Practitioners Guide to the Transitional Water Phytoplankton Tool
Water Framework Directive: Transitional Waters

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

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*. 2007a, Devlin and Best, *in press*). 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 (Fig. 1). Once the EQR score and ecological status class have been calculated an assessment must be made to consider the certainty of the classification (i.e. confidence in the assigned class).

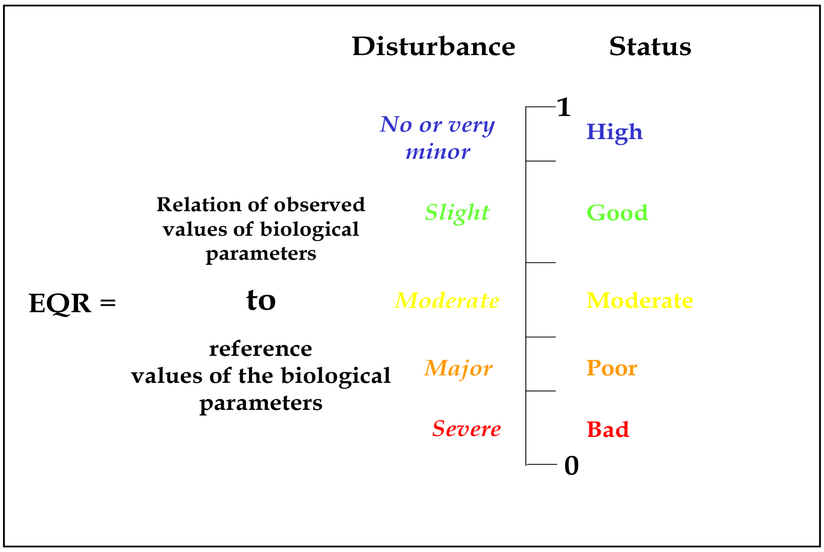

Fig. 1: Illustration of the Ecological Quality Ratio and how it relates to the level of disturbance and ecological status during a classification. The class band widths relate to biological changes as a result of disturbance.

1. Key Facts

1.1 Tool Overview: TW 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 multimetric indices:
(i) chlorophyll multimetric
(ii) elevated count multimetric

The **chlorophyll multimetric** incorporates compliance assessment of five statistics of chlorophyll biomass:
i) mean
ii) median
iii) percentage compliance under two separate thresholds
iv) percentage exceedance over a maximum threshold.

Measurements for the chlorophyll 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 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 multimetric, an assessment can be made from one index or with data from one salinity zone. 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) due to insufficient available data at that time.

The phytoplankton tool (and component indices) operates over an Environmental Quality Ratio (EQR) from zero 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, 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 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 nine months data across a single year are recommended for appropriate data confidence for the elevated count multimetric. For chlorophyll 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 (at least 3 years of data will be required in any 6 year reporting period, although most TW will require more data due to high inherent levels of variability).

Response to pressure: The phytoplankton tool has been designed to identify the impact on phytoplankton from nutrients and organic enrichment, 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 from different time periods.

1.3. Key Documents

The documents marked * will be hosted on the UK technical advisory group (UKTAG) website www.wfduk.org.

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

Devlin, M. J., M. Best, D. Coates, E. Bresnan, S. O'Boyle, R. Park, J. Silke, J. Skeats & J. Barry, 2007a. 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.*

*Devlin, M and Best, M. (in press). Water Framework Directive: The development and status of phytoplankton tools for ecological assessment of coastal and transitional waters. United Kingdom. Update to Report to Scottish and Northern Ireland forum for environmental research (SNIFFER) for the Environment Agency.

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

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 indicate the excessive growth of undesirable phytoplankton species. Persistent, high cell counts of any algae may lead to excess algal production and impact 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 (Bellin *et al.*, 1995).

The TW phytoplankton tool combines the outcomes of two indices which describe the phytoplankton community; in terms of chlorophyll 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).

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).

Bio Element	Our Interpretation Structural & functional relevance	Reference Conditions	High	Good	Moderate
Phytoplankton	Composition & 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 CWs. Nutrient ratios (N/P, N/Si, and P/Si) follow natural seasonal fluctuations. Chl-a used as a proxy for phytoplankton bloom biomass and is controlled by type-specific physico-chemical conditions. This is elevated c.f. CWs. Nuisance or potentially toxic species naturally bloom at key times in the year.	1.1 The composition & 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 & 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. 1.3Planktonic 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. 1.3Planktonic blooms occur at a frequency and intensity which is consistent with type-specific physico-chemical conditions.	1.1 The composition & 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.
		Prone to higher levels of production compared with Coastal Waters – possibly tempered by light availability, salinity & hydrological effects. Species richness high with a “normal spring bloom” usually dominated by	Species richness high, diatom domination persists throughout growth-period. Nuisance/toxic species @ persistently low levels compared with local background levels. Peaks in chlorophyll infrequent	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 chl-a above background. Elevated cell count numbers of flagellates. Increasing presence of nuisance /toxic species.

		diatoms. Patterns of seasonal growth & succession mirror coastal dynamics, but demonstrate greater variability, in peak, duration & composition.	**&winter-bloom periods low cf. local background.**		

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, Devlin and Best, *in press*.

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 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 (e.g. Bellin *et al.*, 1995). Devlin *et al*., (2007a) 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 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).

Understanding the individual indices within the TW phytoplankton tool

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

The inherent variability of chlorophyll in transitional waters is far greater than coastal waters. Preliminary analysis of UK transitional data indicated that the calculation of chlorophyll *a* concentrations 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 assessment must be structured as a multimetric tool, to encompass the natural and extreme levels of variability inherent in the estuarine system.

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

i) mean
ii) median

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

Chlorophyll 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 (ideally monthly values over a 6 year period) measurements. 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 the mean chlorophyll 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 (Bellin *et al*., 1995; Borja *et al*., 2004), thresholds accepted in previous directives (CSTT, 1997; 2002), expert knowledge (UKTAG MPTT) and investigations of outputs between water bodies at low and high risk of eutrophication (Devlin *et al*., 2007).

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). 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 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 multimetric. Thresholds are presented for the two salinity zones (inner and outer).

Metric\Salinity zone	Inner zone (salinity 1-25 ppt) thresholds	Outer zone (salinity > 25 ppt) thresholds
Mean	15 µg l^{-1} Chl	10 µg l^{-1} Chl
Median	12 µg l^{-1} Chl	8 µg l^{-1} Chl
% samples < 10 µg l^{-1} Chl	70%	75%
% samples < 20 µg l^{-1} Chl	80%	85%
% samples > 50 µg l^{-1} Chl	5%	5%

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. Thresholds for elevated counts were adapted from Bellin *et al*., (1995) and tested through the outcomes of low, moderate and high risk water bodies (Devlin *et al*., 2007).

The face value reference (% exceedances) for the elevated count multimetric is zero. 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 (Table 4). Note the tables show the one step process with just the rescaled equidistant range.

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

Multimetric:		Lower Face Value range value (the measurements towards the "bad" end of this class range)	Upper FV range value (the measurements towards the "High" end of this class range)	FV class range	Lower 0-1 equidistant range vale	Upper 0-1 equidistant range value	Equidistant class range
TW chlorophyll statistics Compliance of: 1.Mean 2.Median 3. % Samples <10 ug l^{-1}Chl 4. % Samples <20 ug l^{-1} Chl 5. % Samples >50 ug l^{-1} Chl Reference for **two** salinity zones: **10** (ie all statistics in both zonescompliant with threshold)	Two Zones	Numbers of statistics	Numbers of statistics	Numbers of statistics			
	High	9	10	-1	≥0.8	1	0.2
	Good	7	8	-1	≥0.6	<0.8	0.2
	Moderate	5	6	-1	≥0.4	<0.6	0.2
	Poor	3	4	-1	≥0.2	<0.4	0.2
	Bad	0	2	-2	0	<0.2	0.2
TW elevated counts 1. Single taxa exceedance 2. Total taxa exceedance		% 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

3. Undertaking an assessment

3.1 Summary of the process

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

Work Area	*Considerations*
Monitoring Design	• Is TW phytoplankton an appropriate assessment for the survey/investigation? (Consider level of natural variability, turbidity and expected response to pressure) • Set appropriate sample numbers in the two required salinity zones
Sample collection	• Use of standardised methods (BS EN 15204:2006) • Collect chlorophyll and phytoplankton • Collect required supporting parameters
Sample analysis	• Use of standard laboratory methods • Full enumeration of phytoplankton • Taxa identified to the Reduced Taxon List • Quality assurance procedures
Calculation: Face value	• *Chlorophyll statistics– count of compliance with thresholds (inner and outer salinity zones respectively) for* *mean, (15 µg l^{-1},10 µg l^{-1})* *median, (12 µg l^{-1},8 µg l^{-1}),* *% samples under 10 µg l^{-1} Chl (70%, 75%)* *% samples under 20 µg l^{-1} Chl (80%, 85%)* *% samples over 50 µg l^{-1} Chl (5%, 5%)* • *Elevated cell counts* – count (as %) of (i) individual taxa exceeding 500,000 cells l^{-1} (ii) count (as %) of total taxa exceeding 10^6 cells l^{-1} Average combined % exceedance
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 scores
Water body classification	• Derive WB average IQI (EQR) • Assign Class Status (use defined class boundaries) • Calculate Confidence of Class and Risk of Misclassification

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

3.2 Data requirements

Calculation of the phytoplankton indices requires measurement of chlorophyll a concentration (chlorophyll statistics) and phytoplankton cell counts (full enumeration of taxa as defined in the Reduced Taxa 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 6 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 this is logistically difficult so it should be noted that a minimum of nine months in any one year for phytoplankton counts is recommended for appropriate data confidence. For chlorophyll assessment, the number of sampling sites should be based on the size of the WB and that of its ‘salinity zones (in E&W for example this is generally 3-5 sites per salinity zone) with at least 10 sampling occasions (months) in the waterbody per year. The chlorophyll 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 5 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 and processing (EN 15204:2006 and EN 15972:2011).

Surface sampling is carried out for phytoplankton and chlorophyll. The surface sample is collected, mixed and sub samples processed for (i) chlorophyll 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 (EA, NIEA, SEPA) have their own operating procedures and instructions (please refer to the relevant Agency for further details).

3.5 Sample Analysis

The contents of the 25ml phytoplankton cell sample are settled into a Utermöhl Chamber for microscopic analysis of phytoplankton composition and abundance.
Phytoplankton cells should be identified to the lowest practical taxonomic level possible. UK WFD authorities use a reduced taxa list (RTL, please refer to the relevant WFD Agency for current list).

Chlorophyll a concentrations are generally analysed through fluorescence to obtain an estimate of biomass as µg l^{-1} (*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 zero to one (Fig. 3).

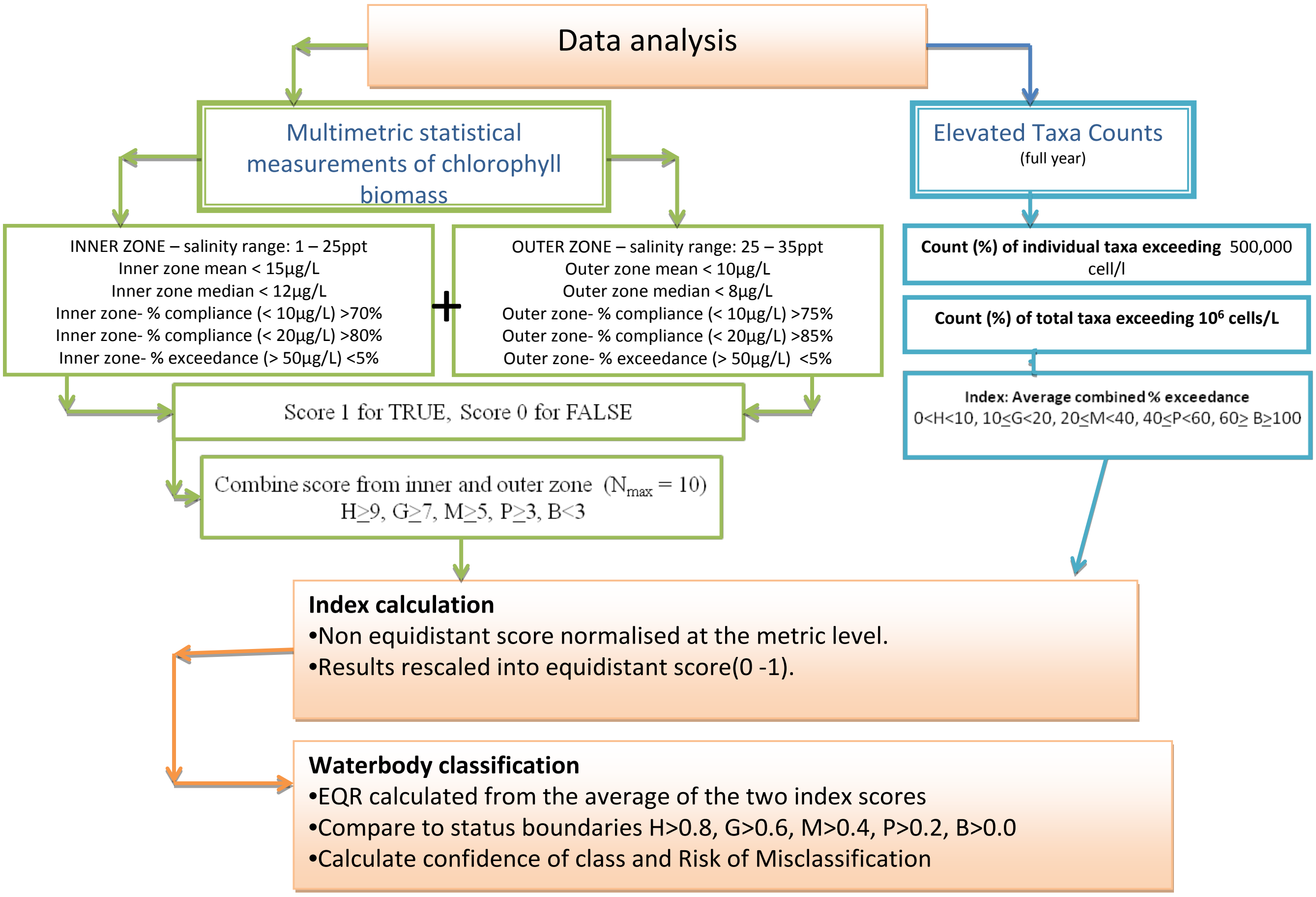

Fig. 3: Conceptual diagram illustrating how the TW phytoplankton indices are combined to calculate a water body classification:

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 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 4 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 4.999’.

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 biological community across a whole water body continuously throughout 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.

When assigning discrete ecological status classes, variability means that, depending on the proximity of the water body assessment result to a class boundary, there is a likelihood that the "true" status (i.e. that status if the EQR for the total population was known with zero error) is different to that assigned. This is termed the 'risk of misclassification' (RoM). Conversely, the statistical confidence that the status assigned from the sample population falls into each of the five ecological status classes is referred to as the 'confidence of class' (CofC) – the CofC is the reciprocal value of RoM.

The approach developed to define and report the CofC and RoM for WFD TraC benthic invertebrates is described by Ellis and Adriaenssens (2006) in terms of its' general application to the WFD ecological quality elements. The approach to CofC and RoM requires the following information for a given assessment:

- Mean EQR
- Ecological class status boundaries
- Standard error (SE) of the assessment data

The TW Phytoplankton CofC tool, called CUTLASS (Chlorophyll Uncertainty Tool - Likelihood Analysis of Salinity Sub-metrics), calculates the face value EQR and confidence of class for the two multimetrics - chlorophyll 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 statistics compliance 9
- Elevated counts 7.65%

TW chlorophyll
The face value 9 for the chlorophyll compliant statistics falls within the "High" class band. The values for this row in Table 4 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 4 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

Belin, C., Beliaeff, B., Raffin, B., Rabia, M., Ibanez, F., 1995. Phytoplankton time-series data of the French Phytoplankton Monitoring Network: toxic and dominant species. In: Lassus. P., Arzul, G., Erard Le Denn. E., Gentien, P., Marcaillou Le Baut, C. (Eds.), Harmful marine algal blooms – prolife´rations d'algues nuisibles, Paris–France Lavoisier, pp. 771–776

Borja, A., Franco, F., Valencia, V., Bald, J., Muxika, I., Belzunce, M.J., Solaun,O. (2004). Implimenting the European Water Framework Directive from the Basque country (northern Spain): a methodological approach. Mar. Poll. Bull. 48 (3-4), 209-218.

Council of European Communities (CEC) (1991a) Council Directive of 21 May 1991 concerning urban waste water treatment (91/271/EEC). Off J Eur Commun L135:40–52 (30.5.91)

Council of European Communities (CEC) (1991b) Council Directive of 31 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources (91/676/EEC). Off J Eur Commun L375:1–8 CSTT, 1994.

CSTT, (1997). Comprehensive studies for the purposes of Article 6 & 8.5 of DIR 91/271 EEC, the Urban Waste Water Treatment Directive, second edition. Published for the Comprehensive Studies Task Team of Group Coordinating

CSTT 2002. Comprehensive studies for the purposes of Article 6 of DIR 91/271 EEC, the Urban Waste Water Treatment Directive. Published for the Comprehensive Studies Task Team of Group Coordinating Sea Disposal Monitoring by the Forth River Purification Board, Edinburgh

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

Davey, A. (2010) CUTLASS: Chlorophyll Uncertainty Tool - Likelihood Analysis of Salinity Sub-metrics. WRC project note for the Environment Agency 15412-0.

Devlin, M. J., M. Best, D. Coates, E. Bresnan, S. O'Boyle, R. Park, J. Silke, J. Skeats & J. Barry, (2007a). 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.

Devlin MJ, Painting S, Best M (2007b) Setting nutrient thresholds to support an ecological assessment based on nutrient enrichment potential primary production and undesirable disturbance. Mar Pollut Bull 55:65–73.

Devlin, M and Best, M. (in press). Water Framework Directive: The development and status of phytoplankton tools for ecological assessment of coastal and transitional waters. United Kingdom. Report to Scottish and Northern Ireland forum for environmental research (SNIFER).

Water Framework Directive, 2000. Directive 2000/60/EC of the European Parliament and of the council of 23 October 2000 establishing a framework for community action in the field of water policy. Official Journal of the European Communities L 327: 1–72.

EN 15204:2006 Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique);

EN 15972:2011 Water quality - Guidance on quantitative and qualitative investigations of marine phytoplankton).

EN 16161:2012 Water quality - Guidance on the use of in vivo absorption techniques for the estimation of chlorophyll-a concentration in marine and fresh water samples.

Ellis, J. A, (2006). Uncertainty estimation for monitoring results by the WFD biological classification tools. WRC report to the Environment Agency.

ELLIS, J.A. AND ADRIAENSSENS, V., 2006. Uncertainty estimations for monitoring results by the WFD biological classification tools. Environment Agency WFD Report. 32pp.

Foden J, Devlin MJ, Mills DK, Malcolm SJ (2011) Searching for undesirable disturbance: an application of the OSPAR eutrophication assessment method to marine waters of England and Wales. Biogeochemistry 106:157–175. doi:10.1007/s10533-010-9475-9.

ISO 10260:1992 Water quality Measurement of biochemical parameters -- Spectrometric determination of the chlorophyll-a concentration Oslo Paris Convention for the Protection of the North Sea (OSPAR) (2002) Common assessment criteria their assessment levels and area classification within the Comprehensive Procedure of the Common Procedure. OSPAR Commission for the Protection of the Marine Environment of the North-East Atlantic. http://www.ospar.org

OJEC (2000) DIRECTIVE 2000/60/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 October 2000 establishing a framework for Community action in the field of water policy.

Tett, P, Gowen, R., Mills, D. Fernandes, T., Gilpin, L., Huxham, M., Kennington, K., Read, P., Service, M., Wilkinson, M., Malcolm, S. (2007). Defining and detecting undesirable disturbance in the contex of marine eutrophication. Mar Poll Bull 55, 282-297.