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Occurrence of UVA- and UVB-absorbing compounds in 152 species (206 strains) of marine microalgae

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ABSTRACT: Marine microalgae (152 species, 206 strains) from 12 classes were examined for the presence of UVA- and UVB-absorbing compounds. Cultures were grown under white fluorescent light without supplementary UVA or UVB radiation and were extracted after harvest in tetrahydrofuran:methanol (20:80, v/v). Ratios of UV absorbance (280 to 390 nm) to chlorophyll *a* (chl *a*) (665 nm) obtained by spectrophotometry ranged from 0.18 to 6.75. Three groups of species were distinguished: those with low UV:chl *a* ratios (0.18 to 0.9, diatoms, green algae, cyanophytes, euglenophytes, eustigmatophytes, rhodophytes, some dinoflagellates, some prymnesiophytes), those with intermediate ratios (0.9 to 1.4, chrysophytes, some prasinophytes, some prymnesiophytes) and those with very high ratios (1.4 to 6.75, surface bloom-forming dinoflagellates, cryptomonads, prymnesiophytes and raphidophytes). UV-absorbing pigments varied across species of the same algal class and strains of the same species. HPLC analysis of extracts of 5 species (1 diatom, 2 bloom-forming raphidophytes and 2 bloom-forming dinoflagellates) showed suites of mycosporine-like amino acids in 4 of them, which included mycosporine-glycine, asterina-330, shinorine, porphyra-334 and palythine. The dinoflagellate *Gymnodinium catenatum* also contained major quantities of unknown UV-absorbing compounds.

KEY WORDS: Ultraviolet radiation · Microalgae · Dinoflagellates · Cryptomonads · Prymnesiophytes · Raphidophytes Mycosporine-like amino acids Bloom-forming species · *Gymnodinium catenatum*

INTRODUCTION

Ultraviolet-B radiation (UVB) reaching the earth's surface is increasing as a result of anthropomorphic damage to the stratospheric ozone layer. First detected over the Antarctic by Farman et al. (1985), seasonal ozone depletion has now also been documented above the Arctic and mid-latitudes (north and south) (e.g. Frederick & Snell 1988, Roy et al. 1990, Stolarski et al. 1992, Kerr & McElroy 1993). Of immediate concern to this expanding global problem are the potential threats to biological ecosystems (Calkins 1982). UVB (280 to 320 nm) has a high energy level per photon and is effectively absorbed by nucleic acids (affecting the genome), proteins and pigments. Not only are terrestrial ecosystems at risk (Caldwell 1981, Bornman 1989, Bornman & Teramura 1993, Rozema et al. 1997), with evidence that UVB affects plant morphology, biomass production and photosynthesis, but marine ecosystems may also be threatened (Worrest 1982, El-Sayed & Stephens 1992).

Even before the recent ozone concerns were apparent, it was known from direct measurements (Jerlov 1950, 1976) and from inhibition studies of *in situ* phytoplankton productivity (Steeman Nielsen 1964, Jitts et al. 1976, Lorenzen 1979, Smith et al. 1980, Worrest et al. 1980) that, in aquatic systems, UVB penetrates to ecologically significant depths (up to 20 m). More recent *in situ* measurements by Smith et al. (1992) have shown that UV penetrates to 70 m depth in oligotrophic waters, and that direct inhibition of phyto-

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plankton productivity in Antarctic waters (0 to 12%) is correlated with ozone depletion (Worrest & Häder 1997).

Recent research has highlighted a variety of UVB damage effects in phytoplankton, expanding on early concepts (Halldal 1967). These include damage to nuclear DNA (dimer formation, Karentz et al. 1991a, Buma et al. 1995, 1996) with consequent inhibition of cell growth rates, inhibition of photosynthesis with lesions in the Photosystem II-D1 reaction centre protein (Jordan et al. 1991), loss of, and changes to, the key photosynthetic enzyme, ribulose bis-phosphate carboxylase (Rubisco) (Strid et al. 1990, Lesser et al. 1994, 1996, Wilson et al. 1995, Lesser 1996), decreased nitrogen uptake, metabolism and protein synthesis (Döhler et al. 1987, 1991, Goes et al. 1995, Döhler 1996), and loss of photo-orientation and motility in dinoflagellates, diatoms and other species (e.g. Häder & Häder 1988, Ekelund 1990, Donkor et al. 1993, Nielsen & Ekelund 1993, Sundbäck et al. 1996, 1997). The severity of these effects depends on the length and intensity of UVB exposure, the nutrient status of the cells, individual species responses, and supplementary radiation involved in repair processes (e.g. photosynthetically active radiation (PAR), UVA or red/blue radiation).

To counteract harmful UV effects, terrestrial and aquatic plants have evolved UV-protective, anti-oxidant, avoidance and repair mechanisms to aid survival (see Dunlap & Shick 1998). These include rapid DNA repair mechanisms (Sancar & Sancar 1988, Buma et al. 1995), avoidance reactions (e.g. photo-orientation in diatoms and dinoflagellates, see above), protective sheaths in cyanobacteria and protective cell walls in freshwater green microscopic algae (Garcia-Pichel & Castenholz 1991, Xiong et al. 1997), and synthesis of cytoplasmic protective pigments with a UV-absorbing sunscreen function. These last compounds include flavonoids in higher plants (Caldwell 1981) and mycosporine-like amino acids (MAAs) in marine biota (Nakamura et al. 1982, reviewed by Dunlap & Shick 1998).

UV-absorbing MAAs have been found in marine plants and animals from Antarctic regions to the tropics (Tsujino et al. 1980, Nakamura et al. 1981, 1982, Dunlap & Chalker 1986, Dunlap et al. 1986, 1989, Karentz et al. 1991b, Shick et al. 1991, 1992, 1995, Stochaj et al. 1994, Dionisio-Sese et al. 1997, Dunlap & Shick 1998). MAA-like compounds were studied in a small number of microalgae—the 4 dinoflagellates *Noctiluca miliaris* (Balch & Haxo 1984), *Alexandrium excavatum* (Carreto et al. 1990), *Prorocentrum micans* and *Gonyaulax polyedra* (Vernet et al. 1989), colonial strains of the prymnesiophyte (=haptophyte) *Phaeocystis pouchetii* (Marchant et al. 1991), Antarctic diatom mats (Karentz et al. 1991b), a number of Antarctic diatom cultures (Helbling et al. 1996, Riegger & Robinson 1997), and the tropical cyanobacterium *Trichodesmium* sp. (Shibata 1969). Some cyanobacteria are known to have the UV-absorbing extracellular sheath pigment scytonemin (Garcia-Pichel & Castenholz 1991), whereas in freshwater green algae sporopollenin in cell walls acts as a UV absorber (Xiong et al. 1997). MAAs have also been found in 20 strains of cyanobacteria (13 genera) isolated from habitats receiving high natural solar radiation (Garcia-Pichel & Castenholz 1993), 20 isolates of *Microcoleus* (cyanobacteria) (Karsten & Garcia-Pichel 1996), and in a community of halophilic cyanobacteria (Oren 1997).

UV-absorbing compounds have long been suggested to protect cells from UV damage (see Shibata 1969, Dunlap et al. 1986, Vernet et al. 1989, Carreto et al. 1990, Helbling et al. 1996), but experimental evidence was unclear until recently. Adams & Shick (1996) provided the first direct experimental evidence for the UV-photoprotective function of MAAs in the eggs of sea urchins during reproductive development. More recently Neale et al. (1998) have shown that UV sunscreens (MAAs) in the dinoflagellate *Gymnodinium sanguineum* clearly protect against UV inhibition of photosynthesis.

In the present work we investigated the occurrence of UV-absorbing compounds in 152 species (= 206 strains) of cultured marine microalgae from 12 classes, a greater variety than had previously been examined, in order to gain further understanding of MAA distribution as a possible protective mechanism against UV radiation in the phytoplankton. The strains came from our extensive bank of cultured microalgae in the CSIRO Algal Culture Collection and were isolated from a variety of tropical, temperate and polar habitats, both in Australia and overseas (Jeffrey 1980, Jeffrey & LeRoi 1997, CSIRO 1998). Particular attention was given to surface bloom-forming species that might normally live in a UV-rich environment (dinoflagellates, prymnesiophytes, cryptomonads and raphidophytes). In addition, we examined natural surface phytoplankton samples, containing mainly dinoflagellates and diatoms, that had received ambient solar radiation.

MATERIALS AND METHODS

Algal cultures. Cultures (152 species, 206 strains) from 12 algal classes were obtained from the CSIRO Algal Culture Collection (Jeffrey 1980, CSIRO 1998). Full details including dates of isolation and deposition into the Collection are given in CSIRO (1998).

The cultures were grown according to Jeffrey & LeRoi (1997), and were maintained in stationary 125 ml

pyrex Erlenmeyer flasks containing 75 ml medium. Names of species examined, CSIRO culture code numbers, culture media and growth temperatures are listed in Table 1 Growth temperatures were 25 to 27°C for tropical species, 12 to 17.5°C for temperate species and 5°C for Antarctic species. Photosynthetically active radiation was provided by banks of Philip's Daylight fluorescent tubes (TL20W47 de Luxe), beneath glass shelves supporting the culture flasks. Philip's Daylight fluorescent lamps produce a more uniform spectrum than cool white lamps and therefore simulate the natural daylight spectrum more closely (see Behrenfeld et al. 1994). Dinoflagellates received overhead illumination. No supplementary UV radiation was provided. Light irradiances (12 h light:12 h dark cycles) were generally 70 to 80 µE m^{-2} s^{-1}, or 20 µE m^{-2} s^{-1} for picoplanktonic cyanobacteria isolated from the deep euphotic zone. Light measurements were obtained in the culture flasks with a Biospherical Optics light meter before algal innoculation.

Field samples. Natural phytoplankton field samples having received natural incident radiation were collected with a 33 µm mesh plankton net from a depth of 1 m at the CSIRO Marine Laboratories wharf station (Derwent River Estuary, water depth 5 to 6 m). Dates of collection were 11 March 1991, 16 May 1991, 22 July 1992, and 15 September 1992. This station is usually rich in dinoflagellates (*Ceratium*, *Dinophysis*, *Gymnodinium* spp.) alternating with diatoms (mainly *Chaetoceros* spp., Hallegraeff et al. 1989). Each sample was examined microscopically to determine major cell types before extraction. The samples were extracted by the same techniques as the cultured samples, described below.

Culture harvest and pigment extraction for spectrophotometry. Cultured microalgae, which had been maintained in the absence of supplementary UV radiation for between 2 and 35 yr, were harvested in mid to late log phase. Before extraction each culture was checked microscopically to ensure good cell morphology. Duplicate 10 ml samples were withdrawn from each culture, usually 2 to 4 h into the light cycle, and cells were harvested by centrifugation at 2000 *g* for 3 to 5 min. The pellet was extracted with 1 ml cold (5°C) tetrahydrofuran : methanol (20:80, v/v), a solvent mixture which provided maximum extraction efficiency for both MAAs and lipophilic pigments (Chalker & Dunlap 1982, Dunlap et al. 1986). Cells were chilled in an ice bath during extraction and were sonicated for 60 s in a sonicator bath, using a Bransonic Model 52 sonicator. The solvent mixture extracted both UV-absorbing mycosporine amino acids and photosynthetic pigments from most algal cells, leaving a colourless pellet. Some of the chlorophytes with tough cellulose walls were extremely difficult to extract. In these cases cell pellets were frozen in a small volume of distilled water before solvent extraction, to weaken the cell walls for enhanced extraction efficiency. After centrifugation in a Sorvall refrigerated centrifuge, the absorption spectrum of the supernatant was taken between 190 and 700 nm with a Shimadzu UV-240 recording spectrophotometer, the baseline of which had been previously set to zero. No drift was encountered. The relative proportion of UV absorbance to chl *a* was calculated as the ratio of absorbance intensity at the UV maxima between 280 and 390 nm to that of chlorophyll *a* at 665 nm. All extraction procedures were carried out in a darkened fume cupboard, to minimize photo-oxidative pigment breakdown of chlorophylls and carotenoids during analysis.

Extraction of samples for HPLC analysis of mycosporine amino acids. Extracts of 5 species of microalgae were examined by HPLC to see if they contained UV-absorbing compounds similar to the MAAs found in other marine plants and animals. Cultures examined were the diatom *Chaetoceros affinis*, the raphidophytes *Heterosigma carterae* and *Fibrocapsa* sp., and the dinoflagellates *Woloszynskia* sp. and *Gymnodinium catenatum*. For each species 450 ml late log phase cultures were used for these extractions. Cultures were centrifuged in a Sorvall refrigerated centrifuge at 5000 *g* for 5 min. The cell pellets were extracted with 1 to 2 ml of cold (5°C) tetrahydrofuran:methanol (20:80, v/v) in an ice bath, using a Braun Labsonic Model 1510 sonicator with a 4 mm needle probe (1 min), and left for 30 min at 5°C to fully extract. MAAs are stable in wet methanol under the following conditions: 1 to 2 d at room temperature (e.g. 20°C), 1 wk at 5°C, 2 to 3 wk at −20°C and 2 mo at −80°C. Since stability rapidly decreases with storage temperatures greater than 30°C care was taken to keep temperatures around 5°C during experimental treatments. When dry, samples are stable at room temperature for several months.

After centrifugation of the combined extracts (about 5 to 6 ml), a small aliquot (0.1 to 0.2 ml) was diluted for spectrophotometry, and equal volumes of the remaining extract were placed in 4 Eppendorf tubes and evaporated in a Savant evaporator for several hours at ambient temperature (0.9 to 1.5 ml). Samples could be left overnight at −20°C if evaporation was not complete, and evaporations were then completed the following day. The quadruplicate tubes of the lyophilized algal extracts of each species were then dispatched to Dr W. Dunlap at the Australian Institute for Marine Science (AIMS) for HPLC analysis. MAAs are stable under these conditions (see above).

HPLC analyses. Analyses of MAAs were performed according to published procedures (Dunlap & Chalker 1986, Dunlap et al. 1989, Karentz et al. 1991b, Stochaj

Table 1. UV-absorption characteristics of tetrahydrofuran:methanol (20:80, v/v) extracts of 152 species (206 strains) of cultured unicellular marine algae. Ratios of UV absorption to chl *a* absorption are listed with UV maxima in parentheses. For geographic origin of strains see the CSIRO Algal Culture Collection Strain List (CSIRO 1998)

Class and species	CSIRO culture code	Culture medium[a]	Growth temp. (°C)	Abs. ratio of major UV peaks:chl *a* (665 nm) 280–320 nm	320–340 nm	340–390 nm
BACILLARIOPHYCEAE						
Centrales						
Chaetoceros affinis Lauder	CS-78	G_2	17.5		0.74 (337.9)	
Chaetoceros calcitrans (Paulsen) Takano	CS-178	fE_2	17.5		0.64 (337)	
Chaetoceros didymus Ehrenberg	CS-2	f_2	17.5		0.80 (337)	
Chaetoceros muelleri Lemmermann	CS-176	f_2	17.5		0.74 (337.5)	
Chaetoceros cf. *mitra* (Bail.) Cleve	CS-70	G	17.5		0.48 (337.9)	
Chaetoceros cf. *radians* Schutt	CS-68	G	17.5		0.81 (337.3)	
Chaetoceros socialis Lauder	CS-236	G	17.5		0.83 (337.1)	
Coscinodiscus cf. *wailesii* Gran. et Angst.	CS-238	f_2	17.5		0.52 (338.1)	
Coscinodiscus sp.	CS-151	f_2	17.5		0.69 (338)	
Detonula pumila Schutt	CS-103	f_2	17.5		0.78 (337.1)	
Ditylum brightwellii (T. West) Grunow	CS-74	G	17.5		0.49 (337.3)	
Ditylum brightwellii (T West) Grunow	CS-131	f_2	17.5		0.72 (337.9)	
Extubocellulus spinifer (Hargraves et Guillard) Hasle, Von Stosch et Syvertsen	CS-136	G_2	17.5		0.82 (337.9)	
Lauderia annulata Cleve	CS-30	G	17.5		0.71 (337.5)	
Minutocellus polymorphus (Hargraves et Guillard) Hasle, Von Stosch et Syvertsen	CS-3	f_2	17.5		0.74 (337.7)	
Odontella aurita (Lyngbye) de Brebisson	CS-19	f_2	17.5		0.61 (337.7)	
Odontella mobiliensis (Bail.) Grunow	CS-82	f_2	17.5	0.54 (300.9)	0.71 (336.9)	
Odontella mobiliensis (Bail.) Grunow	CS-133	f_2	17.5		0.57 (336.3)	
Rhizosolenia setigera Brightwell	CS-62	f_2	17.5		0.74 (337.5)	
Skeletonema pseudocostatum Medlin	CS-76	G_2	17.5		0.68 (336.7)	
Skeletonema costatum (Greville) Cleve	CS-167	G_2	17.5		0.70 (337)	
Skeletonema costatum (Greville) Cleve	CS-181	f_2	17.5		0.72 (336.7)	
Stephanopyxis turris (Greville) Ralfs in Pritchard	CS-31	G	17.5		0.82 (337.5)	
Stephanopyxis turris (Greville) Ralfs in Pritchard	CS-100	G	17.5		0.83 (336.9)	
Streptotheca tamesis Shrubsole	CS-81	G_2	17.5		0.77 (338.3)	
Streptotheca tamesis Shrubsole	CS-130	G_5	17.5		0.93 (337.7)	
Thalassiosira eccentrica (Ehrenb.) Cleve	CS-148	f_2	27		0.66 (338.1)	
Thalassiosira oceanica Hasle	CS-67	f_2	17.5		0.55 (338.1)	
Thalassiosira pseudonana (Hust.) Hasle et Heimdal	CS-20	f_2	17.5		0.64 (337.3)	
Thalassiosira pseudonana (Hust.) Hasle et Heimdal	CS-173	fE_2	17.5		0.68 (336.7)	
Thalassiosira rotula Meunier	CS-32	G	17.5		0.58 (338.7)	
Thalassiosira rotula Meunier	CS-77	G_2	17.5		0.79 (336.7)	
Thalassiosira rotula Meunier	CS-102	G_2	17.5		0.72 (337.7)	
Thalassiosira stellaris Hasle et Guillard	CS-16	G	17.5		0.64 (337.7)	
Pennales						
Amphiprora hyalina Eulenstein	CS-28	f_2	17.5		0.65 (337.7)	
Amphora sp.	CS-10	f_2	17.5		0.63 (337.9)	
Amphora sp.	CS-361	fE_2	27		0.66 (337.1)	
Asterionella glacialis Castracane	CS-90	f_2	17.5		0.75 (337.3)	
Asterionella glacialis Castracane	CS-135	f_2	17.5		0.66 (338.1)	
Cylindrotheca fusiformis Reimann et Lewin	CS-13	f_2	17.5		0.75 (337.5)	
Delphineis sp.	CS-12	f_2	17.5		0.77 (338.5)	
Fragilaria pinnata Ehrenb.	CS-121	f_2	27		0.68 (338.6)	
Grammatophora oceanica Ehrenb.	CS-84	f_2	17.5		0.57 (338.5)	
Haslea ostrearia Bory	CS-250	f_2	15		0.88 (337.1)	
Navicula jeffreyi Hallegraeff et Burford	CS-46	f_2	17.5		0.85 (337.1)	
Nitzschia cf. *bilobata* W. Smith	CS-47	f_2	17.5		0.76 (337.9)	
Nitzschia closterium (Ehrenb.) W. Smith	CS-1	f_2	17.5		0.18 (338.3)	
Nitzschia closterium (Ehrenb.) W. Smith	CS-5	f_2	17.5		0.72 (337.9)	
Nitzschia closterium (Ehrenb.) W. Smith	CS-114	f_2	27		0.72 (337.3)	
Nitzschia closterium (Ehrenb.) W. Smith	CS-111	f_2	27		0.85 (337.3)	
Nitzschia cf. *frustulum* (Kutz.) Grun.	CS-258	G_2	27		0.86 (336.7)	
Nitzschia cf. *frustulum* (Kutz.) Grun.	CS-115	f_2	27		0.75 (337.3)	
Nitzschia cf. *constricta* (Greg.) Grun.	CS-106	G	27		0.86 (337.1)	
Nitzschia sp. (Prydz Bay, Antarctica)		f_2	5		1.22 (336.9)	
Phaeodactylum tricornutum Bohlin	CS-29	f_2	17.5		0.75 (333.6)	
Thalassionema nitzschioides Hustedt	CS-146	f_2	27		0.86 (337.8)	
Thalassiothrix heteromorpha Karsten	CS-132	f_2	17.5		1.02 (337.7)	

Table 1 (continued)

Class and species	CSIRO culture code	Culture medium[a]	Growth temp. (°C)	Abs. ratio of major UV peaks:chl *a* (665 nm) 280–320 nm	320–340 nm	340–390 nm
CHLOROPHYCEAE						
Chlamydomonas reinhardii Dangeard	CS-51	MBL/NB_2	17.5	0.42 (295.2)	0.63 (338.7)	
Chlorella protothecoides Krüger	CS-41	MBL/NB_2	17.5		0.62 (337.5)	
Chlorella vulgaris Beijerinck	CS-42	MBL/NB_2	17.5		0.65 (338)	
Chlorella sp.	CS-122	D	25		0.73 (339.5)	
Chlorella-like	CS-195	fE_2	25		0.78 (339.3)	
Chlorella-like	CS-247	G_2	17.5		0.80 (338.6)	
Chlorella-like	CS-248	G_2	17.5		0.87 (330.9)	
Dunaliella salina (green form) Teodoresco	CS-265	f_2	25		0.71 (339.3)	
Dunaliella salina (orange form) Teodoresco	CS-265	f_2	30		1.35 (340.4)	
Dunaliella tertiolecta Butcher	CS-175	f_2	17.5	0.34 (296.7)	0.58 (338.9)	
Dunaliella sp. (Burton Lake, Antarctica)		f_2	5		0.63 (339.8)	
Dunaliella sp. (Ace Lake, Antarctica)		f_2	5		0.66 (338.1)	
Nannochloris atomus Butcher	CS-183	f_2	17.5		0.56 (338.5)	
Nannochloris atomus Butcher	CS-184	f_2	17.5		0.56 (338.8)	
Stichococcus sp.	CS-92	f_2	17.5		0.71 (338.2)	
CHRYSOPHYCEAE						
Pelagococcus subviridis Norris	CS-58	f_2	17.5		1.08 (337.9)	
Pelagococcus subviridis Norris	CS-99	f_2	17.5		1.06 (337.3)	
CRYPTOPHYCEAE						
Rhodomonas salina (Wislouch) Hill et Wetherbee	CS-24	fE	17.5		0.66 (339.1)	
Rhodomonas salina (Wislouch) Hill et Wetherbee	CS-174	fE	17.5	0.47 (288.3)	0.58 (339.4)	
Chroomonas placoidea Butcher	CS-200	G	17.5		0.95 (335)	
Rhodomonas maculata Butcher ex Hill et Wetherbee	CS-85	fE	17.5		0.68 (339.3)	
Rhodomonas baltica Karsten	CS-201	G	17.5	2.96 (284.8)	0.73 (337.2)	
STX-157 (*Chroomonas* sp.?)	CS-48	fE	17.5	2.07 (280.0)	0.68 (337.9)	
Geminigera cryophila (Taylor et Lee) Hill	CS-138	f_2	5		0.63 (339)	
Chroomonas sp. (MB-3)	CS-204	G	17.5	2.44 (283.5)	0.67 (337.9)	
Rhodomonas sp.	CS-202	G	17.5		0.9 (345.9)	
Rhodomonas sp.	CS-215	G	17.5	2.42 (290.7)	0.67 (338.8)	
CYANOPHYCEAE						
Anabaena cylindrica Lemmermann	CS-53	MM11	17.5		0.67 (338.2)	
Anabaena cylindrica Lemmermann	CS-172	MM11	17.5		0.63 (338.5)	
Oscillatoria sp.	CS-52	f_2	17.5		0.6 (339.0)	
Oscillatoria sp.	CS-180	f_2	27	0.89 (293.7)	0.56 (339.7)	
Synechococcus sp.	CS-94	fE	17.5		0.72 (338.1)	
Synechococcus sp.	CS-197	f_2	17.5		0.61 (339.9)	
Synechocystis sp.	CS-95	fE	17.5	0.50 (293.8)	0.81 (340.2)	
DINOPHYCEAE						
Alexandrium affine (Inoue et Fukuyo) Balech	CS-312 (AABB01)	GSe	15		1.21 (333.9)	
Alexandrium catenella (Whedon et Kofoid) Balech	CS-313/1 (ACPP01)	GSe	15		1.17 (336.1)	
Alexandrium catenella (Whedon et Kofoid) Balech	CS-316/1 (ACJP01)	GSe	15		1.70 (332.6)	
Alexandrium catenella (Whedon et Kofoid) Balech	CS-316/1 (ACCA01)	GSe	15		2.47 (334.4)	
Alexandrium margalefi Balech	CS-322 (AMADE01)	GSe	15		3.00 (337.4)	2.3 (364)
Alexandrium minutum Halim	CS-323/2 (AMAD02)	GSe	15		1.42 (337.2)	
Alexandrium minutum Halim	CS-323/4 (AMAD04)	GSe	15		1.55 (336.7)	
Alexandrium minutum Halim	CS-324/13 (AMAD13)	GSe	15		1.06 (337.3)	
Alexandrium minutum Halim	CS-324/17 (AMAD17)	GSe	15		1.81 (336.5)	
Alexandrium tamarense (Lebour) Balech	CS-298 (ATBB01)	GSe	15		1.18 (337)	
Alexandrium tamarense (Lebour) Balech	CS-299 (ATJP01)	GSe	15		1.19 (336.9)	
Amphidinium carterae Hulburt	CS-21	G	17.5	0.18 (280.6)	0.79 (337.5)	

(Table continued on next page)

Table 1 (continued)

Class and species	CSIRO culture code	Culture medium[a]	Growth temp. (°C)	Abs. ratio of major UV peaks:chl *a* (665 nm) 280–320 nm	320–340 nm	340–390 nm
Amphidinium carterae Hulburt	CS-212	G	17.5	0.59 (280.6)	0.88 (337.8)	
Amphidinium klebsii Kofoid et Swezy	CS-33	G	17.5		0.93 (338.3)	
Amphidinium sp.	CS-109	G_2	25		0.93 (337.7)	
Amphidinium sp.	CS-259	G_2	25		1.24 (338)	
Gymnodinium catenatum Graham	CS-301/2 (GCDE02)	GSe	17.5		2.17 (336.3)	
Gymnodinium catenatum Graham	CS-301/8 (GCDE08)	GSe	17.5		2.07 (336.7)	1.69 (380.5)
Gymnodinium catenatum Graham	CS-302/20 (GCHU20)	GSe	17.5		1.84 (337.4)	1.62 (385.4)
Gymnodinium catenatum Graham	CS-302/22 (GCHU22)	GSe	17.5		1.83 (335.7)	1.63 (384.3)
Gymnodinium catenatum Graham	CS-302/9 (GCHU09)	GSe	17.5		2.48 (339.4)	2.57 (374.7)
Gymnodinium catenatum Graham	CS-304/2 (GCHA02)	GSe	17.5		1.49 (337.6)	1.56 (390)
Gymnodinium catenatum Graham	CS-305 (GCJP10)	GSe	17.5		3.5 (337.1)	1.5 (377.4)
Gymnodinium catenatum Graham	CS-306/4 (GCSP04)	GSe	17.5		2.94 (338.8)	2.6 (373.9)
Gymnodinium catenatum Graham	CS-309/1 (GCPT01)	GSe	17.5	4.0 (319.7)		4.68(345) 6.75(370.3)
Gymnodinium catenatum Graham	CS-309/3 (GCPT03)	GSe	17.5		2.65 (340)	3.46 (372.3)
Gymnodinium galatheanum (Braarud) Taylor	CS-214	GSe	17.5		0.96 (337.5)	
Gymnodinium sanguineum Hirasaki	CS-35	G	17.5	0.22 (281.8)	1.24 (335.8)	
Heterocapsa niei (Loeblich) Morrill et Loeblich	CS-36	G	17.5		1.33 (333.7)	
Heterocapsa niei (Loeblich) Morrill et Loeblich	CS-89	G	17.5		1.65 (323.8)	
Katodinium cf. *rotundatum* (Lohmann) Loeblich	CS-290/2	GSe	15		1.14 (332.1)	
Kryptoperidinium foliaceum (Stein) Lindemann	CS-37	G	17.5		0.70 (337.9)	
Peridinium balticum (Lev.) Lemm	CS-38	G	17.5	0.67 (295.9)	0.59 (338.2)	
Prorocentrum compressum (Bailey) Abé ex Dodge	Proro 1	GSe	17.5		1.32 (336.2)	
Prorocentrum gracile Shutt	CS-80	G	17.5		1.33 (337.3)	
Prorocentrum micans Ehrenberg	CS-27	G	17.5		0.97 (336)	
Scrippsiella sp.	CS-168	G	17.5		0.99 (336.3)	
Scrippsiella sp.	CS-295/1	GSe	17.5		1.79 (336.8)	
Scrippsiella sp.	CS-297	GSe	17.5		1.10 (336.8)	
Symbiodinium microadriaticum Freud.	CS-73	G	27		0.88 (337.5)	
Symbiodinium microadriaticum Freud.	CS-153	f_2	27		0.88 (336.9)	
Symbiodinium microadriaticum Freud.	CS-154	f_2	27		1.17 (336.9)	
Symbiodinium microadriaticum Freud.	CS-156	f_2	27		0.93 (337.3)	
Symbiodinium microadriaticum Freud.	CS-158	f_2	27		0.93 (336.6)	
Symbiodinium microadriaticum Freud.	CS-164	f_2	27		0.90 (337.3)	
Woloszynskia sp.	CS-341 (Wol 1)	GSe	27		3.40 (339.7)	
Woloszynskia sp. (Shearwater ballast)		GSe	27		1.56 (335)	
EUGLENOPHYCEAE						
Euglena gracilis Klebs	CS-66	MBL/NB_2	17.5		0.58 (339.1)	
EUSTIGMATOPHYCEAE						
Eustigmatos vischeri Hibberd	CS-144	BB	17.5		0.53 (336.7)	
Nannochloropsis oculata (Droop) Green	CS-179	f_2	17.5		0.47 (338.1)	
Nannochloropsis oculata (Droop) Green	CS-189	fE_2	17.5		0.52 (337.5)	
Nannochloropsis oculata (Droop) Green	CS-216	fE_2	17.5		0.69 (340.5)	
Nannochloropsis salina Hibberd	CS-190	f_2	17.5		0.52 (337.8)	
Nannochloropsis salina Hibberd	CS-191	fE_2	17.5		0.51 (337.8)	
Nannochloropsis sp.	CS-192	fE_2	17.5		0.48 (337.5)	
Nannochloropsis-like	CS-246	f_2	27		0.64 (337.7)	
Vischeria helvetica (Vischer et Pasher) Hibberd	CS-143	BB	17.5		0.59 (338.1)	
Vischeria punctata Vischer	CS-142	BB	17.5		0.72 (330)	
PRASINOPHYCEAE						
Mantoniella squamata (Manton et Parke) Desikachary	CS-199	f_2	17.5		0.91 (339)	
Micromonas pusilla (Butcher) Manton et Parke	CS-86	G	17.5		0.97 (338.9)	
Micromonas pusilla (Butcher) Manton et Parke	CS-170	G	27		0.99 (339.5)	
Micromonas pusilla (Butcher) Manton et Parke	CS-222	fE_2	17.5	0.81 (295.7)	0.81 (339.3)	

Table 1 (continued)

Class and species	CSIRO culture code	Culture medium[a]	Growth temp. (°C)	Abs. ratio of major UV peaks:chl *a* (665 nm) 280–320 nm	320–340 nm	340–390 nm
Nephroselmis minuta (Carter) Butch.	CS-207	G	17.5		0.72 (339.4)	
Nephroselmis rotunda (Carter) Fott	CS-223	fE_2	17.5	0.70 (294.7)	0.91 (340)	
Pseudoscourfieldia marina (Throndsen) Manton	CS-208	G	17.5		0.97 (338.3)	
Pycnococcus provasolii Guillard	CS-185	f_2	17.5		0.96 (339.4)	
Pyramimonas cordata McFadden	CS-140	G_2	17.5		0.97 (334.8)	
Pyramimonas gelidicola McFadden, Moestrup et Wetherbee	CS-139	f_2	5		0.74 (339.2)	
Pyramimonas oltmansii Schiller	CS-225	fE_2	17.5		0.88 (339.9)	
Pyramimonas propulsa Moestrup et Hill	CS-226	fE_2	17.5		0.76 (338.5)	
Tetraselmis chuii Butcher	CS-26	f_2	17.5		0.67 (339.3)	
Tetraselmis suecica (Kylin) Butcher	CS-56	f_2	17.5		0.72 (339.3)	
Tetraselmis suecica (Kylin) Butcher	CS-187	f_2	17.5	0.47 (290)	0.63 (339.1)	
Tetraselmis sp.	CS-91	f_2	17.5		0.68 (340.6)	
Unidentified prasinophyte (CCMP-BT5)	CS-211	f_2	17.5		1.2 (339.3)	
PRYMNESIOPHYCEAE						
Chrysochromulina camella Leadbeater et Manton	CS-268	GSe	15		1.27 (337)	
Chrysochromulina hirta Manton	CS-228	GSe	15		1.31 (337.1)	
Chrysochromulina kappa Parke et Manton	CS-269	GSe	15		1.20 (337)	
Chrysochromulina minor Parke et Manton	CS-270	GSe	15		1.38 (337)	
Chrysochromulina strobilus Parke et Manton	CS-271	GSe	15		1.65 (337)	
Chrysochromnulina strobilus Parke et Manton	CS-231	GSe	15		1.49 (337.3)	
Chrysochromulina sp.	CS-219	fE_2	15		1.37 (337.2)	
Chrysotila lamellosa Anand	CS-272	GSe	15		0.88 (337)	
Cricosphaera carterae (Braarud et Fagerland) Braarud	CS-40	D	17.5		0.85 (339)	
Crystallolithus hyalinus Gaarder et Markali	CS-273	GSe	15		1.05 (337)	
Diacronema vlkianum Prauser	CS-266	GSe	15		0.67 (337)	
Dicrateria inornata Parke	CS-267	GSe	15		1.23 (337)	
Emiliania huxleyi (Lohmann) Hay et Mohler	CS-57	f_2	17.5		1.49 (337.7)	
Emiliania huxleyi (Lohmann) Hay et Mohler	CS-274	GSe	15		1.24 (337)	
Emiliania huxleyi (Lohmann) Hay et Mohler	CS-275/2	GSe, K	15		1.38 (337)	
Imantonia rotunda Reynolds	CS-194	fE_2	17.5		1.34 (337.1)	
Isochrysis galbana Parke	CS-22	f_2	17.5		0.76 (338)	
Isochrysis sp.	CS-177	f_2	17.5	1.04 (279.1)	0.84 (337.8)	
Ochrosphaera neopolitana Schussing	CS-285	GSe	15		1.05 (337)	
Pavlova gyrans Butcher	CS-213	f_2	17.5		0.73 (337.9)	
Pavlova lutheri (Droop) Green	CS-23	f_2	17.5		0.81 (338.9)	
Pavlova lutheri (Droop) Green	CS-182	f_2	17.5		0.57 (338.9)	
Pavlova pinguis Green	CS-286	GSe	15		0.76 (337)	
Pavlova salina Carter (Green)	CS-49	f_2	17.5		0.72 (337.5)	
Pavlova sp.	CS-63	f_2	17.5		0.79 (337.3)	
Pavlova sp.	CS-50	f_2	17.5		0.70 (337.6)	
Phaeocystis pouchetii (Hariot) Lagerheim	CS-239	GSe	15		1.14 (336.9)	
Phaeocystis pouchetii (Hariot) Lagerheim	CS-240	GSe	15		1.15 (337.8)	
Phaeocystis pouchetii (Hariot) Lagerheim	CS-241	GSe	15		1.34 (337.2)	
Phaeocystis pouchetii (Hariot) Lagerheim	CS-243	G_5	5		1.36 (336.3)	
Phaeocystis pouchetii (Hariot) Lagerheim	CS-244	G_5	5	2.89 (315.7)	2.32 (337)	
Prymnesium parvum Carter	CS-288	GSe	15		0.87 (337)	
Pleurochrysis aff. *carterae* (Braarud et Fagerland) Christiansen	CS-287	GSe	15		0.68 (337)	
Prymnesiophyte 120	CS-120	f_2	27	1.46 (296)	0.96 (335.9)	
Pseudoisochrysis paradoxa Ott	CS-186	f_2	17.5	0.75 (279)	0.71 (338.3)	
RAPHIDOPHYCEAE						
Chattonella antiqua (Hada) Ono	CS-171	G	17.5		1.43 (333.3)	
Fibrocapsa sp.	CS-220	fE_2	17.5		1.86 (325)	
Heterosigma carterae (Hulbert) Taylor	CS-39	D	17.5		0.69 (337)	
Heterosigma carterae (Hulbert) Taylor	CS-169	G	17.5		0.66 (337)	
RHODOPHYCEAE						
Porphyridium purpureum (Bory) Drew et Ross	CS-25	f_2	17.5		0.54 (335)	

[a]Media codes: f = medium from Guillard & Ryther (1962); f_2 = 50:50 dilution of f-medium with seawater; fE = f + EDTA (Jeffrey 1980); G = GPM medium (Loeblich & Smith 1968); G_2 and G_5 = 50:50 and 20:80 dilution of GPM medium with seawater; GSe = G + selenium (CSIRO medium); D = D medium (Provasoli et al. 1957); MBL/NB_2 (Nichols 1973); BB = Bold's Basal medium (Nichols & Bold 1965); K = Keller medium (Keller et al. 1987; modified by CSIRO)

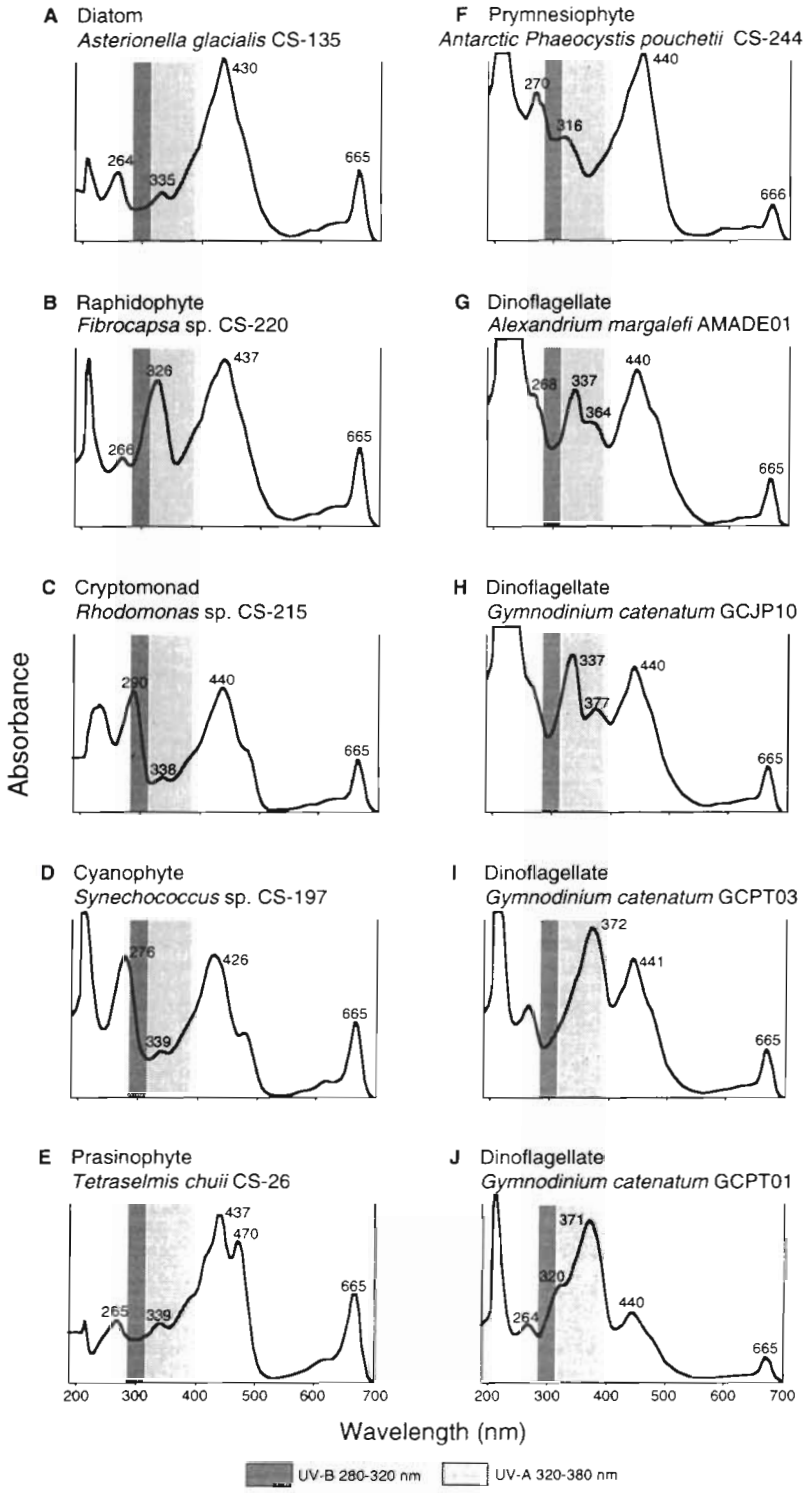


Fig. 1. UV-visible absorption spectra of tetrahydrofuran:methanol (20:80, v/v) extracts of selected microalgae. (A) *Asterionella glacialis*, CS-135 (diatom); (B) *Fibrocapsa* sp., CS-220 (raphidophyte); (C) *Rhodomonas* sp., CS-215 (cryptomonad); (D) *Synechococcus* sp., CS-197 (cyanophyte); (E) *Tetraselmis chuii*, CS-26 (prasinophyte); (F) *Phaeocystis pouchetii*, CS-244 (prymnesiophyte); (G) *Alexandrium margalefi*, AMADE01 (dinoflagellate); (H) *Gymnodinium catenatum*, GCJP10 (dinoflagellate); (I) *G. catenatum*, GCPT03 (dinoflagellate); (J) *G. catenatum*, GCPT01 (dinoflagellate)

et al. 1994, Carroll & Shick 1996, Helbling et al. 1996). Lyophilized algal extracts were reconstituted in 80% aqueous methanol (1/5 volume for *Chaetoceros affinis* and *Heterosigma carterae*, equi-volume for *Fibrocapsa* sp., *Woloszynskia* sp. and *Gymnodinium catenatum*). Individual MAAs were separated and quantified by isocratic HPLC on a reverse-phase, Brownlee RP-8 column (Spheri-5, 4.6 i.d. × 250 mm) protected with a RP-8 guard column (Spheri-5, 4.6 i.d. × 30 mm) with a mobile phase consisting of aqueous 25% methanol containing 0.1% acetic acid which was delivered at a flow rate of 0.8 ml min^{-1}. MAAs were identified by comparison and co-chromatography (where applicable) with authenticated standards and quantified by dual wavelength absorbance at 313 and 340 nm (Waters Model 440 dual wavelength detector) and peak area integration (Spectra-Physics 4400 dual channel integrator). Chromatographic standards were previously prepared (W. Dunlap) from the macroalgae *Porphyra tenera* (porphyra-334: Dunlap & Yamamoto 1995) and *Mastocarpus stellatus* (shinorine: Carroll & Shick 1996), the zoanthid *Palythoa tuberculosa* (mycosporine-glycine, palythine and palythinol: Dunlap & Chalker 1986), the ascidian *Lissoclinum patella* (mycosporine-glycine and shinorine: Dunlap & Yamamoto 1995), the sea anemone *Anthopleura elegantissima* (mycosporine-taurine, shinorine, porphyra-334 and mycosporine-2glycine: Stochaj et al. 1994) and the ocular lens of the coral trout *Plectropomus leopardus* (palythine, asterina-330, palythinol and palythene: Dunlap et al. 1989).

RESULTS

The relative proportions of UVA- and UVB-absorbing compounds (280 to 390 nm) compared to chl *a* (665 nm) in extracts of microalgae from 12 classes are shown in Table 1, together with the culture conditions for each of the 152 species (= 206 strains). A total of 85 genera were represented, with 26 strains from tropical waters, 173 from subtropical and temperate waters and 7 from Antarctic waters. These included surface-living, bloom-forming strains

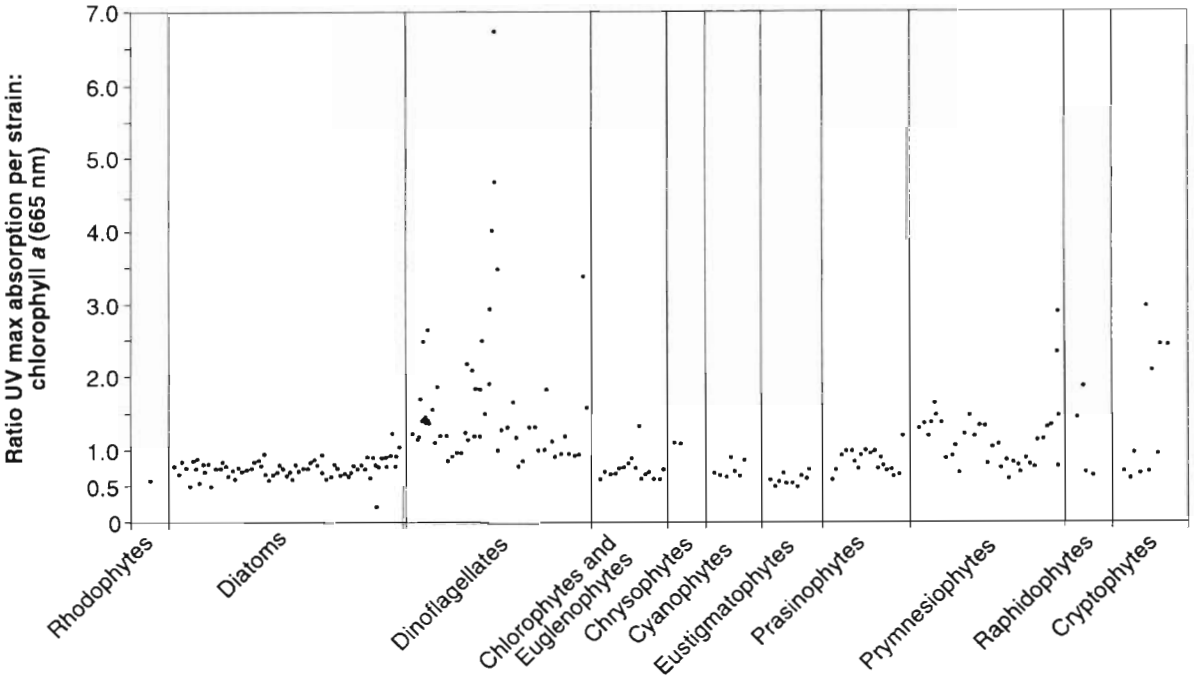


Fig. 2. Scatter plot of ratios of the maximum UV absorbance (290 to 390 nm) to chl *a* absorbance (665 nm) for each algal strain examined in Table 1. Data are grouped according to algal class

(dinoflagellates, prymnesiophytes, cryptomonads and raphidophytes), many species from coastal waters and some from the deep euphotic zone (e.g. picoplanktonic cyanobacteria).

Extracts of microalgae showed environmentally relevant, ultraviolet absorption maxima in 3 main regions: UVB (280 to 320 nm), UVA (320 to 340 nm) and near-UVA (340 to 390 nm). All strains, without exception, had maxima in the UVA region between 320 and 340 nm (most were close to 337 nm), 22 strains showed strong absorption in the UVB region (280 to 320 nm), and 9 strains of the dinoflagellate *Gymnodinium catenatum* had high absorbance in the near-UVA (340 to 390 nm) (Table 1).

UV-visible spectra of tetrahydrofuran-methanol extracts of 10 selected strains are shown in Fig. 1. The diatom *Asterionella glacialis* (Fig. 1A) and the prasinophyte *Tetraselmis chuii* (Fig. 1E) were 2 examples of species with very low absorbance in UVA and UVB regions. In contrast, the raphidophyte *Fibrocapsa* sp. (Fig. 1B), the prymnesiophyte *Phaeocystis pouchetii* (Fig. 1F), and the dinoflagellates *Alexandrium margalefi* (Fig. 1G) and *Gymnodinium catenatum* (Fig. 1H, I, J) were rich in UVA- and UVB-absorbing compounds, with some species showing multiple peaks in the UVA and UVB regions.

Fig. 2 presents a scatter plot of the ratio of the maximum UV absorbance to that of chl *a* at 665 nm for each strain listed in Table 1, grouping the data according to algal class. Strains rich in UV-absorbing compounds (ratios 1 to 6.75) came mostly from the bloom-forming flagellate classes: dinoflagellates, prymnesiophytes, raphidophytes and cryptomonads. The lowest ratios (<0.90) were found in the diatoms, eustigmatophytes, chlorophytes, prasinophytes, cyanophytes, chrysophytes, euglenophytes and rhodophytes.

A summary of all UV to chl *a* ratios by algal class is shown in Table 2. The majority of microalgae had UV:chl *a* ratios less than 1 (range 0.5 to 0.9, see Fig. 2). A second group had slightly higher ratios (0.9 to 1.4). It included the chrysophytes, and some of the prasinophytes and prymnesiophytes. A third group had the highest ratios (1.4 to 6.75). It included many bloom-forming members of the cryptomonads, dinoflagellates, prymnesiophytes and raphidophytes. Exceptions to these generalisations for each class were found, but the main trends were as specified (see Fig. 2). These data show that algal extracts differ across classes and strains of the same species (Table 3), having a range of UV-absorbing peaks with different patterns of absorption maxima and intensities, in both UVA and UVB regions.

The dinoflagellates were a special group, showing the greatest variations in UV-absorbing compounds across species and across strains of the same species (Tables 1 & 3, Figs. 1 & 2). Some dinoflagellates had very low UV:chl *a* ratios (e.g. *Amphidinium carterae*). The 2 endosymbiont-containing species—*Peridinium balticum* and *Kryptoperidinium foliaceum*—also had low ratios, possibly reflecting the presence of their

Table 2. Summary of the number of strains studied from 12 algal classes with their range of ratios of maximum UV absorbance (280 to 390 nm) to chlorophyll *a* absorbance (665 nm)

Algal class	No. of strains	Range of absorbance ratios of max. UV_{abs} (280–390 nm) to chl *a* (665 nm)
Bacillariophyceae	57	0.18 – 1.22
Chlorophyceae	15	0.34 – 1.35
Chrysophyceae	2	1.06 – 1.08
Cryptophyceae	10	0.47 – 2.96
Cyanophyceae	7	0.50 – 0.89
Dinophyceae	47	0.18 – 6.75
Euglenophyceae	1	0.58
Eustigmatophyceae	10	0.47 – 0.72
Prasinophyceae	17	0.47 – 1.20
Prymnesiophyceae	35	0.57 – 2.89
Raphidophyceae	4	0.66 – 1.86
Rhodophyceae	1	0.54
Total	206	0.18 – 6.75

chrysophyte or diatom endosymbionts (Tomas & Cox 1973, Jeffrey et al. 1975, Jeffrey & Vesk 1976). Other dinoflagellates, such as the surface bloom-forming species *Gymnodinium catenatum*, were rich in UV-absorbing compounds, with ratios of up to 4.0 at 319.7 nm, 4.6 at 345 nm and 6.75 at 370 nm (CS-309/1, GCPT01). Table 3 shows that strains of the same species either had very similar UV:chl *a* ratios (e.g. *Amphidinium carterae*, *Symbiodinium microadriaticum* and *Heterocapsa niei*) or varied widely (e.g. *Alexandrium catenella*, *Alexandrium minutum* and *Gymnodinium catenatum*). These data indicate that even in species rich in UV-absorbing compounds, significant strain variations are found.

The UV-absorbing properties of natural phytoplankton field populations obtained by net tows from the CSIRO wharf station (Derwent River Estuary) are shown in Fig. 3. When dinoflagellates were the main component of the phytoplankton (Fig. 3A, B), high values were obtained for UV:chl *a* absorbance ratios (e.g. 2.4 at 337 nm and 3.1 at 339 nm for the 2 samples, respectively). When diatoms were the main component (Fig. 3C, D), lower ratios (1.4 at 335 nm and 1.5 at 336 nm) were seen. The UV-visible spectrum of a field sample in which *Gymnodinium catenatum* was dominant (Fig. 3A) closely resembled that of one maintained culture of *Gymnodinium catenatum* (Fig. 1H), originally isolated from a local estuary.

Five microalgal cultures were examined by isocratic HPLC to check for the presence of MAAs. The strains were selected on the basis of their UV:chl *a* absorbance ratios obtained by spectrophotometry to represent a range of UV-absorbing properties (Fig. 4). The diatom *Chaetoceros affinis* had a low ratio (UV_{337nm} : chl *a* = 0.75), the raphidophyte *Heterosigma carterae* ratio was also low (UV_{338nm} : chl *a* = 0.66), the raphidophyte *Fibrocapsa* sp. had a high ratio (UV_{323nm} : chl *a* = 2.33), the ratio of the dinoflagellate *Gymnodinium catenatum* was also high (UV_{370nm} : chl *a* = 6.56) and that of the dinoflagellate *Woloszynskia* sp. was moderately low (UV_{365nm} : chl *a* = 1.13).

HPLC analyses of the 5 species are shown in Fig. 5. *Chaetoceros affinis* with a low UV:chl *a* ratio (0.75) gave no trace of MAAs, but the other 4 species had various combinations of MAA components. *Heterosigma carterae*, with a low UV:chl *a* ratio (0.66) contained both shinorine and asterina-330. The raphidophyte *Fibrocapsa* sp. with a high UV:chl *a* ratio (2.33) showed 2 major fractions, mycosporine-glycine and porphyra-334, as well as 3 minor components (palythine, asterina-330 and 1 unknown). The dinoflagellate *Woloszynskia* sp., with a UV:chl *a* ratio of 1.13, had 2 major components, shinorine and porphyra-334, and 5 minor fractions, mycosporine-glycine, palythine and 3 unknowns. The dinoflagellate *Gymnodinium catenatum* had the highest UV:chl *a* absorbance ratio (6.56), but only 3 MAA fractions could be identified in reason-

Table 3. Variation in the ratios of UV absorption maxima in the 320–340 nm region to chlorophyll *a* absorbance (665 nm) in extracts from selected dinoflagellate species and strains

Dinoflagellate species	No. of strains	Range of abs. ratios UV(320–340 nm): chl *a* (665 nm)
Alexandrium affine	1	1.21
Alexandrium catenella	3	1.17 – 2.47
Alexandrium margelefi	1	2.66
Alexandrium minutum	4	1.06 – 1.81
Alexandrium tamarense	2	1.18 – 1.19
Amphidinium carterae	2	0.79 – 0.88
Amphidinium klebsii	1	0.93
Amphidinium sp.	2	0.93 – 1.24
Gymnodinium catenatum[a]	10	1.49 – 4.68
Gymnodinium galatheanum	1	0.96
Gymnodinium sanguineum	1	1.24
Heterocapsa niei	2	1.33 – 1.65
Katodinium cf. *rotundatum*	1	1.14
Kryptoperidinium foliaceum[b]	1	0.70
Peridinium balticum[b]	1	0.59
Prorocentrum compressum	1	1.32
Prorocentrum gracile	1	1.33
Prorocentrum micans	1	0.97
Scrippsiella sp.	3	0.99 – 1.79
Symbiodinium microadriaticum	6	0.88 – 1.17
Woloszynskia sp.	2	1.56 – 3.40

[a]Note ratio of UV:chl *a* absorbance in the range 340 to 390 nm was 6.75 (370.3 nm) for strain GCPT01 (see Table 1)
[b]Contain chrysophyte-like endosymbionts with fucoxanthin containing chloroplasts (Tomas & Cox 1973, Jeffrey et al. 1975, Jeffrey & Vesk 1976)

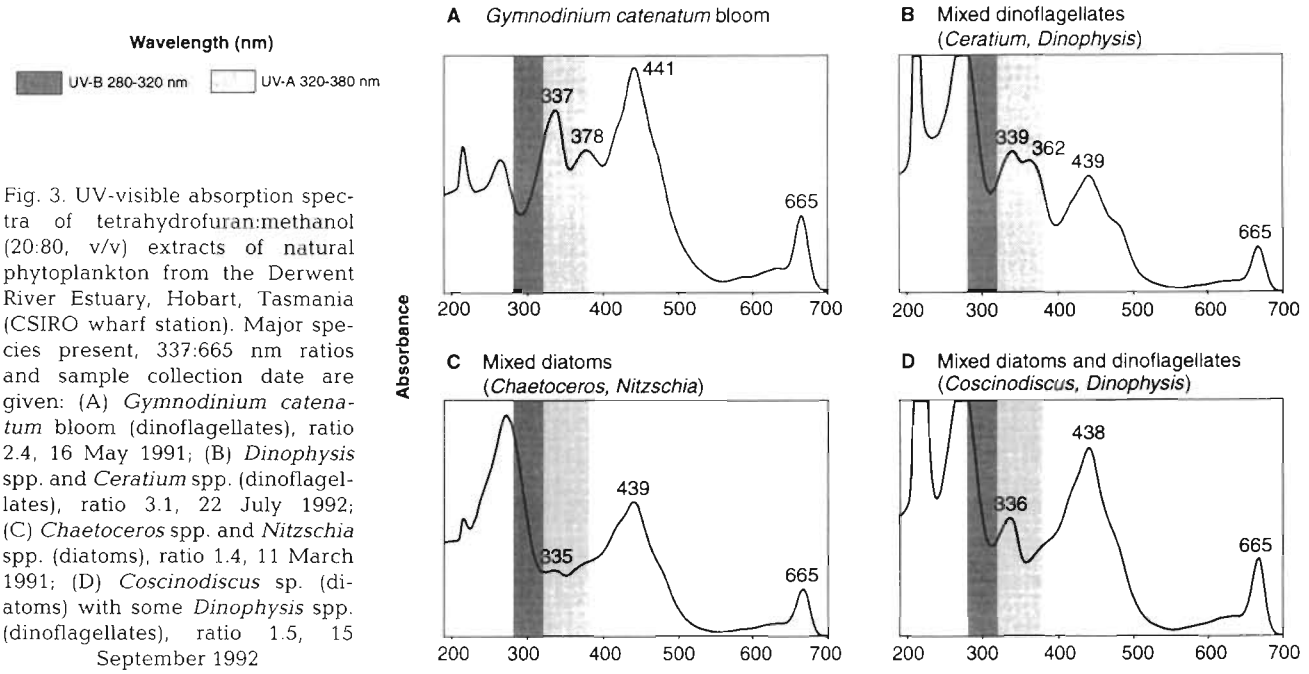
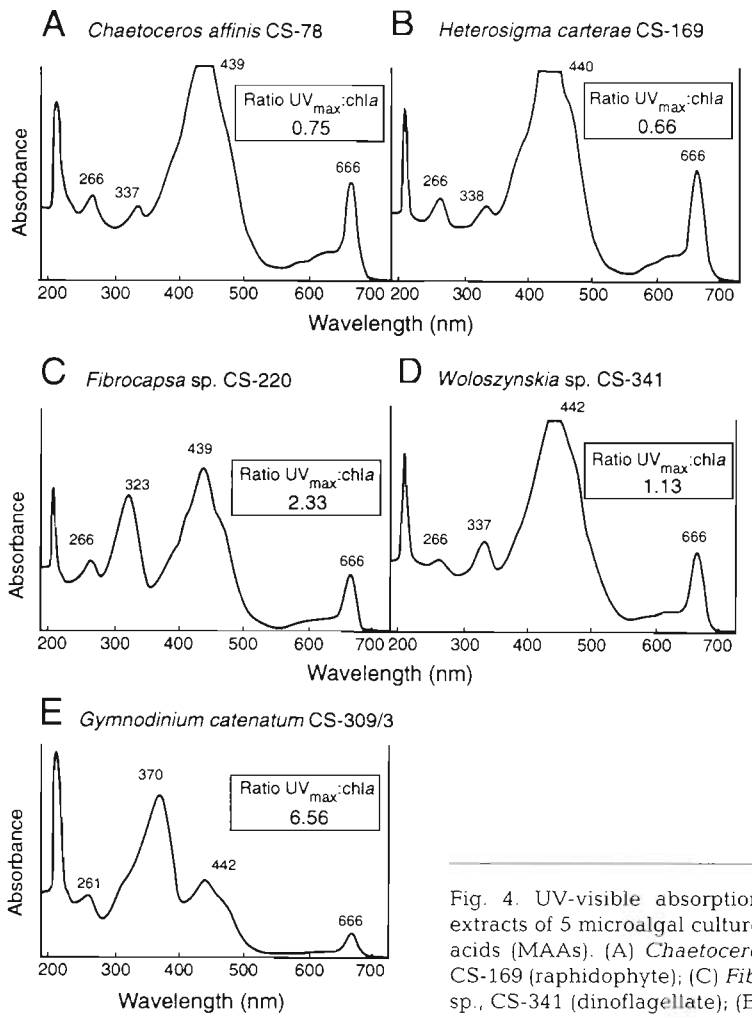


Fig. 3. UV-visible absorption spectra of tetrahydrofuran:methanol (20:80, v/v) extracts of natural phytoplankton from the Derwent River Estuary, Hobart, Tasmania (CSIRO wharf station). Major species present, 337:665 nm ratios and sample collection date are given: (A) *Gymnodinium catenatum* bloom (dinoflagellates), ratio 2.4, 16 May 1991; (B) *Dinophysis* spp. and *Ceratium* spp. (dinoflagellates), ratio 3.1, 22 July 1992; (C) *Chaetoceros* spp. and *Nitzschia* spp. (diatoms), ratio 1.4, 11 March 1991; (D) *Coscinodiscus* sp. (diatoms) with some *Dinophysis* spp. (dinoflagellates), ratio 1.5, 15 September 1992



able yield—mycosporine-glycine, porphyra-334 and shinorine. The major peak (retention time ~9.4 min) was an unknown compound, which occurred together with several unknown minor fractions. The major UV-absorbing component responsible for the large 370 nm absorption peak in the extract of *G. catenatum* (Fig. 4E) was not detected by this method of HPLC analysis (detection was at 313 and 340 nm, see Fig. 5), and may not be an MAA compound.

The relative proportions of MAAs analyzed in each of the 5 selected species are given in Table 4. Concentrations per unit cell were not determined; concentrations per unit chlorophyll could not be assessed because the extinction coefficients of chlorophylls *a* and *c* in the extraction solvent had not been determined (tetrahydrofuran:methanol = 20:80, v/v, see Jeffrey & Welschmeyer 1997). The most noteworthy features are the dominance of porphyra-334 and mycosporine-glycine in *Fibrocapsa* sp. (with palythine and asterina-330 minor components), the dominance of porphyra-334 and shinorine in *Woloszynskia* sp., and

Fig. 4. UV-visible absorption spectra of tetrahydrofuran:methanol (20:80, v/v) extracts of 5 microalgal cultures used for HPLC analysis of microsporine-like amino acids (MAAs). (A) *Chaetoceros affinis*, CS-78 (diatom); (B) *Heterosigma carterae*, CS-169 (raphidophyte); (C) *Fibrocapsa* sp., CS-220 (raphidophyte); (D) *Woloszynskia* sp., CS-341 (dinoflagellate); (E) *Gymnodinium catenatum*, CS-309/3 (dinoflagellate)

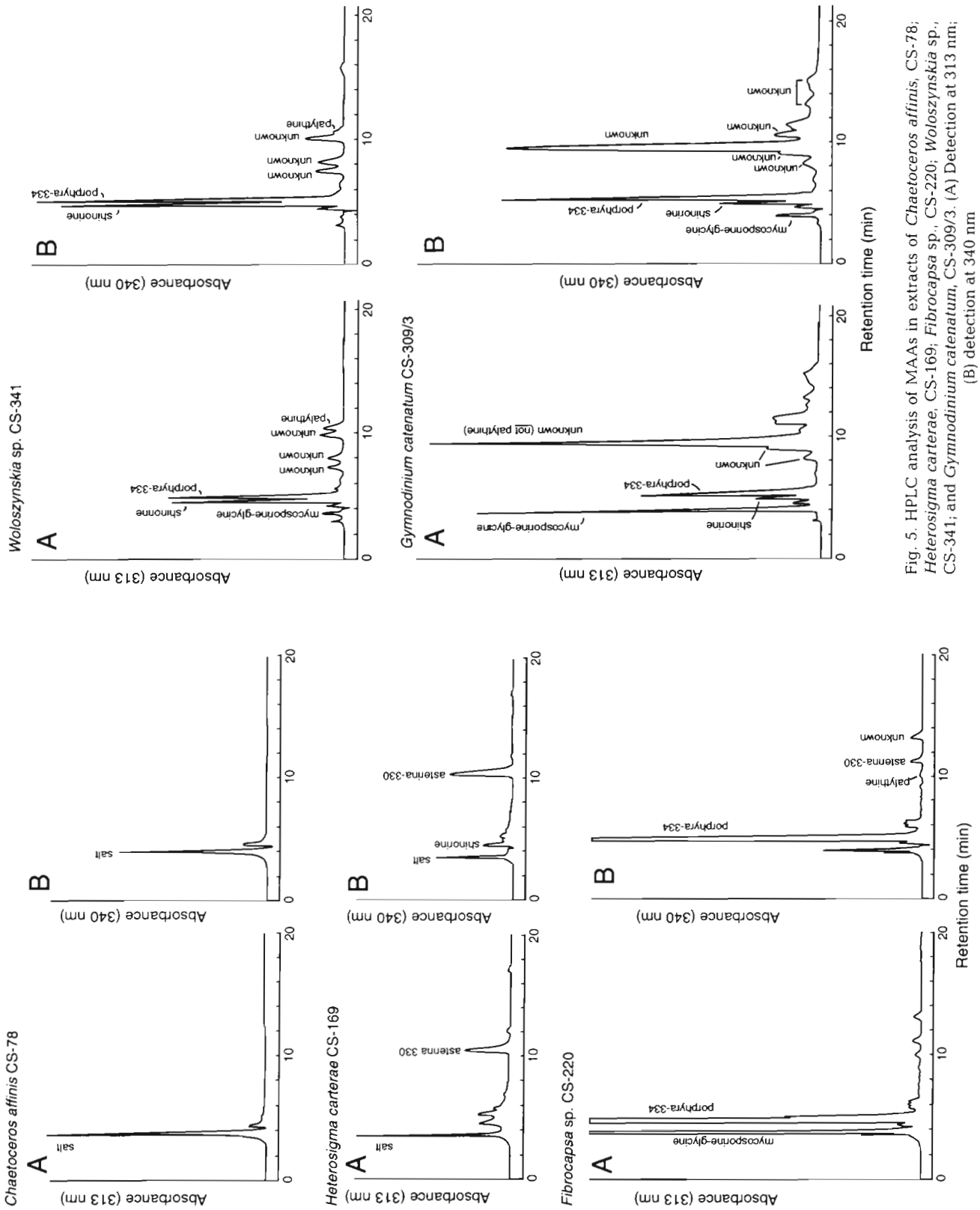


Fig. 5. HPLC analysis of MAAs in extracts of *Chaetoceros affinis*, CS-78; *Heterosigma carterae*, CS-169; *Fibrocapsa* sp., CS-220; *Woloszynskia* sp., CS-341; and *Gymnodinium catenatum*, CS-309/3. (A) Detection at 313 nm; (B) detection at 340 nm

Table 4. Relative proportions of mycosporine-like amino acids (MAAs) in quadruplicate analyses of tetrahydrofuran:methanol (20:80, v/v) extracts of 5 cultures of microalgae

Species	CSIRO culture code	MAAs (nmol ml^{-1} extract)[a]					
		Mycosporine -glycine	Shinorine	Porphyra-334	Palythine	Asterina-330	Unknown MAA (RT ~9.4 min)
Chaetoceros affinis	CS-78	–	–	–	–	–	–
Heterosigma carterae	CS-169	–	0.76 ± 0.42	–	–	1.54 ± 0.04	–
Fibrocapsa sp.	CS-220	74.86 ± 1.20	–	156.66 ± 4.77	1.23 ± 0.89	1.31 ± 0.16	–
Woloszynskia sp.	CS-341	2.34 ± 0.79	18.51 ± 0.45	23.78 ± 0.99	4.11 ± 0.35	–	–
Gymnodinium catenatum	CS-309/3	37.11 ± 1.01	7.36 ± 1.25	31.42 ± 3.35	–	–	Major component (not quantified)

[a]Not related to unit of tissue mass

a major unidentified MAA in *Gymnodinium catenatum*, which co-occurred with lesser amounts of mycosporine-glycine, porphyra-334 and shinorine.

DISCUSSION

The 206 strains of cultured marine microalgae examined from 12 classes showed a wide variation in the distribution of putative UV-screening pigments as grown under visible light conditions. The UV-screening capacity was judged on the basis of ratios of UV-absorbance (maxima 280 to 390 nm) to chl *a* absorbance (665 nm) in extracts of algal cells grown without supplementary UV radiation (see Table 1 & Fig. 2). The highest UV-screening capacity in both cultured microalgae and phytoplankton field samples was found in surface bloom-forming species of dinoflagellates, particularly strains of *Gymnodinium catenatum*, *Alexandrium*, *Heterocapsa*, *Scrippsiella* and *Woloszynskia* (see Table 3). Other bloom-forming flagellates had less, but still significant, UV-absorbing potential: the prymnesiophytes *Chrysochromulina*, *Emiliania*, *Imantonia* and *Phaeocystis*, the cryptomonads *Chroomonas*, *Rhodomonas* (including several unidentified strains), and the raphidophytes *Chattonella* and *Fibrocapsa*. Other algal classes showed UV:chl *a* absorbance ratios of less than 0.9 (see Table 1 & Fig. 2): they included diatoms, chlorophytes, chrysophytes, eustigmatophytes, planktonic cyanophytes, prasinophytes, 1 euglenophyte and 1 rhodophyte.

The small spectral differences in *Fibrocapsa* sp. in Fig 1B and a later culture used for HPLC analysis (Fig. 4C) could have been due to slightly different growth conditions or harvest times. The cause of small variations in absorption characteristics of individual strains were not studied here.

Because some of our microalgae were cultured for many years in the absence of supplementary UV radiation (see CSIRO 1998) the UV:chl *a* absorbance ratios should be considered the minimum achievable for the strains examined (Table 1). There is good evidence from the literature that UVA and/or UVB radiation can stimulate MAA synthesis in some microalgae: e.g. cyanobacteria (Garcia-Pichel & Castenholz 1993), Antarctic marine diatoms (Helbling et al. 1996), *Phaeocystis antarctica* (Riegger & Robinson 1997) and natural Antarctic phytoplankton field samples (Villafañe et al. 1995). Future work needs to examine if supplementary UV and blue radiation (370 to 460 nm) during growth can induce synthesis of UV-absorbing compounds in cultured microalgae across the algal classes, as it does for MAAs in some cultured Antarctic marine diatoms (Riegger & Robinson 1997). It is also necessary to examine whether algal species capable of MAA synthesis under conditions of visible light exposure alone may have an adaptive advantage, given the greater penetration of light of increasing wavelengths, for competitive survival of these species within a well-mixed photic zone.

Our HPLC analyses of MAAs in 2 cultures with low UV:chl *a* absorbance ratios (*Chaetoceros affinis* [0.75] and *Heterosigma carterae* [0.66]) while showing no MAAs in *C. affinis*, clearly showed the presence of small amounts of shinorine and asterina-330 in *H. carterae*. The lack of MAA peaks on the HPLC trace for *C. affinis*, which showed a small spectrophotometric peak at 337 nm in Fig. 4A, may have been due to the very low signal to noise ratio in the HPLC response. If MAA-like or other unidentified compounds are indeed present in algal species showing low UV:chl *a* absorbance ratios, then the capacity for their synthesis might be more widely distributed across algal classes than this study would suggest, needing only an external environmental trigger, such as UV or blue radiation exposure, for activation. These radiation effects may be even more complex, since UVA can inhibit photosynthesis in mixed assemblages of Antarctic phytoplankton (Holm-Hansen 1997), whereas UVA also reduces the effects of UVB inhibition in Antarctic cyanobacteria (Quesada et al. 1995).

While the range of species examined for the occurrence of MAAs by HPLC was small (5 species from 3 algal classes), the results showed suites of MAAs clearly evident in species with moderate to high UV:chl *a* absorbance ratios: *Fibrocapsa* sp. (UV:chl *a* = 2.33, porphyra-334, mycosporine-glycine, palythine and asterina), *Woloszynskia* sp. (UV:chl *a* = 1.13, porphyra-334, shinorine, palythine and mycosporine-glycine) and *Gymnodinium catenatum* (UV:chl *a* = 6.56, major and minor unknown MAAs, plus mycosporine-glycine, porphyra-334 and shinorine).

Gymnodinium catenatum consistently showed UV absorption by spectrophotometry at 370 to 372, 377 to 378 nm (see Figs. 1H, I, J, 3A & 4E, respectively). This absorption does not match that of any known MAA, and may be due to a new MAA derivative with extended conjugation, a new class of UV-absorbing compounds or perhaps the *cis*-peak of a carotenoid. Further work is needed to secure the identification of the *G. catenatum* unknowns.

On the basis of these limited results it is expected that most microalgae with moderate to high UV:chl *a* absorbance ratios would be likely to contain significant amounts of MAAs, and good correlation between UV absorption recorded by spectrophotometry and MAA content determined by HPLC analysis has previously been observed (Dunlap et al. 1995). Indeed, the presence of unknown MAAs, as well as compounds absorbing in the near UVA region (370 to 378 nm, see Fig. 4E, *Gymnodinium catenatum*) may indicate a rich source of new UV-absorbing compounds in dinoflagellates. These MAAs and related compounds may well act as effective UV screens to ensure the success of surface bloom-forming species in UV-rich environments.

Natural phytoplankton field samples obtained locally also showed high UV:chl *a* absorbance values for dinoflagellate-rich samples, and lower values when diatoms predominated. A natural bloom of *Gymnodinium catenatum* (Fig. 3A) showed absorption characteristics similar to those of 1 local strain maintained in culture (Fig. 1H), but differed from 2 other *G. catenatum* strains (Fig. 1I, J). Strain differences were a characteristic of species examined in this study (see Table 3).

The present investigation adds to the body of work provided by other authors (e.g. Vernet et al. 1989, Carreto et al. 1990, Helbling et al. 1996, Riegger & Robinson 1997) to document the distribution of UV-protective pigments in microalgae. Our screen of 152 microalgal species (206 strains) showed that most algal classes had representatives with both low and high UV-absorptive capacity (Table 2). Bloom-forming species, especially dinoflagellates, were particularly enriched, and our study of *Fibrocapsa* sp. (raphidophyte), *Woloszynskia* sp. (dinoflagellate) and *Gymnodinium catenatum* (dinoflagellate) bear similarities to the results of Carreto et al. (1990). These authors reported a suite of MAAs in the red-tide dinoflagellate *Alexandrium excavatum*, whose UV-absorbing compounds spanned an absorption range from 310 to 360 nm, to include MAAs with absorption at 310 nm (mycosporine glycine), 320 nm (palythine), 330 nm (asterina-330), 332 nm (palythinol), 334 nm (shinorine, porphyra-334), 337 nm (pathythenic acid), 357 (*cis*-usujirene) and 360 nm (palythene). These screening compounds would be expected to give broad cover from UVB (280 to 320 nm) and UVA (320 to 380 nm) radiation.

The successful bloom-forming dinoflagellate *Gymnodinium catenatum* contained previously known MAAs (mycosporine-glycine, porphyra-334 and shinorine) together with unidentified UV-absorbing components in the UVB (280 to 320 nm), UVA (320 to 360 nm) and the near UVA (>360 nm) regions (see Figs. 4E & 5). The value of both spectrophotometric and HPLC techniques used in the present work is clearly seen, since the former picks up most UV-absorbing compounds, and the latter, as currently used, identifies known MAAs. Unidentified UV-absorbing components as found in *G. catenatum* will need further analytical study (eg. nuclear magnetic resonance/mass spectrometry) to secure identification.

It is clear from this survey that certain bloom-forming microalgae are 'sun-adapted' and have the capacity to synthesize sunscreen pigments whose chemistry, stimulation of biosynthesis by light of particular wavelengths and protective function warrant further investigation. While the most recent evidence shows a global levelling off of ozone-depleting emissions (Houghton 1996), indicating that the Montreal Protocol is beginning to work, the ozone hole over Antarctica formed in 1998 is the largest ever recorded (Soloman 1998). A heightened global UV flux is forecast to continue for several decades (Van der Leun et al. 1998), and could alter the biomass and floristic composition of marine phytoplankton species in certain habitats to favour those surface bloom-forming species most adapted to UV stress. Further study should determine whether this is a permanent or reversible threat to particular marine ecosystems.

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