1972, Ackman and McLachlan 1977, Johns *et al.* 1979, Ackman 1981, Stefanov *et al.* 1988, Miralles *et al.* 1990, Banaimoon 1992, Mishra *et al.* 1993, Fleurence *et al.* 1994, Vaskovsky *et al.* 1996). In *Ulva lobata* the major FA were 16:0, $C_{16}$ polyunsaturated fatty acids (PUFA), 18:1(n-7), 18:2(n-6), 18:3(n-3) and 18:4(n-3) (Table II), as seen in previous research on green algae (Jamieson and Reid 1972, Ackman and McLachlan 1977, Johns *et al.* 1979, Ackman 1981, Stefanov *et al.* 1988, Dembitsky *et al.* 1990a, Aknin *et al.* 1992, Banaimoon 1992, Khotimchenko 1993, Fleurence *et al.* 1994, Dunstan *et al.* 1996, Mai *et al.* 1996).

Palmitic acid (16:0) was a major FA common to all algal samples (21–42 % of total FA). *Ulva lobata* contained the highest amounts of the *trans*-monoene 16:1(n-13)t (2.4–3.9 %), which is derived from chloroplasts and commonly present in higher plants (Nichols *et al.* 1982). *Ulva lobata* also contained considerable amounts of $C_{16}$ PUFA (15–20 %). The $C_{16}$ PUFA were mainly 16:4(n-3) and 16:3(n-3), which are frequently elevated in chlorophytes (Stefanov *et al.* 1988, Dembitsky *et al.* 1990a, Aknin *et al.* 1992, Khotimchenko 1993), and were suggested to have chemotaxo-

Table II. Mean percentage fatty acid composition of macroalgae.

| Fatty acid | *Egregia menziesii* | | | | *Chondracanthus canaliculatus* | | | | *Ulva lobata* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Winter | Spring | Summer | Autumn | Winter | Spring | Summer | Autumn | Winter | Spring | Summer | Autumn |
| 14:0 | 6.0 ± 0.2 | 7.1 ± 0.0 | 6.0 ± 0.0 | 6.3 ± 0.4 | 4.9 ± 0.3 | 4.9 ± 0.3 | 5.4 ± 0.7 | 6.1 ± 0.7 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.5 ± 0.1 |
| 15:0 | 0.4 ± 0.0 | 0.2 ± 0.0 | 0.1 ± 0.0 | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.5 ± 0.1 | 0.5 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 |
| 16:0 | 21.6 ± 1.2 | 25.8 ± 0.3 | 25.3 ± 0.0 | 23.1 ± 1.3 | 39.5 ± 3.3 | 39.1 ± 1.1 | 41.7 ± 1.5 | 38.5 ± 0.5 | 22.0 ± 0.4 | 20.7 ± 0.1 | 28.0 ± 0.8 | 25.4 ± 1.3 |
| 18:0 | 0.9 ± 0.1 | 1.6 ± 0.0 | 1.3 ± 0.0 | 1.0 ± 0.2 | 1.5 ± 0.2 | 1.3 ± 0.1 | 1.6 ± 0.1 | 1.6 ± 0.3 | 0.2 ± 0.0 | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.3 ± 0.1 |
| 20:0 | 0.7 ± 0.0 | 0.9 ± 0.0 | 1.2 ± 0.0 | 0.8 ± 0.0 | – | – | – | – | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.2 ± 0.0 | 0.3 ± 0.2 |
| 22:0 | – | – | – | – | – | – | – | – | 0.6 ± 0.0 | 0.6 ± 0.0 | 0.6 ± 0.0 | 0.7 ± 0.1 |
| Sum SFA | 29.6 ± 1.5 | 35.6 ± 0.4 | 34.1 ± 0.1 | 31.4 ± 2.0 | 46.1 ± 3.9 | 45.6 ± 1.5 | 48.8 ± 2.3 | 46.4 ± 1.6 | 23.8 ± 0.5 | 22.6 ± 0.2 | 29.7 ± 0.9 | 27.6 ± 1.7 |
| 14:1(n-7)c | 0.2 ± 0.1 | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 | 1.4 ± 0.0 | 1.7 ± 0.3 | 2.1 ± 0.2 | 0.6 ± 0.4 | tr | tr | tr | 0.1 ± 0.1 |
| 16:1(n-9)c | 0.7 ± 0.1 | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.3 ± 0.1 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.8 ± 0.0 | 0.9 ± 0.1 | 0.6 ± 0.1 | 0.5 ± 0.2 |
| 16:1(n-7)c | 1.2 ± 0.0 | 1.2 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.1 | 2.2 ± 0.1 | 2.8 ± 0.3 | 1.2 ± 0.2 | 2.6 ± 0.0 | 0.8 ± 0.0 | 0.7 ± 0.0 | 1.4 ± 0.1 | 1.3 ± 0.1 |
| 16:1(n-5)c | 0.4 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.2 ± 0.0 | tr | 0.1 ± 0.1 | tr | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 |
| 16:1(n-13)t | 0.9 ± 0.1 | 1.1 ± 0.0 | 1.0 ± 0.0 | 0.8 ± 0.0 | 0.5 ± 0.0 | 0.6 ± 0.0 | 0.4 ± 0.1 | 0.4 ± 0.2 | 3.1 ± 0.1 | 3.9 ± 0.1 | 2.4 ± 0.1 | 3.0 ± 0.0 |
| 18:1(n-9)c | 9.9 ± 0.3 | 14.5 ± 0.2 | 14.1 ± 0.2 | 13.7 ± 0.2 | 10.6 ± 0.7 | 10.9 ± 0.2 | 11.7 ± 0.2 | 10.7 ± 0.1 | 1.0 ± 0.0 | 0.8 ± 0.0 | 1.2 ± 0.0 | 0.9 ± 0.1 |
| 18:1(n-7)c | tr | tr | tr | tr | 3.0 ± 0.0 | 2.1 ± 0.1 | 1.9 ± 0.3 | 4.0 ± 0.2 | 8.2 ± 0.0 | 7.0 ± 0.0 | 9.2 ± 0.1 | 9.0 ± 0.1 |
| Sum MUFA | 13.3 ± 0.6 | 17.5 ± 0.3 | 16.7 ± 0.2 | 16.3 ± 0.4 | 18.1 ± 1.0 | 18.7 ± 1.0 | 17.9 ± 1.1 | 19.1 ± 1.1 | 14.0 ± 0.2 | 13.5 ± 0.3 | 14.9 ± 0.5 | 15.0 ± 0.7 |
| C16 PUFA | 2.8 ± 0.3 | 1.2 ± 0.0 | 1.0 ± 0.0 | 1.6 ± 0.2 | 1.4 ± 0.1 | 1.4 ± 0.0 | 1.4 ± 0.0 | 1.8 ± 0.0 | 19.7 ± 0.0 | 20.2 ± 0.2 | 15.4 ± 0.4 | 17.0 ± 0.7 |
| 18:3(n-6) | 0.1 ± 0.2 | 0.3 ± 0.0 | 0.3 ± 0.0 | 0.2 ± 0.2 | 0.4 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.0 | 0.6 ± 0.1 | tr | tr | 1.0 ± 0.1 | tr |
| 18:4(n-3) | 10.9 ± 0.6 | 8.1 ± 0.2 | 8.4 ± 0.0 | 10.4 ± 0.7 | 0.5 ± 0.0 | 0.3 ± 0.2 | 0.2 ± 0.0 | 0.5 ± 0.0 | 11.8 ± 0.1 | 13.9 ± 0.1 | 7.0 ± 0.1 | 11.6 ± 0.4 |
| 18:2(n-6) | 9.4 ± 0.1 | 8.7 ± 0.2 | 8.1 ± 0.3 | 7.7 ± 0.3 | 2.0 ± 0.3 | 1.3 ± 0.1 | 1.3 ± 0.1 | 1.9 ± 0.1 | 4.1 ± 5.9 | – | 10.5 ± 0.5 | – |
| 18:3(n-3) | 8.4 ± 0.8 | 7.3 ± 0.5 | 6.6 ± 0.3 | 8.2 ± 0.0 | 0.6 ± 0.2 | 0.8 ± 0.1 | 0.3 ± 0.1 | 1.0 ± 0.1 | 21.4 ± 6.0 | 24.7 ± 0.1 | 16.9 ± 0.8 | 23.8 ± 0.6 |
| 20:4(n-6) | 18.6 ± 0.2 | 15.2 ± 0.1 | 19.2 ± 0.2 | 20.2 ± 0.9 | 9.9 ± 7.2 | 9.3 ± 0.7 | 10.5 ± 0.1 | 14.2 ± 0.6 | 0.8 ± 0.0 | 0.8 ± 0.0 | 1.3 ± 0.0 | 1.0 ± 0.0 |
| 20:5(n-3) | 5.6 ± 0.6 | 5.1 ± 0.3 | 4.2 ± 0.2 | 3.4 ± 0.2 | 20.6 ± 1.7 | 22.0 ± 0.9 | 19.0 ± 3.0 | 13.9 ± 1.8 | 0.7 ± 0.0 | 0.8 ± 0.0 | 0.6 ± 0.0 | 1.0 ± 0.1 |
| 20:3(n-6) | 0.4 ± 0.0 | 0.5 ± 0.0 | 0.5 ± 0.0 | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.1 ± 0.1 | tr | 0.2 ± 0.0 | 0.3 ± 0.0 | 0.4 ± 0.0 | 0.3 ± 0.0 | 0.3 ± 0.0 |
| 20:4(n-3) | 0.9 ± 0.1 | 0.6 ± 0.0 | 1.0 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.3 ± 0.1 | 0.3 ± 0.0 | 0.5 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.2 ± 0.0 | 0.3 ± 0.0 |
| 22:6(n-3) | – | – | – | – | – | – | – | – | 0.1 ± 0.0 | 0.2 ± 0.0 | 0.1 ± 0.0 | 0.2 ± 0.0 |
| 22:4(n-6) | – | – | – | – | – | – | – | – | 0.7 ± 0.0 | 0.6 ± 0.0 | 0.7 ± 0.0 | 0.5 ± 0.0 |
| 22:5(n-3) | – | – | – | – | – | – | – | – | 2.2 ± 0.0 | 1.9 ± 0.1 | 1.2 ± 0.1 | 1.9 ± 0.1 |
| Sum PUFA | 57.1 ± 2.8 | 46.9 ± 1.4 | 49.3 ± 1.0 | 52.3 ± 2.6 | 35.9 ± 9.8 | 35.8 ± 2.2 | 33.2 ± 3.4 | 34.5 ± 2.8 | 62.3 ± 12.0 | 63.9 ± 0.6 | 55.4 ± 2.0 | 57.4 ± 1.9 |
| Total mg g⁻¹ | 11.2 ± 4.6 | 13.3 ± 0.6 | 6.3 ± 1.2 | 8.8 ± 2.2 | 1.9 ± 0.1 | 1.8 ± 0.1 | 0.9 ± 0.2 | 1.4 ± 0.4 | 21.4 ± 0.8 | 23.8 ± 1.7 | 13.8 ± 1.0 | 13.9 ± 0.3 |
| Ratio AA/ EPA | 3.35 | 2.99 | 4.55 | 6.01 | 0.48 | 0.42 | 0.55 | 1.02 | 1.10 | 1.00 | 2.09 | 1.03 |

Presented as mean ± sd, n = 2; (-), not detected; tr, trace (below integration or coeluted); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; 18:2(n-6) in *U. lobata* (spring and autumn) included with 18:3(n-3).

nomic value (Khotimchenko 1993). *Egregia menziesii* and *Chondracanthus canaliculatus* had elevated relative levels of oleic acid [18:1(n-9)c, 10–15 % and 10–11 %, respectively] and myristic acid (14:0, 6–7 % and 5–6 %), while *Ulva lobata* had very low amounts of these FA (0.8–1.2 %, 0.4–0.5 %). The ratios of oleic acid to *cis*-vaccenic acid [18:1(n-7)] were high in *Egregia menziesii* [18.1(n-7) present in trace amounts only] and *Chondracanthus canaliculatus* (2.7–5.5), but low in *Ulva lobata* (0.1). The predominance of 18:1(n-7) over 18:1(n-9)c has been established as a biomarker for chlorophytes (Ackman and McLackan 1977, Johns *et al.* 1979, Ackman 1981, Stefanov *et al.* 1988, Aknin *et al.* 1992, Khotimchenko 1993, Vaskovsky *et al.* 1996).

*Egregia menziesii* and *Ulva lobata* both had significant amounts of $C_{18}$ PUFA (23–29 % and 34–39 %, respectively), whilst *Chondracanthus canaliculatus* had low amounts (2–4 %). *Egregia menziesii* had higher ratios of 18:4(n-3) to linolenic acid [18:3(n-3)] (1.1–1.3) compared to *Ulva lobata*, which conversely had low ratios (0.4–0.6). Our results are in agreement with previous studies of phaeophyte (Vaskovsky *et al.* 1996) and chlorophyte (Aknin *et al.* 1992, Khotimchenko 1993) species. These PUFA may be important for algae in adaptation to changes of environmental factors (Khotimchenko 1993), however, they may not be essential FA for the abalone *Haliotis fulgens*, unlike the essential $C_{20}$ PUFA (Nelson 1999, Nelson *et al.* 2001). Although present at low levels, 22:0 (0.6 %) and $C_{22}$ PUFA (0.1–2.2 %) were detected only in *Ulva lobata*. Docosapentaenoic [DPA, 22:5(n-3)] and docosahexaenoic [DHA, 22:6(n-3)] acids are common to marine animals, however their significance in *U. lobata* is unknown.

Total FA content (mg g$^{-1}$ dry mass) was highest in *U. lobata* and ranged from 13.8 mg g$^{-1}$ in summer to 23.8 mg g$^{-1}$ in spring (Table II). Likewise, *Egregia menziesii* had highest total FA in spring (13.3 mg g$^{-1}$) and lowest in summer (6.3 mg g$^{-1}$). *Chondracanthus canaliculatus* was consistently low in FA year round (0.9–1.9 mg g$^{-1}$).

*Egregia menziesii* showed a decrease in unsaturated FA from winter (70 %) to spring (64 %; Table II). *Ulva lobata* also had a decrease in unsaturated FA from spring (78 %) to summer (70 %), with a relative increase in 16:0. A marked seasonal change in individual FA occurred in *Chondracanthus canaliculatus*, which had an increase in arachidonic acid [AA, 20:4(n-6)] from 9 to 14 % and concurrent decrease in eicosapentaenoic acid [EPA, 20:5(n-3)] from 22 to 14 % between spring and autumn (Table II). Temperature has a major effect on the FA composition of cell membranes. Low temperature results in elevated levels of unsaturated FA in polar lipids (PL), with the increase in unsaturation lowering melting points and maintaining lipids in a liquid state for normal protoplasmic viscosity (Phleger 1991). This change is best exemplified in the PUFA composition of marine fish, which has been observed to vary between areas of different temperatures, with higher levels of AA in warmer waters

(Sinclair *et al.* 1986, Nichols *et al.* 1998). In macroalgae, the effect on saturation levels in FA was demonstrated by controlled temperature schemes with the rhodophyte, *Palmaria palmata*; highest levels of EPA were observed at 11 °C and AA at 15 °C (Mishra *et al.* 1993). Also, lipid analysis revealed that in the phaeophyte, *Laminaria japonica*, (n-6) PUFA content was maximal during warm months, while (n-3) PUFA content was at a maximum during cold months (Honya *et al.* 1994). Therefore, although temperature changes have a direct impact on growth via the $Q_{10}$ effect (metabolic change due to temperature), it may also dictate essential molecular requirements, such as PUFA.

*Egregia menziesii* had a significant amount of $C_{20}$ PUFA, where proportions of AA to EPA ranged from 3 to 6 (3.4, 3.0, 4.6 and 6.0 for winter, spring, summer and autumn, respectively; Table II) at combined levels ranging from 20–24 % of total FA (1.6–2.7 mg g$^{-1}$). In contrast to *E. menziesii*, *Chondracanthus canaliculatus* had higher proportions of EPA, with AA/EPA ratios reaching 1.0 (0.5, 0.4, 0.6 and 1.0 for winter, spring, summer and autumn, respectively), and slightly higher combined levels (28–31 %, 0.3–0.6 mg g$^{-1}$) of these $C_{20}$ PUFA. Low AA/EPA ratios have been found in other rhodophytes (Fleurence *et al.* 1994, Vaskovsky *et al.* 1996). Interestingly, lipid analyses revealed *Gracilaria asiatica* Zhang et Xia and *G. textori* (Sur.) DeToni to be atypical rhodophytes, comprised of extremely high levels of AA (50–54 % total FA; Vaskovsky *et al.* 1996). Prostaglandins, of which AA is a direct precursor, were found to occur in *Gracilaria lichenoides* (L.) Harv. (Gregson *et al.* 1979). Prostaglandins have been detected in plants and over 100 invertebrate species, and some reports suggest that they function in the fundamental physiology in representatives of many invertebrate phyla (Stanley-Samuelson 1987, Gerwick 1994). Members of the genus *Gracilaria* have been used as a major part of the diet of aquarium-raised abalone, *Haliotis diversicolor* Reeve, in Taiwan, producing individuals more fecund than wild abalone. *Gracilaria* also provided superior growth in *Haliotis iris* Martyn in New Zealand (Stuart and Brown 1994, Marsden and Williams 1996). The low lipid content of *Chondracanthus canaliculatus* may be insufficient for consumers, or there may be physiological perturbation by the high ratio of EPA (Bell *et al.* 1994). It should be noted that the amount of EPA was slightly less in a preliminary analysis of *Egregia menziesii* collected in 1997 (2.8 %; Nelson 1999), a year when gametogenesis in *Haliotis fulgens* was successful, compared to feeding trial *Egregia menziesii* from the following year (3.4–5.6 % EPA; Table II). Combined levels of AA and EPA in *Ulva lobata* were low (2 %, 0.1–0.3 mg g$^{-1}$) with ratios of 1 (except summer, 2). Compared to phaeophytes and rhodophytes, low levels of $C_{20}$ PUFA characterize chlorophytes, including many Ulvales (Aknin *et al.* 1992, Banaimoon 1992, Khotimchenko 1993, Fleurence *et al.* 1994).

## Environmental and food chain implications

This study addressed seasonality in macroalgal lipids, which is related to herbivore physiology and lipid composition. Because PUFA may be most responsive to environmental changes, they have important roles in algal physiology. The tendency to vary may also impact invertebrate herbivores that feed upon them. An example of the influence of temperature was observed during 1997–98, when this study was conducted. There was a 3 °C elevation in sea temperature at the Scripps Institution of Oceanography pier (Scripps Pier 1997/98 Surface Temperature and Salinity data). Elevated temperatures may have contributed to the low local abundance of *Chondracanthus canaliculatus* during 1997–98, as well as the suppressed gonadal development in *Haliotis fulgens* during 1998 (Nelson 1999). Temperature induced changes in specific FA, especially PUFA, or essential FA deficiency in macroalgae may be an important factor in gonadogenesis, consequently affecting recruitment. Both AA and EPA are additionally prostaglandin and eicosanoid precursors, so should be of special consideration, especially to herbivores such as *H. fulgens* (Nelson 1999, Nelson *et al.* 2001). Knowledge concerning climate-ocean variations in the northeastern Pacific Ocean have increased dramatically in recent years (McGowan *et al.* 1998). The results of this preliminary study are also significant in furthering the understanding of possible aquaculture trophic transfer as well as knowledge of these species in the wild. More information about the nutritional requirements of abalone is needed, especially to establish a basis for formulation of artificial diets (Fleming *et al.* 1996). Furthermore, interactions with temperature and effects of lipids in both diet and metabolism requires further research, but this study should serve to supplement our growing knowledge, particularly concerning herbivorous species which breed by broadcast spawning.

## Conclusions

Our results for macroalgae revealed highest lipid content in winter and spring, which coincided with highest unsaturated FA in *Egregia menziesii* and *Ulva lobata*. Highest levels of TAG were in spring and summer, although *Chondracanthus canaliculatus* contained low lipid and low TAG. Unlike the red alga, the brown and green algae contained high levels of $C_{18}$ PUFA. *Ulva lobata* also contained high levels of $C_{16}$ PUFA, but was low in $C_{20}$ PUFA. *Egregia menziesii* and *Chondracanthus canaliculatus* contained highest relative levels of $C_{20}$ PUFA, of which AA was lowest in spring. The ratio of AA to EPA was low in *C. canaliculatus* and high in *Egregia menziesii*. Because the brown alga contained the highest absolute amounts of $C_{20}$ PUFA, its role as a potential diet for aquacultured *Haliotis fulgens* should be considered.

## Acknowledgements

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