UKTAG Transitional Water Assessment Method

Phytoplankton

Transitional Water Phytoplankton Tool

by

Water Framework Directive – United Kingdom Technical Advisory Group (WFD-UKTAG)

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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 Transitional Water Phytoplankton Tool
Water Framework Directive: Transitional Waters

Purpose of document: To provide an overview of the transitional water (TW) phytoplankton tool, informing Practitioners of how to monitor, assess and classify suitable phytoplankton data according to Water Framework Directive (WFD) requirements in transitional 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 reports (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).

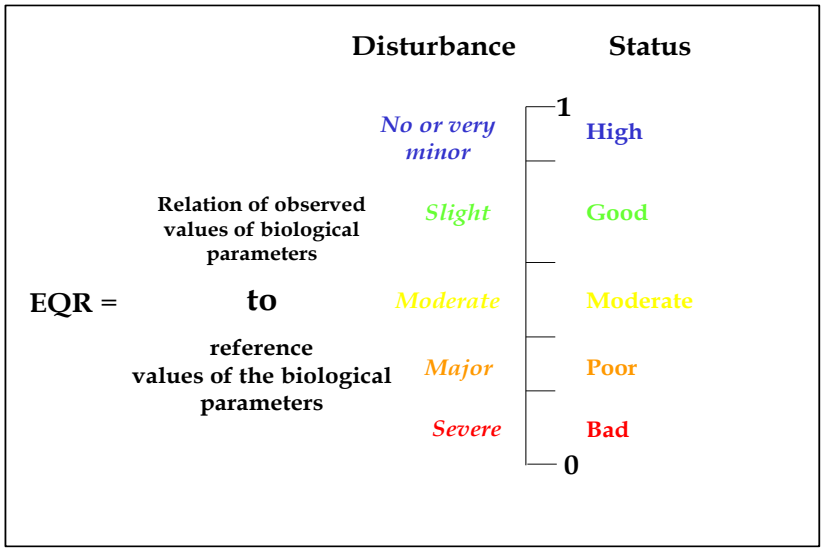

Figure 1: Illustration of the Ecological Quality Ratio and how it relates to the level of disturbance and ecological status during a classification. The class band widths relate to biological changes as a result of disturbance (WFD CIS Guidance Document No. 5, 2003).

1. Key Facts

1.1 Tool Overview: Transitional Water Phytoplankton Tool

The TW phytoplankton tool enables an assessment of the condition of the quality element, "phytoplankton", as listed in Table 1.2.3 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 transitional waters is formed of two separate multimetrics:

(i) chlorophyll-a[1] multimetric
(ii) elevated count multimetric.

The **chlorophyll-a multimetric** incorporates compliance assessment of five statistics of chlorophyll-a biomass:

(i) mean
(ii) median
(iii) percentage compliance under a threshold (10 µg l^{-1} chlorophyll-a)
(iv) percentage compliance under a threshold (20 µg l^{-1} chlorophyll-a)
(v) percentage exceedance over a maximum threshold.

Measurements for the chlorophyll-a multimetric are delineated into two salinity zones, inner (salinity 1 - 25) and outer (salinity > 25), with thresholds for assessing compliance of the statistics, salinity zone specific.

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

(i) percentage exceedance of **single** taxa threshold (measured as cells l^{-1})
(ii) percentage exceedance of **total**[2] taxa threshold (measured as cells l^{-1}).

The two multimetric indices are averaged to provide an overall phytoplankton assessment. *Note*: although a phytoplankton water body assessment is designed to be an average of the two indices, with data from both salinity zones for the chlorophyll-a multimetric, a low confidence assessment can be made with data from only one salinity zone or from one index. It is important to understand the implication, and potential risk of misclassification, when interpreting an assessment where only a partial assessment is made.

An assessment of TW phytoplankton was not reported for the first River Basin Management Plans (2009).

[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 product.
[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 an Environmental Quality Ratio (EQR) from zero (severe disturbance) to one (reference/minimally disturbed conditions). The four class boundaries are:

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

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

1.2 Applicability

The TW phytoplankton tool has been developed to operate at a whole estuary scale, incorporating data from both the inner and outer estuary. An assessment can be made considering data from a single salinity zone but the implication on the water body assessment must be considered. *Note*: these salinity zones are not necessarily defined as separate water bodies under WFD.

Where: The tool can be applied to all UK transitional waters. (There are no geographical differences for reference conditions or boundary thresholds.) However, the tool 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, e.g. where there are naturally high levels of turbidity or flushing, or 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. *Note*: some transitional water bodies may only have a single salinity zone that can be assessed and this needed to be taken in to account within the assessment.

When: The phytoplankton indices have been developed to classify data from monthly samples across the year. *Note*: a *minimum* of 10 months data across a single year are recommended for appropriate data confidence for the elevated count multimetric. For chlorophyll-a assessment, at least 10 sampling occasions are required over the year for each salinity zone.

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

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 (UKTAG) website www.wfduk.org.

*Chlorophyll Uncertainty Tool - Likelihood Analysis of Salinity Sub-metrics. (CUTLASS v1.8) - *Excel workbook to estimate the precision of the assessment.*

*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.

*UKTAG Biological Status Methods: Transitional 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-a biomass is seen as an easily measurable, repeatable parameter that can detect periods of excessive phytoplankton growth. 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).

The TW phytoplankton tool combines the outcomes of two indices which describe the phytoplankton community; in terms of chlorophyll-a biomass and 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 (1.2.3) 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:

(i) composition
(ii) abundance
(iii) biomass
(iv) planktonic bloom frequency and intensity.

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

2.3 Development of the TW phytoplankton tool

The TW phytoplankton tool combines two 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) 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 (measurement of chlorophyll-a through statistical measurements) and taxa counts (exceedances of taxa counts above a threshold).

Initial ideas were 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 (ND, CEC 1991b) and existing scientific literature (Beliaeff *et al*., 2001; Belin, 1998; Gailhard *et al*., 2002). Devlin *et al*., (2007) summarises the early stages of development.

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 (e.g. percentage exceedance)
(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).

Our Interpretation: Structural & functional relevance	Reference Conditions	High	Good	Moderate
Composition and abundance – there is a high degree of species richness and a natural pattern of seasonal species succession, dependent on nutrient availability. Leads to natural seasonal changes in diatom: dinoflagellates and autotrophic: heterotrophic ratios. Variability in all parameters naturally greater than for coastal waters. Chlorophyll-a used as a proxy for phytoplankton bloom biomass and is controlled by type-specific physico-chemical conditions. This is elevated compared to coastal waters. Nuisance or potentially toxic species naturally bloom at key times in the year.	1.1 The composition and abundance of phytoplanktonic taxa are consistent with undisturbed conditions. 1.2 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. 1.3 Planktonic blooms occur at a frequency and intensity which is consistent with type-specific physico-chemical conditions.	1.1 The composition and abundance of phytoplanktonic taxa are consistent with undisturbed conditions. 1.2 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. 1.3 Planktonic blooms occur at a frequency and intensity which is consistent with type-specific physico-chemical conditions.	1.1 There are slight changes in the composition and abundance of phytoplanktonic taxa. 1.2 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. 1.3 Planktonic blooms occur at a frequency and intensity which is consistent with type-specific physico-chemical conditions.	1.1 The composition and abundance of phytoplanktonic taxa differ moderately from type-specific conditions. 1.2 Biomass is moderately disturbed and may be such as to produce a significant undesirable disturbance in the condition of other biological quality elements. 1.3 A moderate increase in the frequency and intensity of planktonic blooms may occur. Persistent blooms may occur in summer months.
	Higher levels of production compared with coastal waters – moderated by light availability, salinity and hydrological effects. Species richness high -the “normal spring bloom” usually dominated by diatoms. Patterns of seasonal growth and succession mirror coastal dynamics, but demonstrate greater variability, in peak, duration and composition.	Species richness high, diatom domination persists throughout growth-period. Nuisance blooms at persistently low levels compared with local background levels. Peaks in chlorophyll-a infrequent and winter-bloom periods low compared to local background.	Slight decline in species richness and evidence of minor disturbance from High status, indicated by slightly higher biomass and /or longer / more frequent blooms	Prolongation of spring bloom with elevated chlorophyll-a above background. Elevated cell count numbers of flagellates. Increasing presence of nuisance /toxic species.

Understanding the individual indices within the TW phytoplankton tool

Chlorophyll-a multimetric (WFD criteria compliance – biomass, planktonic blooms)

The inherent variability of chlorophyll-a in transitional waters is far greater than coastal waters. Preliminary analysis of UK transitional data indicated that the calculation of the chlorophyll-a concentration should be based on commonly observed peaks, rather than a single exceptional one, and must reflect significant events in space and/or time. To encompass this, the chlorophyll-a assessment must be structured as a multimetric, to encompass the natural and extreme levels of variability inherent in the estuarine system.

The multimetric index is based on data collected over monthly intervals for the reporting period, with descriptive statistical measurements of chlorophyll-a biomass:

i) mean
ii) median
iii) percentage **compliance** under a threshold (10 µg l^{-1} chlorophyll-a)
iv) percentage **compliance** under a threshold (20 µg l^{-1} chlorophyll-a)
v) percentage **exceedance** over a maximum threshold (50 µg l^{-1} chlorophyll-a).

Chlorophyll-a measurements are delineated into two salinity zones; the inner zone (salinity of 1 – 25) and the outer zone (salinity >25). Numbers of compliance/ exceedances (%) are calculated as a percentage of samples under/over the threshold against all sample data measurements (ideally monthly values over a six year period). These are then compared to a threshold value (Section 2.4, Table 2).

If the metric is compliant, i.e. the output falls below the threshold, the output is scored as “1”. If the metric output is above the threshold, the output is scored as “0”. For example, if mean chlorophyll-a value within the inner salinity zone equals 11 µg l^{-1}, this measurement is less than threshold of 15 µg l^{-1}, so it is scored as “1”.

The final classification is based on a score out of 10 for the two combined salinity zones (five metric scores per salinity zone). If only one salinity zone exists/is measured within water body the final classification is based on a score out of 5.

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

The multimetric is based on the number of occasions that phytoplankton counts exceed an established threshold over the reporting period. Phytoplankton thresholds exist for single taxa and for total taxa counts based on all the monthly samples from sites within the water body.

The multimetric face value is calculated as the average exceedance of the two metrics (%).

2.4 Reference conditions

Reference conditions (thresholds and class boundary thresholds) for each index were constructed based on a combination of scientific review (Beliaeff *et al*., 2001; Belin, 1998; Gailhard *et al*., 2002; Borja *et al*., 2004), thresholds accepted in previous directives (CSTT, 1997), expert knowledge (MPTT) and investigations of outputs between water bodies at low and high risk of eutrophication (Devlin *et al*., 2007; 2013).

Thresholds were confirmed by empirically assessing all TW data against salinity normalised nutrients and comparing them with the lowest quartile of the dataset and with the nutrient risk assessments. A risk index of water bodies based on the level of nutrient enrichment and susceptibility of the water body to enrichment was established, allocating a risk factor to water bodies (see Devlin *et al*., 2007; *2013*). Water bodies assessed as low risk from nutrient enrichment were more likely to be high status. Low risk water bodies showed good correlations with the proposed thresholds with the majority (91%) of water bodies being identified as high to good status.

For the chlorophyll-a multimetric, where two salinity zones are assessed, the reference score is 10. (If only one zone is assessed, the reference value is five). The compliance thresholds are shown in Table 2.

Table 2: Thresholds associated with each statistical measurement for the assessment of the TW chlorophyll-a and elevated cell count multimetric. Chlorophyll-a thresholds are presented for the two salinity zones (inner and outer), whereas the elevated counts threshold applies across the whole waterbody.

Metric\Salinity zone	Inner zone (salinity 1-25) thresholds	Outer zone (salinity > 25) thresholds
Mean	15 µg l^{-1} chlorophyll-a	10 µg l^{-1} chlorophyll-a
Median	12 µg l^{-1} chlorophyll-a	8 µg l^{-1} chlorophyll-a
% samples < 10 µg l^{-1} chlorophyll-a	70%	75%
% samples < 20 µg l^{-1} chlorophyll-a	80%	85%
% samples > 50 µg l^{-1} chlorophyll-a	5%	5%
Single taxa count	500,000 cells l^{-1}	
Total taxa count	1,000,000 cells l^{-1}	

Normative definitions describe the reference condition as the abundance 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 (Table 1). Thresholds for elevated counts were adapted from 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; 2013).

The face value reference (% exceedances) for the elevated count multimetric is 0.
The thresholds for assessing compliance are (i) individual taxa 500,000 cells l^{-1} and (ii) total taxa 10^6 cells l^{-1}.

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) against risk assessments and using expert judgement. The overall class boundaries for the phytoplankton tool are shown in Table 3.

Table 3: Overall ecological status boundaries for the TW 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 multimetrics are shown below for chlorophyll-a (Table 4) and elevated counts (Table 5).

Table 4: Class status boundaries for the TW chlorophyll-a multimetric assessments. Face value (FV) and equidistant multimetric ranges are shown.

	Number of compliances (Face Value Range)	Metric Range (0 - 1)	Class	Equidistant class range
Multimetric - 2 salinity zones (10 statistics)				
TW chlorophyll-a statistics Compliance of: 1. Mean 2. Median 3. % Samples <10 ug l^{-1}chlorophyll-a 4. % Samples <20 ug l^{-1} chlorophyll-a 5. % Samples >50 ug l^{-1} chlorophyll-a **Reference for <u>two</u> salinity zones: <u>10</u>** (i.e. all metrics in both zones compliant with threshold)	≥ 9 - ≤ 10	≥ 0.8 - ≤ 1.0	High	0.2
	≥ 7 - < 9	≥ 0.6 - < 0.8	Good	0.2
	≥ 5 - < 7	≥ 0.4 - < 0.6	Moderate	0.2
	≥ 3 - < 5	≥ 0.2 - < 0.4	Poor	0.2
	≥ 0 - < 3	0 - < 0.2	Bad	0.2
Multimetric - 1 salinity zone (5 statistics)				
TW chlorophyll-a statistics Compliance of: 1. Mean 2. Median 3. % Samples <10 ug l^{-1}chlorophyll-a 4. % Samples <20 ug l^{-1} chlorophyll-a 5. % Samples >50 ug l^{-1} chlorophyll-a **Reference for <u>one</u> salinity zone: <u>5</u>** (i.e. all metrics in only one zone compliant with threshold)	> 4 - ≤ 5	≥ 0.8 - ≤ 1.0	High	0.2
	> 3 - ≤ 4	≥ 0.6 - < 0.8	Good	0.2
	> 2 - ≤ 3	≥ 0.4 - < 0.6	Moderate	0.2
	> 1 - ≤ 2	≥ 0.2 - < 0.4	Poor	0.2
	≥ 0 - ≤ 1	0 - < 0.2	Bad	0.2

Table 5: Class status boundaries for the TW elevated counts multimetric assessments. Face value (FV) and equidistant multimetric ranges are shown.

Multimetric: 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

3. Undertaking an assessment

3.1 Summary Flow Chart

The process for undertaking a water body assessment of transitional water phytoplankton is summarised below (Figure 2).

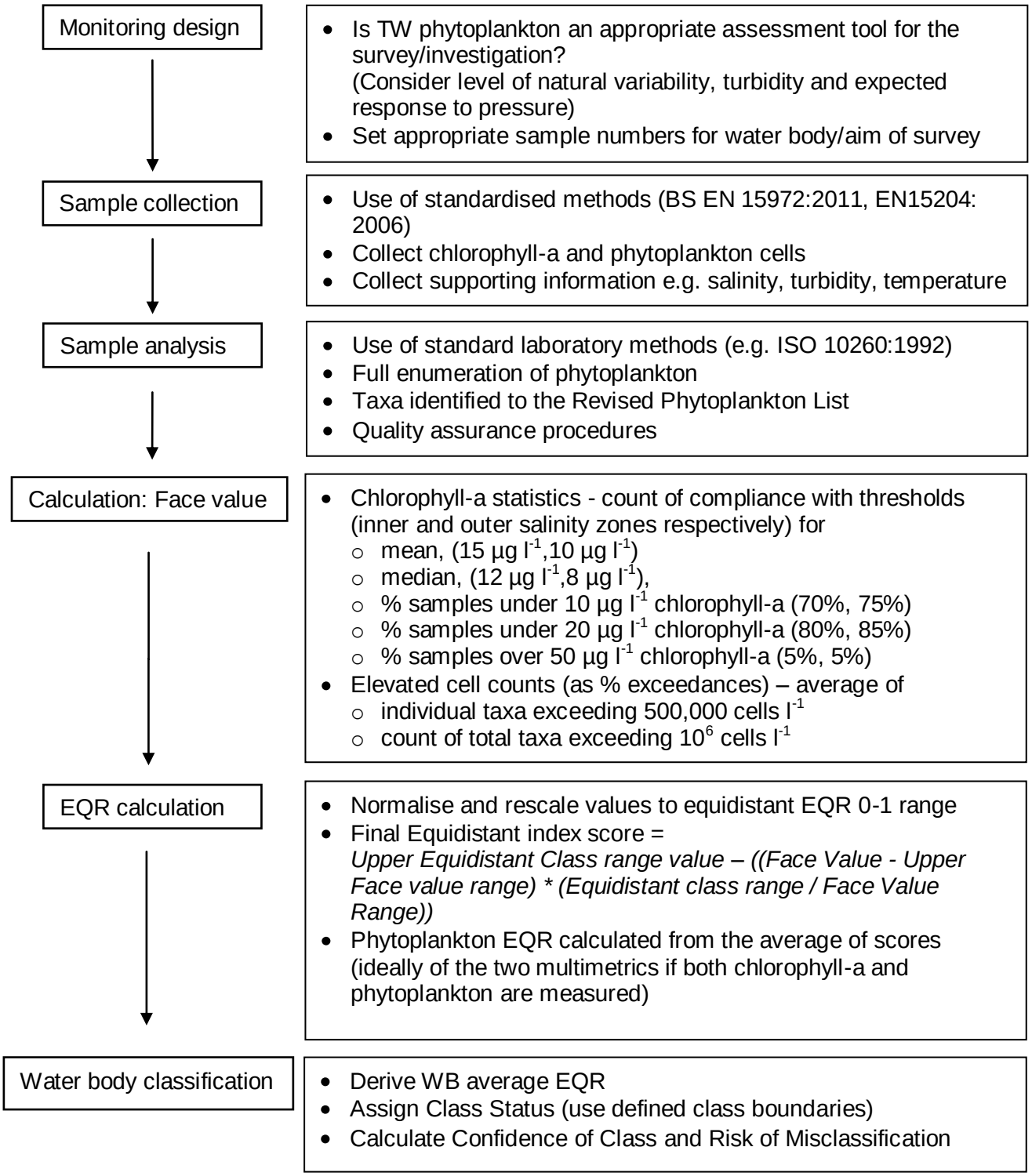

Figure 2: Flow chart summarising the main stages involved in undertaking a TW phytoplankton assessment.

3.2 Data requirements

Calculation of the phytoplankton indices requires the measurement of chlorophyll-a concentration (chlorophyll-a statistics) and phytoplankton cell counts (full enumeration of taxa as defined in the revised phytoplankton list). Salinity measurements are also required.

3.3 Sampling strategy

The TW 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-a and phytoplankton are required throughout the full year. It is recognised that this is logistically difficult so it should be noted that a minimum of ten months in any one year for phytoplankton counts is recommended for appropriate data confidence. For chlorophyll-a assessment, the number of sampling sites should be based on the size of the water body and that of its salinity zones (in England and Wales for example this is generally three to five sites per salinity zone) with at least ten sampling occasions (months) in the waterbody per year. The chlorophyll-a metric will not be biased by more sampling in one salinity band than the other so long as both high and low salinity bands have the minimum data required to calculate the various metrics. It is recommended that no less than five sampling occasions (months) per year for a salinity zone are used.

WFD assessments generally use single surface samples with suitable replication within the two required salinity zones; inner zone (salinity of 1-25) and the outer zone (salinity > 25). Sampling within the zones should be at the appropriate number of sites in respect to the size of the water body (and salinity zones) and the natural variability seen in the phytoplankton community. *Note*: some transitional waters do not have the two salinity zones described and therefore sampling and assessment need to be based on a single salinity zone.

3.4 Sampling methodology

The UK monitoring authorities follow the British standards for phytoplankton sampling (EN 15972:2011) and processing (EN 15204:2006).

Surface sampling is generally carried out for phytoplankton and chlorophyll-a. 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

Data should be checked to ensure it complies with the salinity and the data frequency requirements.

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 Figure 3.

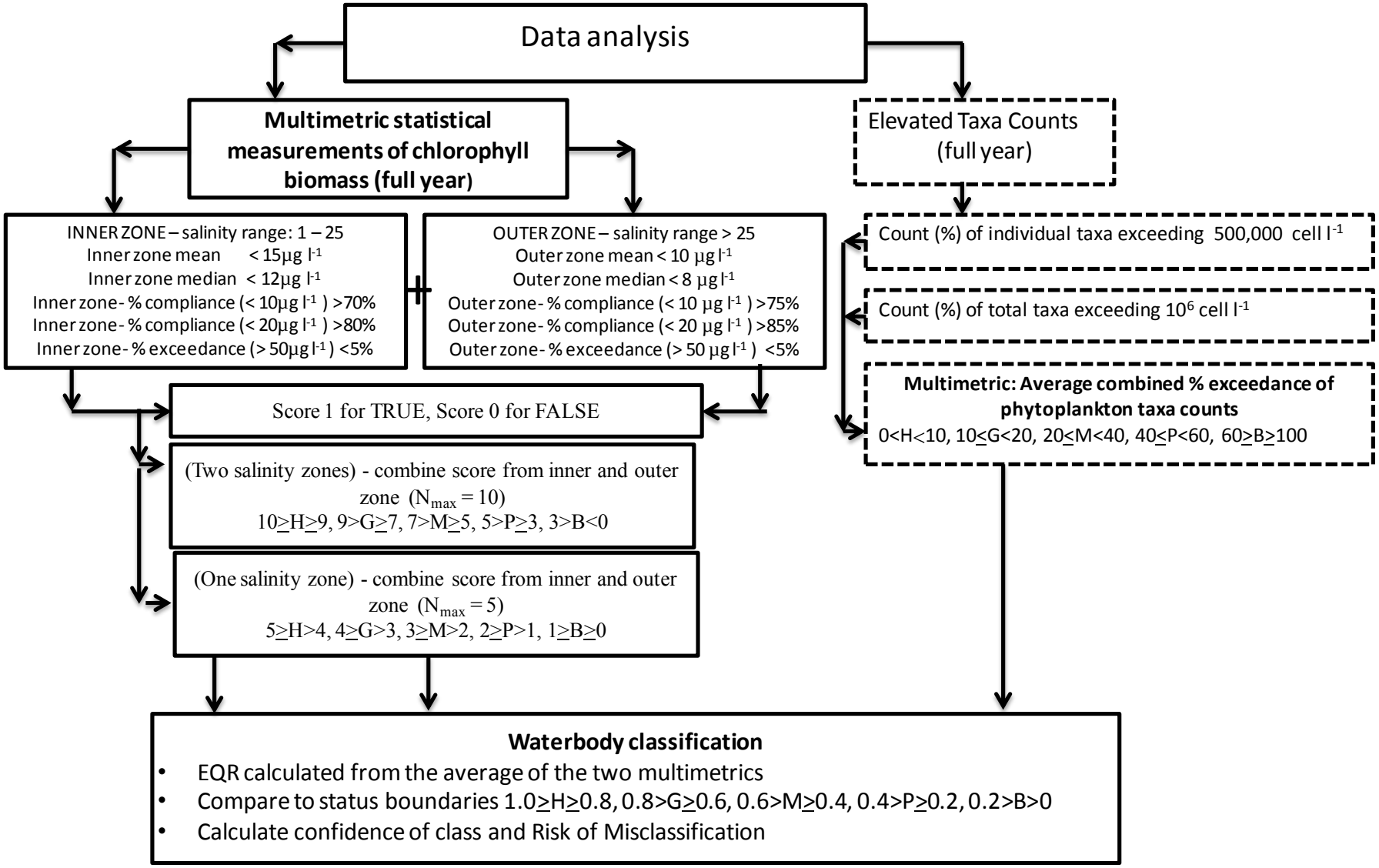

Figure 3: Conceptual diagram illustrating how the TW phytoplankton indices are combined to calculate a water body classification.

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 two 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 TW phytoplankton EQR, i.e. average of equidistant index scores.

Calculation of face values

The *chlorophyll-a multimetric* face value uses a count of compliance of five statistical attributes:

(i) mean
(ii) median
(iii) percentage of samples under 10 µg l^{-1}
(iv) percentage of samples under 20 µg l^{-1}
(v) percentage of samples over 50 µg l^{-1}.

Thresholds are specific for salinity zone: inner (salinity 1-25) and outer (salinity >25) (see Table 2).

The *elevated count* index is calculated as the arithmetic mean of:

(i) fraction of all samples where measured individual taxa exceeds the threshold (500,000 cells l^{-1}) and
(ii) the fraction of all samples whose measured total taxa concentrations exceed the threshold (10^6 cells l^{-1}).

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 indices. Initially this was carried out in a two step process, 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 mathematically combined 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 6 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.

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.

Multimetric:		Lower Face Value range value (measurements towards the "bottom" end of this class range)	Upper Face Value range value (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
TW chlorophyll-a statistics (2 salinity zones) Compliance of: 1. Mean 2. Median	Two Zones	Numbers of metrics "passed"	Numbers of metrics "passed"	Numbers of metrics "passed"			
3. % Samples <10 ug l^{-1} chlorophyll-a	**High**	9.00	10.00	-1.00	≥0.8	1.00	0.2
4. % Samples <20 ug l^{-1} chlorophyll-a	**Good**	7.00	8.99	-2.00	≥0.6	0.80	0.2
5. % Samples >50 ug l^{-1} chlorophyll-a	**Moderate**	5.00	6.99	-2.00	≥0.4	0.60	0.2
Reference for **two** salinity zones:	**Poor**	3.00	4.99	-2.00	≥0.2	0.40	0.2
10 (statistics in both zones compliant with threshold)	**Bad**	0.00	2.99	-3.00	0	0.20	0.2
TW chlorophyll-a statistics (1 salinity zone) Compliance of: 1. Mean 2. Median	One Zone	Numbers of metrics "passed"	Numbers of metrics "passed"	Numbers of metrics "passed"			
3. % Samples <10 ug l^{-1} chlorophyll-a	**High**	4.5	5	-0.5	≥0.8	1	0.2
4. % Samples <20 ug l^{-1} chlorophyll-a	**Good**	3.5	4.49	-1	≥0.6	0.8	0.2
5. % Samples >50 ug l^{-1} chlorophyll-a	**Moderate**	2.5	3.49	-1	≥0.4	0.6	0.2
Reference for **one** salinity zones:	**Poor**	1.5	2.49	-1	≥0.2	0.4	0.2
5 (statistics in one zone compliant with threshold)	**Bad**	0	1.49	-1.5	0	0.2	0.2
		% Exceedances	% Exceedances	% Exceedances			
TW elevated counts	**High**	<10	0	10	≥0.8	1	0.2
1. Single taxa exceedance	**Good**	<20	≥10	10	≥0.6	<0.8	0.2
2. Total taxa exceedance	**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

Table 6: Values for the normalisation and rescaling of face values to EQR metric ranges for transitional waters.

TW phytoplankton assessment – site level
The overall phytoplankton assessment is the average of the calculated equidistant index values.

TW phytoplankton EQR = (Chlorophyll-a equidistant index value + Elevated count equidistant index value)/2

3.8 Water body level classification

The overall TW water body classification is based on the arithmetic mean score (EQR) of the two multimetrics calculated for the water body.

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, water bodies 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 water bodies 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.

The TW Phytoplankton CofC tool, Chlorophyll-a Uncertainty Tool - Likelihood Analysis of Salinity Sub-metrics (CUTLASS), calculates the face value EQR and confidence of class for the two multimetrics - chlorophyll-a and elevated counts. For both indices, calculations are performed for multiple water bodies simultaneously and give the confidence of class over the whole reporting period. The results from the two indices are then combined to give an overall status assessment.

A technical note (Davey, 2010) explains and documents the statistical methodology used by CUTLASS.

4. Worked Example

Using an example water body we have the Face Value results

- Chlorophyll-a statistics compliance 9
- Elevated counts 7.65%

TW chlorophyll-a
The face value 9 for the chlorophyll-a compliant statistics falls within the "High" class band. The values for this row in Table 6 are input into the equation:

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

= 1- ((9-10) * (0.2/-1)) = 0.8

(*Note*: because of the linear equidistant nature of the face values of this metric almost all scores can be read directly from the equivalent equidistant column. Only a face value score of 1 has to be interpolated.)

TW elevated counts
The face value of 7.65% falls within the "High" class band. The values for this row in Table 6 are input into the equation:

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 / 9.999) = 0.8470 = **0.85**

The overall TW water body classification is based on the arithmetic mean score (EQR) of the two multimetrics calculated for the water body.

EQR = (0.8 + 0.85) / 2 = 0.825 = **0.83**

= **High** status

5. References

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