DoE Reference PECD 7/7/079
Automatic Identification and Enumeration
of Algae

Contract Extension Report
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CONTENTS

Introduction and programme of work

A good understanding of the population dynamics of algal communities is vital in many ecological and pollution studies of freshwater and oceanic systems. Present methods require manual counting and identification of algae and can take up to 90 min to obtain a statistically reliable count on a complex population. Several alternative techniques to accelerate the process have been tried on marine samples but none have been completely successful because insufficient effort has been put into verifying the technique before field trials. The objective of the present study has been to assess the potential of in vivo fluorescence of algal pigments as a means of automatically identifying algae. For this work total fluorescence spectroscopy was chosen as the observation technique.

The papers herein constitute the final report for extended contract PECD7/7/079 - 141-82, due to finish in September 1987. The full programme of work was as follows:

1. Modify the spectrometer as necessary for the investigation of total fluorescence spectra.

2. Develop computer programmes for the reduction of raw data.

3. Obtain replicate spectra for a series of different algae and compare differences statistically.

4. Obtain replicate spectra for a limited number of algae species under different stress regimes such as nutrient, light etc and study effects on spectra.

5. Provide a full report at the end of the investigation, to include recommendation for further development if appropriate.

The research programme

1. The modifications to the spectrometer are given in the final report (March 1986) to the initial contract. Minor modifications were made in the present year to enable more rapid capture of excitation spectra at a fixed emission wavelength.

2. Computer programs for initial data presentation were developed during the initial contracts and are given in final report (March 1986). New programmes have been written to carry out statistical analysis on the total data set. The objectives of these programs are discussed in appendix A.

3. Total fluorescence and mean excitation spectra at an emission wavelength of 680 nm were obtained for 33 algal cultures covering the seven most common algal groups in fresh waters. Spectra were normalised in four different ways and compared statistically to assess the information content of the spectra in their different normalised forms. It was concluded that all the algal groups could be identified with 95% certainty. Within groups some species could be separated from others. At this level, identification was more limited but probably still useable. These aspects are discussed more fully in Appendix A.

4. Replicates of 13 of the 33 algal cultures used were grown in both light and dark conditions. Most of the groups showed no spectral changes, except for increases in fluorescent output from low light cultures. Some blue green algae and all the chryptophytes showed major spectral changes associated with increased production of phycoerythryn in low light conditions. This behaviour can be used to give information on the recent light history of the cells and increases the power of separation of some groups. However, it may make it more difficult to actually name a species without some operator intervention. Details of this work are given in Appendix B.

The future

The results of this study have applications in two directions:

a) Simple field instruments for the location of blue-green algal maxima in lakes and reservoirs. Tastes, odours and toxic substances from blue-green algal blooms can be a serious problem to fish farmers and to operators of potable water supply reservoirs. At present there is no simple means of obtaining a warning that populations of these organisms are increasing, particularly if they form mid water horizons. Proposals for a further one-year extension to the present contract have been submitted to DoE in order to design and build an instrument capable of making such observations in the field. The design is likely to use an electronic flash gun as the source and photo diodes as detectors.

b) Fully automatic identification and enumeration of algae. For the last two years of this contract we have kept in close contact with a group at Strathclyde University under the direction of Dr Alex Cunningham. They have developed a flow cytometer (an instrument which measures the optical properties of individual cells, removing the deconvolution problem) specifically for use in algal identification studies. The results of our fluorescence studies have given an insight into the reasons for the limitations of earlier attempts to combine the techniques and show the way forward. We are now working together to find funds from industry to develop and test a final pre production model of the proposed instrument.

Conclusions

i) The information contained in the in-vivo fluorescence (em 680 nm) excitation spectrum of algae is very high. All algal classes can be separated with a 95% certainty and most with a 99% certainty.

ii) Changes in environmental conditions can change the fluorescence spectrum. This is not a disadvantage when sorting algae on the basis of their fluorescence properties and in fact increases the power of separation. However, it does mean that an individual species will have a range of fluorescence spectra depending on their post environment which will make it more difficult initially to automatically name algae with these properties.

iii) The results of this study can be applied to the development of both simple field instruments, capable of identifying blue-green algal blooms and sub surface maxima, and to sophisticated flow cytometers with the ability to count, size and identify the components of mixed algal populations.

Recommendations

a) Funds should be made available for the one-year extension in order to produce a simple, robust field instrument to identify blue-green algal blooms in situ.

b) Although funds are being sought from industrial sources for the development of a flow cytometer based system, it may be necessary to top them up with LINK funds to enable work to proceed. Some involvement by the new NRA would also be helpful, particularly, by transferring the DoE's insight of the potential for such an instrument to future users in the water authorisation.

Appendix A

Algal identification using in vivo fluorescence spectra

Introduction

For many years attempts have been made to identify algae automatically using their fluorescence characteristics. Two main techniques have dominated: the algal ratio techniques of Yentsch et al. (1979, 1984, 1985a,b), particularly when used with flow cytometry (Yentsch et al., 1983, 1984), and total fluorescence spectral recognition (Oldham et al., 1985). Work to date has been carried out on marine algae with some encouraging results but recently, Hilton et al. (in press) have shown that the picture for freshwater blue-green algae and cryptophytes is much more complex than was apparent from other work. Photoadaptation can have a major effect on algae from these groups, changing the pigment content, and hence the fluorescent response, enormously. This is not necessarily a disadvantage. A more serious problem is the implication from marine work that some classes cannot be distinguished from each other, e.g. diatoms and chrysophytes; green algae and xanthophytes, etc. In this paper we will use the excitation spectra of a range of freshwater planktonic algae to assess the potential for differentiation between algal species using their fluorescence spectra.

Methods

Excitation spectra were recorded at an emission wavelength of 680 nm on a Perkin Elmer 201 fluorescence spectrometer which had been modified 'in-house' to enable control of excitation and emission wavelengths by computer controlled stepping motors. The large capacitors on the instrument output were disconnected to facilitate rapid response to the photomultiplier output. Signal to noise was maintained by using the A/D convertor to integrate over short time periods of about 40 mSec. In vivo spectra were run of suspensions of 30 algal cultures covering planktonic classes commonly occurring in freshwaters. Culture conditions are given elsewhere (Hilton et al., in press). A full spectrum over the excitation range 220-780 nm in approximately 3 nm steps (instrument band width = 10 nm) was collected and stored on disc in about 30 seconds. Five separate replicates of each dark adapted culture were obtained and the mean and standard deviation of the intensity at each wavelength step was calculated. All further analyses were carried out using these parameters.

In order to obtain an estimate of fluorescence per cell in each culture, the instrument gain was calibrated using a standard perspex block containing rhodomine. Turbidity was measured on a Turner Designs, Model 40, Nephelometer and manual counts of cell concentrations were made using standard methods (Lund et al., 1958). The appropriate choice of statistical techniques used to assess significant spectral differences will be discussed later.

Results

The spectra were normalised in four different ways: (a) the maximum intensity in a spectrum was made equal to 100% and the rest of the spectrum normalised accordingly. This will be referred to as max. intensity normalisation; (b) the

intensity at a constant wavelength, in this case 450 nm, was made equal to 100% and the rest of the spectrum normalised accordingly. This will be referred to as wavelength normalisation; (c) intensities were rescaled so that they were all reported at the same instrumental sensitivity and then divided by turbidity to give a surrogate estimate of fluorescence per cell; (d) intensities were rescaled as in (c) and divided by the concentration of cells per ml. For the rest of this paper, (c) and (d) will not be differentiated as the results were the same and will be referred to as fluorescence per cell.

Average max. intensity normalised spectra from five replicates of each of the 30 algal species used in this work are given in figure 1. Although a stirrer was used when necessary to reduce settling, the inhomogeneity of large colonial algal suspensions increased the variance of estimates of mean intensities for these cultures. The large range of variances, many of which were significantly different from others, excluded the possibility of using analysis of variance on the original replicates. After trying a number of potentially useful methods, two different techniques were chosen for use in the analysis. The intensity difference at each wavelength, for all pair combinations from two algal groups, were tested for significance using Welch's Test (Cooper, 1969). The data sets were then combined to show regions where all pairs were not significantly different. This technique was used on both normalised and per cell fluorescence spectra. The results of analysis of normalised spectra using this technique will not be shown here as they were the same as results from the more rigorous second test. For max. intensity and wavelength normalised spectra the mean spectrum for each group was calculated with appropriate variances and the significance of intensity differences at each wavelength was estimated using Welch's Test.

Spectral comparisons were only made in the region 360-670 n, to exclude interference from primary and secondary scatter, but the amount of information outside this region was limited. In all cases presented here, high light conditions (100 µEinst. $m^{-2}.s^{-1}$) were used to culture algae so that phycoerythrin fluorescence, ex. 550 nm, em 580 nm, was not observed in either cryptophytes or blue-green algae, i.e. the worst case for algal identification. Regions of significant differences are indicated in Table 1 for maximum intensity normalisation, Table 2 for wavelength normalised data and Table 3 for fluorescence per cell. Max. intensity and wavelength normalisation showed essentially the same patterns. Spectral comparisons using fluorescence per cell showed different discrimination ranges compared to max. intensity and wavelength normalisation. Many differences between groups which were apparent using the latter two representations were no longer significant, but within groups many species were distinguishable from other species where max. intensity and wavelength normalisation revealed no differences. The majority of algal classes studied could be differentiated over at least one reasonably wide wavelength range.

Discussion

The data sets produced in this work can be used to assess the effectiveness of some automatic identification strategies. Yentsch et al. (1979, 1985) have suggested that the Chlorophyll:Accessory Pigment (CAP) ratio, i.e. F (ex 530 , em 685) divided by F (ex 450, em 685), where F is the fluorescence intensity, is a good indicator of algal class in samples of marine algae. Ranges of the CAP estimated from the averaged spectra of species used in our study are given

in Table 4. Four of the seven ranges are reasonably close: the green algae; the xanthophytes which are not distinguishable from them; the chrysophytes excluding the data from Syncrypta globosa, and the blue-green algae (there is some confusion in Yentsch's papers as to whether the CAP for blue-greens will be very low or very high. From their own reasoning it should be greater than 1, tending to infinity). The observed range differences are not due to chromatic adaptation (Hilton et al., in press) and at this stage the only conclusion is that freshwater algae cannot be uniquely classified by a single ratio of fluorescence intensity stimulated at 450 nm and 530 nm.

Both maximum intensity normalisation and wavelength normalisation show essentially the same level of differentiation between groups. All the pairs can be distinguished in one or more wavelength ranges except for green algae from xanthophytes. Distinctions are less clear (only 95% probability) for chrysophytes from xanthophytes, diatoms from chrysophytes and diatoms from xanthophytes. The spectrum can be split into 6 regions (+ a region 435-460 nm which contains the normalisation wavelength). The regions which differentiate the algal groups are shown for the wavelength normalised spectra in Table 5 (max. intensity normalisation shows the same patterns). Closer inspection reveals that four of these regions actually carry redundant information, so that the minimum number of ranges required for maximum group differentiation is two: 360-390, 510-580, of which only two pairs of algae, blue-green vs. cryptophyte and diatom vs. chrysophyte, require the range 360-390. Apparently, even for freshwaters, Yentsch et al.'s choice of ex 530 nm, em 685 should be very discriminating, although the CAP ranges may not be applicable. Hence, using wavelength normalisation, the comparative range (435-460 nm) and two other ranges (360-390, 510-580) should be sufficient to differentiate the maximum number of groups, although inclusion of extra groups would probably improve the clarity of group separation and may even give some separation of species within groups. Max. intensity normalisation, on the other hand, would require information in all ranges so as to identify the spectral maximum prior to normalisation.

Per cell fluorescence, or in our case surrogate measurements, are apparently much less discriminating than ratio methods (Table 3). However, it is possible to distinguish phycocyanin-rich blue-green algae from other groups because of the low fluorescence output after excitation in the region 400-500 nm, and the xanthophytes are apparently more easily distinguished from chrysophytes. But many pairs which were differentiated by ratio techniques are no longer significantly different. On the other hand, it is now apparent that there are regions of significant difference within algal classes, something which was not recorded in Tables 1 and 2 as no significant differences were found in the ratio tests. Apparent from the data from blue-green algae these results may be slightly misleading. The fluorescence per cell is not just a function of algal class or species but reflects the long and short term light history of the algal cells. Algae are known to increase the amount of pigments per cell (Beers et al., 1975; Kiefer et al, 1976) when grown under low light conditions and the cultures used here showed the same effects (Hilton et al., in press). Similarly, the chloroplasts can move within the cell in response to short term changes in light intensity with consequent changes in fluorescence output (Kiefer, 1973a,b; Loftus and Seliger, 1975; Heaney, 1978). However, except for some blue-green algae and the cryptophytes (Hilton et al., in press) we have observed no changes in the pigment content of algal groups in response to light intensity changes so that, in these cultures, light history appears to effect

the absolute light output but not the spectral distribution of stimulation. This is consistent with our observation that all the algal species studied here showed an overall increase in output but no spectral changes with the addition of DCMU (Hilton, unpublished results). It is likely that many of the significant differences within classes are due to differences in light history, caused by differences in handling, rather than by class or species differences.

It is unlikely that algal fluorescence spectroscopy, either excitation or total, will be used successfully by itself to identify the algal class or species in a natural mixed algal population. The peaks tend to be broad and it would be difficult to deconvolute the spectra of the algal species making up the population. Oldham et al. (1985) could not distinguish the components of a two species culture except at either end of the mixture range as the constituents approach a monoculture. If cells can be separated and analysed individually, as in a flow cytometer, then fluorescence becomes a powerful tool in identification (Yentsch et al., 1983; 1984). Under these circumstances, per cell fluorescence can be used to identify blue-green algae and split other species into arbitrary groups depending on their light history. If it is possible to obtain an estimate of the excitation spectrum of each algae, then the majority of the algal classes can be separated with a 99% certainty using a minimum of three wavelength bands, including one excitation band, and all classes with a 95% certainty. Although completely automatic counting and identification of algal populations remains a long term aim, the combination of flow cytometry with fluorescence excitation spectra should allow a very efficient means of subdividing mixed populations for counting, descriptions of each group being sufficiently accurate that identification by an operator is much simplified.

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C.M. Yentsch, L. Cucci, D.A. Phinney (1984) Flow cytometry and cell sorting: Problems and promises for biological ocean science research. In: Lecture Notes on Coastal and Estuarine Studies. Vol. 8. Marine Phytoplankton and Productivity. O. Holm-Hansen, L. Bolis, R. Gilles (Eds). Springer-Verlag, 141-155.

C.S. Yentsch, A. Phinney (1984) Observed changes in spectral signatures of natural phytoplankton populations: the influence of nutrient availability. In: Lecture Notes on Coastal and Estuarine Studies. Vol. 8. Marine Phytoplankton and Productivity. O. Holm-Hansen, L. Bolis, R. Gilles (Eds), Springer-Verlag, 129-140.

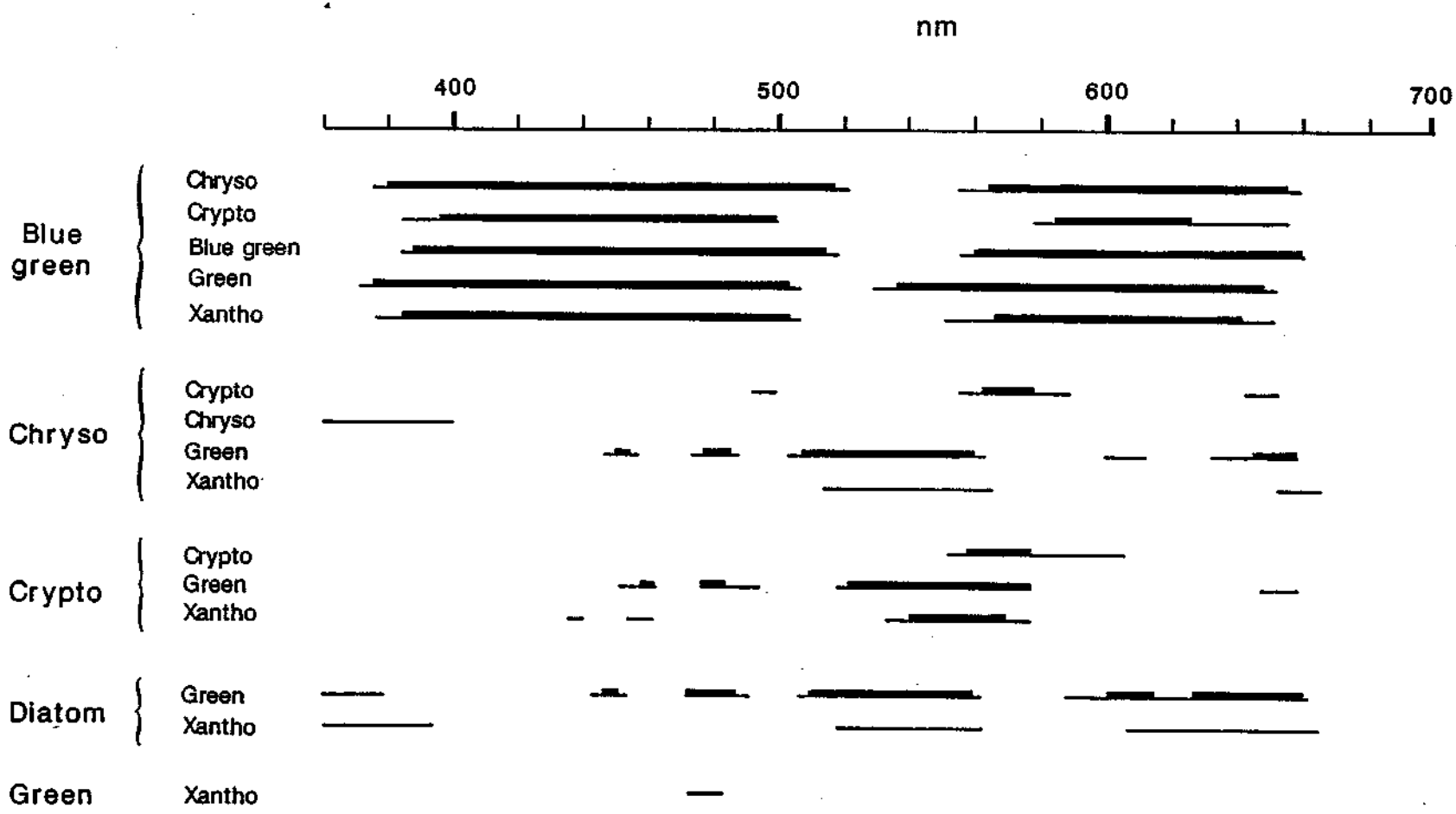
C.S. Yentsch, D.A. Phinney (1985) Spectral fluorescence: an ataxonomic tool for studying the structure of phytoplankton populations. J. Plankton Res. 7; 617-632.

C.S. Yentsch, D.A. Phinney (1985) The use of fluorescence spectral signatures of marine phytoplankton, In: Mapping Strategies in Chemical Oceanography. A. Zimino (Ed), 259-274.

Table 1. Regions of significant differences between mean spectra of algal groups after max. intensity normalisation. Thick lines denote 99% and thin lines 95% probability level.

Table 2. Regions of significant differences between mean spectra of algal groups after wavelength normalisation at 450 nm. Thick lines denote 99% and thin lines 95% probability level.

Table 3. Regions of the fluorescence excitation spectrum, normalised to fluorescence per cell, where all pair combinations of algal species in a group comparison show significant differences with at least 95% probability.


nm
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500
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700
Blue green
Chryso
Crypto
Blue green
Green
Xantho
Chryso
Crypto
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Crypto
Green
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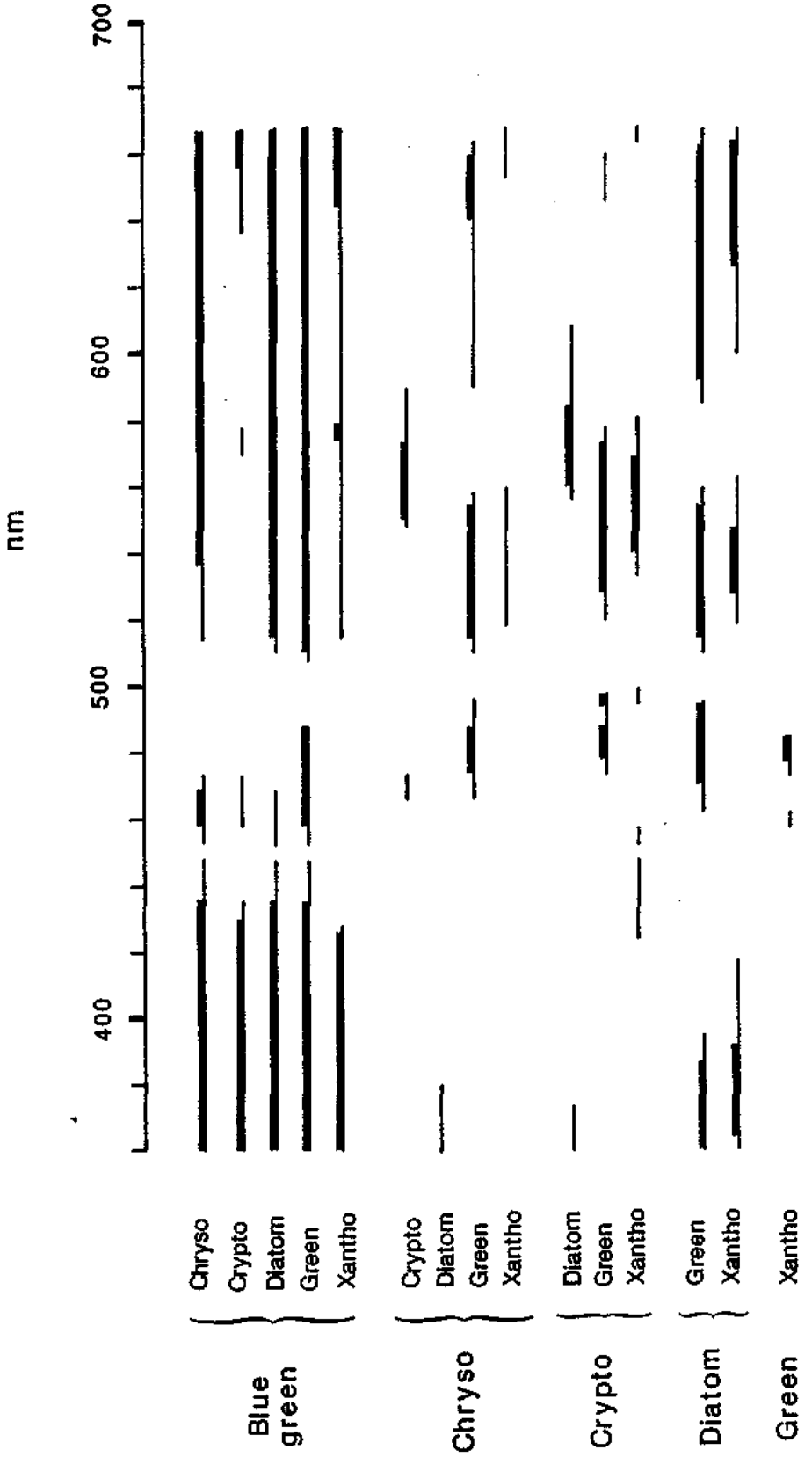
Diatom
Green
Xantho
Green
Xantho

Table 1

Table 2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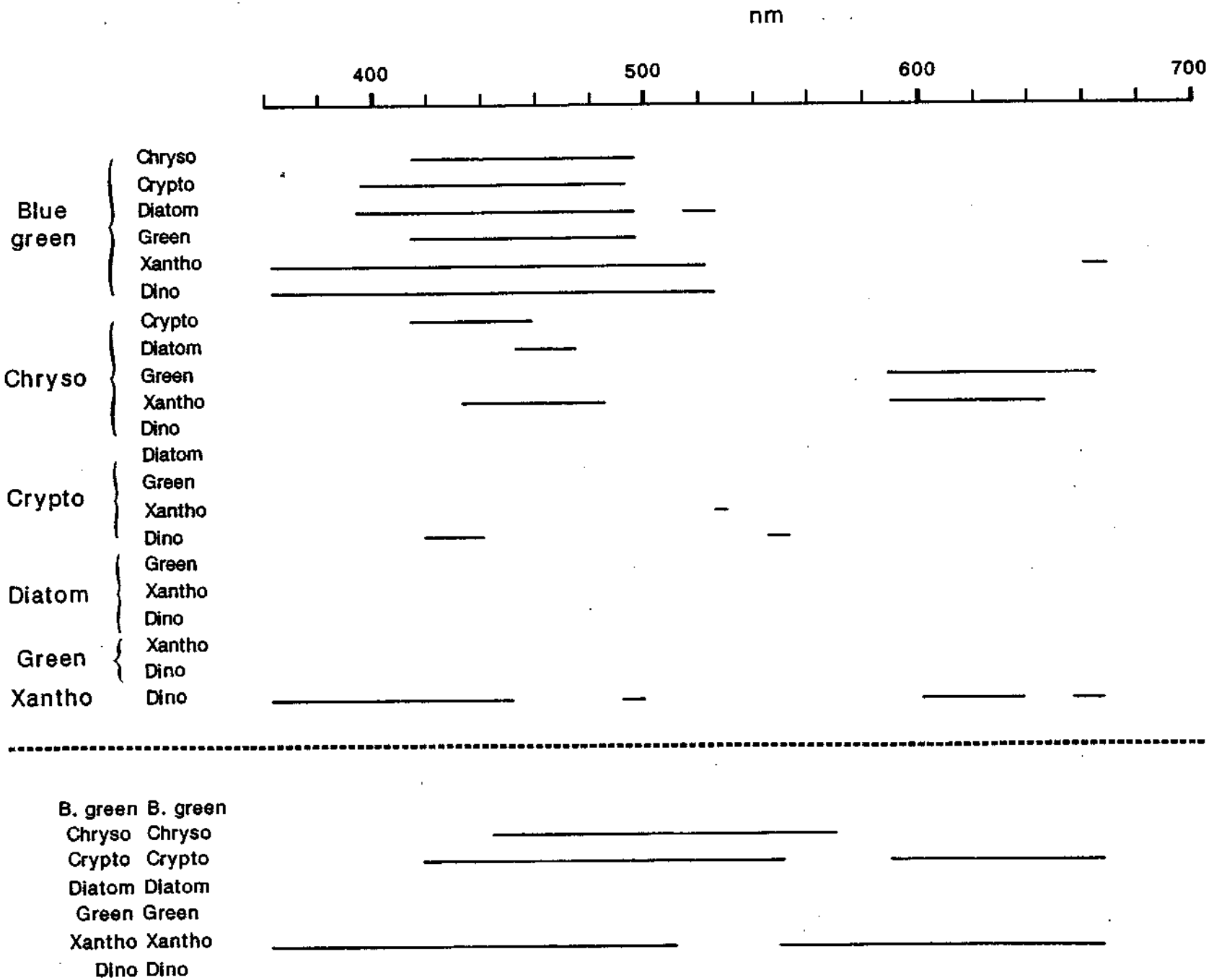
Green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Crypto Crypto
Diatom Diatom
Green Green
Xantho Xantho
Dino Dino

Table 3

Table 4 A comparison of CAP ratios for marine algae with those for freshwater algae obtained in this work.

	no. of freshwater species	CAP range	CAP range excluding one outlier	CAP for marine algae Yentsch *et al*.
Blue-green	7	0.33 - 2.4	1.15 - 2.4	>>1
Chrysophyte	5	0.32 - 0.76	0.32 - 0.49	0.3 - 0.4
Cryptophyte	2	0.33 - 0.34		0.7 - 0.8
Diatom	6	0.33 - 0.62	0.33 - 0.48	0.8 - 0.9
Dinoflagellate	1	0.45		0.7 - 0.8
Green	7	0.12 - 0.23		0.1 - 0.2
Xanthophyte	3	0.15 - 0.25		none given

Table 5 Wavelength ranges which show significant differences between different algal groups.

	BG	Chryso	Crypto	Diatom	Green	Xantho
Chryso	360–390					
	390–435+					
	460–510					
	510–580					
	580–630					
	630–665					
Chrypto	360–390	–				
	390–435+	–				
	(460–510)	–				
	–	510–580				
	–	–				
	630–665	–				
Diatom	360–390	(360–390)	(360–390)			
	390–435+	–	–			
	(460–510)	–	–			
	510–580	–	510–580			
	580–630	–	(580–630)			
	630–665	–	–			
Green	360–390	–	–			
	390–435+	–	–	360–390		
	460–510	460–510	460–510	–		
	510–580	510–580	510–580	510–580		
	580–630	(580–630)	–	580–630		
	630–665	630–665	(630–665)	630–665		
Xantho	360–390	–	–	360–390	–	
	390–435+	–	–	–	–	
	–	–	–	–	–	
	(510–580)	(510–580)	510–580	510–580	–	
	(580–630)	–	–	(580–630)	–	
	(630–665)	(630–665)	–	630–665	–	

( ) values in brackets show 95% differences; all others 99%

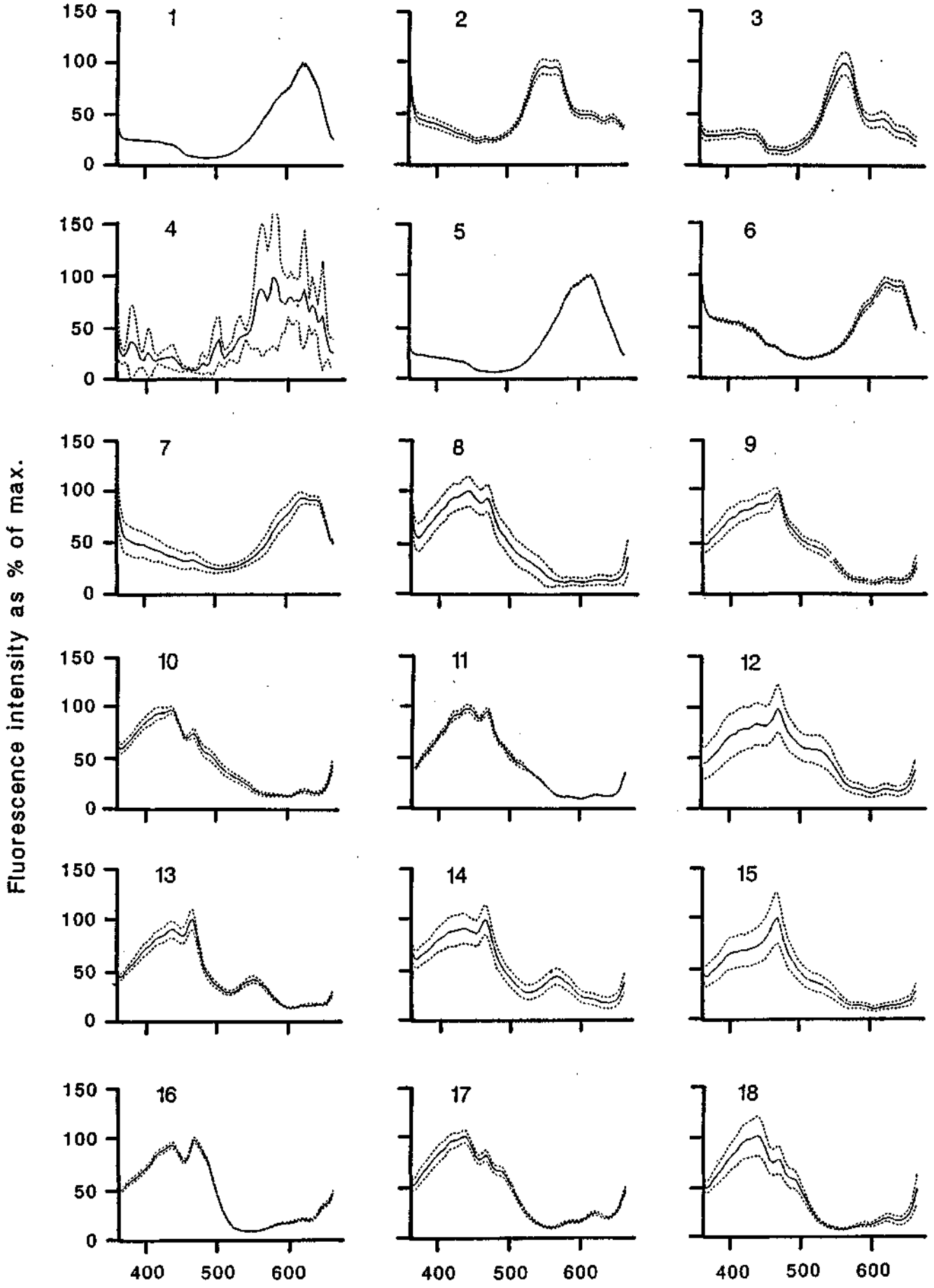
+ 390–460 for max. intensity normalisation

Figure 1.

Excitation spectra of *in vivo* algal flourescence emitted at 680 nm. Dotted lines give ± 1 standard deviation on the mean of five measurements.

1) Synecococcus sp.
2) Oscillatoria agardhii Gomont
3) Oscillatoria bourrellyi Lund
4) Nostoc sp.
5) Microcystis aeruginosa Kutzing emend. Elenkin
6) Aphanizomenon flos aquae Ralfs.
7) Anabaena solitaria klebahn.
8) Dinobryon divergans Imhof
9) Mallomonas minima Rekfous
10) Mallomonas caudata Iwanoff
11) Ochromonas sp.
12) Syncrypta glubosa
13) Rhodomonas lacustris var. nannoplanctica (Skuja) Jaworicky
14) Cryptomonas sp.
15) Woloszynskia coronata (Woloszynska) Thompson
16) Monodus sp.
17) Botrydium granulatum Greville
18) Tribonema sp.
19) Asterionella formosa Hassall
20) Aulacoseira granulata var. angustissima (O. Müller) Simonsen
21) Fragilaria crotonensis var. prolongata Grunow ex. Van Heurck.
22) Stephanodiscus hantzchii Grunow ex Cleve & Grunow
23) Tabellaria flocculosa var. asterionelloides (Grunow) Knudson
24) Synedra delicatissima var. angustissima Grunow ex Van Heurck
25) Botryococcus braunii Kutzing
26) Chlorella sp.
27) Dictyosphaerium ehrenbergianum Naegeli
28) Gaminella minor (Naegeli) Heering
29) Eudorina unicocca G.M. Smith
30) Pediastrum duplex Meyem
31) Volvox aereus Ehrenberg

Figure 1


1
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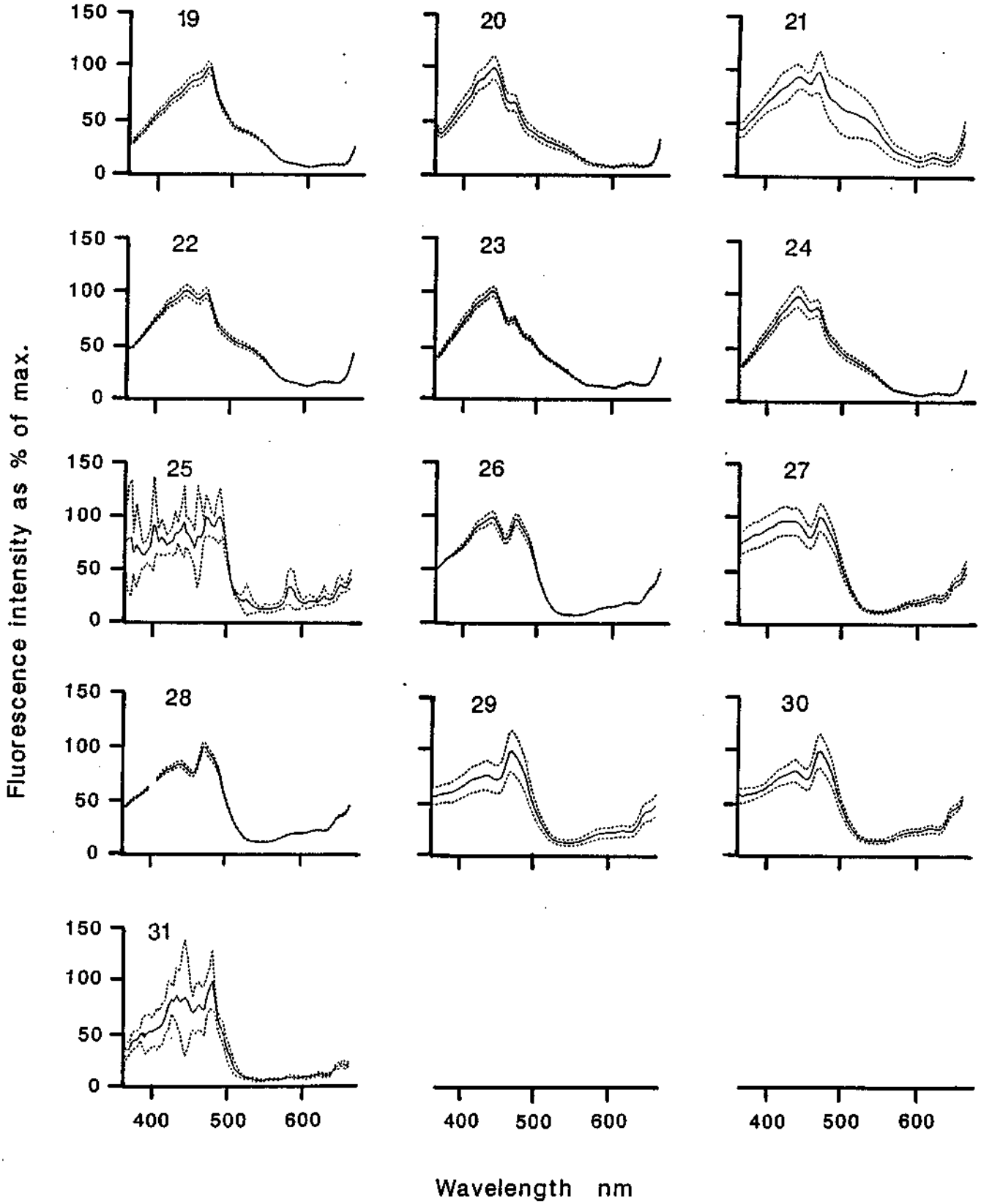
16
17
18
150
100
50
0
400
500
600
Fluorescence intensity as % of max.
Wavelength nm

Figure 1 cont



Appendix B

In vivo algal fluorescence, chromatic adaptation and the automatic characterisation of algae.

Introduction

Many attempts have been made to automate time-consuming manual methods of counting and identification of algae in water. In adapting to diverse environmental niches, different algal groups have developed different combinations of photo-pigments to make the most of the light conditions which they are likely to encounter. The determination of these pigments by in vivo fluorescence has been used to characterise algae of different groups (e.g. Yentsch and Phinney, 1985; Oldham *et al*. 1985). The method is becoming increasingly used in studies of marine phytoplankton and has been particularly successful when used in conjunction with flow cytometry (Yentsch *et al*., 1984). Although work on the fluorescent properties of algae has been carried out on both marine and freshwater species, its extension to algal characterisation has been applied mainly to marine phytoplankton. In this paper we will present the results of a study of the *in vivo* total fluorescence spectra of freshwater algae and its consequences for automatic algal identification.

Methods

Total fluorescence spectra were obtained from algal suspensions using a Perkin Elmer 204 fluorimeter which had been modified, in-house, to allow computer control of the excitation and emission wavelengths. Digital intensity values at each pair of wavelengths were stored on disc and processed later to produce contour plots. All spectra were run over the excitation wavelength range 220-737 nm in 7.46 nm steps and an emission range of 263-780 nm in 7.17 nm steps. (The fixed slit width of the instrument gave a spectral band width of 10 nm). The start wavelength of emission scans at each excitation wavelength was offset by 43 nm to protect the photomultiplier from the high light intensities caused by the scatter peak.

After dark adaption for at least 30 min, (Heaney, 1978) total fluorescence spectra were obtained for cultures of 32 freshwater algae from either the FBA or CCAP (Culture Collection for Algae and Protozoa) collections (Table 1). The algae covered the seven most common groups found in freshwater plankton. The media composition (see Table 1) is described in the CCAP catalogue of strains (in press). Non axenic cultures were grown in pyrex flasks incubated at 20 ± 1°C in a temperature controlled cabinet (Droop, 1969) and illuminated continuously from below using 40W daylight fluorescent lamps. The irradiance was measured with a spherical quantum sensor (Biospherical Instruments Inc., San Diego, USA). Replicates of those species marked with an asterisk in Table 1 were grown under a 12/12 hr light/dark cycle at either 388 or 21 µEinst. m^{-2}. s^{-2}. The lower irradiance was obtained by placing layers of plastic netting between the base of the flasks and the light source. All the other samples were cultured in light of intermediate intensity (100 µEinst. $m^{-2}.s^{-1}$) conditions. In general spectra were recorded sufficiently quickly (10-20 min) so that settlement was not important. However, for some of the large colonial algae a small stirrer was included in the fluorescence cell.

Results

A typical spectrum of the full scan is shown in figure 1a. The peak at about ex. 280 nm, em. 353 is common to all spectra. Its cause is unknown but it is a true fluorescence emission over and above the high background scatter observed in this region (N.B. Hetherington, Perkin Elmer. Personal Communication). The diagonal peak from approximately ex. 240; em 490 to approx. ex 385; em 778 is due to secondary scatter from the instrument gratings.

Spectra typical of each group are presented in figure 1a-e. For convenience only the emission range from 621-740 is shown for four groups. The spectra from only 5 of the total of 32 species, contained information outside this region and will be discussed later. The essential feature of all these spectra is that the excitation spectrum of fluorescence emission at 680 nm contains all the available information. In other words, we are not observing the fluorescence of individual accessory pigments directly. The energy they absorb is efficiently transferred to chlorophyll a (Papaegeorgou, 1975; Vincent, 1980) from which the fluorescence eminates. Some general properties of the fluorescence spectra from different algal groups can be observed and are listed in Table 2. Blue-green algae showed little or no fluorescence when excited at 450 nm. The majority of these wavelength ranges are consistent with data for marine algae given by Yentsch and Phinney (1985). More detailed comparisons will be made in a further publication.

From studies of natural populations of marine algae, Yentsch and co-workers (1985a, b; 1979) have suggested, that blue-green algae and cryptophytes have a characteristic absorbance at about ex 550 n, due to phycoerythrin which stimulates flouresence of phycoerythrin itself at 580 nm and of chlorophyll a at 680 nm. However, all the blue green algae studied in the first part of this work showed no phycoerythrin absorbance or emission. The only fluoresence peak observed was due to phycocyanin (ex 600-620 nm, em 680 nm). Similarly cryptophytes only showed chlorophyll a fluoresence at 680 nm stimulated by excitation at 400-480 nm. All the cultures used up to this point had been grown in light saturating conditions (100 μEinst.cm^{-2}.s^{-1}) and preliminary experiments suggested that the observed spectral differences may be the result of chromatic adaptation of the algal pigment systems. In order to test this hypothesis, replicates of all classes of the algae previously investigated were cultured in either saturating light (388 μ Einst m^{-2}s^{-1}) or low light (21 μ Einst m^{-2}s^{-1}) conditions.

In low light all algal species showed enhanced fluoresence over the whole spectrum compared to high light conditions. The majority of classes showed no other changes in spectral features, although the marine literature suggested that they should exhibit chromatic adaption (e.g. Neori et al., 1984). Conversely many blue-green algae and all the cryptophytes showed large spectral changes. Under low light conditions the 680 nm fluorescence excitation spectrum of all the cryptophytes (Fig. 2) studied showed an increased absorbance of light in the 550 nm region indicating the production of phycoerythrin. However, the fluorescence of phycoerythrin itself, at em 580 nm, was of very low intensity.

Changes shown by the blue-green algae were more complex. Of the six species cultured under different light conditions, three (Anabaena solitaria, Synechococcus and Oscillatoria agardhii, Fig. 3a) showed no spectral change under different light conditions; two (Microcystis aeruginosa and Anabaena flos aquae, Fig. 3a, b) showed a slight increase in phycoerythrin absorption and fluoresence in low light conditions and one (Oscillatoria bourrellyi, Fig. 3b, c) showed very low phycoerythrin fluoresence in high light conditions with most of the fluorescence coming from phycocyanin but nearly all the fluorescence from phycoerythrin at em 680 and ex 580 under low light incubation.

Discussion

These data, and data from the marine literature, can be rationalised in terms of each species ability to maintain a defined position in the water column. Utkilen et al. (1985) have defined three basic types of the species Oscillatoria: type 1 contain gas vacuoles which can be collapsed completely by the internal osmotic pressure imbalance (turgor pressure). They remain green at all times; type 2 contain gas vacuoles which can withstand such high pressures that they cannot be collapsed by cell turgor pressure and are red, i.e. high phycoerythrin, all the time; type 3 contain no gas vacuoles and change colour to optimise their use of available light. From data presented above it would appear that with only slight modification, these groupings can be extended to cover all blue-green algae and rationalise all the observations of chromatic adaptation in this work and in the literature. Anabaena solitaria, Synechoccus and the strain of Oscillatoria agardhii which we have studied show photo adaptation consistent with type 1 species. Critical and turgor pressures are only available for Oscillatoria agardhii but they were used to classify the algae in Utkilen et al's., original paper (1985). Our Oscillatoria bourrellyi has been shown to be the same as Skulberg's bornetti (Skulberg, 1982) and is obviously type 3. Anabaena flos aquae, and presumably Microcystis aeruginosa, has a cell turgor pressure which is about the same as its critical pressure (Walsby, 1971). As such it is on the borderline between type 1 and type 2, hence its limited ability to chromatically adapt. It is likely that a continuum exists between type 1 and type 2 where at low critical pressure/turgor pressure ratios type 1 characteristics dominate; as the ratio approaches 1 from below, photo adaptation occurs so that in low light conditions small quantities of phycoerythrin are formed but most of the light capture is done by phycocyanins. As the ratio increases above 1, phycoerythrin becomes the dominant pigment, but at high light intensities phycocyanin will make a contribution and when the ratio $>> 1$ phycoerythrin dominates. Data in the literature for marine algae are consistent with this rationale. Yentsch and co-workers (1985a, b; 1979) studied natural populations of blue-green algae which are small and have no gas vacuoles. Assuming that these algae came from either a relatively low light or a green light environment, they have the typical attributes of type 3. We have only located one example of a type 1 marine algae. Oldham et al. (1985) showed the total fluoresence spectrum of Oscillatoria woronchini a gas vaculate marine algae. The major fluoresence occurred at ex $>$ 600 em 670 nm with only a very minor peak at ex 550, em 580. The typical spectrum expected for a gas vaculate blue-green alga with its turgor pressure approaching, but greater than its critical pressure.

In conclusion it can be stated that chromatic adaptation increases the potential for automatic recognition of some species within the same class. Although this phenomenon may make it more difficult to actually name a species

from its fluorescence spectrum, it increases the separation of species on a multiparameter basis and may also give information on the previous light history of the body of water enveloping the cells.

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Table 1.

Class		Alga	Strain No.	Medium
CYANOPHYCEAE	1 *	Anabaena solitaria Klebahn	FBA L446	JM
	2	Aphanizomenon flos aquae Ralfs	FBA L319	JM
	3 *	Microcystis aeruginosa Kutzing emend. Elenkin	FBA L155	JM
	4	Nostoc sp.	CCAP 1453/30	JM
	5 *	Oscillatoria agardhii Gomont	FBA L101	JM
	6 *	Oscillatoria buorellyi Lund	FBA L159	JM
	7 *	Synechococcus sp.	CCAP 1479/5	JM
	8 *	Anaebaena flos aquae Brébisson ex. Bornet & Flahault	FBA L102	JM
CHRYSOPHYCEAE	9	Dinobryon divergens Imhof	FBA L143	DM
	10	Mallomonas caudata Iwanoff	FBA L428	DM
	11	Mallomonas minima Rekfous	FBA L433	DM
	12	Synura uvella Stein emend. Korschikov	FBA L316	DM
	13	Ochromonas sp.	FBA L455	DM
	14	Syncrypta globosa (Schiller) Bourrelly	FBA L422	DM
CRYPTOPHYCEAE	15 *	Cryptomonas sp.	FBA L315	DM
	16 *	Rhodomonas lacustris var. nannoplanctica (Skuja) Javornicky	FBA L164	DM
BACILLARIOPHYCEAE (diatoms)	17	Asterionella formosa Hassall	FBA L374	DM
	18	Aulacoseira granulata var. angustissima (O. Muller) Simonsen	FBA L384	DM
	19	Fragilaria crotonensis var. prolongata Grunow ex. Van Heurck	FBA L378	DM
	20 *	Stephanodiscus hantzchii Grunow ex Cleve & Grunow	FBA L382	DM
	21	Synedra delicatissima var. angustissima Grunow ex. Van Heurck	FBA L395	DM
	22	Tabellaria flocculosa var. asterionelloides (Grunow) Knudson	FBA L430	DM
DINOPHYCEAE	23 *	Woloszynskia coronata (Woloszynska) Thompson	CCAP 117/2	DM
CHLOROPHYCEAE	24	Botryococcus braunii Kutzing	FBA L420	JM
	25 *	Chlorella sp.	FBA L289	JM
	26	Dictyosphaenium ehrenbergianum Naegeli	FBA L114	JM
	27	Eudorina unicocca G.M. Smith	FBA L371	JM
	28	Geminella minor (Naegeli) Heering	FBA L357	JM
	29	Pediastrum duplex Meyeri	FBA L387	JM
	30	Volvox aereus Ehrenberg	FBA L451	JM
XANTHOPHYCEAE	31	Botrydium granulatum Greville	CCAP 805/3B	JM
	32	Monodus sp.	FBA L364	JM
	33	Tribonema sp.	FBA L249	JM

Table 2. Typical excitation wavelength ranges for peak emission at 680 nm for different algal groups.

	peak		peak
Blue-green	400-420 (L)		600-620 (H)
Chrysophyte	400-460 (H)	extending to 560	660 (L)
Cryptophyte	400-480 (H)		660 (L)
Diatom	420-480 (H)	extending to 540	660 (L)
Dinoflagellate	420-480 (H)	" " 540	660 (L)
Green	400-500 (H)		660 (L)
Xanthophyte	400-500 (H)		660 (L)

L = low intensity H = high intensity

Figures

Figure 1.

a) Total fluoresence spectrum over the full wavelength range for Synura uvella, a typical chrysophyte.

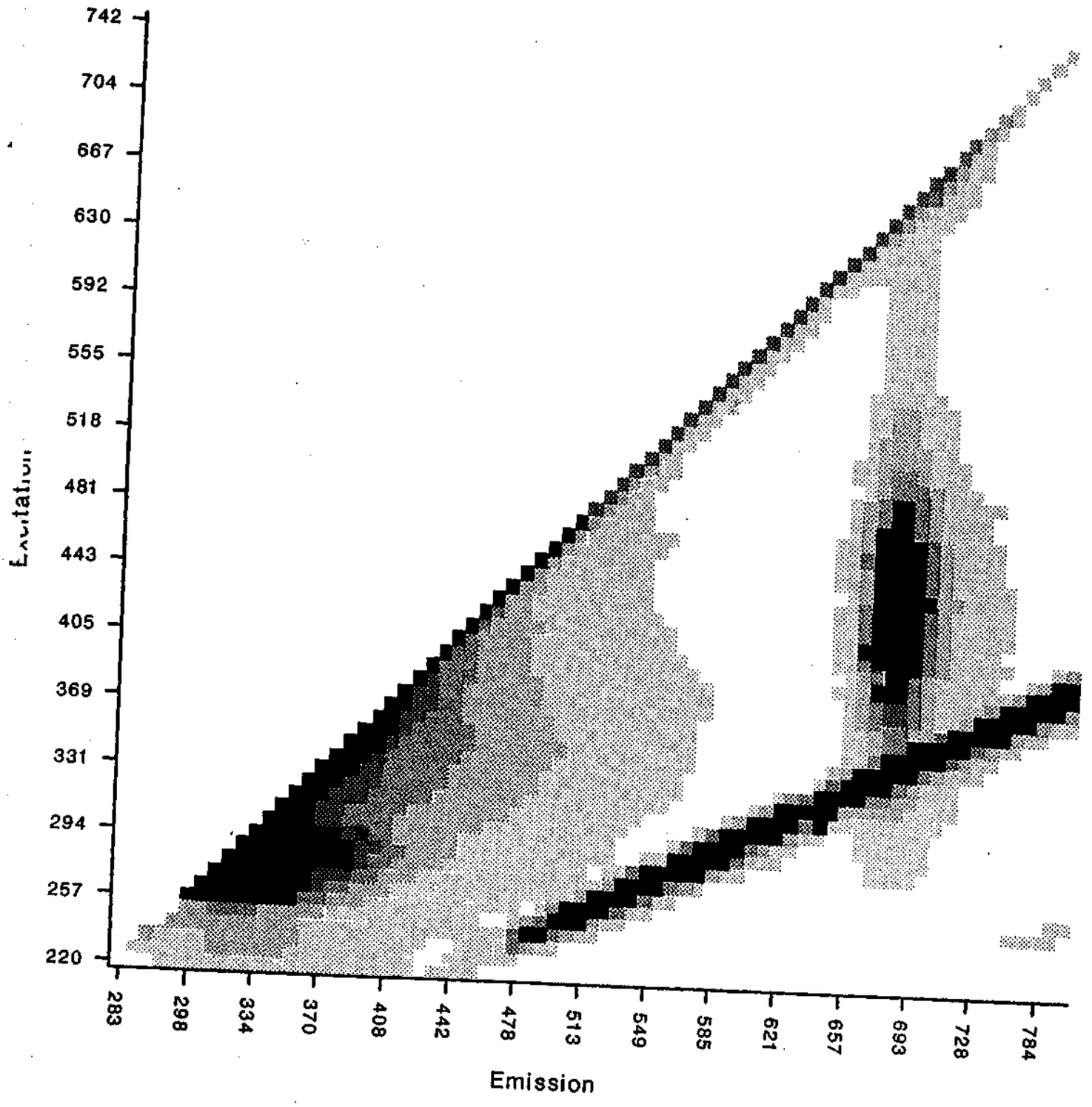
Total fluoresence spectrum over the emission range 621-728 nm typical of classes:

b) bacillariophyceae, Stephanodiscus hantzschii
c) dinophyceae, Woloszynskia coronata
d) chlorophyceae, Chlorella sp.
e) xanthophyceae, Monodus sp.

Figure 2. Total fluoresence spectra over the emission range 549-728 nm of Rhodomonas lacustris, a typical cryptophyte, grown under conditions of (a) low and (b) high light.

Figure 3. Total fluoresence spectra over the emission range 549-728 nm showing the typical range of spectra from (a) phycobilin rich to (c) phycoerythrin rich, blue-green algae.

Fig. 1a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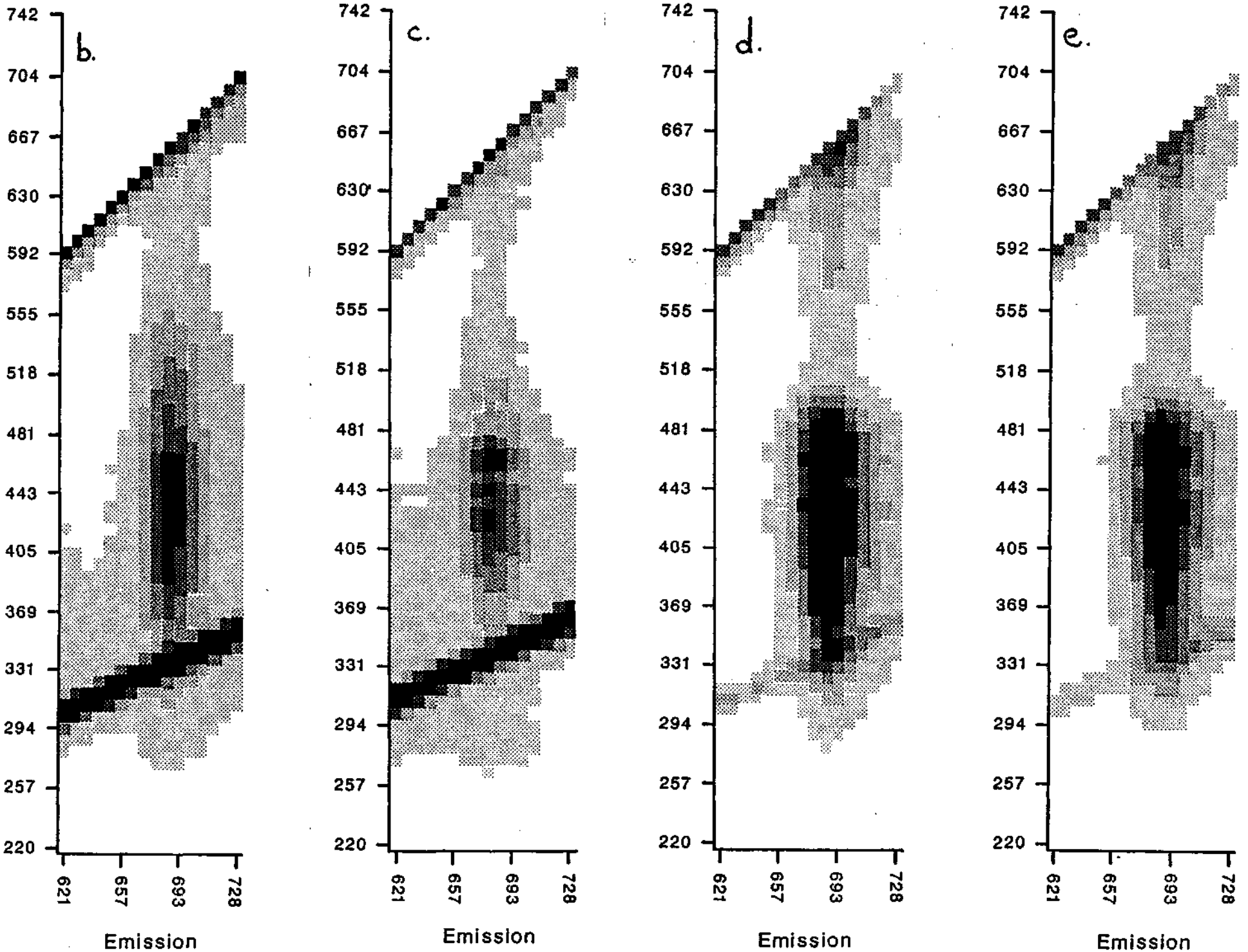
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Fig 1 cont.

Fig. 2.

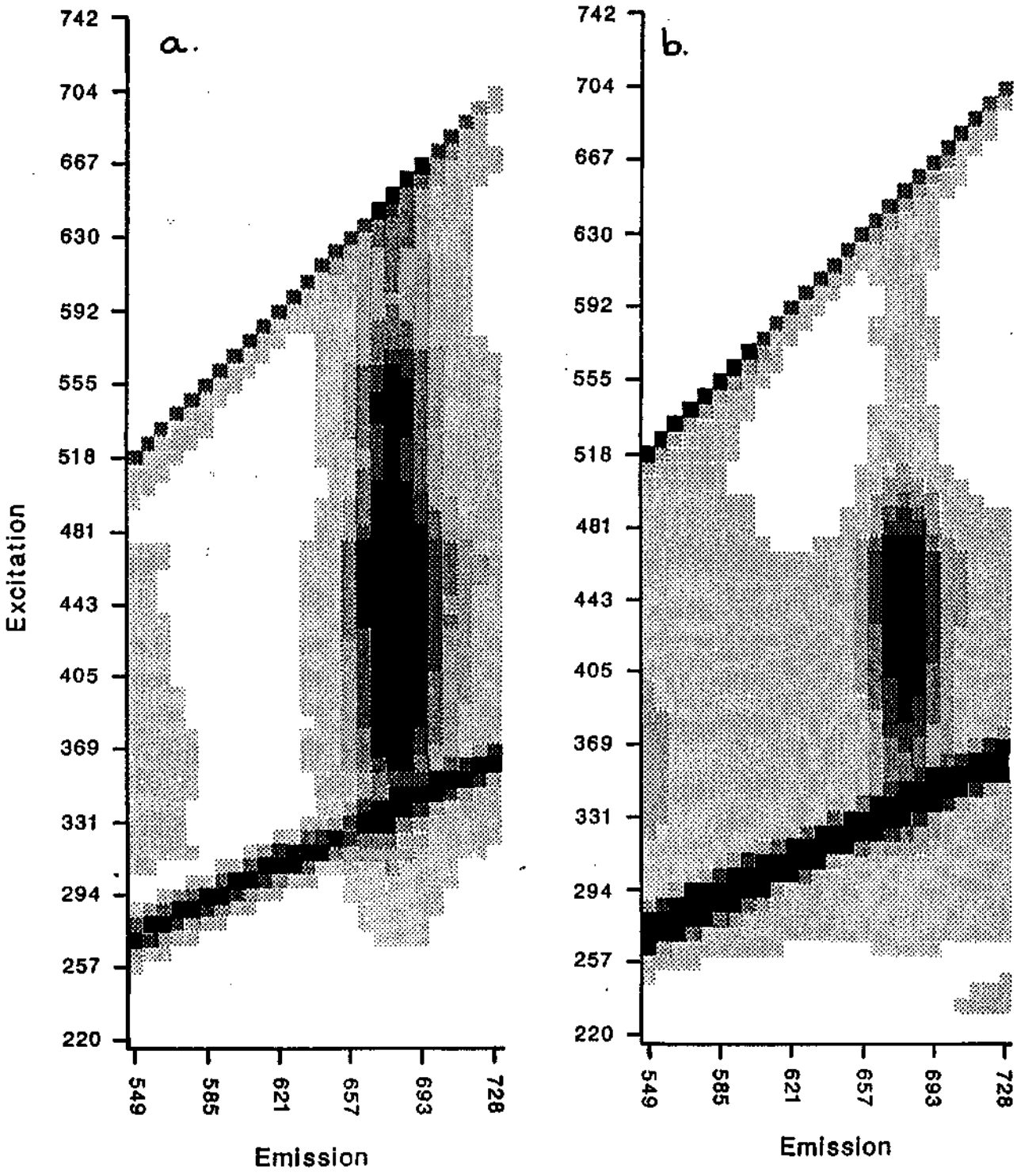
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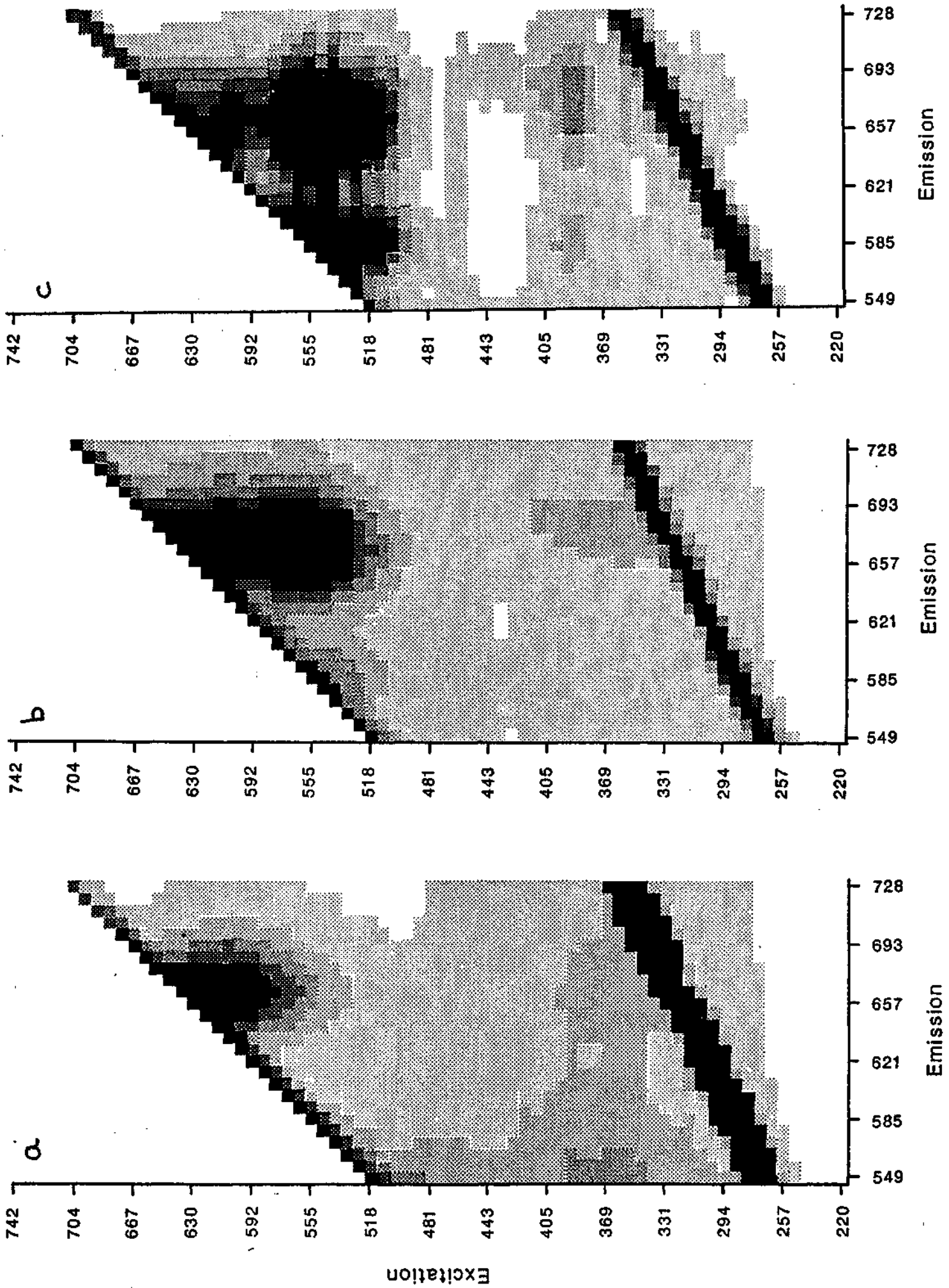
Fig. 3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