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Seasonal changes in the content and composition of lipids in the coral *Goniastrea aspera*

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Introduction

Reef-building corals contain large amounts of storage lipids, which range from 9 to 47% of the dry weight of their tissues (Harland et al. 1993; Yamashiro et al. 1999). These lipids are mainly derived from carbon photosynthetically fixed by symbiotic zooxanthellae (Kelogg and Patton 1983; Harland et al. 1993). Lipid levels in coral tissue appeared to vary with the energy balance between input from zooxanthellae and output as respiration, cell renewal, and release of reproductive materials (Battey and Patton 1984; Rinkevich 1989; Arai et al. 1993; Ward 1995). Lipid levels in corals may show seasonal changes because production of photosynthates varies with light intensity and water temperature (Harland et al. 1992; Al-Moghrabi et al. 1995); however, this has yet to be demonstrated. This study examined the lipid content and composition of the reef-building coral *Goniastrea aspera* throughout a year.

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Materials and methods

Corals

Colonies of the scleractinian coral *Goniastrea aspera* were collected monthly from a 5x20-m area on the reef flat in front of the field station of the University of the Ryukyus, Okinawa, Japan (26°38′N, 127°52′E). Average water depth was 1 m at tidal level. One small round colony approximately 100 mm in diameter was collected each month from December 1996 to November 1997.

Analysis of lipid contents and compositions

Samples were decalcified in a solution of 10% formaldehyde containing 10% acetic acid (Yamashiro et al. 1999) and thoroughly washed with tap water. An area about 30 mm in diameter from the central top of each colony was extracted with approximately 50 ml of chloroform-methanol (C-M, 2:1 by vol.) at room temperature. The solvent was dried under a nitrogen stream at 50 °C. The lipids extracted were weighed, dissolved in C-M to a concentration of 1 mg lipid/ml, and stored at −20 °C.

Aliquots of lipid extract (50 μg) were applied to 100x100-mm high-performance, thin-layer chromatography (HPTLC) plates (Merck, Darmstadt, Germany). The plates were first developed to their full length with hexane and then benzene, and finally to half-length with hexane/ether/acetic acid (70:30:1 by vol.) to separate moderately polar lipid fractions. After drying in a stream of air, the plates were immersed in phosphoric acid/33% acetic acid/sulfuric acid/0.5% copper sulfate (5:5:0.5:90 by vol.) for 40 s, dried, and heated at 130 °C for 12 min (Brod et al. 1991) to visualize the chromatogram. Gray-scale images of the chromatograms were obtained using an image scanner (GT-9000, Epson) running Adobe Photoshop (Adobe Systems Inc., California, USA). Relative amounts of the different lipids were estimated from the densities of their gray-scale images using an image analysis program (NIH image, National Institute of Mental Health, Bethesda, USA).

Results and discussion

The lipid content of *G. aspera* showed a seasonal fluctuation (Fig. 1). It was low in winter (December–January) and high in summer (June–September). Lipid content was correlated with light intensity ($r = 0.87$) and sea surface temperature (SST; $r = 0.90$; see also Fig. 2).

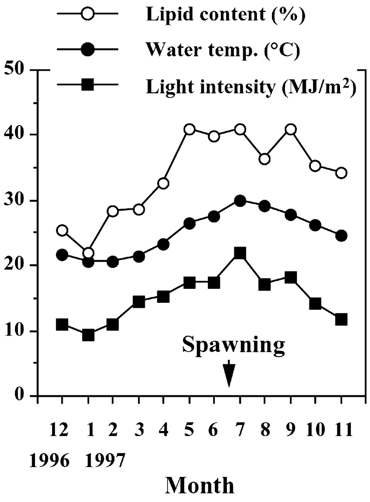


Fig. 1 Seasonal changes in total lipid content of *Goniastrea aspera* and sea surface temperature at the field station of the University of the Ryukyus from December 1996 to November 1997. Light intensity was measured at the Naha local weather forecast station 45 km south of the field station

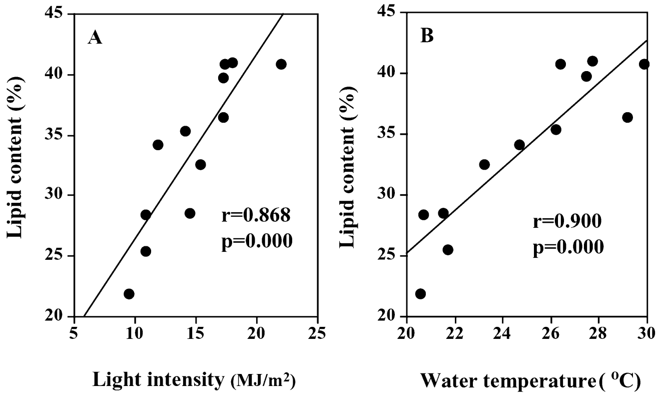


Fig. 2 Relationships between lipid content of *Goniastrea aspera* and **A** light intensity and **B** sea surface water temperature

These results suggest that the lipid content is linked to light intensity or water temperature or both.

G. aspera is a hermaphroditic spawner with one or two spawning peaks per year (Hayashibara et al. 1993). In 1993, *G. aspera* from this site spawned once (Sakai 1997) with oocytes first seen in February. Their diameter increased linearly until May and stayed the same until spawning in late June. In 1997, *G. aspera* spawned once around 24 June (K. Sakai, personal communication) and we assume that oocyte development was the same. We found lipid content to increase linearly from January to May and remain high throughout summer. Thus lipid biosynthesis may be linked with oocyte development. Oocytes, like other coral tissues, increased in lipid content during spring, and hence spawning might carry away only a fraction of the total lipid.

Lipids in the tissues of *G. aspera* are mainly derived from carbon fixed by photosynthesis of zooxanthellae (Kelogg and Patton 1983; Harland et al. 1993). Higher lipid levels in summer could be due to greater photosynthetic production of lipids at higher summer light levels. However, highest densities of zooxanthellae and pigment (chlorophyll) content occur in winter (Fitt et al. 2000), and there is a significant negative correlation between algal numbers and levels of photosynthetically active radiation (Brown et al. 1999). We did not measure algal number in our colonies. However, in the light of seasonal photoadaption seen in other corals, it seems unlikely that higher lipid levels in summer in our shallow-water corals is due to higher summer photosynthetic activity. We believe that seasonal variations in lipid levels are probably linked with changes in metabolic rate due to seasonal variations in SST, in the same way that seasonal variations in calcification in shallow-water corals is linked with changes in SST rather than changes in light intensity. (Lough and Barnes 2000).

Lipids in *G. aspera* comprised nine lipid classes, including two unknown components, UK1 and UK2 (Fig. 3). Wax ester was the largest component forming 20–30% of total lipids. Levels of non-polar lipids were higher in summer than in winter (Fig. 4A), while polar lipids showed the opposite trend (Fig. 4B).

There was a strong positive correlation between lipid composition and lipid content. The ratio of waxes to sterols showed the highest correlation against absolute lipid content determined by the gravimetric method (Fig. 5). We have reported similar correlation in tumor in coral tissues (Yamashiro et al. 2001), in which the decrease in the absolute lipid content accompanied loss of waxes, and hence led to a lower ratio of waxes to sterols. Sterols were largely stable in their composition throughout the year; thus, the ratio of waxes to sterols

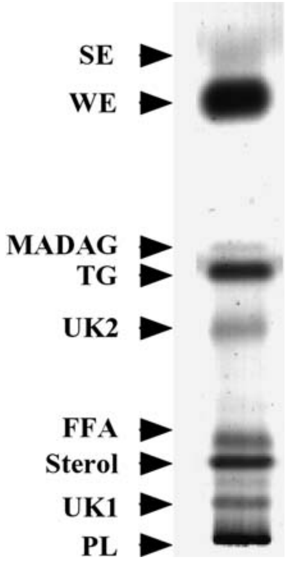
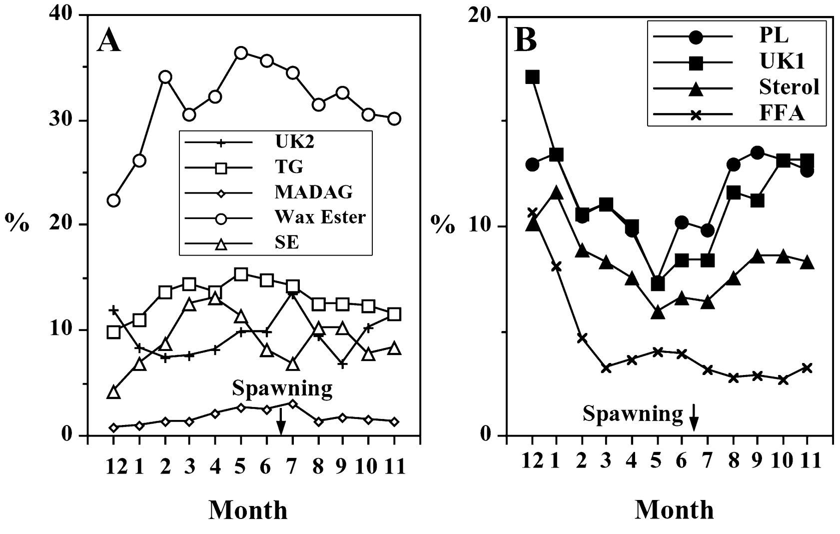


Fig. 3 HPTLC chromatogram showing lipids from a colony of *Goniastrea aspera* collected in February 1997. *UK1* Unknown 1; *FFA* free fatty acids; *UK2* unknown 2; *TG* triacylglycerol; *MADAG* monoalkyldiacylglycerol; *WE* wax esters; *SE* sterol esters. The polar lipid (*PL*) remaining at the origin is mostly phospholipids

Fig. 4 Seasonal changes in the amounts of **A** non-polar lipids and **B** polar lipids. Data are means of triplicate analyses



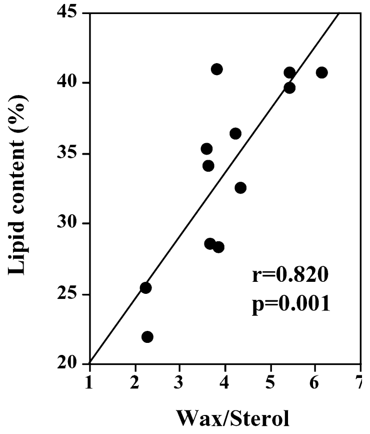


Fig. 5 Relationship between total lipids in *Goniastrea aspera* and the ratio of wax esters to sterols

could be used to estimate the net lipid content. Measurement of the ratio of waxes to sterols using our method requires only a few micrograms of total lipid. Thus, an index based upon the ratio would be especially useful for estimating the total lipid content of small specimens and samples.

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