UKTAG COASTAL WATER ASSESSMENT METHODS
PHYTOPLANKTON

PHYTOPLANKTON MULTI-METRIC TOOL KIT

By
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(WFD – UKTAG)

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HEALTH AND SAFETY STATEMENT

WARNING— working in or around water is inherently dangerous; persons using this standard should be familiar with normal laboratory and field practice. This published monitoring system does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national regulatory guidelines.

It is also the responsibility of the user if seeking to practise the method outlined here, to gain appropriate permissions for access to watercourses and their biological sampling.

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1. INTRODUCTION

This method statement describes a system for monitoring, assessing and classifying coastal waters in accordance with the requirements of Article 8; Section 1.3 of Annex II; and Annex V of the Water Framework Directive (2000/60/EC).

1.1. Geographic application of the method

The method can be applied to coastal waters in England, Northern Ireland, Scotland and Wales.

1.2. Quality element assessed by the method

The method enables an assessment of the condition of the quality element, "phytoplankton", listed in Tables 1.2.3 and 1.2.4 of Annex V to the Water Framework Directive.

The following parts of the tool have been intercalibrated in the first round of European intercalibration: phytoplankton biomass; bloom frequency - *Phaeocystis*; and bloom frequency - individual taxa.

1.3. Pressures to which the method is known to be sensitive

The method has been designed to detect the impact on the quality element of nutrient enrichment.

1.4. Parameters indicative of the quality element

The method uses a multi-parameter index, “Phytoplankton Multi-metric Toolkit Index” for the purpose of assessing the condition of the quality element. The Index is based on three parameters:

Phytoplankton biomass during the growing season;

Bloom frequency in respect of chlorophyll, individual taxa, total taxa and *Phaeocystis*;

Seasonal succession of phytoplankton functional groups.

2. SAMPLING AND ANALYSIS

2.1. Sampling method

Within this method, “sampling” means the collection and preservation of sets of water samples for the purpose of measuring the composition, abundance, biomass and blooms of phytoplankton so as to estimate the Ecological Quality Ratio.

The sampling method should conform to international standard EN 15204.

2.1.1. Sampling sites

Sampling sites should be chosen in waters representative of phytoplankton biomass concentration, phytoplankton composition, salinity and ancillary characteristics.

2.1.2. Sample number, timing and frequency

Samples of chlorophyll-a and phytoplankton should be collected each month in numbers sufficient to be representative of a water body.

Samples not taken in any calendar month may be collected up to, but not exceeding, 7 days before or after that month. There must be an interval of at least 10 days between sampling.

Sampling should be done in daylight.

2.1.3. Method – introduction to phytoplankton and chlorophyll-a

Separate samples should be collected for the analysis of chlorophyll-a and phytoplankton composition.

In waters 5 metres deep or less at the time of sampling, samples should be taken with a simple container close to the water surface, avoiding any surface film and without disturbing bottom sediments.

In waters deeper than 5 metres, an integrated sample should be taken with a hose across a depth range representative of the waters, avoiding any surface film and without disturbing bottom sediments.

Accompanying measurements of the salinity should be made.

2.1.4. Method – phytoplankton

Phytoplankton samples should be preserved with Lugol's iodine solution and kept in the dark. Preserved samples may be stored at room temperature, provided that they are analysed within 3 weeks. If not to be analysed within 3 weeks, samples should be kept between 1ºC and 4ºC and should be analysed within 3 months.

2.1.5. Method – chlorophyll-a

Samples should be kept cool and dark. They should be micro-filtered (47µm filter paper) within four hours of collection. The filtered residue should be analysed as soon as possible and, if possible, within four hours. If not analysed within four hours, the filter papers should be frozen and kept dark until analysed.

2.1.6. Method – salinity

The salinity at the site should be measured or water should be sampled for salinity determination.

2.2. Analytical method

2.2.1. Salinity

The salinity of water should be measured by a contemporary quality-controlled reproducible method traceable to the salinity of IAPSO standard seawater.

2.2.2. Chlorophyll-a samples

The concentration of chlorophyll-a should be estimated in micrograms per litre ($\mu g.l^{-1}$) by a contemporary quality-controlled reproducible method involving fine algal filtration, solvent extraction and quantitative fluorimetric measurement of chlorophyll-a.

2.2.3. Phytoplankton samples

Phytoplankton should be identified to the lowest possible taxonomic level (e.g. species) according to a contemporary standard taxonomic list.

The count of taxonomically identified phytoplankton cells per litre ($cell.l^{-1}$) of sample should be estimated with light microscopy.

3. DERIVATION OF THE ECOLOGICAL QUALITY RATIO FOR THE PARAMETERS

3.1. Calculation of the observed value for each parameter

Three indicative parameters should be estimated from the measurements over a period of a year: phytoplankton biomass during the growing season; bloom frequency; and seasonal succession of phytoplankton functional groups.

3.1.1. Phytoplankton biomass during the growing season

The observed value of the parameter, phytoplankton biomass during the growing season, is the 90-percentile phytoplankton biomass; this should be estimated as the chlorophyll-a concentration in micrograms per litre ($\mu g.l^{-1}$) below which lie ninety percent (90%) of the measured chlorophyll-a concentrations made during the phytoplankton growing season from March to October inclusive.

3.1.2. Bloom frequency of phytoplankton functional groups

The observed value of the parameter, bloom frequency of phytoplankton functional groups, should be composed from four indicators of bloom frequency that should be estimated from samples: chlorophyll; individual taxa; total taxa; and *Phaeocystis*.

3.1.2.1. Chlorophyll Bloom Frequency Indicator

The chlorophyll bloom frequency should be calculated as the fraction of all samples whose chlorophyll-a has been measured in which the chlorophyll-a concentration exceeds ten micrograms per litre (10 $\mu g.l^{-1}$).

3.1.2.2. Individual Taxa Bloom Frequency Indicator

The individual taxa bloom frequency should be calculated as the fraction of all samples whose individual taxon concentrations have been measured in which the concentration of any individual taxon other than *Phaeocystis* exceeds a quarter of a million cells per litre (250,000 cell.l^{-1}).

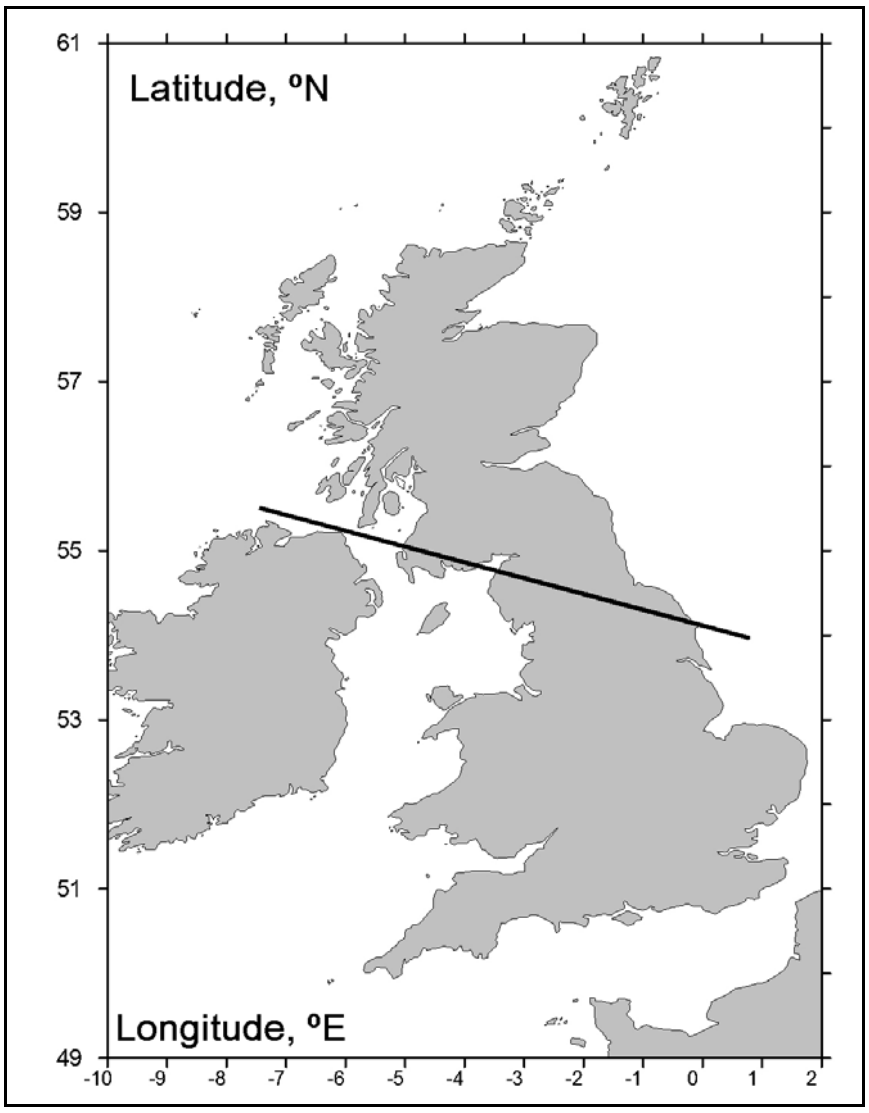
3.1.2.3. Total Taxa Bloom Frequency Indicator

The total taxa bloom frequency should be calculated as the fraction of all samples whose total taxa concentrations have been measured in which the total taxa concentration exceeds one million cells per litre (10^6 cell.l^{-1}) in the southern ecozone of Figure 1 or ten million cells per litre (10^7 cell.l^{-1}) in the northern ecozone of Figure 1.

3.1.2.4. *Phaeocystis* Bloom Frequency Indicator

For the southern ecozone and the intercalibrated southern North Sea water bodies of Figure 1, the *Phaeocystis* bloom frequency should be calculated as the fraction of all samples whose *Phaeocystis* concentrations have been measured in which the concentration of *Phaeocystis* exceeds one million cells per litre (10^6 cell.l^{-1}).

Figure 1: Northern and southern ecozones for the total taxa and *Phaeocystis* bloom indicators, The boundary coordinates at the west coast are E/N 206728 580323 near Ballantrae and at the east coast are E/N 525836 470449 near Flamborough Head.



3.1.2.5. Bloom Frequency as a combination of the indicators

For the northern ecozone of Figure 1, the observed value of the parameter, bloom frequency of phytoplankton functional groups, should be estimated as the arithmetic mean of the three indicators estimated according to 3.1.2.1, 3.1.2.2 and 3.1.2.3.

For the southern ecozone and the intercalibrated southern North Sea water bodies of Figure 1, the observed value of the parameter, bloom frequency of phytoplankton functional groups, should be estimated as the arithmetic mean of the four indicators estimated according to 3.1.2.1, 3.1.2.2, 3.1.2.3, and 3.1.2.4.

3.1.3. Seasonal succession of phytoplankton functional groups

The observed value of the parameter, seasonal succession of phytoplankton functional groups, should be estimated from the measurements of phytoplankton in all samples from relevant years, having regard to functional group and geographical region.

For the waters of Scottish lochs and coasts and Northern Ireland, three functional groups should be used: diatoms, dinoflagellates and microflagellates.

For the waters of English and Welsh coasts two functional groups should be used: diatoms and

dinoflagellates.

3.1.3.1. Functional Group Concentrations

For each functional group, estimates of concentration (cells per litre) should be made by arithmetic addition of the concentrations of those species relevant to the functional group as measured according to section 2.2.3.

3.1.3.2. Functional Group Seasonal Series

For each functional group, a seasonal series of twelve (January, February … December) monthly concentrations (C_1, C_2 … C_{12} cell.l^{-1}) should be estimated as the arithmetic average of all estimates falling in the corresponding calendar months. The averages C_1, C_2 … C_{12} should be composed from all data at the site (averaging all January values into C_1, all February values into C_2 … all December values into C_{12}).

3.1.3.3. Functional Group Seasonal Z-scores

For each functional group, each monthly concentration (C_i cell.l^{-1}) estimated according to 3.1.3.2 should be converted to a monthly Z-score calculated according to the algorithm in Table 1:

Table 1: Algorithm for the computation of Z-scores

Compose twelve logarithmically transformed values Y_1, Y_2, … Y_{12} by using natural logarithms (Ln) to calculate:
$Y_1 = Ln(C_1)$ … $Y_{12} = Ln(C_{12})$
Compare these Y_i with the reference set mean P and the reference set standard deviation S to give Z_i scores by calculating:
$Z_i = (Y_i - P) \div S$
where i takes values from 1 to 12
The values of P and S should be taken from the geographically relevant Table 2 or Table 3.

Table 2: English and Welsh coasts, reference set values for the functional groups

Functional group	**Diatoms**	**Dinoflagellates**
Mean P	5.90	5.00
Standard deviation S	1.89	1.54

Table 3: Scottish lochs and coasts, and Northern Ireland, reference set values for the functional groups

Functional group	**Diatoms**	**Dinoflagellates**	**Microflagellates**
Mean P	Mean of all transformed concentrations Y_i	Mean of all transformed concentrations Y_i	Mean of all transformed concentrations Y_i
Standard deviation S	Standard deviation of all transformed	Standard deviation of all transformed	Standard deviation of all transformed

	concentrations Y_i	concentrations Y_i	concentrations Y_i

3.1.3.4. The Functional Group Indicator

In the waters of the English and Welsh coasts, for each functional group a functional group indicator should be estimated as the proportion of monthly Z-scores calculated according to 3.1.3.3 that lie below the appropriate functional group reference envelope delimited by the upper bound in Column 2 of Table 4 or Table 5.

Table 4: Waters of the English and Welsh coasts - seasonal reference bounds for diatoms

Column 1	**Column 2**
Month	**Upper bound**
1	-0.12
2	-0.16
3	-0.06
4	0.39
5	0.95
6	1.43
7	1.26
8	1.07
9	0.58
10	0.05
11	-0.17
12	-0.17

Table 5: Waters of the English and Welsh coasts - seasonal reference bounds for dinoflagellates

Column 1	**Column 2**
Month	**Upper bound**
1	-0.11
2	0.05
3	0.06
4	0.44
5	0.63
6	0.88
7	0.86
8	0.92
9	1.18
10	0.48
11	0.15

12	-0.19

In the waters of Northern Ireland, and the Scottish coast and sea-lochs, for each functional group a functional group indicator should be estimated as the proportion of monthly Z-scores calculated according to 3.1.3.3 that lie within the appropriate functional group reference envelope delimited by the upper and lower bounds in Columns 2 and 3 of Table 6, Table 7, Table 8, Table 9, Table 10 or Table 11.

Table 6: Scottish sea-lochs, seasonal reference bounds for diatoms

Column 1	**Column 2**	**Column 3**
Month	**Lower bound**	**Upper bound**
1	0.19	-1.76
2	-0.24	-2.07
3	1.08	-0.20
4	0.82	0.48
5	1.00	0.56
6	1.37	-0.90
7	1.43	-0.68
8	0.74	0.16
9	1.22	-0.76
10	0.85	-0.23
11	0.55	-1.23
12	-0.33	-2.15

Table 7: Scottish sea-lochs, seasonal reference bounds for dinoflagellates

Column 1	**Column 2**	**Column 3**
Month	**Lower bound**	**Upper bound**
1	0	0
2	-0.25	-1.52
3	0	0
4	0.76	0.52
5	1.44	-0.30
6	0.94	0.55
7	1.08	0.72
8	1.45	0.86
9	1.33	-1.07
10	0.18	-1.43
11	0.72	-0.91
12	0.07	-1.46

Table 8: Scottish sea-lochs, seasonal reference bounds for microflagellates

Column 1	Column 2	Column 3
Month	**Lower bound**	**Upper bound**
1	0.16	-0.38
2	-0.68	-1.32
3	0.47	0.06
4	0.51	-0.07
5	0.44	-0.08
6	0.74	0.08
7	0.73	0.13
8	0.56	-0.40
9	0.42	-0.12
10	0.56	0.05
11	-0.65	-1.31
12	0.26	-0.42

Table 9: Waters of Northern Ireland, and the Scottish coast other than sea-lochs, seasonal reference bounds for diatoms

Column 1	Column 2	Column 3
Month	**Lower bound**	**Upper bound**
1	0.12	-1.27
2	0.53	-1.54
3	0.77	-0.97
4	0.85	0.01
5	1.49	-0.65
6	1.59	-0.53
7	1.08	0.38
8	1.14	0.27
9	0.83	0.04
10	0.56	-1.86
11	0.30	-1.92
12	0.28	-1.59

Table 10: Waters of Northern Ireland, and the Scottish coast other than sea-lochs, reference bounds for dinoflagellates

Column 1	Column 2	Column 3
Month	**Lower bound**	**Upper bound**

1	-0.35	-1.40
2	0.26	-1.36
3	-0.01	-1.42
4	0.94	-1.12
5	0.94	-1.12
6	1.47	-0.30
7	1.24	0.83
8	1.37	0.87
9	1.46	-0.33
10	0.90	-0.92
11	0.79	-1.11
12	-0.44	-1.37

Table 11: Waters of Northern Ireland, and the Scottish coast other than sea-lochs, seasonal reference bounds for microflagellates

Column 1	**Column 2**	**Column 3**
Month	**Lower bound**	**Upper bound**
1	0.30	-1.88
2	1.46	-2.24
3	1.36	-1.88
4	0.61	-0.95
5	1.20	-0.07
6	1.05	0.01
7	0.72	-0.36
8	1.21	-0.62
9	0.76	-0.15
10	0.58	-0.56
11	0.47	-0.80
12	0.69	-1.08

3.1.3.5. The Seasonal Succession Indicator

The observed value of the parameter, seasonal succession of phytoplankton functional groups, should be estimated as the arithmetic mean of the two (English and Welsh coasts) or three (Scottish coasts and sea-lochs, Northern Ireland) functional group indicators calculated according to sections 3.1.3.1, 3.1.3.2, 3.1.3.3 & 3.1.3.4.

3.2. Reference values for each parameter

Reference conditions were derived using a combination of ranked sites (i.e. identifying those approaching reference) and historical data.

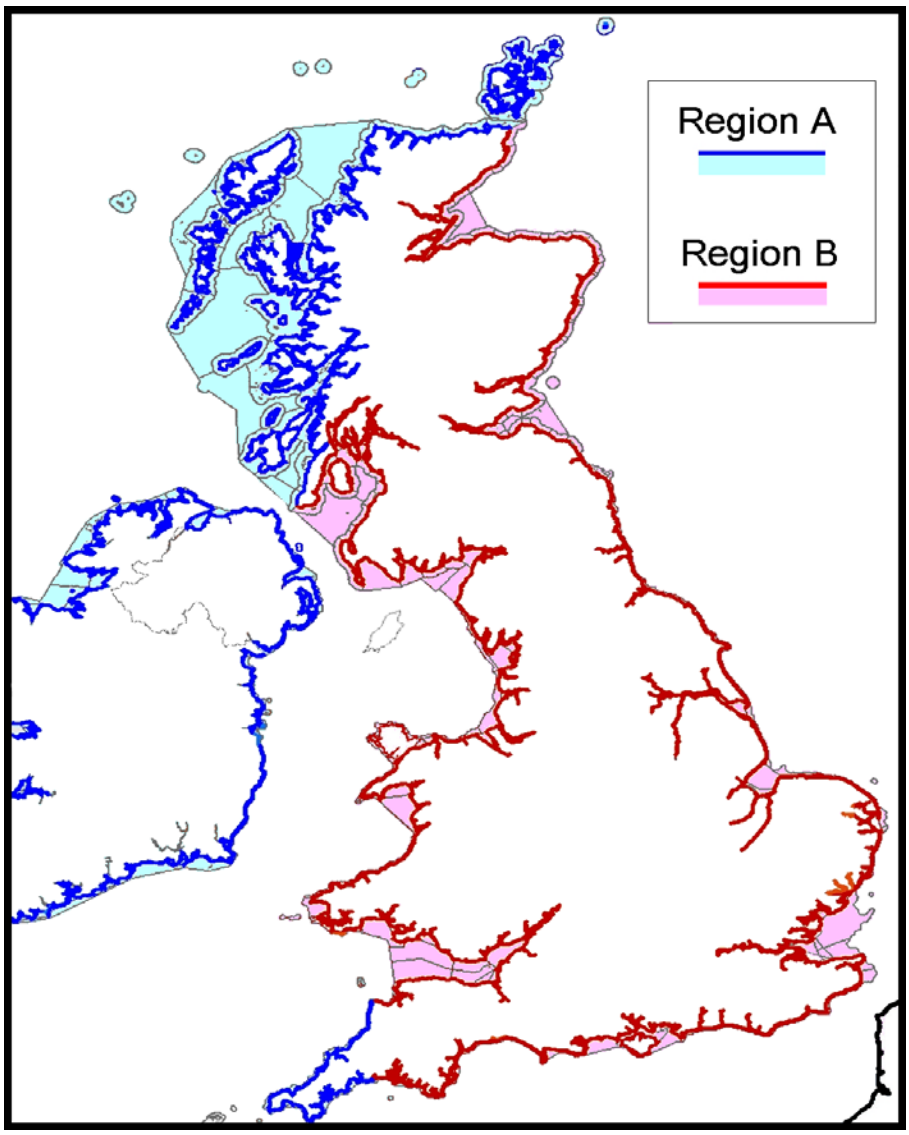
The values in Column 2 of Table 12 should be applied as the reference conditions for the corresponding parameters listed in Column 1 of that Table.

Table 12: Reference values for each parameter

Column 1	**Column 2**	
Parameter	**Reference values**	
	Sea Region A	**Sea Region B**
Phytoplankton biomass (PB) as chlorophyll-a concentration ($\mu g.l^{-1}$)	3.33	6.67
Bloom frequency (BF) (%)	10	10
Seasonal succession of functional groups (SS) (%)	80	80

Figure 2: Sea Regions for the phytoplankton biomass parameter.

Region A includes all Irish waters, and Scottish waters between the Mull of Kintyre (E/N 158913 607187) and Duncansby Head (E/N 340614 972891), including Orkney, Shetland and the Western isles. Region A also includes the following English water bodies; St Austell, Fal/ Helford, Cornwall South, Land's End to Trevose Head, Cornwall North. All other waters are found in sea region B.



3.3. Calculation of the ecological quality ratio

The ecological quality ratio (EQR_{PB}) for the parameter, phytoplankton biomass, should be calculated using the following equation:

$$EQR_{PB} = \text{reference value for parameter} \div \text{observed value for parameter}$$

Where the calculated value of EQR_{PB} is greater than "1", EQR_{PB} should be assigned a value of "1".

The ecological quality ratio (EQR_{BF}) for the parameter, bloom frequency, should be calculated using the following equation:

$$EQR_{BF} = [100 - \text{observed value for parameter}] \div [100 - \text{reference value for parameter}]$$

Where the calculated value of EQR_{BF} is greater than "1.06", EQR_{BF} should be assigned a value of "1.06".

The ecological quality ratio (EQR_{SS}) for the parameter, seasonal succession of functional groups, should be calculated using the following equation:

$$EQR_{SS} = \text{observed value for parameter} \div \text{reference value for parameter}$$

Where the calculated value of EQR_{SS} is greater than "1.06", EQR_{SS} should be assigned a value of "1.25".

4. DEFINITION OF TERMS

IAPSO – International Association of Physical Sciences of the Ocean

Annex A LISTING INDICATORS

Phaeocystis is an indicator species for the bloom frequency indicator.

Annex B WORKED EXAMPLE

A notional set of samples from the English coast in the southern ecozone of Figure 1 and region A of was analysed with the following results.

4.1.1. B.1 Phytoplankton biomass during the growing season

The 90-percentile phytoplankton biomass was measured as 6 $\mu g.l^{-1}$ chlorophyll-a.
The ecological quality ratio (EQR_{PB}) for the parameter, phytoplankton biomass, should be calculated using the following equation:

EQR_{PB} = [3.33] ÷ [6] = 0.56

4.1.2. B.2 Bloom frequency of phytoplankton functional groups

4.1.2.1. Chlorophyll Bloom Frequency

The fraction of samples whose chlorophyll-a concentration exceeded 10 $\mu g.l^{-1}$ was 16.9%.

4.1.2.2. Individual Taxa Bloom Frequency

The fraction of all samples whose concentration of any individual taxon other than *Phaeocystis* exceeded a quarter of a million cells per litre (250,000 $cell.l^{-1}$) was 18.2%.

4.1.2.3. Total Taxa Bloom Frequency

The fraction of all samples whose total taxa concentration exceeded ten million cells per litre (10^7 $cell.l^{-1}$) was 6.1%.

4.1.2.4. *Phaeocystis* Bloom Frequency

The fraction of all samples whose *Phaeocystis* concentration exceeded one million cells per litre (10^6 $cell.l^{-1}$) was 12.7%.

4.1.2.5. Bloom Frequency as a combination of the four indicators
The bloom frequency indicator was estimated as the arithmetic mean of the four indicators as (16.9% + 18.2% + 6.1% + 9.6%), or 12.7%.

The ecological quality ratio (EQR_{BF}) for the parameter, bloom frequency, should be calculated using the following equation:

EQR_{BF} = [100 – 12.7] ÷ [100 - 10] = 0.97

4.1.3. B.3 Seasonal succession

4.1.3.1. Functional Group Concentrations

Estimates of the functional group concentration (cell.l^{-1}) were made by arithmetic addition of the relevant species concentrations measured according to section 2.2.3.

4.1.3.2. Functional Group Seasonal Series (diatoms and dinoflagellates)

A seasonal series of twelve (January, February … December) diatom concentrations (C_1, C_2 … C_{12}) was composed from the arithmetic means of all estimates falling in the corresponding calendar months and was analysed according to section 3.1.3.4 and Table 4 as in Table 13.

Table 13: Exemplary calculations for functional group - diatoms

	Diatom measurements	**Algorithm from Table 1**		**Seasonal reference bounds from Table 4**	**Is Z below the bound?**
Month	**Functional group concentrations**	**Y_1 … Y_{12}**	**Z-scores**	**Upper**	
	C_1 … C_{12}	**$Ln(C_1)$ … $Ln(C_{12})$**	**Z_1 … Z_{12}**		
January	100	4.61	-0.69	-0.12	Yes
February	430	6.06	0.09	-0.16	No
March	670	6.51	0.32	-0.06	No
April	1100	7.00	0.58	0.39	No
May	1200	7.09	0.63	0.95	Yes
June	3578	8.18	1.21	1.43	Yes
July	5000	8.52	1.38	1.26	No
August	1000	6.91	0.53	1.07	Yes
September	1000	6.91	0.53	0.58	Yes
October	250	5.52	-0.20	0.05	Yes
November	230	5.44	-0.24	-0.17	Yes
December	200	5.30	-0.32	-0.17	Yes
P	5.9	Value taken from Table 2 (for southern ecozone)			
S	1.89	Value taken from Table 2 (for southern ecozone)			
Diatom indicator:		Proportion within bounds = 8 ÷ 12 = 67%			

Similar working of dinoflagellate data gave a dinoflagellate indicator value of 42%. The microflagellate indicator is not applicable to the English coast.

The seasonal succession indicator was estimated as the arithmetic mean of the two functional group indicators = (67% + 42%) ÷ 2 = 54%.

The ecological quality ratio (EQR_{SS}) for the parameter, seasonal succession of functional groups, should be calculated using the following equation:

EQR_{SS} = 54 ÷ 80 = 0.67

Annex C FURTHER READING

Devlin, M, M Best, D Coates, E Bresnan, S O'Boyle, R Park, J Silke, C Cusack & J Skeats (2007) Establishing boundary classes for the classification of UK marine waters using phytoplankton communities. Marine Pollution Bulletin 55: 91–103

BS EN 15204:2006 Water quality. Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermoehl technique). British-Adopted European Standard / 29-Sep-2006 / 46 pages ISBN: 0580489345