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STEROLS OF THE PHYTOPLANKTON—EFFECTS OF ILLUMINATION AND GROWTH STAGE*

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Key Word Index—Phytoplankton; marine sterols; illumination; growth stage.

Abstract—The sterol compositions of different species of cultured phytoplankton, (two diatoms—*Phaeodactylum tricornutum* and *Skeletonema costatum*, two green algae—*Danaliella minuta* and *Tetraselmis tetrathele* and a brown alga—*Monochrysis lutheri*) were compared with that of a diatom field population (>98% *Thalassionema nitzschioides*) using GC–MS techniques. The effect of culture age in the cultured specimen; was examined by harvesting in both the exponential and stationary growth phases and was found to produce considerable differences in the sterol composition in some species. The influence of the intensity and different spectral illumination on a cultured specimen of a green alga (*Danaliella minuta*) was also examined and found to produce changes in the sterol composition.

INTRODUCTION

There has been increased interest in the sterols of phytoplankton during recent years for a number of reasons. Plants, particularly diatoms and unicellular flagellates, are the primary producers of organic carbon in the marine ecosystem and will constitute the major source of sterols in the marine system for subsequent geochemical and biochemical cycling. As a result of their position at the base of the marine food web, phytoplankton are also likely to be the primary dietary source of sterols for the majority of other marine organisms, either directly, as in the case of the herbivores, or indirectly for the carnivores.

The sterol compositions of marine phytoplankton vary widely, some groups containing quite simple sterol mixtures, whilst in others very complex sterol assemblages are found, including occasionally novel sterols [1, 2]. Because of this range in compositions, it has been suggested by Patterson [3] that a knowledge of sterol make-up may aid in solving the many problems of algal taxonomy.

A major problem in studying the compositions and biosynthesis of marine phytoplankton sterols is that it is seldom possible to obtain field samples containing only a single species. Consequently most work has been carried out on laboratory cultured samples. In assessing the analytical results from such samples, it is essential to consider the possible effects of culture condition (temperature, light quality and quantity, nutrition and carbon source) and the stage of culture (exponential growth phase, stationary growth phase) on the biochemical composition of the species involved.

Unfortunately there is little good relevant data available. Orcutt and Patterson [4] have stressed the need to relate compositional data of diatoms to chemically defined growth media. Pugh [5] reports changes in the biochemical composition of a marine diatom with culture age and salinity, of particular relevance to lipid studies being the observation that lipid appeared to accumulate during the stationary growth phase. In the diatom *Nitzschia alba*, hydrocarbon and sterol compositions were unaffected by the stage of growth but changes were seen in fatty acid composition [6]. The stage of growth is also reported to determine the photosynthetic rate in the diatom *Phaeodactylum tricornutum* [7], the rate per cell being at a maximum in early or middle exponential phase and at a minimum in the stationary phase.

There have been few studies on the effect of culture temperature on phytoplankton composition, although Betouhim-El *et al.* [8] report the phytoflagellate *Ochromonas danica* to have a higher sterol content when cultured at low temperatures (15–18°) than when cultured at higher temperatures (35–37°).

Other recent studies suggest that the biochemical composition of cultured phytoplankton is dependent on both light quantity [9, 10] and light quality [11–15], whilst the rate and efficiency of photosynthesis is also dependent upon light quantity [16, 17] and quality [12]. These results may be compared with field observations of changes in photosynthetic efficiency and pigment composition of phytoplankton with depth in the water column and hence changing light regimes [18–20].

The present work firstly investigates the effects of culture age on the sterol composition of a number of laboratory cultured marine diatoms and algae under standard culture, temperature and light conditions; secondly monitors the sterol composition of a marine algal species cultured under various light conditions using a well-characterized light source whose relative spectral radiance in the visible range closely follows that of natural daylight; and thirdly presents a detailed sterol analysis of a single species of a marine diatom taken from a single natural field population.

* Part X in the series "Marine Sterols". For Part IX see Ballantine, J. A., Williams, K. and Morris, R. J. (1978) *J. Chromatogr.* **166**, 491.

Table 1. Sterol fractions of the phytoplankton

	Sterol quantities (% of total lipid)		
	Exponential phase		Stationary phase
	Free sterol	Steryl ester	Free sterol*
Phaeodactylum tricornutum	0.55	—	0.87
Skeletonema coastatum	—	—	1.8
Danaliella minuta	0.34	0.12	—
Tetraselmis tetrathele	—	—	0.6
Monochrysis lutheri	1.29	—	1.7
Thalassionema nitzschioides (field population)	2.42% of total lipid		

* No steryl esters were detected.

RESULTS

Yields of lipids and sterols

The quantitative data for the sterol extractions are summarized in Table 1. Note that it was not possible to quantify the sterols in the experiments in which *D. minuta* was grown under differing light conditions owing to the small quantities available.

Sterols of the diatom

The results of the sterol analyses of the diatoms are given in Table 2, where the retention times of each of the sterol TMSi derivatives on two column systems are reported as well as the molecular ions of each compound and the percentage of each sterol in the organism. In the two cases where the diatoms were harvested during the

Table 2. Sterol compositions of diatoms. GC–MS data for sterol TMSi ethers

Sterol	Identity*	RRT Dexsil	RRT Silar	M^+	*Phaeodactylium tricornutum* Exponential GC–MS%	*Phaeodactylium tricornutum* Stationary GC %	*Skeletonema costatum* Stationary GC %	*Thalassionema nitzschioides* Free living GC–MS %
D1	26C 5,22*E*	0.62	0.64	442	—	—	—	1.5
D2	27C 5,22*E*†	0.85	0.89	456	—	—	—	trace
D3	27C 5,22*E*	0.88	0.95	456	—	—	—	22.7
D4	27C 5	1.00	1.00	458	1.5	1.5	24.6	59.6
D5	27C 5,24(25)	1.10	1.31	456	—	—	—	5.0
D6	28C 5,22*E*	1.10	1.09	470	91.1	94.4	—	3.6
D7	27C 7	1.10	1.12	456	—	—	—	trace
D8	28C 5,24(28)	1.28	1.49	470	—	—	39.7	2.2
D9	28C 5	1.28	1.32	472	3.6	3.7	16.9	2.9
D10	29C 5,22*E*	1.38	1.30	484	3.2	—	—	—
D11	29C 5	1.61	1.61	486	1.5	1.5	16.8	2.0
D12	29C 5,24(28)*Z*	1.64	1.82	484	—	—	—	0.8
D13	29C 7	1.91	1.94	486	—	—	—	trace
D14	?	1.44	1.77	?	—	—	1.4	
D15	?	1.79	1.91	?	—	—	1.8	

* The shorthand notation for the 3β-sterols used in this and other tables refers to the number of carbon atoms (C) followed by the position of any double bonds and an indication of their geometrical isomerism, e.g. 29C 5,22*E* is (22*E*)(24α)-24-ethylcholesta-5,22-dien-3β-ol (i.e. either stigmasterol or its C_{24} epimer poriferasterol).

† Occelasterol.

Table 3. Sterol compositions of chlorophytes. GC–MS data for sterol TMSi ethers

Sterol	Identity*	RRT Dexsil	RRT PZ-176	M^+	*Danaliella minuta*† Exponential GC–MS % (Free sterols)	*Danaliella minuta*† Exponential GC–MS % (Sterol esters)	*Danaliella minuta*† Stationary GC %	*Tetraselmis tetrathele* Stationary GC %
C1	27C 5,22*E*	0.89	0.95	—	—	—	—	0.5
C2	27C 5	1.00	1.00	458	trace	8.0	36.0	5.0
C3	27C	1.04	1.00	460	—	trace	—	—
C4	28C 5,22*E*	1.09	1.09	—	—	—	—	1.6
C5	28C 5	1.29	1.29	472	37.6	6.3	2.0	34.0
C6	28C 5,24(28)	1.28	1.40	470	35.0	29.4	7	57.7
C7	28C 7,22*E*	1.29	1.28	470	—	trace	—	
C8	29C 5,22*E*	1.38	1.30	484	12.7	—	26	—
C9	28C 7	1.55	1.61	472	6.8	22.1	12	—
C10	29C 5,7,22*E*	1.55	1.69	482	3.4	—	—	—
C11	29C 5	1.61	1.55	486	4.5	—	8†	—
C12	29C 7,22*E*	1.64	1.64	484	—	20.0	—	—
C13	29C 7	1.92	1.92	486	trace	14.3	4.5	—

* For identity code see Table 2.

† Warm white fluorescent lamp, 65–80 W.

‡ And/or 29C 7,22*E*.

Table 4. Sterol compositions of *Dunaliella minuta* grown under differing illumination conditions (TMSi derivatives)

Sterol identity*	White light† %	Red light %	Amber light %	Yellow light %
27C 5	8	9	5	10
28C 5	2.5	2	7	2
28C 5,24(28)	10	10	13.5	12
29C 5,22*E*	31	29	32	28
28C 7	36	41	35	37
29C 5‡	7	10	8	3.5
29C 7	5	5	5	6

* For identity code see Table 2.
† Xenon CSX 450 W.
‡ And/or 29C 7,22*E*.

stationary phase, insufficient material was available for GC–MS investigation and the quoted analyses were obtained by GC only and must be considered as less accurate.

The diatom sterols D1–D13 (Table 2) were all identified by comparison of their GC retention data on the two column systems, and whenever possible, by comparisons of their MS with those of authentic marine sterol TMSi ethers [21].

Sterols of the green algae

The results of the sterol analyses of the chlorophytes are given in Table 3. It was found again that in the two cases where the organisms were harvested during the stationary phase of growth insufficient material was present for GC–MS analyses. Sufficient material was available however from the exponential growth phase of *D. minuta* to enable us to separate the free sterols from the sterol esters and to analyse them separately, to establish whether there were any differences between the bound and free sterols in this organism.

The chlorophyte sterols C1–C13 (Table 3) were all identified by comparison with authentic marine sterols [21].

Effect of differential illumination on D. minuta *sterol production*

The sterol compositions of *D. minuta* samples, which were cultured under differing illumination conditions, are tabulated in Table 4.

Sterols of the brown alga

The sterol analyses on the samples of *M. lutheri* from the exponential and stationary growth phases are given in Table 5. The quantity of material available from the stationary phase proved insufficient for complete analysis and the results are based on GC–MS data obtained from the Dexsil column only.

A number of compounds could not be identified precisely but a considerable quantity of structural information could be deduced from the GLC retention times and the GC–MS data. Details on these unknown compounds are as follows.

Sterol M-9 had an MS with peaks at *m/e* (rel. int.): 486 (100) M^+; 471 (20) M-15; 388 (35) M-98; 359 (45) M-sc.-2H; 353 (63) M-133; 271 (80) M-sc-90, indicated that this compound was a C_{29} mono-unsaturated sterol and the ions at *m/e* 271, 359 and 388 clearly established that it contained a C_{20} saturated stanol nucleus and a C_9 side

Table 5. Sterol compositions of the chrysophyte *Monochrysis lutheri*. GC–MS data for sterol TMSi ethers

Sterol	Identity*	RRT Dexsil	RRT PZ-176	M^+	Exponential %	Stationary %
M1	27C 5	1.00	1.00	458	26.7	0.6
M2	28C 5,22*E*	1.10	1.09	470	1.1	0.7
M3	27C 5,24(25)	1.10	—	456	—	trace
M4	28C 5	1.31	1.32	472	17.9	10.3
M5	28C 5,24(28)	1.31	—	470	—	trace
M6	28C	1.36	—	474	—	trace
M7	29C 5,22*E*	1.39	1.32	484	31.1	17.3
M8	29C 22*E*	1.41	—	486	—	1.9
M9	29C 22?	1.47	—	486	—	1.9
M10	29C 5	1.61	1.55	486	22.6	17.3
M11	29C	1.64	—	488	—	1.4
M12	30C 22?	1.86	—	500	—	15.0
M13	30C ?	2.17	—	502	—	1.7
M14	?	2.26	—	488	—	3.0
M15	29C (OH)?	2.58	—	504	—	27.2
M16	30C (OH)?	3.20	—	518	—	1.8

* For identity code see Table 2 and text.

chain with a Δ^{22} double bond [22, 23]. Sterol M-9 was therefore a 24-methyl-5α-cholest-22-en-3β-ol with an extra nuclear methyl group, which was probably in the C-4 position from biosynthetic grounds.

Sterol M-12 had the following MS: *m/e* (rel. int.): 500 (100) M^+; 485 (17) M-15; 457 (14) M-43; 388 (44) M-112; 373 (10); 367 (78) M-43-90; 360 (35); 359 (53) M-sc-2H; 346 (10); 299 (18); 271 (98) M-sc-90; 269 (26) M-sc-90-2H; 243 (12); 283 (11); 231 (20) and 229 (19). The molecular ion established that sterol M-12 was a monounsaturated C_{30} sterol and the ions at *m/e* 271, 359 and 388 clearly established that it contained a C_{20} saturated 5α-stanol nucleus and a C_{10} sidechain with a Δ^{22} double bond [22, 23]. Sterol M-12 was therefore either a 24-ethyl- or a sidechain dimethyl-5α-cholest-22-en-3β-ol compound with an extra nuclear methyl group, probably at the C-4 position. Two marine sterols have been reported with these substitution patterns. Djerassi and Tan [24] have isolated a 4α-methyl-24-ethyl compound (4α-methyl-24*ε*-ethyl-5α-cholest-22-en-3β-ol) from an ascidian *Eudistoma psammion* and both Shimizu *et al.* [25] and Withers *et al.* [26] have reported on a 4α,23,24-trimethyl compound (4α,23,24-trimethyl-5α-cholest-22-en-3β-ol; dinosterol) from dinoflagellates.

The MS of both compounds are very similar to that of sterol M-12, with prominent sidechain cleavages between carbon atoms 24/25, 20/21 and 19/20 being a feature of all three compounds. However, dinosterol has a characteristic sidechain cleavage between 20/22 to give an M-71 ion which is absent in both the spectra of sterol M-12 and the 24-ethyl compound and hence it seems probable that M-12 is a 24-ethyl compound similar to that isolated by Djerassi and Tan [24].

Sterol M-13 had the following MS: *m/e* (rel. int.): 502 (100) M^+; 487 (91) M-15; 414 (86); 412 (60) M-90; 313 (86) M-123; 371 (49) M-129; 245 (14) M-257. The molecular ion corresponded to a fully saturated C_{30} stanol but the usual ions which characterize a 3β-cholestanol nucleus were missing and the compound was not identified.

Sterol M-14 was a minor compound which had very few ions at the high end of its MS and was not identified; *m/e* (rel. int.): 488 (38) M^+; 473 (100) M-15.

Sterol M-15 was a very intense peak in the chromatogram and provided the following MS: *m/e* (rel. int.): 504 (34) M^+; 489 (35) M-15; 486 (7) M-18; 473 (18); 471 (4) M-18-15; 414 (8) M-90; 399 (5) M-105; 397 (7); 396 (2) M-18-90; 381 (2) M-18-105; 375 (20); 373 (16); 357 (100) M-18-129; 331 (6); 329 (5); 247 (6); 217 (7). The molecular ion at *m/e* 504 could correspond to a C_{29} saturated stanol which also contained an underivatized hydroxyl group, which must be either a tertiary or hindered secondary alcohol as it did not react with BSA/pyridine during the derivatization step. This suggestion is supported by the presence of an M-18 ion at *m/e* 486 which would correspond to a monounsaturated C_{29} compound. The large ion at *m/e* 357 (i.e. M-18-129) would seem to suggest that a double bond has been introduced in the Δ^5 position during thermal loss of water and might give a clue to the position of the hydroxyl group, but the compound could not be identified.

Sterol M-16 was a minor sterol which provided the following MS: *m/e* (rel. int.): 518 (31) M^+; 503 (25) M-15; 473 (13); 428 (5) M-90; 389 (14) M-129; 371 (100) M-18-129. The molecular ion at *m/e* 518 could correspond to a C_{30} saturated stanol containing an underivatized hydroxyl group. The very intense ion at *m/e* 371 corresponds to M-18-129 and suggests that after elimination of water a double bond is introduced into the Δ^5 position. Sterol M-16 would therefore seem to be a homologue of M-15.

DISCUSSION

Sterols of the diatoms

The sterol compositions of the diatom *P. tricornutum* (Table 2) did not vary significantly with the growth phase at which it was harvested and the results obtained compared favourably with those of Orcutt and Patterson [4] and Rubinstein and Goad [27] in that the major sterol (>90%) was 24-methylcholesta-5,22-dien-3β-ol. However, the results differ from those of Orcutt and Patterson [4] with respect to the minor sterols as these workers found small quantities of Δ^7 sterols but did not detect any cholesterol.

Nine species of the sub-order to which *P. tricornutum* belongs have been investigated so far and six of them have been found to contain 24-methylcholesta-5,22-dien-3β-ol as their major sterol. Surprisingly, however, those species in the same family contain stigmasterol as their major sterol with little or no 24-methylcholesta-5,22-dien-3β-ol being present [4].

No one sterol was dominant in the profile of *Skeletonema costatum*, the major sterols being 24-methylenecholesterol, cholesterol, 24-methyl- and 24-ethyl-cholesterol. The other organism of the same family (*Thallasiosira pseudonana*) was reported by Orcutt and Patterson [4] to contain six sterols with 28C 5,22(*E*) and 28C 5 predominating.

The free living *Thalassionema nitzschioides* has a complex sterol composition with twelve sterols being detected with cholesterol predominating. It is interesting that a C_{26} sterol is present as this is found to be widely distributed in marine invertebrates. Furthermore, occelasterol, which has been suggested to be an intermediate in the biosynthesis of C_{26} sterols [28], is also present in the extracts.

No 5α-stanols were detected in any of the diatom species.

The classification of diatoms is based at present on the physical characteristics of the siliceous skeletal parts of the cell wall [29] and using this classification, the work so far performed on the analysis of sterols in closely related organisms does not suggest any similarity of sterol patterns. However, it has been noted [4] that colonial diatoms tend to have a more complex sterol profile than those which usually exist in a solitary state. The present work substantiates this observation; *P. tricornutum* which normally exists as solitary cells, has over 90% of its total sterols in a single compound whereas the other two organisms, which have tendencies to form colonies, have a more diverse sterol pattern.

Sterols of the green algae

The free sterols of *Danaliella minuta*, harvested in the exponential growth phase, consisted of eight components of which 24-methylene-cholesterol and 24-methylcholest-5-en-3β-ol were the major sterols comprising 73% of the sterol mixture. It is interesting that one of the minor sterols was 24-ethylcholesta-5,7,22-triene-3β-ol. This is a rare sterol that had also been detected by Patterson [3] in *Chlomydomonas rheinhardi* where it comprised 56% of the

sterol mixture. As both *D. minuta* and *C. rheinhardi* are members of the same family, it is possible that the presence of this rare sterol is a feature of this group.

In the sterols obtained from the sterol ester by alkaline saponification, the major components were 24-methylenecholesterol, 24-methylcholest-7-en-3β-ol and 24-ethylcholesta-7,22-dien-3β-ol. The composition as a whole was different from that of the free sterols, underlining the fact that saponification of the total lipid should be avoided wherever possible so as not to mix the sterols from these two sources. In the free sterols, those sterols with a Δ^7 double bond comprised 10% of the mixture whereas in the bound sterols they comprised 57% of the total. Also C_{28} compounds comprised a higher proportion (80%) of the free sterols whereas the C_{27} and C_{29} compounds were correspondingly higher in the bound sterols. Cholestanol was only detected in the bound sterols, and then merely in trace amounts.

The *D. minuta* extracts from the stationary growth phase were insufficient for GC–MS investigation and the identifications must be regarded as tentative. There seemed to be two major sterols, 28C 7 and 29C 5,22 and five other components. Thus there were observable differences between the free sterols extracted from the exponential and stationary phases of this organism.

The sterol composition of *Tetraselmis tetrathele* (Table 3) consisted of two major sterols, 24-methylenecholesterol and 24-methylcholest-5-en-3β-ol, together comprising 92% of the sterol mixture, and three minor sterols. In this there were some similarities to the free sterols from the exponential growth phase of *D. minuta*, but C_{29} sterols were absent in the former organism. The very large quantities of 24-methylenecholesterol may be significant, as Goad *et al.* [30] have discussed the intermediacy of this sterol in the biosynthesis of C_{29} sterols in the chlorophytes.

Effects of different illumination on the sterols of D. minuta

A number of variables will alter the spectral composition of light in the marine water column. Weather conditions will determine the spectral composition of light incident upon the water surface. Then, except in the immediate surface layers, this incident light will be subjected to selective absorption of certain wavelengths by suspended particulate matter both living and dead ('blue' wavelengths mainly), dissolved organic matter (range of wavelengths) and the water molecules themselves ('red' wavelengths), e.g. see Jerlov [31]. It is therefore difficult to know with any certainty the normal light conditions experienced by a particular field population of marine algae and hence hard to mimic these conditions for laboratory cultures. It is certainly a formidable problem to determine the important wavelengths responsible for controlling the major cellular biochemical/physiological processes in marine algal species (see for example Dring [32]).

As a preliminary attempt to ascertain the extent to which light quality might affect sterol composition in green algae, *D. minuta* was laboratory cultured under four light conditions (a) white; (b) white minus blue/green; (c) white minus blue/green and yellow and (d) white minus blue/green, yellow and amber (Fig. 1). The cells were harvested in the stationary phase of growth and as very small quantities of material were obtained the analyses were performed by GC only.

The results (Table 4) should first be compared with the

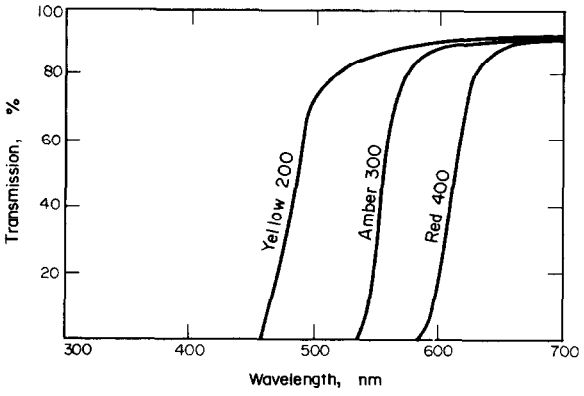

Fig. 1. Spectral transmission of the acrylic filters (3.2 mm thickness).

results for the stationary growth phase of *D. minuta* (Table 3) where a different type of white light was employed. The sterols, which were produced under the different white sources, were identical but there were very interesting differences in the amounts of two sterols. The warm white fluorescent lamp produced a higher relative percentage of cholesterol (36% of total sterol) than the CSX lamp (8% of total sterol), whereas the CSX lamp produced relatively more 24ϵ-methylcholesta-7-en-3β-ol, (36% of total sterol) than the fluorescent lamp (12% of total sterol). The relative percentages of all other sterols were substantially unchanged. Hence it appears that the differences in irradiance and spectral composition of the white light have produced a marked change in the quantities of these two major sterols.

The results (Table 4) of the experiments carried out using the filters show that the different spectral composition seems to have very little effect on the sterols produced by *D. minuta*. Minor differences were observed in that the relative percentage of cholesterol decreased under amber light whilst that of 24-methylcholest-7-en-3β-ol increased under red light. Also the relative percentage of 24-ethylcholest-5-en-3β-ol was lower under yellow light but higher under red light. However, these changes were very minor compared with the effect of the two white sources.

Sterols of the brown alga

Four sterols, 24-ethylcholesta-5,22-dien-3β-ol, cholesterol, 24-ethylcholest-5-en-3β-ol and 24-methylcholest-5-en-3β-ol, comprise 98% of the sterols composition of *Monochrysis lutheri* (Table 5) when harvested in the exponential growth phase. Previous work on the *Ochromonas* species, which belong to the same class as *M. lutheri*, also reported 24-ethylcholesta-5,22-dien-3β-ol as the major sterol in *O. malhomensis* [33] and *O. sociablis* [34] and *O. danica* contained four of the five sterols identified in *M. lutheri*. However, it is surprising that none of the *Ochromonas* species have been found to contain cholesterol although *M. lutheri* has a considerable quantity of this compound in its sterol make-up.

The sterols, which were harvested during the stationary phase, were very complex, ten sterols being easily identified but there were also six unknown sterols which could not be positively identified (Table 5). Two of these were major sterols; sterol M-15 was thought to be a C_{29}

stanol with an extra hindered hydroxyl group in the nucleus and sterol M-12 was thought to be a C_{30} sterol with a saturated nucleus containing an extra nuclear methyl group and a Δ^{22} double bond.

A major difference between the extracts was that the stationary phase extracts contained a number of stanols comprising some 50% of the total sterol content, whereas stanols were not observed in the exponential growth extracts of *M. lutheri* or indeed in any of the other phytoplankton species examined, except in trace quantities of cholestanol in *D. minuta*. However, Nishimura and Koyama [35] have reported on high proportions of stanols being isolated from lake sediments and an associated diatom *Melosira granulata*.

Other differences in the two extracts related to the possible occurrence of oxygenated sterols and nuclear methylated sterols in the stationary phase extracts. The hydroxylated sterols may be produced by auto-oxidation and it is possible that they represent one of the first stages in the degradation of cholesterol are its conversion to 7-hydroxycholesterol and then to a 7,12-dihydroxystanol [36]. Sterols with nuclear methylation at positions C-4 and C-14 are biosynthetic intermediates in the production of the usual membrane sterols, but it is possible that the enzymes of the stationary phase cells are not functioning normally so that control over sterol biosynthesis is not so efficient and by-products are formed. It is possible that factors such as the exhaustion of essential nutrients and the build-up of toxic products produce a loss of metabolic control which results in the abnormal sterol compositions which are seen in these stationary phase cells.

There are no easily defined differences between the sterol compositions of the species of diatoms, green algae and brown algae analysed in this work, although the composition of the diatom species sampled 'in the wild' was far more complex than that of any of the cultured species, irrespective of family.

These results suggest that the sterol chemistry of diatoms is certainly complex and it is obvious that, for phytoplankton species as a whole, more work is necessary if the relative importance of inherent species difference as compared with growth conditions is to be evaluated in relation to sterol composition.

Comparison of the sterol composition of a species of diatom, green and brown algae, all sampled in their exponential growth phase and their stationary growth phase, suggests that the stage of growth/culture age may be a very important factor in determining the sterol composition of a phytoplankton species. Thus the stage of growth must be considered in any attempt to relate sterol composition with phytoplankton species type. From the present work, culture age appears to be especially important with respect to the species of green algae examined (increase in C_{27} sterols at the expense of C_{28} sterols with increased culture age) and the species of brown algae examined (C_{26} and C_{27} sterols being replaced by higher molecular weight sterols, stanols and hydroxylated compounds), although the diatom exhibited little change in sterol composition with culture age.

In the analysis of component sterols from phytoplankton, care must be exercised in order to discriminate between 'free sterols' and 'bound sterols' as esters as the spectrum of sterols in the two fractions may be quite different.

No major changes were seen in the sterol compositions of the green algae *D. minuta* as the blue-green, yellow and finally amber parts of the continuous light spectrum under which it was grown were removed. Unfortunately, insufficient material was available to quantify the total sterols produced during the experiment so it has not been possible to relate this work with that of Boutry *et al.* [15] who found that for a diatom the presence of UV light resulted in an increase in the levels of total sterol. What is of interest, however, is a comparison of the sterol compositions found in the stationary phase of *D. minuta* grown under the 'warm white' fluorescent light with those found when the organism was grown under the xenon continuous light spectrum. A major change in composition has occurred with a switch between 27C C sterol in the former (minimal red and blue light) and 28C 7 sterol in the latter (continuous spectrum).

The total light intensity in the photosynthetic range which was received by the two cultures was different (1200–2000 $\mu W/cm^2$ for the former and 4770 $\mu W/cm^2$ for the latter) although the intensities are essentially in the same order of magnitude. They are both considerably less than bright sunlight (*ca* 3×10^4 $\mu W/cm^2$) but are in the range expected for the upper few metres of the oceanic water column. The work of Orcutt and Patterson [9] suggests that levels of total sterol in a marine diatom were not affected by changes in light intensity although, of course, this does not mean that qualitative changes could not occur. Thus the variation in light intensity may have been an important factor contributing to the observed changes in sterol composition.

The two cultures were also subjected to different light regimes, the former was essentially without either blue or red light whereas the latter received a continuous light spectrum. The results of the controlled light experiment suggest that removing the blue-green wavelengths does not affect the sterol composition of this green algae. Therefore it would appear that the absence of red light could be the other important factor responsible for the observed differences in sterol composition.

From these results it is clear that a careful evaluation must be made of (a) the effects of the changes in light intensity and (b) the effects of qualitative changes in the light spectrum on the sterol composition of cultured marine phytoplankton.

EXPERIMENTAL

Diatoms. Phaeodactylum tricornutum. The cells are solitary, or sometimes united in chains. It is a marine and brackish water species. The organism was laboratory cultured on two occasions, once being harvested in the exponential phase of growth after 6–7 days, and once in the stationary phase after 11–12 days.

Skeletonema costatum. The cells are lens-shaped with parallel spines around the margins which serve to unite the cells into straight chains. It is a very common pelagic diatom. It was laboratory cultured and harvested during the stationary phase of growth after 11–12 days.

Thalassionema nitzschioides. The rod-like cells unite to form stellate or zig-zag colonies. It is a common neritic species and was not laboratory cultured, but was collected by net during a biological cruise of the RRS Discovery at a depth of 30–37 m at a location 44°05′ N 12°44′ W on 9 April, 1975. The purity of the diatom sample was estimated as *ca* 98% of a single species. The sample was kept frozen at −30° in an atmosphere of N_2 until analysis.

Green algae: (division Chlorophyta). Danaliella minuta is a

motile, unicellular biflagellate which lacks a true cell wall and was cultured under ordinary illumination on two occasions, once being harvested during the exponential growth phase and once during the stationary phase. This organism was also grown under several different illumination conditions before being harvested in the stationary growth phase after 11–12 days.

Tetraselmis tetrathele. This is a flagellate and is characterized by one or two plate-like scales on the flagella. The organism was laboratory cultured and harvested during the stationary phase of growth after 11–12 days.

Brown algae: (division Chrysophyta). Monochrysis lutheri. This is a unicellular flagellate alga. It was laboratory cultured on two occasions, once being harvested during the exponential phase of growth after 6–7 days and once during the stationary phase after 11–12 days.

Culture conditions. Standard illuminations. Two species of diatom, two species of chlorophyte and one species of chrysophyte were grown in 5 l. Guillard's medium f/2 [37] under continuous white light (Mazda, Osram Cryselco 65–80 W, warm white fluorescent tubes) at an intensity of 1200–2000 $\mu W/cm^2$ and at 18 $\pm$ 2°. This light source was known to give over 95% of its light output in the 510–660 nm range, output in the blue/green (350–510 nm) range being low.

Different illumination conditions. One species of chlorophyte was grown in 4 identical cultures (f/2 medium, 1.5 l.) at 17 $\pm$ 3° and each culture was subjected to a different light regime. The light source was a Phillips Xenon CSX ozone free 450 W lamp with a continuous emission spectrum in the visible range (350–700 nm) closely resembling natural daylight. The lamp was mounted in a ~1 m square housing with reflectors and mirrors directing the light beneath a plate glass table divided into 4 equal sections. Squares of translucent, coloured acrylic (3.2 mm, ICI Perspex, yellow 200, amber 300 and red 400) were used to modify the light incident onto 3 of the 4 sections (Fig. 1). Light intensities, as measured with a logarithmic irradiation meter [38], were as follows: white (unfiltered), 4770; yellow, 3830; amber 3480 and red, 1250 $\mu W/cm^2$. The cultures were sampled after 11 days in the stationary phase.

Extraction of the sterols. The total lipids were extracted with $CHCl_3$–MeOH (2:1) and the free sterols and steryl esters were isolated by PLC as previously described [39].

GC–MS investigation and quantification of the sterol mixtures. The sterol fractions were converted to their TMSi ethers and subjected to GC–MS analysis on Dexsil 300GC, Silar 5CP and PZ-176 columns, and the sterols identified and quantified as previously described [21, 40–42].

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