# UKTAG Coastal Water Assessment Method

# Phytoplankton

# Coastal Water Phytoplankton Tool


**by**

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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 water courses and their biological sampling

# UKTAG Guide to the Coastal Water Phytoplankton Tool
# Water Framework Directive: Coastal Waters

**Purpose of document:** To provide an overview of the coastal water (CW) phytoplankton tool to inform Practitioners of how to monitor, assess and classify suitable phytoplankton data according to Water Framework Directive (WFD) requirements in coastal waters.

*Note:* this document does not fully describe all aspects of the phytoplankton tool development and application; for this please refer to the full technical report (Devlin *et al*., 2013). A summary of key documents and references is provided within this document.

**Introduction to WFD Terminology and Assessment:** This guide describes a system for classifying in accordance with the requirements of Article 8; Section 1.3 of Annex II and Annex V of the WFD (2000/60/EC). Practitioners should recognise that the terminology used in this document is specific to the WFD and as such has a defined meaning.

To carry out a WFD biological assessment, each WFD defined biological quality element (BQE, defined in the WFD) is required to give a statistically robust definition of the 'health' of that element in the defined water body. The 'health' of a BQE is assessed by comparing the measured conditions (observed value) against that described for reference (minimally impacted) conditions. This is reported as an Ecological Quality Ratio (EQR). An EQR of one represents reference conditions and zero represents severe impact. The EQR is divided into five ecological status classes (High, Good, Moderate, Poor, Bad) that are defined by the changes in the biological community in response to disturbance (Figure 1). Alongside the EQR score and class status, any assessment must consider the certainty of the assessment (i.e. confidence in the assigned class).

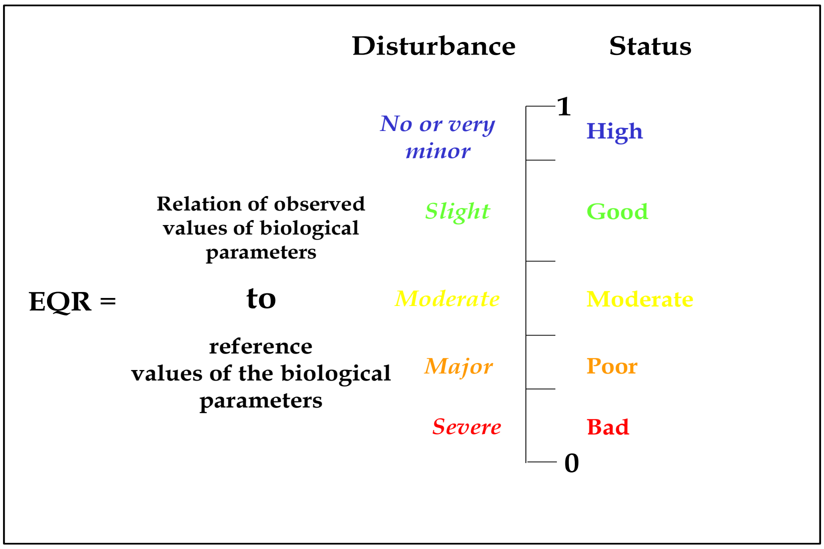


**Figure 1: Illustration of the Ecological Quality Ratio and how it relates to the level of disturbance and ecological status during a classification. The class band widths relate to biological changes as a result of disturbance (WFD CIS Guidance Document No. 5, 2003).**

# 1. Key Facts

## 1.1 Tool Overview: CW Phytoplankton tool

The phytoplankton tool enables an assessment of the condition of the quality element, "phytoplankton", as listed in Table 1.2.4 of Annex V to the Water Framework Directive (2000/60/EC). The WFD requires that the assessment of the phytoplankton quality element considers composition, abundance, biomass and planktonic blooms.

The phytoplankton tool for coastal waters is formed of three separate indices:

(i) Chlorophyll-a[1] 90$^{th}$ percentile metric
(ii) Elevated count multimetric
(iii) Seasonal succession multimetric.

The **chlorophyll-a 90$^{th}$ percentile metric** works by calculating the 90$^{th}$ percentile value of chlorophyll-a biomass over the growing season (March to October inclusive).

The **elevated count multimetric** is based on the number of occasions that phytoplankton counts exceed an established threshold over the reporting period. There are three metrics within this multimetric:

(i) percentage exceedance of chlorophyll-a threshold (measured as $\mu g\ l^{-1}$)
(ii) percentage exceedance of **single** taxa threshold (measured as cells $l^{-1}$)
(iii) percentage exceedance of **total**[2] taxa threshold (measured as cells $l^{-1}$).

The **seasonal succession multimetric** works on the measurement of the two main taxonomic groupings (diatoms and dinoflagellates) falling with a seasonal reference growth curve.

*Note*: index reference conditions are geographically specific.

The three indices are averaged to provide an overall phytoplankton assessment. Although a phytoplankton water body assessment is designed to be an average of the three indices, an assessment can be made from one, or any combination, of the indices. It is important to understand the implication, and potential risk of misclassification, when interpreting an assessment where only a partial assessment is made.

For the first River basin Management Plans in 2009, two indices (chlorophyll-a 90$^{th}$ percentile and elevated counts) were used for assessing CW phytoplankton. *Note*: the *Phaeocystis* metric that was initially incorporated in the elevated count tool has now been removed.

[1] All references to chlorophyll-a in this guide are to chlorophyll which tends to be measured with either HPLC methods or by extraction (hot methanol or cold acetone) and spectrophotometry or flurometry. The latter methods may include a small amount of breakdown products.
[2] The use of the term ‘total taxa’ in this document refers to the taxa, mainly diatoms and dinoflagellates, identified and enumerated using the WFD phytoplankton analysis method. A standardised phytoplankton identification list (referred in this document as the revised phytoplankton list) is used by all laboratories analysing samples for WFD purposes within the UK.

The phytoplankton tool (and component indices) operates over a range from zero (severe disturbance) to one (reference/minimally disturbed conditions). The four class boundaries for the tool are:

- High/Good = 0.80
- Good/Moderate = 0.60
- Moderate/Poor = 0.40
- Poor/Bad = 0.20.

To calculate the phytoplankton indices, the abundance of identified phytoplankton taxa (identified to an agreed practical taxonomic level), measurement of chlorophyll and supporting parameters e.g. salinity and turbidity are required.

### 1.2 Applicability

The phytoplankton tool can be used at different spatial scales, depending on the aims of the survey, but for WFD reporting the tool is applied at a water body scale.

**Where:** The tool can be applied to all UK coastal waters. However, it is not used for assessing saline lagoons due to the particular challenges in setting suitable type-specific reference conditions for these water bodies. For some water bodies, such as where there are naturally high levels of turbidity, or where there is a high level of natural variability in the phytoplankton community, there should be careful consideration of whether phytoplankton can be assessed according to the full requirements of the WFD.

**When**: The phytoplankton indices have been developed to classify data from a minimum of monthly samples across the year. The chlorophyll-a $90^{th}$ percentile metric utilises the monthly data from the growing season only (March to October, inclusive) but the elevated count and seasonal succession indices require monthly data from the whole year (i.e. 12 months). *Note*: a *minimum* of nine months data across a single year is required to run the seasonal succession and elevated counts indices.

Due to the high level of natural variability in phytoplankton communities, several years data will be required before any certainty of assessment can be obtained. Data requirements (i.e. number of years of data) will depend on the level of natural variability seen for the water body type and is likely to be influenced by the hydrodynamic regime (i.e. at least 2-3 years in a 6 year reporting period will be required).

**Response to pressure**: The phytoplankton tool has been designed to identify the impact on phytoplankton from nutrients and should detect signs of eutrophication.

The phytoplankton tool is generally insensitive to hazardous substances or physical modification pressures. However, climate is also a strong driver of phytoplankton community abundance and composition, so indices could reflect a climatic response. This should be considered when interpreting the results.

### 1.3 Key Documents

The documents marked * will be hosted on the UK Technical Advisory Group (UK TAG) website www.wfduk.org.

*Davey, A. (2009). Confidence of Class for WFD Marine Plant Tools. WRC report EA7954. 34 pp.

*Devlin, M. J., Best, M., Bresnan, E., & Baptie, M. (2013). Water Framework Directive: The development and status of phytoplankton tools for ecological assessment of coastal and transitional waters. United Kingdom. Update Report to UK Technical Advisory Group for the Environment Agency.

Devlin, M. J., Best, M., Coates, D., Bresnan, E., O'Boyle, S., Park, R., Silke, J., Skeats J. & Barry, J. (2007). Establishing boundary classes for classification of marine waters using phytoplankton communities - the first step in establishing a link between nutrient pressure and the marine plant community. Marine Pollution Bulletin, 55, 91–104.

*Phytoplankton Uncertainty Gets Worked out And Statistically Handled (v10.7) – *Excel workbook to estimate the precision of the assessment*.

*UKTAG Biological Status Methods: Coastal Waters - Phytoplankton
– *High level non-technical summary*

## 2. Background

### 2.1 Ecological principles

Phytoplankton have routinely been used by UK agencies as an indicator of anthropogenic inputs of nutrients, mainly from inorganic nitrogen (e.g. under the Urban Waste Water Treatment Directive (UWWTD), and the strategies of the OSLO and Paris Commission (OSPAR)).

Chlorophyll biomass is seen as an easily measurable, repeatable parameter that can detect periods of excessive phytoplankton biomass. Persistent observations of high phytoplankton biomass can indicate the potential for impacts on the ecology of the water body. However, in some instances these may also be caused by natural events (such as advection, upwelling or wind driven forcing).

The measurement of elevated taxa counts is designed to assess if the presence, abundance and frequency of occurrence of high counts of algal species correspond to disturbed conditions (Beliaeff *et al.,* 2001; Belin, 1998; Gailhard *et al.,* 2002).

Succession of functional groups can potentially provide an index that represents a healthy planktonic system, with a natural progression of dominant functional groups throughout the seasonal cycle. The structure of the seasonal succession involves the measurement, as cell counts, of the two main taxonomic groupings (diatoms and dinoflagellates). There is an emerging consensus in the literature that climate is shifting the phenology of some phytoplankton groups (Edwards & Richardson, 2004) and the sensitivity of this tool toward climate pressures must also be acknowledged.

The CW phytoplankton tool combines the outcomes of three indices which describe the phytoplankton community; one is a measure of elevated chlorophyll biomass and two focus on the identification of high counts of algae that may result in the decline of ecosystem health or result in an undesirable disturbance (Tett *et al*., 2007). The use of the combined indices enables the phytoplankton tool to reflect changes in the phytoplankton community as described within the normative definitions of the Directive.

## 2.2 Normative definitions

In Annex V (1.2.4) of the WFD, normative definitions describe the aspects of the phytoplankton community that must be included in the ecological status assessment of a water body, these are:

- composition
- abundance
- biomass
- planktonic bloom frequency and intensity.

To facilitate the development of a suitable assessment the WFD normative definitions were further interpreted into expanded normative definitions (Table 1).

## 2.3 Development of the CW phytoplankton tool

The CW phytoplankton tool combines three indices; one is a single metric and two are multimetric indices (a metric is a measure of the biota that changes in some predictable way with increased human influence). The approach has been developed based on expert knowledge, previously accepted criteria (e.g. OSPAR, 2002, 2003, 2005) and use of historical phytoplankton data. Details on the full development process of the phytoplankton tool can be found in Devlin *et al.* (2007, 2013).

The structure of the tools is based around the WFD normative definitions with composition and abundance reflected in the taxa counts, phytoplankton biomass in the measurements of chlorophyll-a concentrations, and planktonic blooms reflected through both biomass (describing chlorophyll-a through statistical measurements) and taxa counts (exceedances of taxa counts above a threshold).

Initial ideas were based on Oslo and Paris Commission (OSPARCOM) work and obtained from expert opinion of the UK Technical Advisory Groups' Marine Plant Task Team (MPTT) to develop a conceptual understanding of how the normative definitions related to current understanding of phytoplankton measurements. This conceptual understanding built on existing directives, including the UWWTD (CEC 1991a) and the Nitrates Directive (CEC 1991b) and existing scientific literature (Beliaeff *et al*., 2001; Belin, 1998; Gailhard *et al*., 2002). The early stages of development are summarised in Devlin *et al*. (2007).

When considering the indices and their expression of the biological community, it is important to understand that there are three numerical scales of data to consider:

(i) the face value i.e. the measurement value such as chlorophyll-a concentration
(ii) the normalised non-equidistant value ('normalisation' is used here to describe the compression/expansion of one scale (face value range) to operate over another scale (0 to 1 EQR scale)
(iii) the rescaled equidistant value (rescaling changes non-equidistant boundaries to equidistant boundaries e.g. adjusting chlorophyll-a metric boundaries to 0.2, 0.4, 0.6 and 0.8 on the 0 to 1 EQR scale).

During early stages of development, calculations were presented in these three separate steps. For practical purposes, steps (ii) and (iii) are now combined mathematically (see Section 3.7. for further details).

**Table 1: Description of the characteristics of the phytoplankton community at the WFD status classes in accordance with the normative definitions (WFD Annex V) and expanded normative definitions (detailed national interpretation).**

| | High | Good | Moderate |
|---|---|---|---|
| **Original normative definitions** | The composition and abundance of phytoplankton taxa are consistent with undisturbed conditions.<br><br>The average phytoplankton biomass is consistent with the type-specific physico-chemical conditions and is not such as to significantly alter the type-specific transparency conditions.<br><br>Planktonic blooms occur at a frequency and intensity which is consistent with type-specific physico-chemical conditions. | The composition and abundance of phytoplankton taxa show slight signs of disturbance.<br><br>There are slight changes in biomass compared to type-specific conditions.<br><br>A slight increase in the frequency and intensity of type-specific planktonic blooms may occur. | The composition and abundance of planktonic taxa show signs of moderate disturbance.<br><br>Algal biomass is substantially outside the range associated with type-specific reference conditions and is such as to impact on other biological quality elements.<br><br>A moderate increase in the frequency and intensity of planktonic blooms may occur. Persistent blooms may occur in summer months. |
| **Expanded normative definitions** | Species richness high. Spring bloom; diatom domination. Diatoms persist throughout growth-period.<br>Increasing numbers of dinoflagellates from late spring. | Slight decline in species richness due to modified nutrient ratios. Evidence of minor disturbance from High status. | Moderate decline in species richness due to modified nutrient ratios. Prolongation of spring bloom with elevated chlorophyll-a above background. Disturbance of natural diatom-dinoflagellate succession. |

***Understanding the individual indices within the CW phytoplankton tool***:

**Chlorophyll-a 90th percentile metric** (WFD criteria compliance – biomass)

This metric works by the calculation of the 90th percentile value of daily averaged chlorophyll-a biomass over the growing season (March to October, inclusive). Boundary conditions are variable for the two geographical areas, Atlantic and North Sea waters (referred to in the NEAGIG intercalibration process as NEA 1/26a and 1/26b respectively). The value of the 90th percentile is compared against boundary conditions to establish classification status.

**Elevated count multimetric** (WFD criteria compliance – composition, abundance, planktonic blooms)

This multimetric is based on three metrics measuring the number of occasions that phytoplankton counts exceed an established threshold over the reporting period. The metrics consider:

- percentage exceedance of a chlorophyll-a threshold (measured as $\mu g\ l^{-1}$)
- percentage exceedance of **single** taxa threshold (measured as cells $l^{-1}$)
- percentage exceedance of **total** taxa threshold (measured as cells $l^{-1}$).

The thresholds for taxa counts are determined by the geographical location, Southern and Northern biogeographical regions.

Each metric value is calculated from the number of times exceedances occur as a proportion of the total number of sampling times. The multimetric face value is calculated as the average exceedance of the three metrics (%).

**CW seasonal succession multimetric** (WFD criteria compliance – composition, abundance, planktonic blooms)

This multimetric uses the measurement of the two main taxonomic groupings falling within a seasonal reference growth curve. The score is based on the proportion of time that the monthly log mean of diatoms and dinoflagellates fall below a reference line for that month.

Monthly Z scores are calculated for diatoms and dinoflagellates from the natural log mean of taxa counts and compared against the Z score (+ 50%) of the reference taxa counts. The index records the percentage frequency of monthly score falling below the upper curve on the diatom and dinoflagellates seasonal envelope. The final value is calculated by the number of points under or within the reference envelope compared to the total number of sampling points.

Original index thresholds were developed by reviewing the outcomes of the proposed indices in water bodies considered to be at low risk from nutrient pressures. As WFD compliant monitoring data have become available, as well as two comprehensive reviews about the relationship between *Phaeocystis* and anthropogenic nutrient enrichment (Gowen *et al*., 2008, 2012), the phytoplankton tool has been modified to better reflect the response of the phytoplankton community to anthropogenic impact.

Specific changes since the first River Basin Management Plans:

(i) removal of *Phaeocystis* as a separate metric in the elevated count index
(ii) elevated count thresholds set for different biogeographical regions
(iii) streamlining of counting and identification of phytoplankton.

(iv) A revised taxa list has been generated which is used by all laboratories analysing samples for WFD purposes within the UK. Identification categories have been consolidated to account for the preservation of samples using Lugol's iodine. In some phytoplankton this preservative obscures the observation of many morphological structures required to identify a cell to species level e.g. thecal plates in dinoflagellates, structures on the frustule of diatoms. Thus in some instances a genus only level identification can be made. This revised taxa list also uses size groupings to differentiate the taxa.

### 2.4 Reference conditions

Reference conditions (and class boundary thresholds) for each index were constructed based on a combination of scientific review (Beliaeff *et al.,* 2001; Belin, 1998; Borja *et al*., 2004; Gailhard *et al.,* 2002), thresholds accepted in previous directives (CSTT, 1997) and international agreements (e.g. OSPAR, Foden *et al.,* 2011; Devlin *et al*., 2007; Painting *et al.,* 2005), expert knowledge (MPTT), and investigations of outputs between water bodies at low and high risk of eutrophication (Devlin *et al*., 2007). The values were also validated and modified by the consensus process of the first phase of the NE Atlantic Intercalibration process (Carletti & Heiskanen, 2009). Reference conditions are geographically specific. (*Note*: the EQR values presented in earlier documentation are often non-normalised so may appear very different from the normalised values presented in this document. It is important to understand whether face values or normalised values are being reported.)

*Chlorophyll*

In previous Directives, the acceptable boundaries for chlorophyll were based on a justified area-specific percentage deviation from background chlorophyll concentrations. These have formed the basis for the WFD defined reference values.

Background chlorophyll conditions for UK waters are based on a deviation from offshore Atlantic shelf break background concentrations (OSPAR, 2003; Gowen *et al*., 2012). For the OSPAR Comprehensive Procedure, appropriate thresholds for assessing chlorophyll concentration were derived from these background nutrient concentrations by making some reasonable assumptions about nutrient conversion to plant biomass using a carbon to nitrogen ratio of 6.6 and a carbon to chlorophyll ratio of 0.012 (Foden *et al*., 2011; Painting *et al*., 2005).

These figures give a background chlorophyll concentration for the more enclosed "North Sea" waters of 6.7 µg $l^{-1}$, which was assumed to be a very conservative reference value for North Sea (NEA 1/26b) waters. OSPAR assumes an allowable increase of 50% above background chlorophyll concentration for nearshore waters where production will be naturally higher. This concentration of 10 µg $l^{-1}$ (6.7 + (6.7*50/100) = 6.7 + 3.3 = 10) was selected as the WFD High/Good boundary. This is also the UK OSPAR coastal reference value (*Note:* "coastal" in the OSPAR sense is further offshore than WFD). A 50% increase on the High/Good boundary produced the Good/Moderate boundary of 15 µg $l^{-1}$ (Table 2).

A similar procedure was used for those areas facing the less nutrient-rich open Atlantic waters, where a chlorophyll reference value was determined as 3.3 µg $l^{-1}$. This gives an inshore High/Good boundary of 5 µg $l^{-1}$. A Good/Moderate boundary of 10 µg $l^{-1}$ was agreed through the NEAGIG intercalibration, reflecting the confidence by experts in the Member States that lower chlorophyll values were representative of Atlantic coastal waters (see Carletti & Heiskanen, 2009) (Table 2).

**Table 2: Chlorophyll-a concentrations (µg $l^{-1}$) for face value class boundaries for Atlantic and North Sea waters.**

| | Atlantic Waters NEA 1/26a (µg $l^{-1}$) | North Sea Waters NEA 1/26b (µg $l^{-1}$) |
|---|---|---|
| Reference | 3.33 | 6.67 |
| High/Good | 5.00 | 10.00 |
| Good/Moderate | 10.00 | 15.00 |
| Moderate/Poor | 15.00 | 20.00 |
| Poor/Bad | 20.00 | 25.00 |

The map below shows the geographical range of the Atlantic and North Sea Waters (Figure 2).

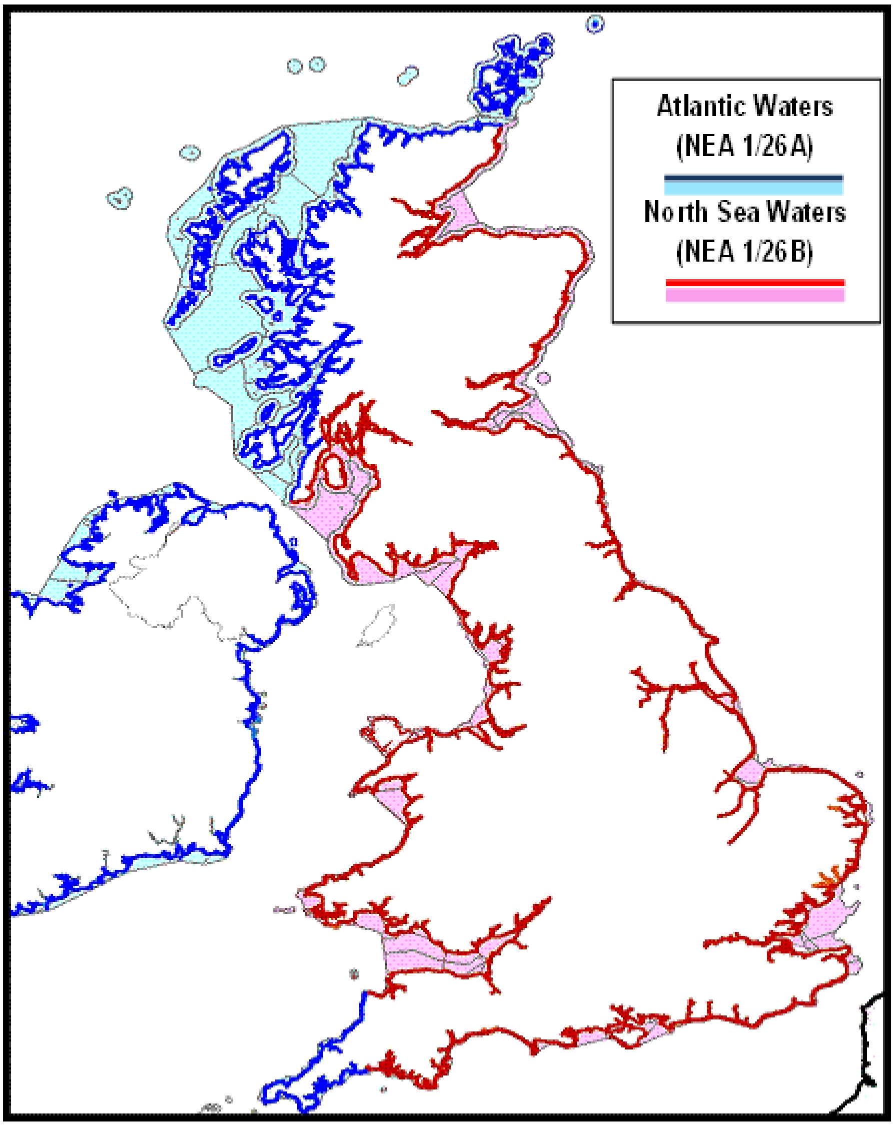


**Figure 2: Map to show the range of the defined Atlantic and North Sea waters for use with the chlorophyll-a 90$^{th}$ percentile metric.**

*Elevated Counts*
Normative definitions describe the reference condition as the abundance (and associated variability) of phytoplankton taxa being consistent with undisturbed conditions and planktonic blooms occurring at a frequency and intensity which is consistent with type-specific physico-chemical conditions. Thresholds for elevated counts were adapted (Beliaeff *et al*., 2001; Belin, 1998; Gailhard *et al.,* 2002) and tested through the outcomes of low, moderate and high risk water bodies (Devlin *et al.,* 2007).

There are two biogeographical regions for phytoplankton cell counts, Southern and Northern (Figure 3. *Note*: these are different to the defined waters used for the chlorophyll-a 90$^{th}$ percentile metric). The Northern region tends to have a shorter summer with longer day length and is more influenced by the North Atlantic drift current and its associated water mass. This tends to be reflected in larger numbers of smaller phytoplankton with a shorter growing season. The Southern region tends to have a longer growing season although with a shorter day length in the growing seasons, which tends to be reflected by lower numbers of larger taxa.

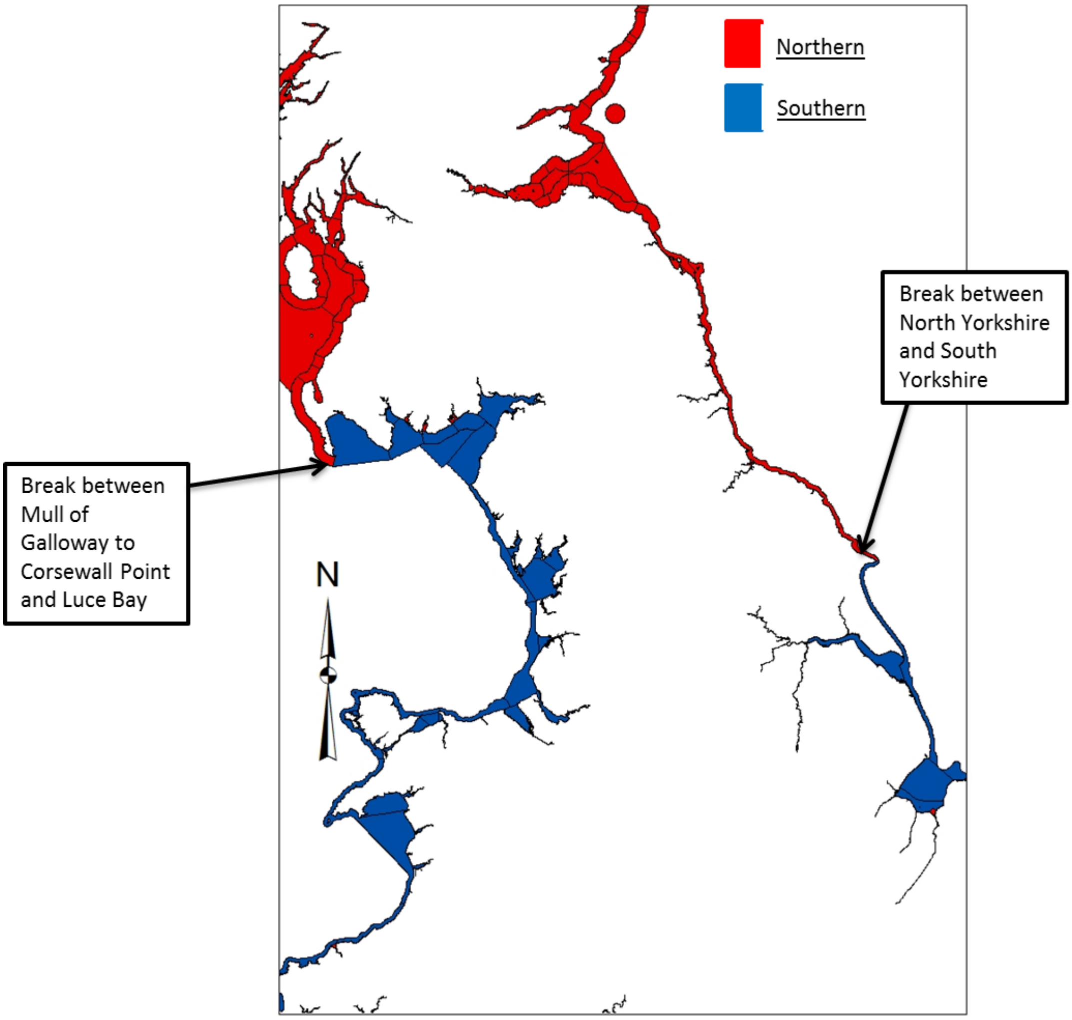


**Figure 3: Delineation of Southern and Northern coastal biogeographical regions for phytoplankton cell count thresholds. West coast split has the Mull of Galloway to Corsewall Point waterbody to the north and Luce Bay to the east. The East coast split has North Yorkshire to the north and South Yorkshire/Lincolnshire to the south.**

Exceedances are counted in response to the following thresholds:

(i) Chlorophyll-a threshold = 10 µg $l^{-1}$ Chl-a
(ii) Individual taxa count threshold = 250,000 cells $l^{-1}$ for the Southern biogeographical region, 500,000 cells $l^{-1}$ for the Northern biogeographical region
(iii) Total taxa count threshold = $10^6$ cells $l^{-1}$ for the Southern biogeographical region, $10^7$ cells $l^{-1}$ for the Northern biogeographical region.

*Seasonal Succession*
The seasonal succession reference condition includes the presence of a spring bloom with high numbers of diatom species in the bloom period and increasing numbers of dinoflagellates from late spring. Reference conditions are locally defined. For England and Wales, generic reference curves were established for coastal water bodies using long term data (from 1991) from a long-term un-impacted monitoring site offshore of Plymouth (Devlin *et al*., 2007, 2013). In addition, there are two reference sites in Scotland, one on the east coast (North Sea) at Stonehaven, the other on the west coast (Atlantic) at Loch Ewe (Marine Scotland Science).

In UK waters, low or no growth conditions are not seen as an issue so the reference curve for both diatoms and dinoflagellates is only one-sided and the tool is based on the number of exceedances of the upper growth curve. The reference condition is 0% exceedance of curve (i.e. 100% compliance). The process of deriving the reference curve for diatoms is illustrated below (Figure 4) and described fully in Devlin *et al*., (2007, 2013).

The reference curves are constructed using the values below (Table 3).

**Table 3: Values used for creating the seasonal succession reference curves for diatoms and dinoflagellates for England, Wales and Scotland.**

| **Diatoms** | | | | **Dinoflagellates** | | | |
|---|---|---|---|---|---|---|---|
| Month | Upper Bound | | | Month | Upper Bound | | |
| | England and Wales | Scotland Stonehaven | Scotland Loch Ewe | | England and Wales | Scotland Stonehaven | Scotland Loch Ewe |
| 1 | -0.12 | -0.62 | -0.85 | 1 | -0.11 | -0.72 | -0.56 |
| 2 | -0.16 | -0.78 | -0.86 | 2 | 0.05 | -1.08 | -0.91 |
| 3 | -0.06 | 0.44 | 0.16 | 3 | 0.06 | -0.25 | -0.59 |
| 4 | 0.39 | 1.47 | 1.85 | 4 | 0.44 | 0.39 | -0.20 |
| 5 | 0.95 | 1.55 | 1.54 | 5 | 0.63 | 1.46 | 1.15 |
| 6 | 1.43 | 1.74 | 1.38 | 6 | 0.88 | 1.74 | 0.90 |
| 7 | 1.26 | 1.18 | 0.89 | 7 | 0.86 | 1.33 | 1.14 |
| 8 | 1.07 | 0.86 | 1.01 | 8 | 0.92 | 1.02 | 1.83 |
| 9 | 0.58 | 0.00 | 0.53 | 9 | 1.18 | 0.48 | 1.77 |
| 10 | 0.05 | 0.19 | -0.13 | 10 | 0.48 | 0.51 | 0.58 |
| 11 | -0.17 | -0.35 | -0.30 | 11 | 0.15 | -0.12 | -0.30 |
| 12 | -0.17 | -0.73 | -0.30 | 12 | -0.19 | -0.12 | 0.28 |

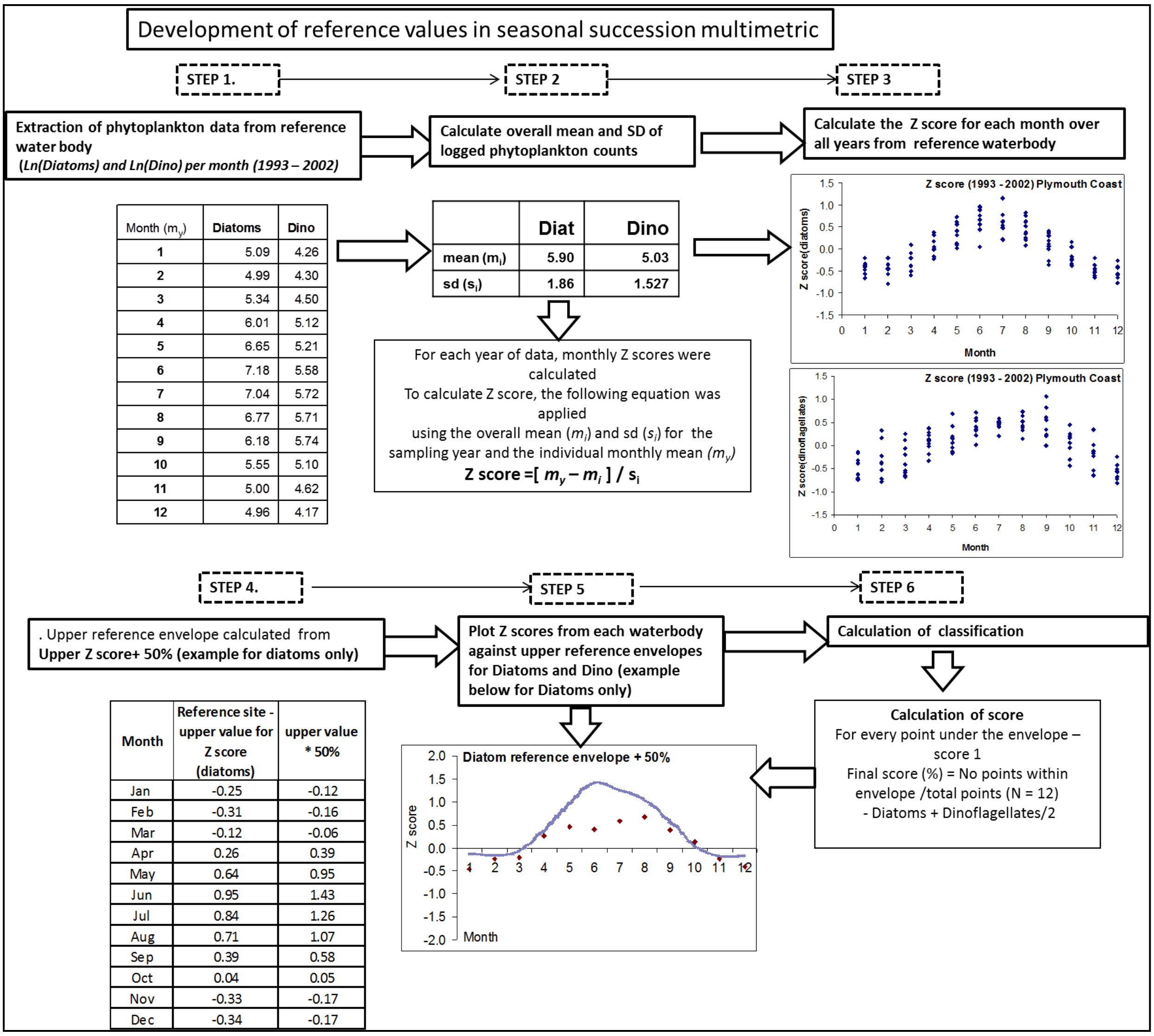


**Figure 4: An example (based on data from English waters) showing the process of deriving the seasonal succession reference curve, shown here for diatoms.**

## 2.5 Class boundaries

Class boundaries have been defined through a UK process by testing of the phytoplankton historical data held by UK monitoring agencies (Devlin *et al*., 2007). Boundaries for the chlorophyll-a metric and some aspects of the elevated count metric were tested and agreed through the Phase 1 Intercalibration process for the NE Atlantic (Commission Decision 2008/915/EC).

The overall class boundaries for the phytoplankton tool are shown in Table 4**.**

**Table 4: Overall ecological status boundaries for the CW phytoplankton tool.**

| Status | EQR |
|---|---|
| High/Good | 0.80 |
| Good/Moderate | 0.60 |
| Moderate/Poor | 0.40 |
| Poor/Bad | 0.20 |

The class boundaries for the individual phytoplankton metrics are shown below for chlorophyll-a (Table 5), elevated counts (Table 6) and seasonal succession (Table 7) for the face value and normalised metrics.

**Table 5: Class status boundaries for the 90$^{th}$ percentile chlorophyll-a metric. Face value and metric (0-1) ranges are shown.**

| **Metric:** CW -90$^{th}$ percentile measure of chlorophyll biomass | 90$^{th}$ percentile chlorophyll-a (µg $l^{-1}$) (Face value range) | Metric range (0-1) | Class |
|---|---|---|---|
| **North Sea waters** Reference: 6.67 µg $l^{-1}$ | > 0 - < 10 | ≥ 0.8 - ≥ 1.0 | High |
| | ≥ 10 - < 15 | ≥ 0.6 - < 0.8 | Good |
| | ≥ 15 - < 20 | ≥ 0.4 - < 0.6 | Moderate |
| | ≥ 20 - < 25 | ≥ 0.2 - < 0.4 | Poor |
| | ≥ 25 - < 50 | 0 - < 0.2 | Bad |
| **Atlantic waters** Reference: 3.33 µg $l^{-1}$ | > 0 - < 5 | ≥ 0.8 - ≥ 1.0 | High |
| | ≥ 5 - < 10 | ≥ 0.6 - < 0.8 | Good |
| | ≥ 10 - < 15 | ≥ 0.4 - < 0.6 | Moderate |
| | ≥ 15 - < 20 | ≥ 0.2 - < 0.4 | Poor |
| | ≥ 20 - < 50 | 0 - < 0.2 | Bad |

**Table 6: Class status boundaries for the elevated count multimetric. Face value and multimetric (0-1) ranges are shown.**

| **Multimetric**: CW-Elevated counts | % exceedances (Face value range) | Metric range (0-1) | Class |
|---|---|---|---|
| **Reference :** 0 | 0 - < 10 | > 0.8 - 1.0 | High |
| | ≥ 10 - < 20 | ≥ 0.6 - < 0.8 | Good |
| | ≥ 20 - ≤ 40 | ≥ 0.4 - < 0.6 | Moderate |
| | ≥ 40 - ≤ 60 | ≥ 0.2 - < 0.4 | Poor |
| | ≥ 60 - ≥ 100 | ≥ 0 - < 0.2 | Bad |

**Table 7: Class status boundaries for the seasonal succession multimetric. Face value and multimetric (0-1) ranges are shown.**

| **Multimetric**: CW – seasonal succession | % compliance (Face value range) | Re-scaled equidistant index range (0-1) | Class |
|---|---|---|---|
| **Reference**: 100 | ≥ 80 - 100 | ≥ 0.8 - 1.0 | High |
| | ≥ 60 - < 80 | ≥ 0.6 - < 0.8 | Good |
| | ≥ 40 < 60 | ≥ 0.4 - < 0.6 | Moderate |
| | ≥ 20 -< 40 | ≥ 0.2 - < 0.4 | Poor |
| | 0 - < 20 | 0 - < 0.2 | Bad |

# 3. Undertaking an assessment

## 3.1 Summary Flow Chart

The process for undertaking a water body assessment of coastal water phytoplankton is summarised below (Figure 5).

**Monitoring design**

- Is CW phytoplankton an appropriate assessment tool for the survey/investigation?
  (Consider level of natural variability, turbidity and expected response to pressure)
- Set appropriate sample numbers for water type/aim of survey

↓

**Sample collection**

- Use of standardised methods (BS EN 15972:2011)
- Collect chlorophyll and phytoplankton cells
- Collect supporting information e.g. turbidity, temperature, salinity

↓

**Sample analysis**

- Use of standard laboratory methods (e.g. ISO 10260:1992; EN 15204:2006)
- Full enumeration of phytoplankton
- Taxa identified to the Revised Phytoplankton List
- Quality assurance procedures

↓

**Calculation: Face value**

- $90^{th}$ percentile chlorophyll-a across growing season (Mar - Oct)
- Elevated cell counts – average combined % exceedance of three metrics
  - (i) count (%) of chlorophyll-a exceeding 10 µg $l^{-1}$
  - (ii) count (%) of individual taxa exceeding 250,000 or 500,000 cells $l^{-1}$
  - (iii) count (%) of total taxa exceeding $10^6$ or $10^7$ cells $l^{-1}$
- Seasonal succession of functional groups – average % exceedance from the reference envelope for diatoms and dinoflagellates grouping

↓

**EQR calculation**

- Normalise and rescale values to equidistant EQR 0-1 range
- Final Equidistant index score =
  *Upper Equidistant Class range value – ((Face Value - Upper Face value range) * (Equidistant class range / Face Value Range))*
- Phytoplankton EQR calculated from the average of index scores

↓

**Water body classification**

- Derive water body average EQR
- Assign Class Status (use defined class boundaries)
- Calculate Confidence of Class and Risk of Misclassification

**Figure 5: Flow chart summarising the main stages involved in undertaking a CW phytoplankton assessment.**

### 3.2 Data requirements

Calculation of the phytoplankton indices requires measurement of chlorophyll-a concentration ($90^{th}$ percentile chlorophyll-a and elevated count of chlorophyll-a) and phytoplankton cell counts (full enumeration of taxa as defined in the revised taxa list).

### 3.3 Sampling strategy

The CW phytoplankton tool was developed expecting that the status of the water body will be classified over a six year reporting period for phytoplankton. (Analysis of WFD compliant data is now allowing for further assessment of data requirements for the tool and implications of reduced sampling periods for water body types.) Monthly measurements of chlorophyll and phytoplankton are required throughout the full year. (It is recognised that logistically this is difficult so it should be noted that an absolute minimum of nine months in any one year over the reporting period for phytoplankton counts is recommended for appropriate data confidence).

WFD assessments generally use single surface samples spread across the water body. Sampling within the water body should be at the appropriate number of sites in respect to the size of the water body and the natural variability seen in the phytoplankton community.

### 3.4 Sampling methodology

The UK monitoring authorities follow the British standards for phytoplankton sampling and processing (EN 15972:2011, EN 15204:2006). Surface sampling or integrated tube sampling is carried out for phytoplankton and chlorophyll. The surface sample is collected, mixed and sub-samples processed for (i) chlorophyll-a by ideally *in situ* filtering and freezing and (ii) preserved with Lugol's Iodine for phytoplankton analysis. Salinity and location measurements should also accompany each biological sample. The WFD competent monitoring authorities have their own operating procedures and instructions (please refer to the relevant Agency for further details).

### 3.5 Sample Analysis

Phytoplankton samples are analysed (identified and counted) using the Utermöhl method.

Phytoplankton cells are identified to the lowest practical taxonomic level possible. UK WFD authorities use a revised phytoplankton list (see footnote 2 on page 2; please refer to the relevant WFD Agency for the current list).

The WFD competent authorities analyse chlorophyll-a concentrations to obtain an estimate of biomass as $\mu g\ l^{-1}$ (e.g. methods are based on ISO 10260:1992).

### 3.6 Data treatment

No specific data treatment is required prior to running the classification. The percentage of exceedances is required for the face value calculation, see section 3.7.

### 3.7 EQR calculation

The Ecological Quality Ratio (EQR) determining the final water body classification ranges between a value of 0-1. The process is illustrated in the conceptual diagram below (Figure 6).

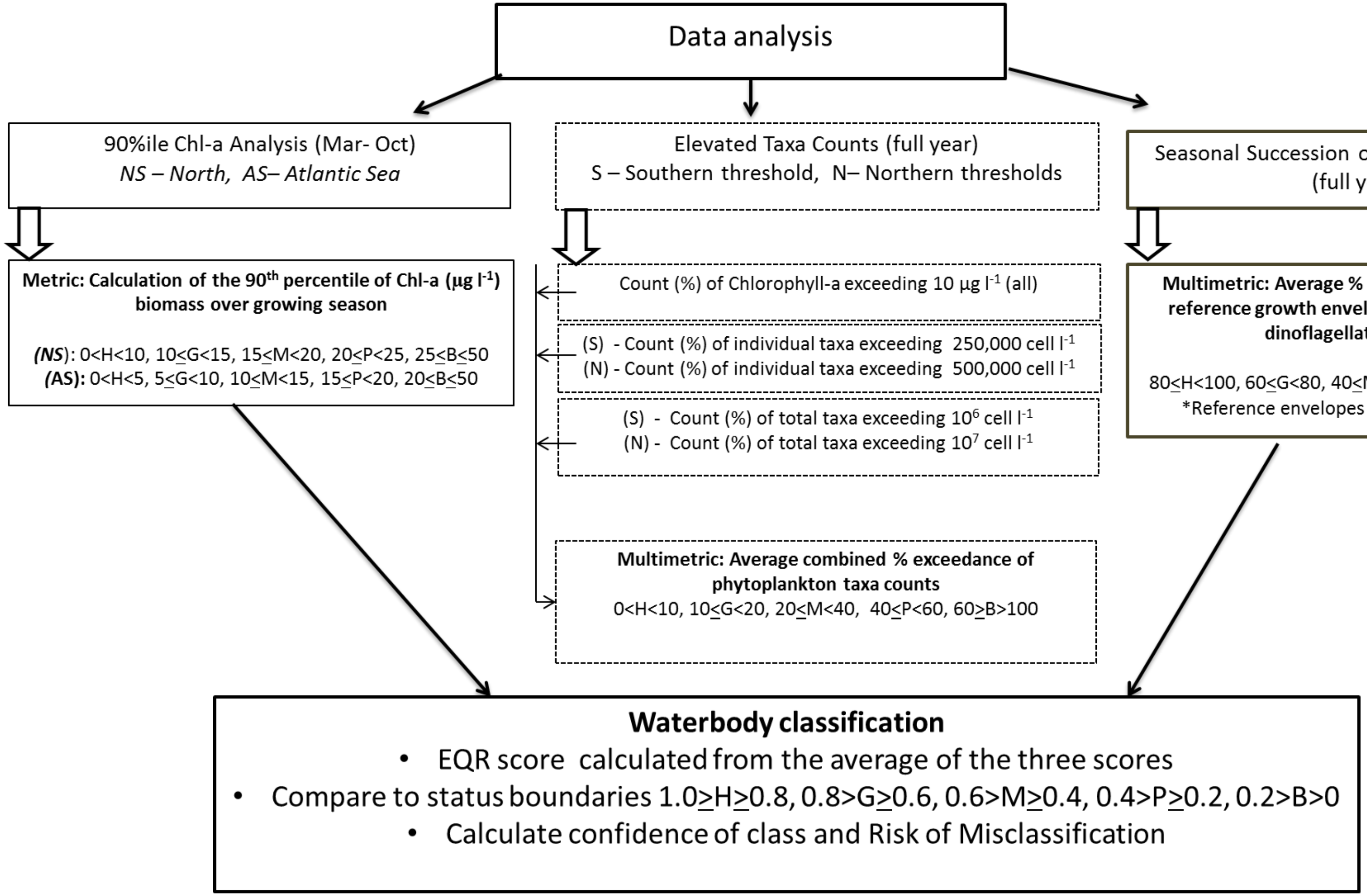


**Figure 6: Conceptual diagram illustrating how the CW phytoplankton indices are combined to calculate a water body classification**.

To calculate the overall water body classification it is necessary to convert the face value measurement to an equidistant EQR scale, in order that the three indices can be combined. A stepwise process is followed:

(i) calculation of the face value (based on the biological measurement e.g. percentage of exceedances) for each index
(ii) normalisation and rescaling to convert the face value to an equidistant index score (0-1 value) for each index (*Note*: this was originally a two-step process but is now combined mathematically into a one-step calculation)
(iii) calculation of CW phytoplankton EQR, i.e. average of equidistant metric scores.

***Calculation of the face values***
The face value for the *chlorophyll-a 90<sup>th</sup> percentile* is calculated from daily averaged chlorophyll-a measurements across the growing season (March to October, inclusive).

The *elevated count* index is calculated as the arithmetic mean of:
(i) the fraction of all sampling occasions where measured chlorophyll-a concentration exceeds the threshold
(ii) the fraction of all sampling occasions where measured individual taxa exceeds the threshold
(iii) the fraction of all sampling occasions where measured total taxa concentrations exceeds the threshold.

The *seasonal succession* index is calculated by
(i) Calculating the natural log mean of cell counts for each month (January, February…December) for both diatom and dinoflagellate taxa. ($C_1$, $C_2$ … $C_{12}$)
(ii) Converting each monthly value ($C_i$) to a Z-score by applying the equation:

$$\text{Z-score}_i = (C_i - P) \div S$$

where: "Ci" = the logarithmically transformed concentration for month "i"

P = the mean of the taxa reference data

S = the standard deviation of the reference data

"P" (the mean of the taxa reference data) and "S" (the standard deviation of the reference data) have different values for diatoms and dinoflagellates (Table 8).

**Table 8: The mean of the taxa reference data (P) and the standard deviation of the reference data (S) for diatoms and dinoflagellates.**

| Region | Statistic | Mean of reference data ("P") | Standard deviation of reference data ("S") |
|---|---|---|---|
| England and Wales | Diatoms | 5.9 | 1.89 |
| England and Wales | Dinoflagellates | 5.0 | 1.54 |
| Scotland - Stonehaven | Diatoms | 7.14 | 1.62 |
| Scotland - Stonehaven | Dinoflagellates | 4.65 | 2.15 |
| Scotland – Loch Ewe | Diatoms | 8.06 | 1.86 |
| Scotland – Loch Ewe | Dinoflagellates | 5.25 | 1.92 |

(iii) Comparing each monthly Z score to the upper boundary reference value (Figure 7 and Table 3)
(iv) Counting the number of points which fall below the reference curve and calculating the percentage value of compliant data points against all data points.

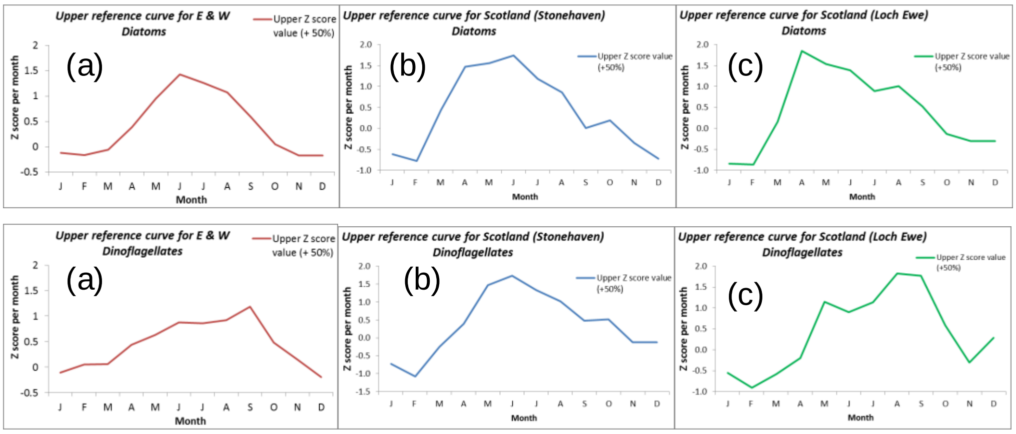


**Figure 7: Upper reference growth envelopes for diatoms and dinoflagellates for England and Wales (a) and Scotland (b and c).**

***Normalisation and rescaling of face values to metric range.***
The face values then need to be converted to an equidistant EQR scale to allow combination of the metrics. Initially this was carried out in a two step process, that is, the normalisation of face values to an EQR (0-1) scale (non-equidistant class boundaries), and then rescaling to an equidistant class EQR scale. These steps have now been combined mathematically in the following equation:

*Final Equidistant index score = Upper Equidistant Class range value – ((Face Value - Upper Face value range) * (Equidistant class range / Face Value Range))*

Table 9 gives the critical values at each class range required for the above equation. The first three numeric columns contain the face values (FV) for the range of the index in question, the last three numeric columns contain the values of the equidistant 0 -1 scale and are the same for each index. The face value class range is derived by subtracting the upper face value of the range from the lower face value of the range, hence the negative values for seasonal succession.

*Note*: the table is “simplified” with rounded numbers for display purposes. The face values in each class band may have greater than (>) or less than (<) symbols associated with them, for calculation a value of < 5 is actually a value of 4.9999.

**Table 9: Values for the normalisation and rescaling of face values to EQR metric ranges for coastal water bodies.**

| | | Lower Face Value range value (the measurements towards the "bottom" end of this class range) | Upper FV range value (the measurements towards the "Top" end of this class range) | Face Value class range | Lower 0-1 equidistant range vale | Upper 0-1 equidistant range value | Equidistant class range |
|---|---|---|---|---|---|---|---|
| **90%ile chlorophyll-a** | | **Chl-a (µg $l^{-1}$)** | **Chl-a (µg $l^{-1}$)** | **Chl-a (µg $l^{-1}$)** | | | |
| **Atlantic Waters (NEA 1/26a)** | High | <5 | 0 | 5 | ≥0.8 | 1 | 0.2 |
| | Good | <10 | ≥5 | 5 | ≥0.6 | <0.8 | 0.2 |
| | Moderate | <15 | ≥10 | 5 | ≥0.4 | <0.6 | 0.2 |
| | Poor | <20 | ≥15 | 5 | ≥0.2 | <0.4 | 0.2 |
| | Bad | ≥50 | ≥20 | 30 | 0 | <0.2 | 0.2 |
| **North Sea Waters (NEA 1/26b)** | High | <10 | 0 | 10 | ≥0.8 | 1 | 0.2 |
| | Good | <15 | ≥10 | 5 | ≥0.6 | <0.8 | 0.2 |
| | Moderate | <20 | ≥15 | 5 | ≥0.4 | <0.6 | 0.2 |
| | Poor | <25 | ≥20 | 5 | ≥0.2 | <0.4 | 0.2 |
| | Bad | ≥50 | ≥25 | 25 | 0 | <0.2 | 0.2 |
| **Elevated Counts** | | **% Exceedances** | **% Exceedances** | **% Exceedances** | | | |
| | High | <10 | 0 | 10 | ≥0.8 | 1 | 0.2 |
| | Good | <20 | ≥10 | 10 | ≥0.6 | <0.8 | 0.2 |
| | Moderate | <40 | ≥20 | 20 | ≥0.4 | <0.6 | 0.2 |
| | Poor | <60 | ≥40 | 20 | ≥0.2 | <0.4 | 0.2 |
| | Bad | 100 | ≥60 | 40 | 0 | <0.2 | 0.2 |
| **Seasonal Succession** | | **% Compliance** | **% Compliance** | **% Compliance** | | | |
| | High | ≥80 | 100 | -20 | ≥0.8 | 1 | 0.2 |
| | Good | ≥60 | <80 | -20 | ≥0.6 | <0.8 | 0.2 |
| | Moderate | ≥40 | <60 | -20 | ≥0.4 | <0.6 | 0.2 |
| | Poor | ≥20 | <40 | -20 | ≥0.2 | <0.4 | 0.2 |
| | Bad | 0 | <20 | -20 | 0 | <0.2 | 0.2 |

***CW phytoplankton assessment – site level***

The overall phytoplankton assessment is the average of the calculated equidistant index values. Ideally this should be the mean score for the three indices.

*CW phytoplankton EQR = (chlorophyll-a 90$^{th}$ percentile equidistant index value + elevated count equidistant index value + season succession equidistant index value)/3*

### 3.8 Water body level classification

Water body classifications are based on the arithmetic mean score (EQR) of all indices calculated for the water body. Ideally this should be the mean score for the three indices.

### 3.9 Understanding the certainty of the assessment

Providing an estimate of the statistical uncertainty of water body assessments is a statutory requirement of the WFD (Annex V, 1.3). In an ideal world of comprehensive monitoring data containing no errors, waterbodies would always be assigned to their true class with 100% confidence. However, estimates of the truth based on monitoring are subject to error because monitoring is not done everywhere and all the time, and because monitoring systems, equipment and people are less than perfect. Understanding and managing the risk of misclassification as a result of uncertainties in the results of monitoring is important on two counts; first, because of the potential to fail to act in cases where a water body has been wrongly classified as being of better status than it is, and secondly because of the risk of wasting resources on waterbodies that have been wrongly classified as worse than they are.

A methodology for calculating a measure of the confidence of class (CofC) for the phytoplankton tools was developed by WRc (Davey, 2009).

For classification purposes, the estimated EQR is translated directly into a face value class (i.e. High - Bad). However, because it is not possible to survey the biological community across a whole water body continuously throughout the whole reporting period, there will always be some sampling error, which will lead to uncertainty in the estimate of the EQR. This uncertainty can be quantified as the expected difference between the observed EQR and the true underlying EQR, which can then be used to calculate the probability of the water body being in each of the five status classes. From this it is possible to determine the most probable class (the one with the highest probability) and state what level of confidence we have that the true status is good or better, and moderate or worse.

The confidence of class tool assumes that surveys for the phytoplankton indices are conducted in such as way as to give a representative and unbiased measure of biological conditions across the whole water body throughout the whole reporting period. Statistical manipulation of the resulting data cannot compensate for poorly planned and executed field sampling; there is no substitute for a sampling scheme that measures directly the spatial and temporal variation in the target population.

An Excel workbook, ‘Phytoplankton Uncertainty Gets Worked out And Statistically Handled’, calculates the confidence of class for the CW phytoplankton tool. It performs calculations for multiple water bodies simultaneously and gives the confidence of class over the whole reporting period. As each metric integrates spatial and temporal variability in the phytoplankton community, the uncertainty in the Final EQR is estimated by combining estimates of the uncertainty within each metric EQR.

The uncertainty calculation workbook approach adopts a bottom-up approach whereby each metric score and its corresponding standard error are first used to compute the confidence of class for each metric. Next, the three metric scores are normalised to produce metric EQRs between 0-1. Finally, the index EQRs are combined to give a final tool EQR, and their standard errors are also combined to produce an overall confidence of class for the Final EQR result (for full details see Davey, 2009).

## 4. Worked Example

Using an example water body from Atlantic waters (NEA 1/26a) and in the Southern region for elevated counts we have the Face Value results:

- 90%$^{th}$ percentile chlorophyll-a 6.0 µg $l^{-1}$
- Elevated counts 7.65 %
- Seasonal succession 5.56 %

*Refer to Table 9 for values to complete the equations:*

**Chlorophyll-a 90$^{th}$ percentile**
The face value of 6.0 µg $l^{-1}$ falls within the "Good" metric class band. Using the normalisation and rescaling equation gives:

Final Equidistant index score = Upper Equidistant Class range value – ((Face Value - Upper Face value range) * (Equidistant class range / Face Value Range))

= 0.7999 – ((6.0 – 5) * (0.1999 / 4.9999)) = 0.7599 = **0.76**

**Elevated counts**
The face value of 7.65% falls within the "High" metric class band. Using the normalisation and rescaling equation gives:

Final Equidistant index score = Upper Equidistant Class range value – ((Face Value - Upper Face value range) * (Equidistant class range / Face Value Range))

= 1 - ((7.65 – 0) * (0.19999 / 9.9999) = 0.8470 = **0.85**

**Seasonal succession**
The face value of 5.56% falls within the "Bad" metric class band. Using the normalisation and rescaling equation gives:

Final Equidistant index score = Upper Equidistant Class range value – ((Face Value - Upper Face value range) * (Equidistant class range / Face Value Range))

= 0.1999 – ((5.56 – 19.9999) * (0.1999 / -19.9999) = 0.05557 = **0.06**

The overall CW phytoplankton assessment is the average (arithmetic mean) of all the calculated equidistant index values:

EQR = (Chl 90$^{th}$ percentile equidistant index value + elevated count equidistant index value + season succession equidistant index value)/3

So, for this example:

EQR = (0.76 + 0.85 + 0.06) / 3 = **0.56**

**= Moderate** class

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