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The Fluorescence of the Black Sea and the Rhodes Gyre, Eastern Mediterranean

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Abstract

Determinations of the ultra violet-visible fluorescence of sea water carefully sampled from precise locations and depths in the Black Sea and the eastern Mediterranean in 1991/1992 are described. Parameters controlling the reproducibility and resolution of the spectra are emphasised and the first fluorescence lifetimes of sea water are recorded. The best excitation spectra are obtained when the emission wavelength is ∼440 nm; the best emission spectra are given with an excitation wavelength of 220-230 nm, and the best synchronous spectra require a Δ of 50 nm. The contributions of humic acids, aromatic amino acids, carotenoids and flavins to the fluorescence spectra are discussed in detail but the fluorescence of chlorophyll has been ignored. Flowing Black Sea and eastern Mediterranean sea waters can be characterised by their fluorescence in the ultra violet and further studies of fluorescence may in future provide information as to the composition of the dissolved organic constituents.

Key words: Black Sea, Eastern Mediterranean, Fluorescence, Excitation, Emission, Synchronous.

Introduction

Whereas UV-visible absorption spectra of sea water are relatively featureless, absorption increasing exponentially as the wavelengths diminish towards 200 nm, the spectra presumably being dominated by scattering, fluorescence spectra show resolved peaks. Fluorescence spectrometry readily analyses materials present in concentrations of parts per billion and is a potentially exciting technique for recognising and determining those organic compounds present in sea water, and, for that matter, for characterising the sea water. Indeed, since Kalle (1938) first observed the fluorescence of 'gelbestoffe', the fluorescence of sea water has been investigated frequently (Duursma, 1974; Karabashev and Agatova, 1984; Hayase *et al.*, 1988; Coble *et al.*, 1990; Coble *et al.*, 1991; Coble and Gagosian, 1991, Mopper and Kieber, 1991; Coble and Bada, 1992; Mopper and Schultz, 1993), in particular the determination of the fluorescence from chlorophyll has become the standard method for quantifying phytoplankton biomass. Currently, chlorophyll fluorescence from the world's surface waters is monitored by the SEAWIFS satellite. In fact, any soluble material or any transparent suspended particles present in sea water and pos-

sessing systems of delocalised electrons may fluoresce. Such material is abundant in sea water, albeit in low concentrations and includes humic material (Thurman *et al.*, 1988; Coble and Gagosian, 1991), chlorophylls, phaeopigments (Neveux and de Billy, 1986; Yunev, 1989), aromatic amino acids, proteins (Starikova and Korzhikova, 1969; Mopper and Kieber, 1991) flavins (Guilgault, 1973; Coble *et al.*, 1991; Mopper and Kieber, 1991), carotenes (Guigault, 1973; Repeta and Simpson, 1991) and polynuclear aromatic hydrocarbons present as pollutants (National Acad. of Sci., 1975; UNEP, 1988). Such compounds will each fluoresce differently and often characteristically.

This paper describes the fluorescence of water from the Black Sea and from the eastern Mediterranean. It describes the ultra violet and visible fluorescence spectra (but excludes chlorophyll fluorescence) which can be obtained, interprets them and demonstrates their utility. Examples are drawn from samples of sea water sampled at precise depths and locations during cruises of R/V Bilim.

Methodology

Samples of sea water collected in a rosette of Niskin bottles during cruises of R/V Bilim were sealed and then stored in a refrigerator until fluorescence measurements were made. All samples were collected at precise locations and depths. Subsequently the samples were filtered through 0.45 μ GF/F filters. The fluorescence of filtered sea water in 1 cm path-length quartz cells was determined by a Hitachi F 3000 spectrofluorometer. Measurements were made with 5 nm band-pass excitation and emission beams and with a response time of 2 s. Save for those from Bosphorus waters all spectra were corrected according to the manufacturer's programme using measurements of concentrated Rhodamine B solutions. The correction was checked weekly. Spectra were generally obtained at laboratory temperature though a few spectra were measured whilst the cell holder was maintained at the temperature of liquid nitrogen. Experiments showed fluorescence spectra to have been unaffected by the storage of the samples.

Time resolved spectra of sea water sealed under nitrogen in 1 cm by 1 cm square quartz cuvettes were determined by single photon counting in an Edinburgh Analytical Instruments CD 900 Fluorescence Spectrometer. A nF900 nanosecond flash lamp provided excitation at 337 nm. The spectral band-width of both excitation and emission was 20 nm. The optical path length was 1 cm. Deconvolution of the decay curves utilised exponential fits derived from a Marquadt algorithm.

Results

Fluorescence spectra, general description

Figures 1, 2 and 3 show excitation, emission and synchronous spectra of Black Sea and eastern Mediterranean sea water. The wavelengths selected to yield spectra are those which we have found to give the greatest number of resolved peaks in the ultraviolet/visible region and thus the most information about the composition of dissolved organic matter. Complete peak shapes require a matrix of spectra (Coble, 1996).

Figure 1 shows typical excitation spectra (emission wavelength: 440 nm). Preliminary experiments demonstrated this emission wavelength to yield spectra displaying several resolvable peaks. Absorption of light occurred throughout the range 230-430 nm, being generally greatest at the shorter wavelengths. The samples show:

i. absorption close to 230 nm. Such absorption is shown by dienes and by substituted naphthalenes, by certain substituted polyaromatics such as chrysenes and by some substituted benzenes where the substituent is conjugated to the ring (West, 1975). The intensity of the absorption in Figure 1 seemed to be enhanced in the region close to 220 nm, which was half of the emission wavelength.

ii. a resolved peak in the vicinity of 240-245 nm where, for example, substituted thiophenols and aceto derivatives of substituted benzenes absorb (West, 1975).

iii. a resolved peak around 265 nm where one expects absorption by substituted benzene rings (West, 1975).

iv. partially resolved absorption between 300 and 350 nm, a region where substituted naphthalenes absorb (West, 1975).

v. resolved absorption above 350 nm, especially at 370 nm, which would be consistent with the presence of polynuclear aromatic hydrocarbons.

vi. a peak at 386 nm due to the Raman scattering of light by water.

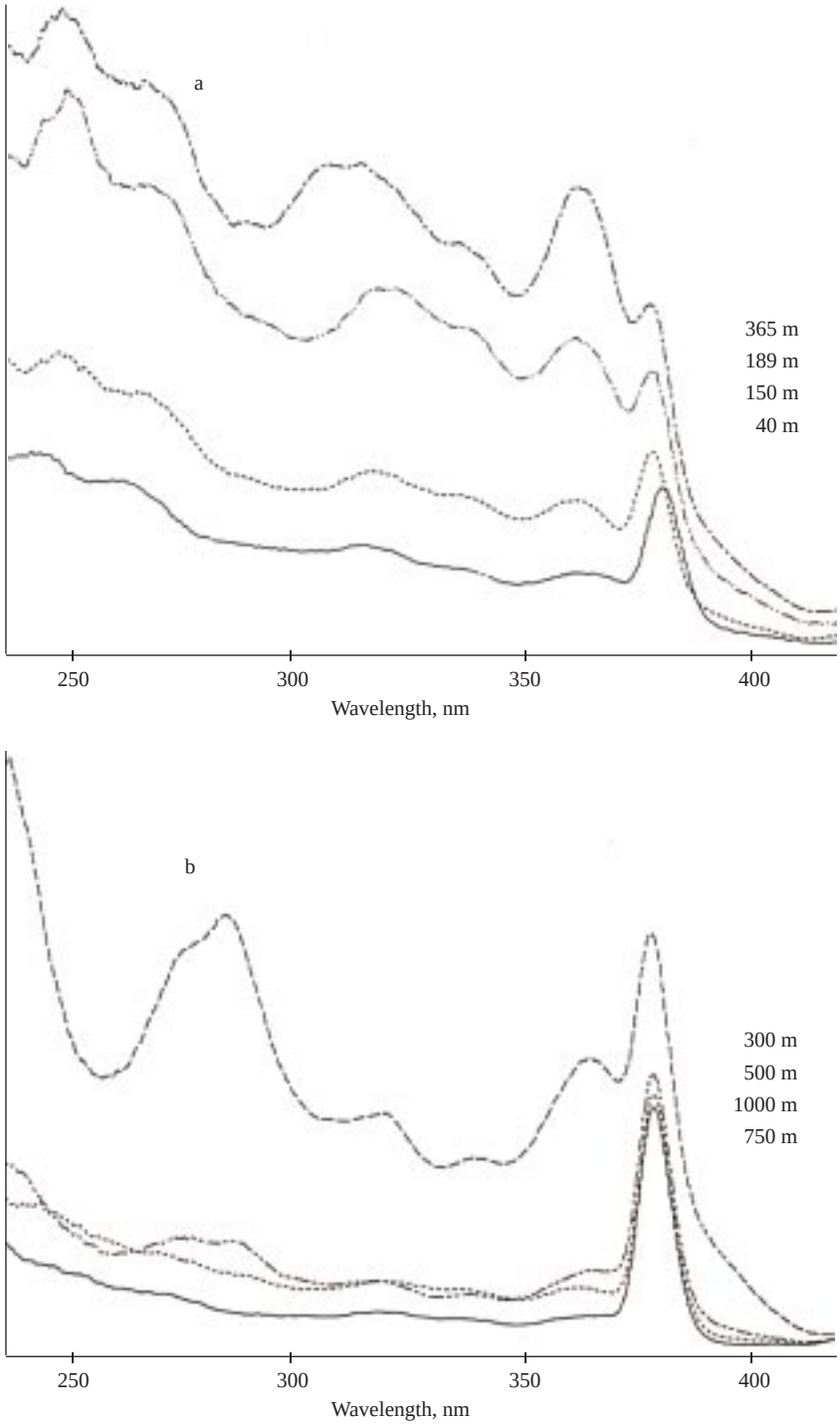

Figure 1. Characteristic excitation spectra (emission wavelength 440 nm; arbitrary units of intensity):
a) Black Sea; Latitude N 41.5; Longitude E 31.5; July 1992.
b) Mediterranean; Latitude N 35.5; Longitude E 28.5; October 1991.
Sampling depths are indicated on the spectra in metres.

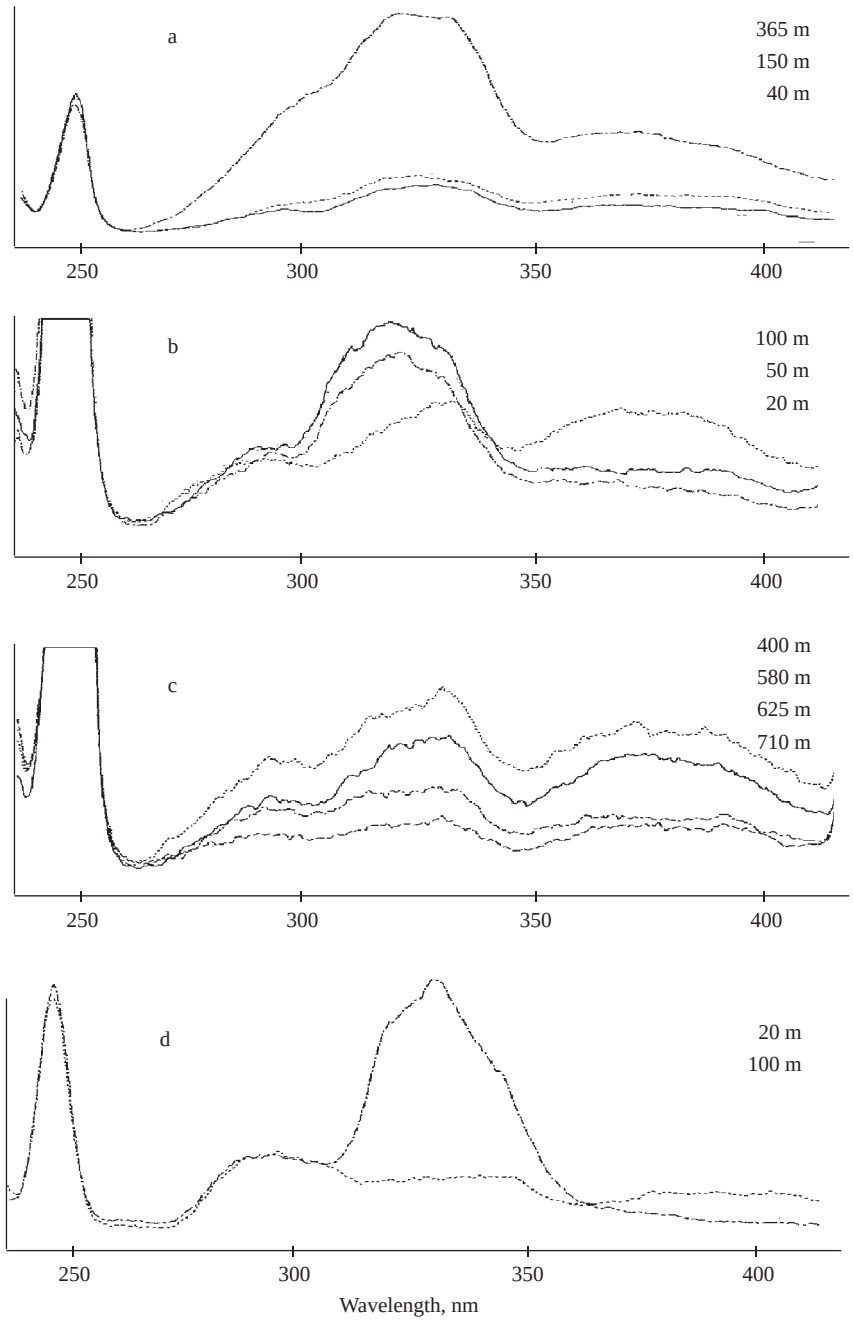

Figure 2. Characteristic emission spectra (Excitation wavelength 230 nm; arbitrary units of intensity):
a) Black Sea; Latitude N 41.5; Longitude E 31.5; July 1992.
b) Mediterranean; Latitude N 34; Longitude E 34; Oct.1991.
c) Mediterranean; Latitude N 34; Longitude E 34; Oct.1991
d) Mediterranean; latitude N 34; Longitude E 34; Oct.1991. Excitation wavelength 220 nm
Sampling depths are indicated on the spectra in metres.

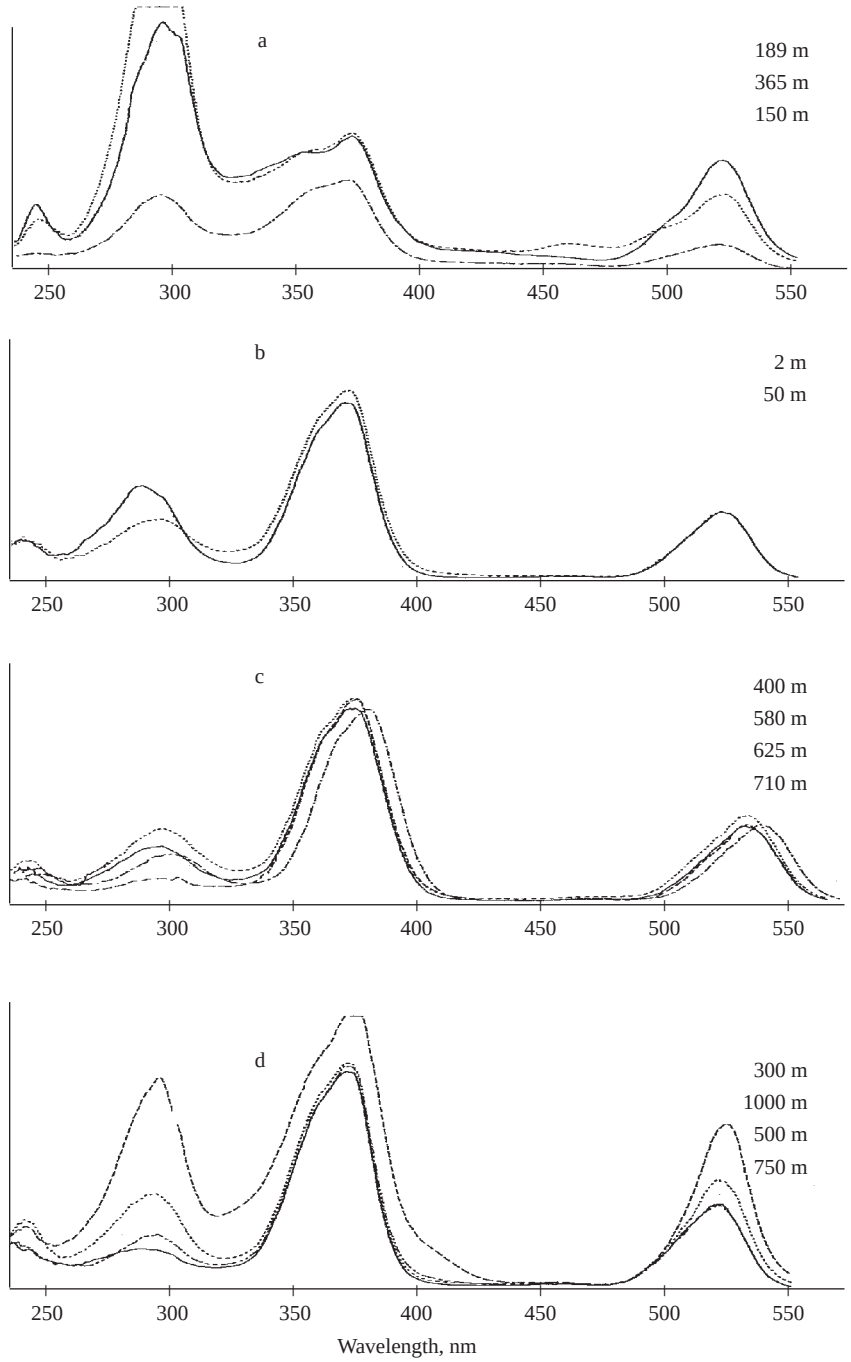

Figure 3. Characteristic synchronous spectra (arbitrary units of intensity) (x axes show excitation wavelengths; $\Delta = 50$ nm):
a) Black Sea; Latitude N 41.5; Longitude E 31.5; July 1992.
Samples from the suboxic and anoxic zones
b) Mediterranean; Latitude N 35.5; Longitude E 28.5; Oct.1991.
c) Mediterranean; latitude N 35.5; Longitude E 28.5; Oct.1991.
d) Mediterranean; Latitude N 34; Longitude E34; Oct.1991.
Sampling depths are indicated on the spectra in metres.

The excitation spectra of the Mediterranean water had generally poorer resolution than the spectra from Black Sea water and the resolution was not improved by decreasing the band-pass of the extinction system from 5 nm to 1.5 nm. Figure 1 shows the fluorescence intensities of Mediterranean water to have decreased with sampling depth, whereas it was the deeper samples from anoxic waters in the Black Sea that gave the greatest fluorescence.

Figures 2 show emission spectra that, perhaps surprisingly, are more structured than the excitation spectra. As Chen and Bada (1992) found, emission appears most intense when the excitation wavelength is at or below 230 nm. It is not practical to use wavelengths as low as 200 nm since dissolved oxygen, chloride, hydroxide and bromide ions absorb strongly in this region. As it is, several inorganic anions, of which the most significant are nitrate, nitrite and iodide (Hellwege, 1951), absorb around 230 nm, consequently lowering the intensities of emission especially from nutrient-rich waters. With 230 nm excitation a small sharp emission maximum is sometimes seen near 240 nm, just below the large peak at 254 nm due to the Raman scattering by water. Broad emission maxima follow in the regions 300-320, ∼350 and 400-450 nm. Figure 2 shows emission obtained from excitation wavelengths of both 220 and 230 nm; consistent with one's understanding of fluorescence, the intensities of emission were more sensitive than the emission wavelengths to the excitation wavelength. Thus, the relative intensity of emission near 300 nm was significantly higher when the excitation wavelength was 220 nm than when it was 230 nm. The peaks seen in the emission spectra are generally the most intense to be found in the fluorescence spectra of sea water and their relative intensities vary from one sea to another.

Figure 3 shows synchronous spectra in which the excitation and emission wavelengths were scanned simultaneously. We find the most useful synchronous spectra are obtained when the excitation and emission wavelengths differ by the rather large amount of 50 nm. The spectra then consist of 4 peaks:

i. A small, rather sharp peak at an excitation wavelength close to 240 nm, probably due to substituted benzene rings.

ii. A larger, broader peak at an excitation wavelength near 280 nm that might arise from substituted benzenes or naphthalenes.

iii. A large peak at excitation wavelengths between 350 and 375 nm which appears to be structured and certainly includes the Raman scattering of water at 345 nm.

iv. A smaller but broad peak centred at 520 nm which might include the fluorescence of flavins (Coble *et al.*, 1991; Mopper and Keiber, 1991).

Investigation of polynuclear aromatic hydrocarbons present as pollutants in sea water is better carried out by synchronous scanning with the excitation and emission wavelengths differing by 20 nm.

Fluorescence spectra, reproducibility

Previous authors appear to have paid little attention to the reproducibility of the fluorescence spectra they have published (Duursma, 1974; Karabashev and Agatova, 1984; Hayase *et al.*, 1988; Coble *et al.*, 1990; Coble *et al.*, 1991; Coble and Gagosian, 1991, Mopper and Kieber, 1991; Coble and Bada, 1992; Mopper and Schultz, 1993). Table 1 shows relative fluorescence intensities (uncorrected) measured in the surface waters at a location in the Bosphorus in May, 1992. The intensities are recorded relative to those of the related Raman scattering by the sea water so as to improve their accuracy. The results are the averages of 6 samplings repeated at the same depth and the calculated standard deviations of an individual result, due to sampling and experimental error, have been included in the Table 1. Generally, an individual result may be in error by 5-10%. The fluorescence of solutions of organic compounds is usually much less than this though care has to be taken when, as often occurs in sea water, the compounds are photoreactive; most of our experimental error arose in sampling.

Usually we have obtained but a single set of fluorescence spectra from each sample of sea water. As it happens, the concentrations of salinity, temperature, nutrients, dissolved oxygen and total organic carbon in the centre of the Rhodes gyre in the eastern Mediterranean are often nearly constant from a depth of about 25 m to a depth of some 1000 m. Table 2 summarises the fluorescence from 4 columns of sea water located near to the centre of the Rhodes gyre in March 1992. At each location the intensities of the major fluorescence maxima have been averaged over 10-14 samples taken at different depths from near the surface down to 1000 m. These average intensities have been collated, together with the standard deviations of an individual result. Table 2

Table 1. Uncorrected fluorescence intensities relative to the intensity of the Raman scattering of Bosphorus water.

Type of Spectrum	Synchronous (Δ = 50 nm)*		Excitation*		Emission*		
Wavelength (nm)	230	280	230	330	300	345	400
Relative intensity (average of 6 readings)	0.23	0.52	1.28	0.66	0.65	1.37	1.11
Standard deviation (of an individual result)	0.04	0.04	0.14	0.03	0.06	0.10	0.09

* Raman peaks at 354, 380 and 254 nm for synchronous, excitation and emission spectra, respectively.

Table 2. Average fluorescence intensities and ratios in the Rhodes Gyre, March, 1992.

Location	N36.5 E28.5	N36.0 E28.5	N35.75 E28.5	N35.5 E29.5
Wavelength of maximum (nm)				
S 230	1.20 ± .04	4.61 ± .36	0.78 ± .08	0.79 ± .28
S 280	1.45 ± .08	4.12 ± .57	1.42 ± .24	0.69 ± .23
S 360*	7.45 ± .05	8.78 ± .15	3.70 ± .06	3.54 ± .07
Ex 230	1.08 ± .01	2.50 ± .11	0.92 ± .07	0.82 ± .06
Ex 300	0.53 ± .01	1.04 ± .08	0.49 ± .05	0.34 ± .03
Ex 384*	3.05 ± .02	3.55 ± .06	1.65 ± .03	1.58 ± .05
Em 254*	25.7 ± .1	41.3 ± .4	10.2 ± .3	10.6 ± .30
Em 300	1.81 ± .10	5.13 ± .46	1.41 ± .16	1.28 ± .23
Em 350	2.19 ± .10	6.47 ± .77	2.17 ± .25	1.69 ± .30
Em 400	1.66 ± .40	4.14 ± .26	1.43 ± .14	1.21 ± .14
Ratios				
S 230/S 360*	0.16	0.52	0.21	0.22
S 280/S 360*	0.19	0.47	0.38	0.19
Ex 230/Ex 384*	0.35	0.70	0.56	0.52
Ex 300/Ex 384*	0.17	0.29	0.30	0.22
Em 300/Em 254*	0.07	0.12	0.14	0.12
Em 350/Em 254*	0.09	0.16	0.21	0.16
Em 400/Em 254*	0.06	0.10	0.14	0.11
S 230/S 280	0.83	1.12	0.55	1.14
Ex 300/Ex 230	0.49	0.42	0.53	0.41
Em 300/Em 350	0.83	0.74	0.65	0.76
Em 400/Em350	0.76	0.64	0.66	0.72

S = Synchronous spectra (Δ =50 nm); Ex = Excitation spectra; Em = Emission spectra.
* Raman scattering by water

also shows the ratios of the average florescence intensities to the intensities of the appropriate Raman scattering by the water as well as the ratios of the average intensities of the major peaks in each type of fluorescence spectrum. One observes that in each water column the standard deviation of an individual result can be as high as 10% of the mean though usually it is only 5% or less of the mean value. The order of magnitude of these errors is similar to that resulting from the repeated sampling of Bosphorus water. Clearly, variations from one location to another have to be greater than this to be significant. The latter 2 columns on the right of Table 2 (at N 37.75 and 35.5) gave essentially identical fluorescence spectra save for the synchronous peak at an excitation wavelength of 280 nm. The variation in the intensities

of the 280 nm peak in the synchronous spectra is a reminder of the patchiness that may occur in distributions of dissolved and particulate organic matter. The other 2 water columns in Table 2 gave significantly different and higher fluorescence intensities. The variation in the Raman scattering by the sea water is particularly obvious. Whereas the fluorescence intensities of individual peaks shown in Table 2 may exhibit as much as a 6-fold range, the range in the ratios of intensities is smaller. In other words ratios of the intensities of fluorescence appear more reproducible than individual intensities. The variation in the individual intensities observed in the different water columns is due to the difference in the overall concentrations of fluorescent material together with smaller differences in the composition of the material.

Fluorescence spectra, resolution

Few of the peaks in Figures 1, 2 and 3 show a resolution limited by the 5 nm band pass used in the excitation and emission beams, and decreasing the band pass to 1.5 nm failed to improve their resolution. Many, probably most, peaks appear to be composites of overlapping fluorescence. We have used 2 techniques to investigate the extent to which the resolution of the fluorescence spectra from anoxic Black Sea water may be improved. First, we have determined the minimum number of Gaussian peaks needed to simulate the spectra. Secondly, the spectra have been repeated with the cell holder at the temperature of liquid nitrogen. At this comparatively low temperature the relative intensities of the fluorescence were changes. Thus, the synchronous spectra showed diminished peaks around 270 nm and enhanced peaks at 520 nm. More importantly, the resolution obtained at the low temperature was generally greater than that at laboratory temperature. The results are summarised in Table 3 and should be compared with the spectra shown in Figures 1a, 2a and 3a. As one would hope, there was good agreement between the wavelengths of the peaks obtained at low temperatures and the major peaks disclosed by simulation of the fluorescence at laboratory temperature. The simulation gave more peaks, however. That is, the simulation technique appears successful in resolving the major peaks observed in the fluorescence spectra. It confirms that the excitation spectra from Black Sea water contain 3 peaks at or below 250 nm, 2 peaks in the 270-280 nm range, 2 peaks above 300 nm and a further peak near 330 nm (compare Figure 1a). Similarly, synchronous spectra are con-

Table 3. Improved resolution of the fluorescence from anoxic Black Sea water a) Low temperature spectra; b) Simulation by Gaussian peaks.

Excitation spectra (Emission at 440 nm)															
Wavelengths of maxima (nm)															
a)	236	247	252	270		290 -295		316	328	345	359	369	386R	401	415
b)	234	244	254	269	279	289	299	319		344	364		384	409	429
%	8.4	5.4	10.8	10.4	3.5	5.8	5.5	20.1		8.5	9.0		9.4	2.0	1.4

Emission spectra (Excitation at 230 nm)																			
Wavelengths of maxima (nm)																			
a)	248	∼ 253 R	260		∼ 284	296	308		322	335	350						397	415	428
b)	244	254	264	274	289		304	314	324	334	349	359	369	379	389	399	419	429	
%	3.0	6.9	0.5	2.2	3.5		9.9	4.1	6.3	12.4	15.2	2.2	4.8	3.6	3.6	6.8	9.2	0.3	

Synchronous spectra (Δ = 50 nm)																	
Wavelengths of maxima (nm)																	
a)		233	240	250 -300				341	367R		418	435	449	455			516
b)	226	230	240	255	270	280	330	345	365	400	410	430	445	455	475	495	520
%	1.6	1.9	2.0	0.5	10.3	29.8	20.2	4.6	9.4	2.1	0.2	2.2	0.4	0.6	0.3	3.4	8.1

% = Relative size of simulated Gaussian peak (area of peak as percentage of the total area of the spectrum)
R = Peak due to the Raman scattering by Black Sea water.

firmed to possess 3 peaks between 250 and 310 nm, 2 peaks and the Raman scattering of sea water around 360 nm and 2 peaks near 500 nm (compare Figure 3a). The resolution of the emission spectra is particularly interesting; it discloses 7 peaks to exist between 290 and 360 nm and, apparently, a further 6 peaks in the region around 400 nm (compare Figure 2a). The simulation technique should be adopted in future determinations of the fluorescence of sea water. It should be noted that further resolution of the major peaks may be possible since at least half of the peaks discerned by simulation possessed widths at half-height which were significantly greater than the sum (10 nm) of the band-widths of the excitation and emission beams.

Fluorescence spectra, lifetimes

The only previous measurements of the fluorescence lifetimes of sea water known to us are those of Inman *et al.* (1990), who discuss the use of lifetime measurements to distinguish polyaromatic hydrocarbons that fluoresce at similar wavelengths. Figure 4a shows the emission spectrum of samples of anoxic

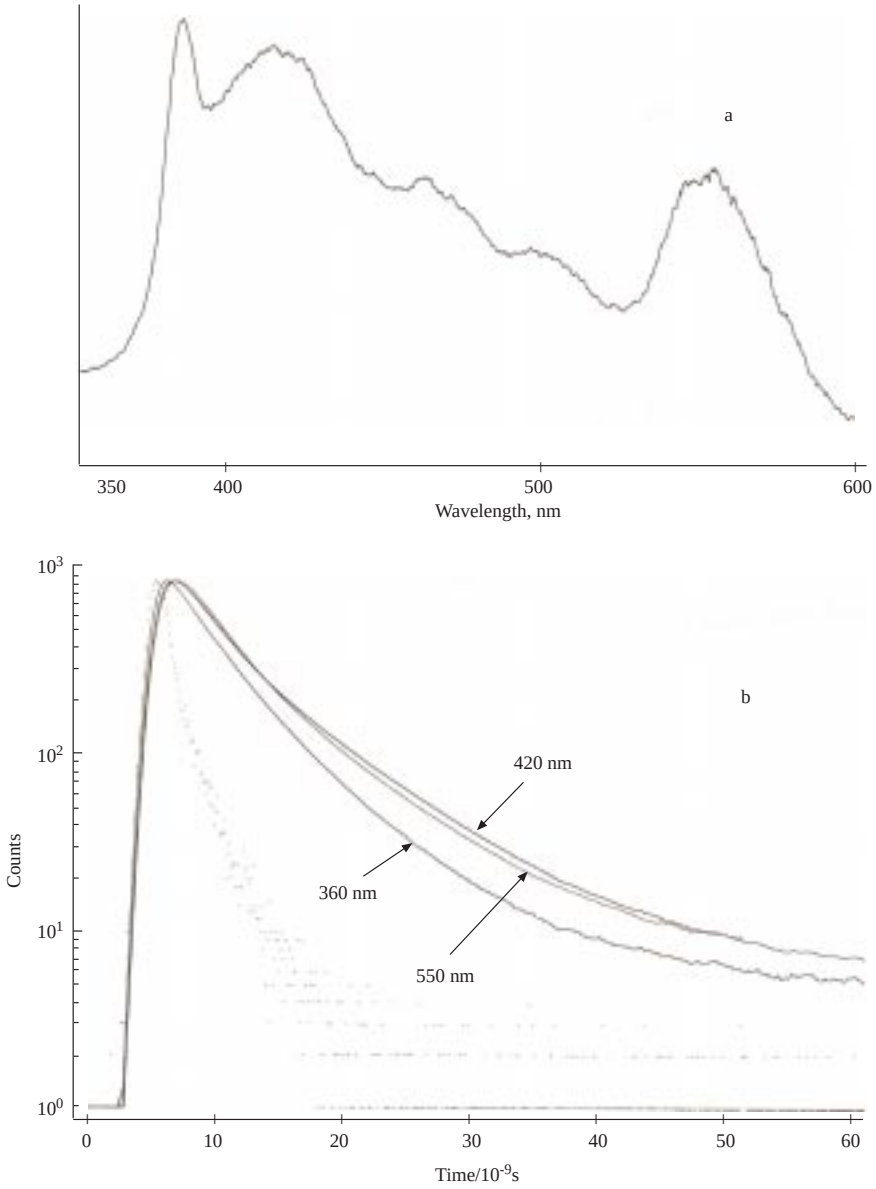

Figure 4. a) The spectrum of samples of anoxic Black Sea water excited by 337 nm light, b) The decay of the fluorescence intensity at emission wave lengths of 360, 420 and 550 nm.

Black Sea water when excited by light of 337 nm wavelength. These samples had been carefully filtered and sealed under nitrogen to eliminate the possibility of quenching by oxygen (or hydrogen sulphide). Figure 4b shows the decay of the fluorescence intensity at emission wavelengths of 360, 420 and 550 nm. The intensities obviously decay monotonically but analysis of the curves shows them to consist not of a single exponential decay, as simple theory would predict, but of a sum of at least 3 exponential decays. Three exponential decays were, in fact, the minimum number required to yield significantly good agreement with the experimental decay. The results for the 420 and 550 nm emissions, the most intense observed in Figure 4a, are summarised in Table 4. Experiments showed that unless great care was taken in the filtering of the samples, the observed fluorescence lifetimes were shorter than those reported. Lifetimes of several nanoseconds duration may be presumed to indicate fluorescence from small molecules. Shorter lifetimes, caused by radiative and non-radiative transfer of energy either between molecules or between parts of the same molecule testify to the presence of molecules with comparatively high molecular weights or to the presence of particles sufficiently minute to escape filtering.

Table 4 suggests, therefore, that part at least of the broadness of the emission at 420 and 550 nm was due to there being at least 3 sources of fluorescence, each source possessing different molecular masses. Less than 50% of the fluorescing material was composed of simple molecules.

Fluorescence spectra, contribution of humic acids

Natural humic acids are organic acids, soluble in aqueous bases, formed by the oxidation of vegetation, usually by air. Their composition and therefore their fluorescence will obviously vary with the degree of oxidation and the composition of the initial vegetation, notably on the presence of lignin. Most humic acids fluoresce characteristically at ∼460 nm when excited at 360 nm and this fluorescence has often been observed from sea water (Ghassemi and Christman, 1958, 1966; Duursma, 1974; Karabashev and Agatova, 1984; Thurman *et al.*, 1988; Coble *et al.*, 1990, 1991; Chen and Bada, 1992; Coble, 1996; Karabashev, 1996). Cronin and Morris (1982a, 1982b) showed humic acids to be formed in marine sediments during the earliest stages of diagenesis and, indeed, Yilmaz *et al.* (1990) showed humic acid fluorescence to be generated by phytoplankton as soon as the cells started dying. Yilmaz *et al.* (1990) found humic acid in coastal regions of the eastern Mediterranean to occur in clumps, often associated with the presence of phytoplankton. Table 5 summarises the fluorescence of a commercial (Aldrich) humic acid formed from peat and a humic acid from the mouth of a small river (Lamas) on the Turkish coast of the eastern Mediterranean. The fluorescence excited by 337 nm light suggests the smaller fluorescence lifetimes measured at 420 nm and 500 nm (cf. the previous section) to be at least partly due to the humic acid in the sea water and that, as one would expect, this humic acid was composed of material having at least 2 different molecular masses.

The humic acid present in a sample of eastern Mediterranean water was isolated and re-dissolved. Figure 4 shows its emission spectrum (excitation at 230 nm) compared to the parent sea water at pHs of 10, 7 and 2. The humic acid showed emission at ∼300, 325 and 425 nm, wavelengths where our 'standard' humic acids also emitted (Table 5). The fluorescence of the sea water increased at pHs of 10 and 7 but became greatly diminished at pH 2. Similar changes with pH were observed in the fluorescence emitted by the re-dissolved humic acid at ∼425 nm though the increase of fluorescence at pHs of 10 and 7 was less marked. However, the pH dependence of fluorescence at ∼300 and ∼350 nm was not the same as that from sea water (Figure 4). Moreover, whereas the most intense emission by the humic acid (excitation at 230 nm) occurred at ∼425 nm and emission at ∼300 and 350 nm was much less intense, emission from the original eastern Mediterranean water at ∼425 nm was significant but unremarkable. Figure 4 shows, therefore, that humic acid made only a modest contribution to the fluorescence of eastern Mediterranean sea water at 425 nm excited by 230 nm light. Little of the significant emission ∼300 and 350 nm can have been due to humic acid. Comparison of Figures 2a, b, c and d indicates one can make a similar statement about the fluorescence from the Black Sea even though the Black Sea is more fertile than the eastern Mediterranean. Monitoring of the fluorescence of sea water at 460 nm (excitation at ∼360 nm) may overemphasise the importance of the contribution by humic acid.

Fluorescence spectra, contribution by aromatic amino acids and proteins

Table 6 shows the fluorescence maxima of aromatic amino acids and a bovine serum albumin under the same experimental conditions used for sea water. The synchronous spectra are similar to those of sea water.

Table 4. Fluorescence lifetimes (nanoseconds) of filtered, anoxic Black Sea water. (203 m depth) (excitation at 337 nm).

Emission (nm)	420			550		
Lifetimes (nanoseconds)	12.0	4.6	1.0	8.9	2.6	0.5
Standard deviation	0.4	0.2	0.04	0.4	0.3	0.2
Relative intensity (%)	25	44	31	42	42	16

Table 5. Wavelengths (nm) of the major peaks in humic acid fluorescence.

Excitation spectra (Emission at 420 nm)							
Humic acid 1	245	265	295	318	342		
Humic acid 2	246	365		317	342		
Excitation spectra (Emission at 504 nm)							
Humic acid 1		279	297	318	342	367	445*
Humic acid 2	267			318	344	368	445*
Excitation spectra (Emission at 230 nm)							
Humic acid 1	308			361		422	
Humic acid 2	309	325	353		397	415	
Excitation spectra (Emission at 337 nm)							
Humic acid 1	422		472	504			
Humic acid 2	420	466		504			

Humic acid 1. From 'Aldrich' obtained from peat
Humic acid 2. Isolated from the Lamas river estuary on the Mediterranean coast of Turkey
* = includes Raman scattering by water

Table 6. Fluorescence maxima (nm) of aromatic amino acids under the experimental conditions used for sea water.

Spectra	Tryptophane			Phenylalanine				Tyrosine			BSA		
Synchronous	287*	362	521	251*	282	364	517	273*	363	535	284*	355	574
Excitation	230	277*	384R	230	270*	370	384R	230	284*	384R	230	281*	314
											338	364	384R
Emission	254R	337	350*		254R	289*	380*		254R	304*	254R	311	334*
											346	383	

* = most intense maximum; R = Raman scattering by water
Synchronous, $\Delta = 50$;
Excitation, Emission wavelength = 440 nm;
Emission, Excitation wavelength = 230 nm.

Mopper and Schultz (1993) ascribed the emission of sea water at ∼300 nm to aromatic amino acid structures. It is tempting to ascribe the emission ∼300 nm and ∼350 nm, which have been seen to contain only small contributions from humic acids, to tyrosine and tryptophan, respectively (cf. Table 6; phenylalanine fluoresces much less intensely than its sisters). Using o-phthalaldehyde/mercaptoethanol as a specific test reagent producing fluorescent substituted isoindoles, we find the concentrations of amino acids in the 1992 Black Sea to have been of the order of 10 mg per litre of sea water, similar to the concentrations previously observed by Starikova and Korzhikova in 1969. However, Mopper and Klieber (1991) found much lower concentrations as a result of direct HPLC analysis on board ship and they question the effect of storage and preservation procedures on amino acid concentrations. Thus it appears that a portion of the fluorescence emitted by sea water at ∼300 and ∼350 nm is indeed due to aromatic amino acids but the magnitude of this proportion is as yet unknown. One notes that aqueous solutions of adenosine triphosphate, calf thymus DNA and histidine fluoresce at ∼300 and ∼350 nm when excited at 230 nm. One also notes that

i. peaks at 270-280 nm are the most intense of those in the excitation spectra of aromatic amino acids (Table 6) but appear less significant than the excitation at 230-250 nm from sea water.

ii. the peak at 376 nm, prominent on the emission spectrum of tryptophan (Table 6) is much less significant in sea water.

iii. Bovine serum albumin gives a clearly resolved peak at 574 nm in its synchronous spectrum (Table 6) that has not been observed from sea water.

One knows that most single ring aromatics, including those present in humic material, together with some naphthalenes, absorb and fluoresce at similar wavelengths and compounds other than aromatic amino acids are clearly contributing to the fluorescence of sea water in these regions of the spectra.

Fluorescence spectra, interpretation of emission ∼550 nm

The weak fluorescence of sea water around 520-550 nm seen, for example, in our synchronous spectra, has been attributed to flavins by Mopper and Kieber (1991), though other compounds also fluoresce weakly in this region. Mopper and Keiber's (1991) results appear to be confirmed by Coble and Gagosian (1991), who separated organic matter from sea water by HPLC and identified 5 flavins by co-injection. Mopper and Kieber's (1991) fluorescence measurements indicated that, with respect to riboflavin, sea water from the Black Sea contained between 1 and 40 picomoles of flavin per litre, an order of magnitude with which we would agree, to interpret the fluorescence of Black Sea samples in the same way. However, it is well established that simple flavins such as riboflavin absorb light at both 350 and 450 nm and fluoresce at 520-560 nm and that when these compounds are reduced – for example by hydrogen gas at 70 ^{o}C in the presence of nickel– the absorption at 450 nm no longer occurs (Matthews and van Holde, 1990). This is confirmed in Figures 5c and d. Figures 5a and b show the excitation and emission spectra of sea water before and after the bubbling of hydrogen gas at 70 ^{o}C in the presence of nickel. There was no diminution of the excitation at 450 nm or of the emission at 555 nm as was seen in the experiments with riboflavin. Again, it was observed that raising the pH of the sea water to 10 and then illuminating it with UV light did not produce the successive decrease and increase in fluorescence that this procedure has been found to induce in solutions of riboflavin (Matthews and van Holde, 1990). Thus, whereas the fluorescence of sea water shows similarities to that of flavins in the 520 nm region, chemistry indicates compounds other than simple flavins were producing this fluorescence at least in the eastern Mediterranean and Black Sea. We suggest the 520-550 nm fluorescence may correspond to that occurring at ∼550 nm from liptinites (for example from the sapropels deposited beneath the eastern Mediterranean and Black Sea), whence the fluorescence may tentatively be ascribed to carotenoids known to be present in microalgae (Blumer *et al.*, 1971; Sinninghe-Damste *et al.*, 1993; Brown *et al.*, 2000). Further investigations should test this hypothesis.

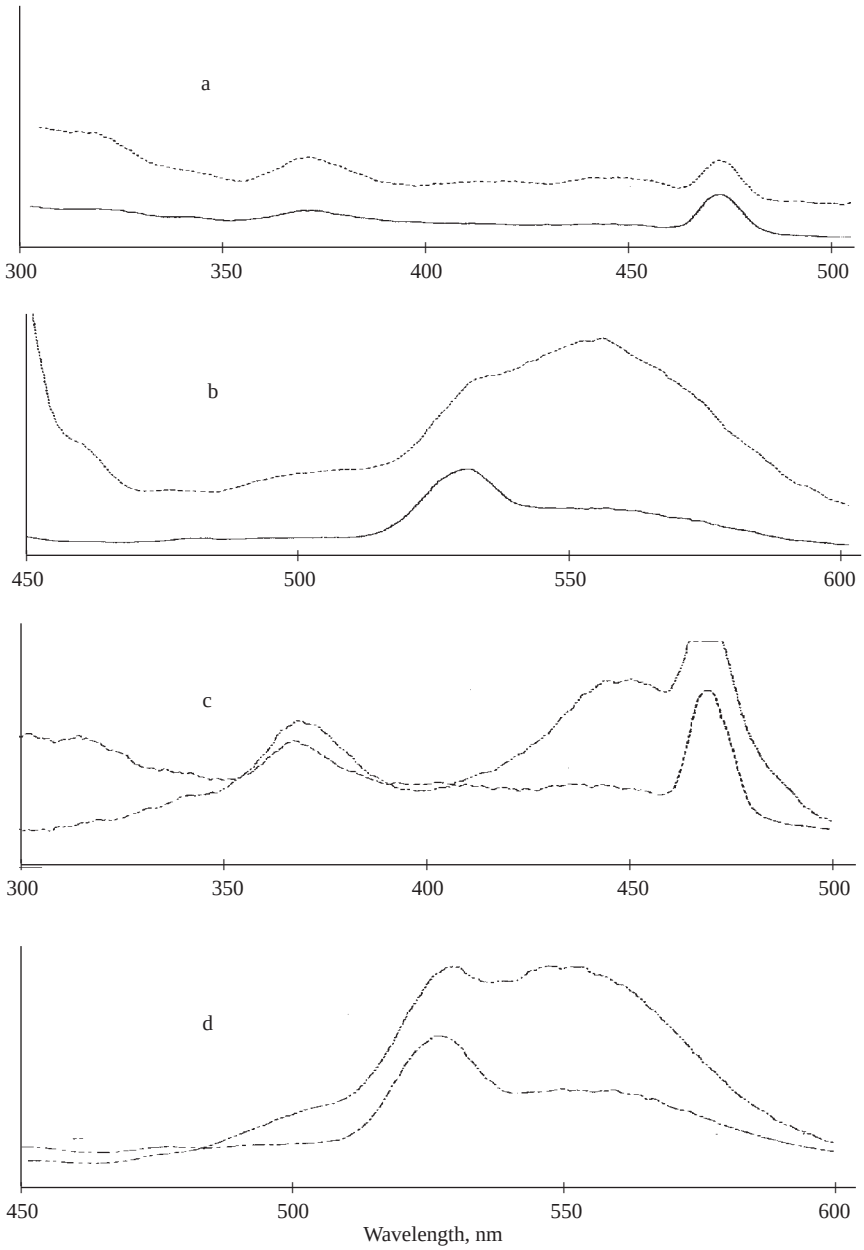

Figure 5. Hydrogenation at 70 °C in the presence of nickel shows the absence of flavins in Mediterranean sea water (fluorescence intensities in arbitrary units)

a) Excitation spectra of sea water (emission wavelength = 555 nm); lower spectrum, before, and upper spectrum, after, hydrogenation.

b) Emission spectra of sea water (excitation wavelength = 446 nm); lower spectrum, before, and upper spectrum, after, hydrogenation.

c) Excitation spectrum of aqueous riboflavin solution (emission wavelength = 555 nm); upper spectrum on the right hand side, before, and lower spectrum, after, hydrogenation.

d) Emission spectrum of aqueous riboflavin solution (excitation wavelength = 446 nm); upper spectrum on the right hand side, before, and lower spectrum, after, hydrogenation.

Note that the hydrogenation of sea water, unlike the hydrogenation of riboflavin, increases rather than decreases absorption at 446 nm.

Chemical oceanography and fluorescence

Whereas the fluorescence intensities Yilmaz *et al.* (1990) determined in eastern Mediterranean coastal waters showed humic acid concentrations to be, like phytoplankton populations, patchy, we now find fluorescence spectra to be quite characteristic of bodies of flowing sea water. This is illustrated in Table 7, which shows the (uncorrected) fluorescence intensities observed in the upper and lower layers of the Bosphorus relative to the appropriate Raman scattering of water. The fluorescence of the upper (Black Sea) layer is significantly greater than the fluorescence of the lower (Mediterranean) layer at all seasons of the year and the 2 layers are easily distinguished by their characteristic fluorescence. Perhaps surprisingly, the fluorescence of the lower layer Mediterranean water showed less seasonal variation than the upper, Black Sea layer.

Our figures show the intensities of the excitation, emission and, to a lesser extent, synchronous spectra obtained from the eastern Mediterranean to decrease below the euphotic zone. In the Black Sea, however, it was the anoxic zone that showed the greatest fluorescence. Clearly, in the eastern Mediterranean the fluorescence from humic material and from aromatic amino acids, for example, is to be associated with phytoplankton and below the euphotic zone these materials – or at least their fluorescence – are destroyed, presumably by oxidation in the comparatively well-oxygenated water. In the stratified Black Sea, however, the humic material and the aromatic amino acids are preserved and accumulate in the anoxic zone.

In this study the major fluorophores that have been discussed are humic acids, aromatic amino acids and proteins and carotenoids. The fluorescence of chlorophyll has not been considered. The discussion in terms of 3 fluorophores is a gross oversimplification; there is undoubtedly a wide range of fluorescent compounds present in the Black Sea and eastern Mediterranean and, as the measurements of fluorescence lifetimes show, many fluorescent structures will be present in compounds having a range of molecular masses. Further study of fluorescence may yield much information about the dissolved organic material present in carefully filtered sea water. Coble and Gagosian (1991) indicate the utility of HPLC in separating dissolved organic matter present in Black Sea water. Our observations suggest that careful detection of HPLC fractions using a variety of fluorescence wavelengths might facilitate the fractionation. The fractions might then be characterised and identified by their mass spectra.

Table 7. Fluorescence of Black Sea surface water and lower layer Mediterranean water in the Bosphorus.

Date	Depth	Syn 230	Syn 280	Exc 230	Exc 330	Em 400	Em 345	Em 300
Aug. 1991	Surface	0.14	0.56	1.72	0.75	1.01	1.21	0.55
Dec. 1991	Surface	0.09	0.50	1.31	0.81	1.26	1.43	0.70
March 1992	Surface	0.10	0.45	1.14	0.65	0.93	1.08	0.52
May 1992	Surface	0.10	0.50	0.93	0.59	0.96	1.30	0.59
Aug. 1991	σ_t 27-29	0.13	0.20	1.14	0.48	0.43	0.37	0.25
Dec. 1991	σ_t 27-29	0.07	0.17	0.65	0.41	0.46	0.42	0.24
March 1992	σ_t 27-29	0.08	0.19	0.71	0.43	0.46	0.35	0.23
May 1992	σ_t 27-29	0.08	0.21	0.41	0.43	0.48	0.44	0.26

σ_t = water density ‰
Syn 230 = Synchronous fluorescence (uncorrected) at excitation of 230 nm relative to Raman scattering at 354 nm
Syn 280 = synchronous fluorescence (uncorrected) at excitation of 280 nm relative to Raman scattering at 354 nm
Ex 230 = Excitation intensity (Em = 440 nm) (uncorrected) at 230 nm relative to Raman scattering at 380 nm
Ex 330 = Excitation intensity (Em = 440 nm) (uncorrected) at 330nm relative to Raman scattering at 380 nm
Em 400 = Emission intensity (Ex = 230 nm) (uncorrected) relative to Raman scattering at 254 nm
Em 345 = Emission intensity (Ex = 230 nm) (uncorrected) relative to Raman scattering at 254 nm
Em 300 = Emission intensity (Ex = 230 nm) (uncorrected) relative to Raman scattering at 254 nm

Conclusions

1. If one ignores the fluorescence generated by chlorophyll and phaeopigments, the most informative fluorescence spectra characterising Black Sea and eastern Mediterranean water are obtained when emission is excited by 220-230 nm ultra-violet light. The most informative excitation spectra are obtained when emission is monitored at about 440 nm and the most informative synchronous spectra are generated when $\Delta = 50$ nm. Characteristic spectra are shown in Figures 1, 2 and 3.

2. The reproducibility of the intensity of a single peak in these fluorescence spectra is 5-10%, the major errors arising from the irreproducibility of sampling at a precise depth and location.

3. The fluorescence spectra consist of overlapping peaks. The resolution, which is not limited by the band pass of the spectrometer, can be improved by working at liquid nitrogen temperatures and by simulating the spectra by a series of Gaussian peaks.

4. Furthermore, studies of fluorescence lifetimes show the presence of both simple and complex molecules with similar fluorophores to contribute to the broadness each peak of the spectra.

5. Previous work has emphasised that the presence of humic acids in sea water can be monitored by their fluorescence at ∼460 nm when excited by 360 nm light. When sea water fluorescence is excited by 230 nm light, however, the most intense emission by humic acids is observed ∼425 nm. Accordingly, humic acid fluorescence made only a modest contribution to the fluorescence of Black Sea and eastern Mediterranean and waters observed here.

6. Tyrosine and tryptophan residues contribute to the emission observed at 300 and 350 nm respectively (excitation = 230 nm) in Black Sea and eastern Mediterranean waters but the magnitude of the contribution remains unknown since other entities, at present unidentified, also fluoresce at these wavelengths.

7. Emission at 520-550 nm observed in Black Sea and eastern Mediterranean waters is not due to simple flavins as previous authors have suggested. We suppose the emission to be similar to that observed at the same wavelengths from sapropels and accordingly attribute it to carotenoids present in lipid structures.

8. There is much opportunity for further studies to utilise fluorescence to identify and fractionate the many different compounds that contribute to the dissolved organic matter in Black Sea and eastern Mediterranean waters.

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