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Chlorophyll and carotenoid pigments in Foraminifera and their symbiotic algae: analysis by high performance liquid chromatography

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ABSTRACT: High performance liquid chromatography has been used to examine the chlorophyll and carotenoid pigments of 11 species, representing 10 genera and 5 orders, of Foraminifera. The presence of symbiotic algae or their chloroplasts in a species is indicated by a high concentration of chlorophyll *a* in relation to its degradation products. The classes of algae present are indicated by the class-specific carotenoids. Both chlorophylls and carotenoids are used in an analysis of the diet of Foraminifera. The method has been successfully applied to a single organism of the species *Elphidium williamsoni*. No significant amounts of pigments were found in *Allogromia laticollaris, Jadammina macrescens, Trochammina inflata, Quinqueloculina dimidiata* and *Ammonia tepida*. *Elphidium oceanense* contains no symbiotic algae but concentrates carotenoids from either algal or animal sources. The pigments found in *Amphistegina lessonii* and *Globigerinoides ruber* are consistent with the known symbionts, a diatom and a dinoflagellate respectively, of these 2 species. The pigments of *E. williamsoni, Haynesina germanica* and *Reophax moniliformis* indicate the presence of symbiotic diatoms or their chloroplasts. *R. moniliformis*, which also concentrates beta-carotene, is the first member of the agglutinating order Lituolida in which symbiosis has been demonstrated. The amount of chlorophyll *a* per individual varied between 27 ng and 0.6 ng, and is related to the size and immediate past history of the specimens examined as well as to the number of chambers containing symbionts.

INTRODUCTION

The symbiosis between Foraminifera and unicellular algae has been reviewed by Lee & McEnery (1983), by Lee (1983) for the so-called larger Foraminifera, and by Bé (1982) and Hemleben & Spindler (1983) for planktonic species. Algal symbionts of the larger Foraminifera have been studied extensively by Lee and coworkers while the life history of at least 1 host, *Heterostegina depressa*, and the means of inheritance of its symbionts by megalospheric juveniles has been elegantly determined by Röttger (1974), Röttger & Spindler (1976) and Röttger & Inst. wiss. Film (1984). Algae belonging to the classes Bacillariophyceae, Dinophyceae, Chlorophyceae and Rhodophyceae have been found in symbiotic association with larger Foraminifera (in spite of the difficulties of determination when diagnostic features such as the flagella of dinoflagellates and the frustules of diatoms are not formed in the host cytoplasm).

Certain planktonic Foraminifera, such as *Hastigerina pelagica*, are commonly associated with large dinoflagellates which live outside the host's test but within the bubble capsule. These are believed to be commensals which are nourished from the diet of their carnivorous hosts. Other planktonic Foraminifera, for example *Globigerinoides sacculifer*, are associated with hundreds of smaller dinoflagellates which spend the daylight hours outside the test and the hours of darkness within it. Such algae are in symbiotic association with the host (Bé et al. 1977, Spindler & Hemleben 1980, Bé 1982). Lopez (1979) added a new dimension to the subject in her demonstration that *Elphidium williamsoni* and *Haynesina germanica*, 2 brackish water species, contain functioning algal chloroplasts but no signs of the rest of the algal cells.

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A quantitative knowledge of the individual pigments of Foraminifera would enable an unambiguous differentiation to be made between animals containing symbiotic algae and herbivorous animals. In the former group such knowledge could also be used in the determination of the endosymbionts (by reference to class-specific pigments), in an assessment of the potential primary production by the symbionts, and to gain an indication of the extent, if any, to which symbiont-bearing species degraded their symbiotic algae. In herbivorous animals the nature of the algal diet could be determined.

Attempts to determine the nature of the pigments have been made by Lee & Zucker (1969) and Lopez (1979) who made a spectroscopic examination of crude aqueous extracts. Lopez (1979) also analysed the extract by thin layer chromatography (TLC). Such studies are limited by the large number of specimens needed for TLC or absorption spectroscopy and their results are limited by the poor differentiation between pigments and by the fact that they are at best semi-quantitative.

We have applied the high performance liquid chromatographic (HPLC) method of Mantoura & Llewellyn (1983) to a study of the chlorophyll and carotenoid pigments in members of 5 orders of Foraminifera (see Haynes 1981 for a classification of Foraminifera and Lee 1980 for a classification of algae). The increase in resolution provided by this method has allowed us to differentiate between various chlorophylls and their degradation products, to resolve a number of carotenoid pigments and to identify some of them. The increase in precision of the HPLC method has enabled us to quantify the pigments better than has hitherto been possible. Moreover, the sensitivity of the method has made it possible for us to quantify the chlorophyll pigments in a single foraminifer.

MATERIALS AND METHODS

Sources and treatment of Foraminifera. The species *Allogromia laticollaris, Reophax moniliformis, Trochammina inflata, Jadammina macrescens, Quinqueloculina dimidiata, Elphidium williamsoni* and *Haynesina germanica* were from the surface mud, both exposed and from the bottom of shallow pools, of a salt marsh in Wacker Lake, a creek off the River Lynher near Antony, Cornwall, UK (Ordnance Survey reference SX 388 550). The mud was gently sieved in seawater collected from beyond the breakwater in Plymouth Sound. This medium was chosen because of the impossibility of securing in all cases adequate quantities of the water to which the specimens were exposed at the time of collection; moreover there could be both rapid and wide variations in salinity throughout a day on the saltmarsh in which the Foraminifera flourished. The fraction between 660 and 90 μm was used. Residues on the sieves were transferred with seawater to Petri dishes and from them Foraminifera were picked by soft-bladed forceps or glass pipettes and placed in Millipore-filtered (0.22 μm) seawater where adherent material was removed with a fine brush. Foraminifera were not used if visible adherent material could not be removed from them. Specimens were washed in a second batch of seawater and only those judged to be living were transferred by forceps to 40 μl of 90 % v/v aqueous acetone contained in a conical polythene vial which was then capped and stored at −18 °C. The vials (Wiedenmann-Plastik AG, Switzerland) were 28 mm long (internal) and consisted of a cylindrical section of length 10 mm and I.D. 6 mm followed by a conical section 18 mm long and tapering to 0.5 mm (I.D.).

Ammonia tepida was from a salt marsh at the mouth of the River Otter, Budleigh Salterton, Devon, UK (Ordnance Survey Reference SY 073 821) and was treated as above. *Amphistegina lessonii* was provided by Professor Rudolf Röttger from clone cultures of specimens deriving from Hawaii (Röttger et al. 1980). The filamentous green alga to which the Foraminifera were adherent was removed, the specimens were washed in filtered seawater and then placed in acetone as above.

An improvement to the technique was made in the case of *Elphidium oceanense* (which was also from the salt marsh in Wacker Lake) cleaned specimens of which were transferred to the vial which contained filtered seawater. When sufficient Foraminifera had been collected the seawater was removed and replaced by 40 μl 90 % v/v aqueous acetone. Specimens of *Globigerinoides ruber,* a spinose planktonic foraminifer, were provided by the late Dr. Allan Bé and were collected by means of nets in October 1983 from a point 2 miles off Port Everglades, Florida, USA.

Extraction of pigments. The following manipulations were carried out away from direct sunlight. Immediately prior to HPLC the pigments were extracted from the benthic Foraminifera by crushing and stirring the specimens which were concentrated at the bottom of the vial, with repeated strokes of a glass rod. The vial was briefly centrifuged (730 g, for 3 min), the supernatant removed into a Hamilton syringe previously wetted with 90 % acetone, and its volume recorded. The efficiency of this first extraction was typically about 90 %. To the vial was added a further 50 μl of 90 % acetone and the crushing, mixing and centrifugation repeated. The 2 extracts were pooled and mixed with 30 μl of ion-pairing agent (Mantoura & Llewellyn 1983). The

efficiency of this procedure in extracting chlorophyll and carotenoid pigments was determined by making a second extraction in an identical manner to the first and comparing the yields. Pigments with the shortest retention time on HPLC (such as chlorophyllide *a*) were extracted with greater efficiency than were the less polar substances (such as β-carotene) which had a longer retention time. In the case of *Globigerinoides ruber* no second extraction was carried out. A known volume (80 to 100 µl) of the mixture was then injected into the chromatograph *via* a 100 µl loop for the analysis of pigments.

HPLC. Chlorophyll and carotenoid pigments were analysed by reverse-phase ion-pairing HPLC using the method of Mantoura & Llewellyn (1983), modified as follows: (1) a 100 × 5 mm column containing 3 µm ODS-Hypersil was used instead of the less efficient 5 µm packing; (2) the 0 to 100 % gradient elution was carried out in 5 min instead of in 10 min; (3) the secondary eluent was 40:60 (instead of 20:80) (v/v) acetone : methanol. The identity and purity of pigments were confirmed by comparison of retention times with those of available standards run at the same time and by absorption spectroscopy using an in-line Hewlett-Packard 1040A diode array HPLC detector. Using these improvements, the detection limits (defined as signal to noise ratio = 2) for chlorophyll *a* and typical carotenoid were 0.16 ng and 1.1 ng respectively.

RESULTS AND DISCUSSION

Allogromia laticollaris, Jadammina macrescens, Trochammina inflata, Quinqueloculina dimidiata, Ammonia tepida

Jadammina macrescens was collected in August 1983 and ranged in diameter from 190 to 330 µm; *Quinqueloculina dimidiata* was collected in November 1983 and ranged in length from 190 to 340 µm, in width from 100 to 150 µm. The 5 species representing 5 genera and 4 orders of Foraminifera contained such small amounts of photosynthetic pigments and their degradation products, that quantitative conclusions must be drawn with care. In all 5 cases (Table 1) degradation products of chlorophyll *a* were measured in excess of the amount of chlorophyll *a* itself, but we do not deduce that the Foraminifera were necessarily responsible for the degradation as such small quantities may have been present in plant debris which had not, in spite of our best endeavours, been removed from the test. This is especially relevant in the case of *Trochammina inflata* (collected in Aug 1983, diameter 350 to 510 µm) which in life harbours a plug of debris which commonly covers the umbilical side of the test and fills the deep umbilicus. Our specimens of *Allogromia laticollaris* (collected in Nov 1983) were grey and showed no sign of the reddish-orange colour of the

Table 1. Chlorophyll and carotenoid pigments in 11 species of Foraminifera. Chlorophylls: Ch *a*, chlorophyll *a*; Chide *a*, chlorophyllide *a*; Phide *a*, phaeophorbide *a*; Phtin *a*, phaeophytin *a*; Ch *a* all, chlorophyll *a* allomer; Ch *b*, chlorophyll *b*; Ch c_1+c_2, chlorophylls c_1+c_2. Carotenoids: PD, peridinin; FX, fucoxanthin; DDX, diadinoxanthin; NFX, neofucoxanthin; DTX, diatoxanthin; NX, neoxanthin; L, lutein; U1, unidentified carotenoid; U2, unidentified mixture of carotenoids; β-C, β-carotene. d, darker specimens; l, lighter specimens

Order and species	Number extracted	Pigment content per Foraminifer (ng)																
		Ch *a*	Chide *a*	Phide *a*	Phtin *a*	Ch *a* all	Ch *b*	Ch c_1+c_2	PD	FX	DDX	NFX	DTX	NX	L	U1	U2	β-C
Order Allogromiida																		
Allogromia laticollaris	23	0.03		0.10	0.02													
Order Lituolida																		
Jadammina macrescens	31	0.01		0.03														
Reophax moniliformis	207	0.65			0.03			0.07		0.32					0.42			3.15
Trochammina inflata	8	0.09	0.15	0.03														
Order Miliolida																		
Quinqueloculina dimidiata	43	0.02		0.03	0.01													
Order Rotaliida																		
Ammonia tepida	80	0.03	0.01	0.05				0.01		0.03								
Amphistegina lessonii	10	20.5	13.1	0.15		4.27		14.3		75.0	5.05	11.7	0.47					1.27
Elphidium oceanense	262	0.14		1.70	0.04										0.27	0.90	0.10	0.25
Elphidium williamsoni d	38	8.82	0.14		0.04	0.07	0.20	0.66	0.03	7.15	1.38	0.42	0.90	0.70				0.23
Elphidium williamsoni l	38	5.49	0.30	0.02	0.02	0.10	0.14	0.59	0.05	4.22	0.90	0.23	0.52	0.23				0.32
Elphidium williamsoni	1	26.54	0.16		0.08	0.36		2.00		15.7	4.88	2.58	1.52	1.78				1.35
Haynesina germanica	31	7.77	0.13	0.13	0.18	1.43		0.82		1.37	0.45	0.90	0.63	1.05				
Order Globigerinida																		
Globigerinoides ruber	12	5.83	0.02			0.34		0.66	3.23	0.43	1.15		0.10					

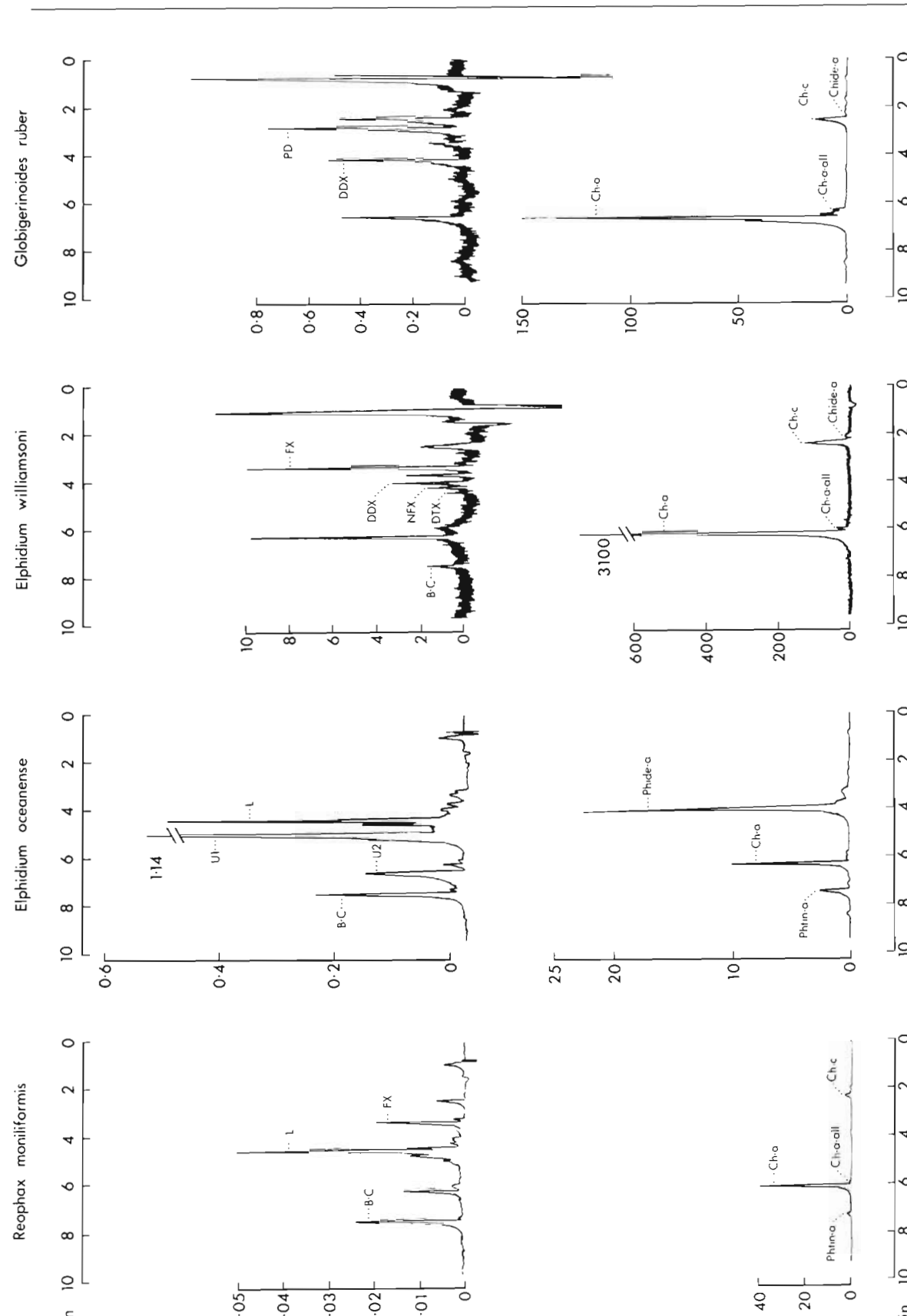

Fig. 1 High performance liquid chromatograms of pigments extracted from 4 species of Foraminifera. Ordinate scales adjusted to represent absorbance and relative fluorescence of single specimens in each case. Measurements of absorbance and relative fluorescence were made successively on the eluate from the chromatographic column. Chromatogram from *Globigerinoides ruber* made according to unmodified method of Mantoura & Llewellyn (1983), others made using the modifications described in the text. Chromatograms for the first 3 species are from the first of the 2 extracts (there was no second extract for *G. ruber*; see text). Noisiness of absorbance chromatograms for *Elphidium williamsoni* and *G. ruber* is related to the small number of specimens used (1 and 12 respectively, compared to 207 and 262 for *Reophax moniliformis* and *Elphidium oceanense*) and the consequent low attenuation used in making the recording. Abbreviations as in Table 1

specimens examined by Laatsch & Schwab (1984) and shown by them to contain 10 free or esterified carotenoids (including 3 previously undescribed as natural products). The small amount of fucoxanthin measured in *Ammonia tepida* (collected in May 1983, diameter 220 to 330 µm) represented less than twice the noise level of the spectrophotometer output and no conclusions can be drawn from it. However, the absence of significant amounts of chlorophyll *a* in any of the 5 species leads to the conclusion that none of them was in symbiotic association with algae or algal chloroplasts when examined.

In the remaining chromatograms much larger quantities of pigments were measured either because many Foraminifera were processed together or because the average concentrations per individual were high (Fig. 1).

Reophax moniliformis

This species was collected in November 1983 and specimens ranged in length from 200 to 730 µm and in width from 100 to 150 µm. The average *Reophax moniliformis* contained 22 times as much chlorophyll *a* as it did of its only degradation product detected, phaeophytin *a*. It also contained a significant amount of chlorophylls c_1 + c_2 (which were not resolved). Chlorophylls are labile compounds which are easily degraded by both chemical and biochemical reactions. The presence of unchanged chlorophylls indicates therefore that *R. moniliformis* is in symbiotic association with algal cells or their chloroplasts and this is supported by the presence of the associated carotenoids, fucoxanthin and β-carotene. Chlorophylls *a*, c_1 and c_2, together with fucoxanthin and β-carotene occur in 4 classes of algae, namely Chrysophyceae, Prymnesiophyceae, Bacillariophyceae and Phaeophyceae. Living members of the Phaeophyceae (brown algae) do not occur at the site from which our foraminiferans were obtained and in any case there are no unicellular representatives of the Phaeophyceae. It is also known from the work of Joint (1978) that Prymnesiophyceae (coccolithophorids) are not abundant on the mud flats close to our site. The Chrysophyceae (golden-brown algae) are largely fresh water. Bacillariophyceae (diatoms) are abundant in the mud from which our foraminiferans were sieved and observations using scanning electron microscopy (R. K. unpubl.) have shown a close association between *R. moniliformis* and several species of diatoms, but an absence of diatom frustules within the tests of specimens fixed, critical point dried and broken open before examination by SEM. For these reasons we conclude that *R. moniliformis* contains symbiotic diatoms which have been removed from their frustules, or chloroplasts which have been removed from the diatoms. Both these conditions are known to occur in other Foraminifera (Lopez 1979, Lee et al. 1980). This is the first reported case of symbiosis in a member of the Textulariida, an order which contains Foraminifera with tests composed partly of particles collected from the environment.

Only 2 of the species examined contained lutein, *Reophax moniliformis* and *Elphidium oceanense*. Lutein is associated with Chlorophyceae (green algae) and with higher plants which also contain chlorophyll *b*. The absence of chlorophyll *b* and its degradation products from either species is consistent with the known persistence of lutein in detritus relative to the labile chlorophyll *b* (R.F.C.M., unpubl.). It is likely, therefore, that *R. moniliformis* feeds on the plant debris which is often seen covering the youngest chamber.

The amounts of chlorophyll pigments in *Reophax moniliformis* (an average of 0.65 ng chlorophyll *a* per foraminifer) are considerably less per specimen than those found in, for example, *Elphidium williamsoni*. However, *R. moniliformis* is smaller than *E. williamsoni*. Moreover, while pigments are never seen in the youngest chamber of either species, in *R. moniliformis* pigments are often absent from the youngest 2 or 3 chambers, and in many specimens are seen to be present only in the oldest half or third of the test.

This species showed more of both β-carotene (3.15 ng) and lutein (0.42 ng) per individual than any other. The oldest chambers of living *Reophax moniliformis* from our site are always yellow/orange when viewed by transmitted light and it is possible that β-carotene and/or lutein are stored by *R. moniliformis*.

Elphidium oceanense

This species was collected in January 1984 and specimens had diameters between 140 and 250 µm. Living specimens of *Elphidium oceanense* appear bright red when isolated from their environment and freed from the plant debris with which they frequently cover themselves. The major breakdown product of chlorophyll *a* in this species is phaeophorbide *a*, and the ratio of the amount of this pigment in *E. oceanense* to that of chlorophyll *a* is typical of that found in the faecal pellets of the copepod *Calanus helgolandicus* (Currie 1962, R.F.C.M. unpubl.). Both lutein and β-carotene are present but in proportions quite different from that found in *Reophax moniliformis*. The major carotenoid (an average of 0.90 ng in each average foraminifer) is represented by pigment U 1 (Fig. 1). This substance is pure as was shown by a plot of the spectrum of the material forming the chromatographic peak being coincidental with plots

on the upslope and the downslope of the peak. It has λ_{max} = 467 nm and, for the purposes of calculating its quantity, is assumed to have a specific extinction coefficient of 2000 ($E^{1\%}_{400\ nm}$, in a 10 mm cell) as suggested by Mantoura & Llewellyn (1983).

A group of less polar carotenoids which have not been resolved is also present in the extract of *Elphidium oceanense* (U 2 in Fig. 1). Making the same assumption as before about the value of $E^{1\%}_{400\ nm}$, they have together an average concentration of 0.10 ng foraminifer^{-1}.

The high value for the ratio of phaeophorbide *a* to chlorophyll *a* indicates that *Elphidium oceanense* is not in symbiotic relation with algae or algal chloroplasts. This foraminifer may be herbivorous and concentrate algal carotenoids (with or without previous chemical modification). The fact that it is frequently found covered in a thin feeding cyst supports this suggestion. Alternatively, the distinctive colour of this species may be in part at least derived from carotenoids obtained from, for example, *Calanus* spp. which are abundant in its immediate environment. In copepods such as *Calanus* the carotenoids may be free (and red) or in complexes with protein (when they are either blue or red). Certain Foraminifera are known to eat copepods (Bé et al. 1977, Anderson et al. 1979).

Elphidium williamsoni

These specimens were collected in November 1983. Diameters of tests of both light and dark individuals ranged from 270 to 380 µm while that of the single specimen was 450 µm. The colour of *Elphidium williamsoni* from our site is variable. Some specimens are dark green mottled with brown while others are light green, with or without brown mottling. The occasional specimen is pale cream and a few specimens have a quite strong orange or red colour neither of which is as intense as the red of *E. oceanense*. Seventy-six individuals were collected from 1 mud sample and were divided into 2 equal groups, darker and lighter green, which were analysed separately. A further single specimen of *E. williamsoni* was also analysed. One chromatographic run designed specifically to reveal the presence of echinenone was made with *E. williamsoni;* none was found.

The small amount of chlorophyll *b* present was about 2 % of the amount of chlorophyll *a* in both light and dark samples. No chlorophyll *b* was found in the single specimen. These small quantities might have derived from living members of the Euglenophyceae or Chlorophyceae contaminating our specimens. The absence of phaeophytin *b*, a degradation product of chlorophyll *b*, and of lutein shows that plant debris, which might have been attached to the tests, was absent.

The presence of substantial amounts of chlorophyll *a* and of lesser amounts of chlorophylls $c_1 + c_2$, together with considerable quantities of the xanthophyll, fucoxanthin, suggest the presence of one or more of the 4 classes of algae: Chrysophyceae, Prymnesiophyceae, Bacillariophyceae, and Phaeophyceae. For the reasons stated in the discussion of the results for *Reophax moniliformis,* and because of the absence of echinenone, we again conclude that the pigments derive from the diatoms (Bacillariophyceae). Only trace amounts of phaeophorbide *a* and phaeophytin *a* were present indicating that the chlorophyll *a* was not being digested and also suggesting the presence of healthy autotrophic algae or their chloroplasts. Chlorophyllide *a* which is also present in trace amounts may have been formed during the internal recycling of chlorophyll *a* and does not necessarily represent a stage in the permanent degradation of chlorophyll *a;* its presence does not imply that *E. williamsoni* digests the chloroplasts which it contains.

The analysis quoted for the single *Elphidium williamsoni* indicates the sensitivity of the method used. The specimen had a mean diameter of 450 µm and contained 26.5 ng of chlorophyll *a* and 15.7 ng of fucoxanthin. One-hundredth this amount of chlorophyll *a* would have been sufficiently above the background, as would one-sixth of the amount of fucoxanthin, to produce a useful analytical result. Thus, assuming that amounts of pigments were proportional to the volume of the Foraminifera, a single specimen with a mean diameter of about 100 µm would yield a useful value for chlorophyll *a* and one with a mean diameter of about 250 µm would be adequate for the analysis of the major class-specific carotenoid, fucoxanthin.

A comparison between our light and dark samples shows that the subjective sorting according to colour corresponded to real differences in pigment content of the 12 pigments estimated. The ratios of the concentrations in the dark and light specimens of 6 of the 12 pigments estimated were between 1.5 : 1 and 1.8 : 1. However, there was no such constancy of ratio between pigments in the single individual and the dark specimens. It is likely, therefore, that there is considerable variation between individuals in the ratios of the concentrations of the various pigments present in each and that this variation has been obscured by taking average values in 38 specimens.

Another feature worthy of note is the variation in the ratios of chlorophyll *a* to chlorophylls $c_1 + c_2$. In both the darker sample and in the single specimen this was about 13 : 1 whereas in the lighter sample the ratio was 8 : 1. This again may be no more than a reflection of the

foraminifer's ability to benefit from whatever algae are available to it (as suggested by Lee [1983] quoting unpublished work of Lopez) or, alternatively, it may represent a photophysiological response on the part of the algae to changes in the intensity and quality of the light.

The ratio between the amounts of chlorophylls and the major carotenoid, in this case fucoxanthin, in the diatoms is fairly constant. Our results imply that the carotenoids of the chloroplasts do not remain in them but are at least partly extracted by the Foraminifera and dealt with by metabolism, excretion or storage within the test, and that the degree to which this happens is variable. The lighter set of *Elphidium williamsoni* contained a small amount of peridinin as have other samples of this species, analyses for which are not reported here. The total amount present in the sample was 1.9 ng which is close to the limit of detection for the carotenoids. For this reason not too much weight must be placed on this result but peridinin is the class-specific carotenoid of the Dinophyceae (dinoflagellates). Because of the variations reported above it is likely that not all the 38 specimens which made up the sample contained peridinin but that this xanthophyll was concentrated in one or a few of the specimens. Murray (1963) showed that *E. crispum* had brown cytoplasm when fed with *Phaeodactylum tricornutum* and green cytoplasm when fed with *Tetraselmis suecica*. Lee (1983) reports the work of Lopez (in progress) as also showing that *E. williamsoni* is relatively non-selective in the algal source of its chloroplasts. Both of these observations are consistent with the present measurements.

Haynesina germanica

Specimens of *Haynesina germanica* were selected from a sample of mud which contained somewhat darker individuals than other samples. They were collected in November 1983, and the diameter of their tests ranged from 220 to 510 µm. There is a wide variation in the appearance of *H. germanica* from dark green mottled with brown through to pale cream but none has as yet been seen which matches the few specimens of *Elphidium williamsoni* which are orange or red.

The considerations which have been discussed above for *Reophax moniliformis* and *Elphidium williamsoni* lead us to conclude that *Haynesina germanica* contains viable diatoms or their chloroplasts. If it digests them at all it does so to a very limited extent as is shown by relatively low concentrations of the degradation products of chlorophyll *a*. Again we conclude that although the chloroplasts remain viable, the class-specific carotenoids and the accessory carotenoids do not maintain their concentrations relative to that of the chlorophylls, which they have in the chloroplasts of intact diatoms.

Amphistegina lessonii

Amphistegina lessonii were cultured in Kiel and demonstrated in London by Professor Rudolf Röttger who kindly allowed us to bring them to Plymouth. Their diameters ranged between 800 and 1100 µm. Some were still attached to filaments of the green alga *Cladophora socialis* var. *hawaiiana* while others were free, and they were transported in an insulated container in the dark in a plentiful supply of seawater. On arrival in Plymouth the Foraminifera were left overnight in the open container and processed early the next morning. They had thus been subjected during a period of 2 d to an unusual regime of temperature and light. The analytical results may, to some extent, be a reflection of their immediate past history of stress and an early opportunity will be taken to examine this species again, together with other larger Foraminifera under controlled conditions. The results are presented here as they differ significantly from those of the other species which contain symbionts, and are suggestive of the known effects of stress on algae (Jensen & Sakshaug 1973). *A. lessonii* from Hawaii is known to harbour the diatoms *Nitzschia frustulum* var. *symbiotica, Amphora roettgeri, Nitzschia laevis* and others (Lee et al. 1980, Lee & Reimer 1984).

The following points are noteworthy: (1) The ratio of the amounts of chlorophyll *a* to chlorophylls $c_1 + c_2$ is unexpectedly low. The commonly occurring ratio in algae is between 8:1 and 12:1 and in 14 samples of Foraminifera which we have examined, not all of which are reported here, and excluding *Amphistegina lessonii*, we have found an average ratio of 10:1, range 5.5:1 to 16.3:1. The present specimens of *A. lessonii* had a ratio of 1.5:1 which is raised only to 2.5:1 if we include chlorophyllide *a* with chlorophyll *a*. (2) The ratio of the amounts of chlorophyll *a* and fucoxanthin is the lowest from any samples we have examined, even if we include chlorophyllide *a* with chlorophyll *a*. (3) There is a high concentration of chlorophyllide *a* relative to that of chlorophyll *a*; this we ascribe to stress. (4) There is a higher than usual concentration of chlorophyll *a* allomer. (5) The ratio of diadinoxanthin to diatoxanthin is high and this may represent the effect of too little light. (6) Peridinin, the class-specific carotenoid of the Dinophyceae, is absent.

Points 1 and 2 are both suggestive of relative lack of chlorophyll *a* in our sample of *Amphistegina lessonii*, but the missing chlorophyll *a* is not represented as

degradation products. Qualitatively, the pigments found in *A. lessonii* are entirely consistent with the known presence in it of the symbiotic diatom, *Nitzschia laevis*.

An analysis of filaments of *Cladophora socialis* var. *hawaiiana* from which some of the specimens of *Amphistegina lessonii* had been removed showed the characteristic chlorophyll *b* and lutein of the Chlorophyceae. The absence of these 2 pigments from our analysis of these Foraminifera indicated that the specimens had been satisfactorily freed of algal filaments.

Globigerinoides ruber

Globigerinoides ruber differs from the other species examined principally in its high concentration of peridinin. This, together with chlorophyll *a* and only a minor amount of degradation product of chlorophyll *a*, indicates that living dinoflagellates are the main source of the pigments of this planktonic species. The relatively small amount of fucoxanthin does not derive from dinoflagellates but *G. ruber* is known not only to contain endosymbiotic dinoflagellates (Bé et al. 1977) but also to prey on both dinoflagellates and diatoms (Anderson et al. 1979).

CONCLUSIONS

Eight of the species examined in this study were from 1 source so it is not possible to comment on whether the occurrence of symbiosis amongst these species is widespread. However, future work using the present method would yield this information because the analysis of photosynthetic pigments in Foraminifera by HPLC is an extremely sensitive method for the determination of the chemotaxonomic status and the biomass (*via* chlorophyll *a*) of algal endosymbionts and their chloroplasts hosted by Foraminifera. In addition, knowledge of the cellular content of chlorophyll *a* and its degradation products can be applied in discriminating between the possible trophic associations (endosymbiotic, commensal, herbivorous) which exist between heterotrophic Foraminifera and photoautotrophic algae.

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