Water Framework Directive:

The development and status of phytoplankton tools for ecological assessment of coastal and transitional waters. United Kingdom

Michelle Devlin, Mike Best, Eileen Bresnan, Clare Scanlan, Malcolm Baptie

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

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2. Executive summary

Phytoplankton of coastal and transitional waters are monitored routinely by UK and ROI agencies as an indicator of ecological and eutrophication status, as required by the Urban Waste Water Directive [UWWTD, CEC, 1991a], the Oslo Paris Convention (OSPAR, 2003a, b), and the Water Framework Directive [WFD, (CEC, 2000)]. Chlorophyll is measured as an indicator of phytoplankton biomass and a range of supporting environmental and chemical measurements are normally taken in support of the biological monitoring (e.g. micronutrient concentrations, phaeophytin, secchi depth, salinity, temperature, evidence of vertical mixing/stratification). The abundance and composition of the phytoplankton is one of the key tools in defining Ecological Quality Status for the Water Framework Directive, particularly in relation to the impact on the ecology of coastal and transitional waters by anthropogenic inputs of nutrient (mainly inorganic nitrogen).

- This report details the development and status of the current UK phytoplankton assessment tools as required under the Water Framework Directive.
- Different variations of the tools exist for transitional and coastal waters due to the different natures of the waters, and the nonlinear responses evident in the more turbid coastal and transitional waters.
- Minimum sampling requirements are set to monthly samples in WFD waterbodies. The numbers of sites per waterbody are dependent on size, but generally 1 to 2 sites per small waterbody, 2-5 sites per medium waterbody and 5 – 10 sites per large waterbody.
- WFD assessment tools for coastal waters using phytoplankton measurements (metrics) consist of
    - The measurement of the 90^{th} value of chlorophyll biomass, based on a recommended minimum of nine monthly samples through the year. The 90^{th} percentile value is compared to baseline or reference value.
    - The frequency of exceedance of phytoplankton counts above a pre-determined threshold of cell counts. Phytoplankton thresholds exist for individual identification groupings and for total taxa count as identified in monthly (minimum) samples from waterbody sites. Chlorophyll thresholds are used to identify the number of occurrences of chlorophyll a measurements that exceed 10µg l^{-1}.
    - Measurement of the two main phytoplankton taxonomic groupings (diatoms and dinoflagellates), normalised to a Z score, based on the proportion of time that the sum of diatoms and dinoflagellates fall below (or above) a reference Z score for each month. This metric also has regional variations, with the reference values being specific to the geographical locations. The metric requires monthly counts for diatoms and dinoflagellates (natural log means) and the calculation of a z score using the waterbody mean against a reference mean and standard deviation.
- WFD assessment tools for transitional waters using phytoplankton measurements (metrics) consist of
    - Statistical measurements of chlorophyll biomass, including mean, median and % exceedances, based on a recommended minimum of monthly sampling. The measurements are delineated into two salinity zones and compared against reference values. Numbers of exceedances are calculated as a percentage of all measurements.
    - The frequency of exceedance of phytoplankton counts above a pre-determined threshold of cell counts. Phytoplankton thresholds exist for single identification categories and for total taxa count as identified in monthly (minimum) samples from waterbody sites.

- **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 chlorophyll 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 are 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 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.

3. Background to Water Framework Directive

3.1 What is the Water Framework Directive?

The Water Framework Directive (hereafter WFD) is a major legislative requirement for Europe and entails broader conceptual thought on whole ecosystems, particularly focusing on ecological monitoring (CEC, 2000, 2008) An innovative approach to monitoring the chemistry and the biology has been required by member states in order to deliver this directive. In the UK, current monitoring was reviewed, gaps noted and new classification tools developed since the implementation of the WFD. Across Europe actions were taken to focus the effort on developing the classification tools and to provide technical support for the unfolding directive.

Classification is a way of reporting the health of the water environment. For a particular point in time, a classification will show us where the quality of the environment is good, and where it may need improvement. It helps identify and focus effort on the parts of the water environment we need to improve. Classification sets a benchmark that can be used to prevent further deterioration and guide management direction.

The WFD provided a more sophisticated way of assessing the whole water environment that helps direct action to where it is most needed (CEC, 2000, 2008). The WFD provides the means to do this by looking at over 30 measures, grouped into ecological status (this includes biology as well as 'elements' like phosphorus and pH) and chemical status ('priority substances'). The WFD covers estuaries, coastal waters, groundwater and lakes as well as rivers. The WFD classifies these surface waters based on the status of a number of "quality elements". For transitional and coastal waters these are biological, hydromorphological and chemical and physico-chemical elements (Vincent et al., 2002). The general physico-chemical elements include: transparency, thermal conditions, oxygen conditions, salinity and nutrient conditions. Specific pollutants are also considered in the directive.

There are two status classifications, ecological and chemical. An ecological status classification can consist of:

- the condition of biological elements, for example fish, benthic invertebrates and marine plants
- concentrations of supporting physico-chemical elements, for example oxygen or ammonia
- concentrations of specific pollutants, for example copper
- and for high status, largely undisturbed hydromorphology

The biological elements are identified as: the composition, abundance and biomass of phytoplankton; the composition and abundance of other aquatic flora; the composition and abundance on benthic invertebrate fauna; and the composition and abundance of fish fauna. The hydromorphological elements supporting the biology are given as: depth variation; quantity, structure and substrate of the bed; structure of the intertidal zone; freshwater flow; and wave exposure and are not detailed in this report.

The directive states, in general terms, what conditions are expected for various states of the water body. These are known as the "normative definitions". Classification tools and schemes have to conform to these normative definitions. For a waterbody to achieve high status all elements must be at high status. In addition, the directive process requires member states to intercalibrate their national methods to ensure consistency of classification across the community.

The overall aim of the Water Framework Directive (CEC, 2000) is to establish good ecological status in all European waters by 2015. Phytoplankton, along with benthic invertebrates, estuarine fish and macrophytes are one of four biological quality elements of the WFD. The WFD directive uses a "classification scheme" for the overall classification of the waterbody which includes some measure of these four biological elements. Classification is a way of reporting the state of the environment and provides a way of comparing waters and looking at changes over time. Classification tools provide a process by which to assess the status of each individual biological quality element against high status (Vincent et al., 2002). Accordingly the ecological status is expressed as a ratio between the values of the biological elements observed by a given body of surface water and the values for these elements in a site with no, or very minor, disturbance from human activities (reference or high ecological status).The WFD provides general definitions for the first three quality conditions or classes (High, Good, Moderate), known as the "normative definitions". Each describes a different degree of impact on the plants and animals. Member states are responsible for further defining these and providing definitions for the Poor and Bad classes

Ecological status class is recorded on the scale of high, good, moderate, poor or bad. 'High' denotes largely undisturbed conditions and the other classes represent increasing deviation from this undisturbed, or reference, condition. The ecological status classification for the water body is determined by the worst scoring quality element. Figure 3-1 illustrates the criteria determining the different ecological status classes for assessment under the WFD. By the rules of the Water Framework Directive, the ecological status is determined by the worst scoring component.

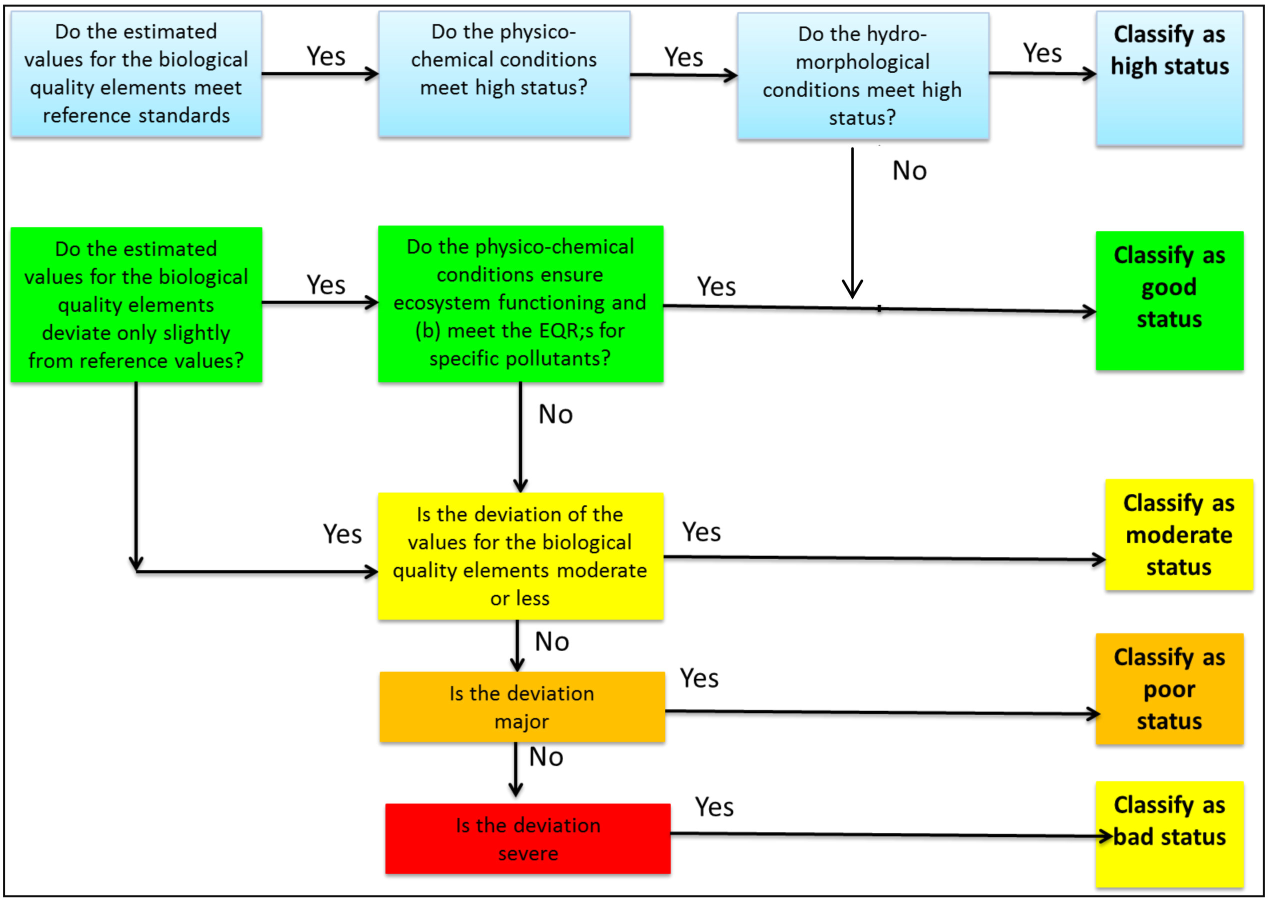

Figure 3-1: The process of classification through ecological and physico-chemical status for the WFD.

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3.2 Assessment of eutrophication

Eutrophication is commonly defined through various EC Directives and obligations as "the enrichment of water by nutrients, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned" (CEC, 1991b, a, 2000, 2008). It is therefore linked directly with anthropogenic activities on land and is mostly identified in estuarine and near shore coastal environments. Land based sources of nutrients are from point sources (e.g. sewage treatment works) and diffuse pollution (e.g. agriculture run-off). Other nutrients inputs such as atmospheric deposition and nutrient enrichment of bays from adjacent coastal waters are not initially considered for control through river basin management plans Eutrophication is limited (prevented) by a supply of nutrients (mostly forms of nitrogen and phosphorus), the availability of light, and the physical nature of the waters (e.g. flushing rates).

Steps have been taken to harmonise eutrophication assessment in the marine environment to ensure international agreement on what constitutes eutrophication impact and risk. For example a "common procedure" has been agreed between OSPAR contracting parties, and "intercalibration" programmes which are taking place to implement the Water Framework Directive, including eutrophication indicators.

A number of indicators of nutrient enrichment are summarised below. These related to indicators of direct impacts:

1. Excessive growth of phytoplankton in the water column
2. Perturbation in characteristic plankton community,
3. Excessive growth of opportunistic macroalgae (green weed) on intertidal sediments and rock.
4. Excessive growth of epiphytic algae (algae growing on other plants), particularly on seagrass and macroalgae.

Indicators of indirect impacts would include:

1. Oxygen depletion in the water column arising from stimulation then die-off of phytoplankton blooms. This could have lethal and sub-lethal impacts on fish and invertebrates.
2. Increased turbidity in the water column leading to reduce photic zone and shading out other plants (eg macroalgae).
3. Reduction of oxygen in surface sediment leading to anoxia. This could have lethal impacts on invertebrates which would also effect birds feeding on them.

3.3 The utilisation of phytoplankton indices as an assessment tool

Assessments relating to phytoplankton are required to encompass taxonomic composition, abundance, biomass and plankton blooms for the ecological classification of transitional and coastal waters (CEC, 2000; Vincent et al., 2002). More specifically the wording of the directive state that if a water body is to attain "high" ecological status "the composition and abundance of phytoplanktonic taxa are to be consistent with undisturbed conditions". Phytoplankton succession and community composition reflect the environmental conditions of the ecosystem, among which nutrient availability plays a significant role (Gowen et al., 2000; Beman et al., 2005; Tett et al., 2007; Tett et al., 2008; Devlin et al., 2009) in structuring that community. The primary biological response to nutrient enrichment in aquatic environments, given suitable environmental conditions (such as light availability and water temperatures), is the growth of phytoplankton and higher plants. Known consequences of marine eutrophication on the phytoplankton community include elevated chlorophyll a levels (Boynton et al., 1996; Harding Jr and Perry, 1997; Bricker et al., 2003a) red tides, water discolouration and foaming - such as that caused by the colonial flagellate *Phaeocystis pouchetii* in the southern North Sea (Lancelot et al., 1987), increased production, which may give rise to extra biochemical oxygen demand (BOD) and hence increased removal of oxygen, in enclosed waters resulting in local anoxia. These include sea-lochs such as Striven (Tett, 1987) and the Baltic Sea (Larsson et al., 1986). Other consequences include shifts in species composition, from diatoms to flagellates (Tett et al., 2008). In general terms, nutrient input is assumed to result in the rapid growth of opportunistic fast growing primary producers and the accumulation of extra biomass which can lead to a negative impact on the ecosystem. Other attributes considered to be symptoms of negative impacts of nutrient enrichment in many ecosystems include blooms of toxic algae, increased growth of epiphytic algae, the growth of nuisance macroalgae, the loss of submerged vegetation due to shading, the development of hypoxic (and anoxic) conditions due to decomposition of the accumulated biomass, and changes in the community structure of benthic animals due to oxygen deficiency (Bricker et al., 1999; Bricker et al., 2003b; Tett et al., 2007; Gowen et al., 2008) The potential ecological ramifications of nutrient enrichment and disturbance also include alterations of the natural phytoplankton community composition, which may in turn change ecosystem food web and nutrient cycling dynamics. For example, if the growth of more readily grazed phytoplankton functional groups, such as diatoms is favoured, trophic transfer and nutrient cycling will take place largely in the water column, with enhanced export of the assimilated algae (as fish) to marine waters. In contrast, if the nutrient loading favours the phytoplankton functional groups that may not be readily grazed, such as dinoflagellates, trophic transfer will be poor and relatively large amounts of unconsumed algal biomass will ultimately settle to the bottom. This unconsumed biomass will stimulate microbial decomposition and oxygen consumption, exacerbating the potential for the development of hypoxia conditions, and alterations in the food chain.

Despite the complexities associated with the phytoplankton community, there are general characteristics of the phytoplankton community which can be explored to identify indicators that measure impact from nutrient enrichment. Phytoplankton biomass has typically been used as indicators of nutrient enrichment (CSTT, 1994; Bricker et al., 1999; Gowen et al., 2000; Bricker et al., 2003b; Painting et al., 2005; Foden et al., 2008; Devlin et al., 2009; Foden et al., 2010; Devlin et al., 2011). Phytoplankton biomass can be used a proxy of the phytoplankton and in UK marine waters; it should reflect low biomass concentration in the winter, high spring concentrations, and variable, periodic summer and autumnal concentrations. Chlorophyll concentrations represent a very simple and integrative measure of the phytoplankton community response to nutrient enrichment. Increase in the phytoplankton biomass can be measured as an increase in the chlorophyll a concentrations. Chlorophyll is a useful expression of phytoplankton biomass and is arguably the single most responsive indicator of N and P enrichment in the marine system (Harding, 1994)

Other common indices or attributes of the phytoplankton population that have been used in ecological assessments across all member states include bulk measurements of biomass and abundance (CSTT, 1994; Painting et al., 2005; Borja et al., 2010; Foden et al., 2010) taxon diversity (Karydis, 1996; Karydis and Tsirtsis, 1996; Devlin et al., 2009), seasonal succession (Hallegraeff and Reid, 1986; Belin et al., 1995; Beliaeff et al., 2001; Gailhard et al., 2002) and indicator species (Edwards and Richardson, 2004; Beaugrand, 2005). Development of these tools should identify anthropogenically induced change from nutrient enrichment within climate change variability. Changes in community must relate to increasing productivity and not driven by a changing climatic conditions. Development and testing of baseline conditions every 6 years enables the tools to develop appropriate phytoplankton assessments across variable conditions driven by climate change (Edwards and Richardson, 2004).

Development of all classification tools under the Directive must relate to the definitions as set out in the Directive guidelines. Phytoplankton tools should encompass

- The composition and abundance of phytoplanktonic taxa,

- Phytoplankton biomass and blooms.

These definitions serve as an anchor on which we have established simple quantitative measurements related to increases in blooms, biomass and phytoplankton abundance. The difficulty lies, as with all of the WFD biological elements, in providing baseline values from which all other measurements are anchored against to deliver a measurement which aligns to WFD ecological boundaries. The directive recognises these potential problems in Europe's modified marine waterbodies and identifies a number of ways to assess reference conditions. They include

- Historical analysis,
- Spatial comparison of type similar waterbodies,
- The use of models and;
- Expert judgement (Vincent et al., 2002)
- Clarification and discussion at expert groups (Marine Plant Task Team, 2001 – 2007)

Classification tools have been developed based on expert knowledge, previously accepted criteria and use of historical phytoplankton data.

Note, that the Directive does not define reference conditions, but does state that they should

- be established,
- be type-specific and
- represent conditions free from anthropogenic influences.

4. The WFD classification process for UK.

4.1 Role of the UK Technical Advisory Group (UK TAG)

On behalf of the UK Administrations, the UKTAG co-ordinates and approves activities and advises UK Administrations on policy for all aspects of WFD compliance in the UK. Classification and classification tool development is co-ordinated through UKTAG Task Teams covering Rivers (RTT), Lakes (LTT), Transitional/Coastal (MTT) and Groundwaters (GWTT).

The development of WFD compliance in the UK is overseen by both the policy and tool developers. The policy owners (i.e. the UK Administrations) provide the strategic business justification for the tools for the WFD competent authorities, and possibly in a European context and steers the funding bids for continued tool development. The tool developers are the technical lead for building and enhancing the classification tools. This involves both developing the scientific understanding of the biological quality element, its' relationship with environmental variables and packaging these relationships into a tool that delivers status classifications according to the WFD rules.

It should be noted that during the development of all tools constraints on monitoring and analysis resources had to be acknowledged which ultimately affects the confidence in which a classification is made.

4.2 Role of the Marine Plant Task Team (MPTT)

Members of the Marine Plant Task Team have guided and directed the progress and development of the marine plant assessment tools, including phytoplankton, macroalgae, angiosperms and saltmarsh. The MPTT works closely with the main tool developer(s) to

- Identify and recommends development needs for the tool.
- Develops scientific understanding of the relationship between the biological quality element and driving environmental variables. This may be carried out in house or through various external routes.
- Documents technical methods for operating the tools and contributes to passing on knowledge of the tool to Operations and Data and Information.
- Provides the technical UK input to the EU intercalibration process.
- Ensures that all UK administrations have joint ownership of the tools and are doing the same thing.

4.3 UK Terminology

In this report, the UK has defined a number of terms that are relevant to the understanding of various components of the phytoplankton assessment in UK waters. 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 for each layer of reporting and assessment (Table 4-1).

Table 4-1: Terminology associated with the UK Water Framework Directive – Marine plants component.

Terminology	Description	Phytoplankton term	Score or Value (examples)
Basic Parameter	Face Value Outcome of the analysis	➢ Chlorophyll l(ug/L) ➢ Cells/L ➢	The value of the analysis. ➢ Biomass value (ug/L) ➢ Percentage ➢ Score (0 – 10)
Metric	Phytoplankton measurement using a single parameter	➢ 90thile chlorophyll ○ *90thile chlorophyll*	Face value (as %) averaged over the metric for CW elevated count and CW seasonal succession Re-scale from face value to non-equidistant 0-1
Multi-metric	Phytoplankton measurement combining multiple parameters	➢ CW – Elevated count (EC) ○ *Single taxa phytoplankton* ○ *total taxa phytoplankton* ➢ CW – seasonal succession ○ *Diatoms within reference envelope* ➢ *Dinoflagellates within reference envelope*	Face value (as %) averaged over the metric for CW elevated count and CW seasonal succession Re-scale from face value to non-equidistant 0-1
Index	The final results of the combination of the metrics for this biological component	➢ 90thile chlorophyll ➢ CW – Elevated count (EC) ➢ CW – seasonal succession	0.0 - 1.0 (transformed equidistant scale) One value assigned to each metric
Tool	Alternative name for the index.	Phytoplankton toolkit	
Biological component	Measurements of phytoplankton community	CW Phytoplankton TW Phytoplankton	0.0– 1.0 (Equidistant scale). One value assigned to the BQE
Biological Quality Element (BQE)	Ecological status of waterbody through biological measurements	Phytoplankton reported at the level of coastal and transitional waters	
Boundary thresholds	The minimum and maximum data range associated with five classes	Elevated count value (%) ranges from 0 to 100%, separated into five boundary classes	High: 0 – 10 Good: 10 – 20 Moderate: 20 – 40 Poor: 40 – 60 Bad: 60 - 100

5. Normative definitions

5.1 UK interpretation of normative definitions.

Measurement of any undesirable disturbance in balance of organisms, such as the change in species composition, abundance, richness which may lead away from defined reference conditions, for example, the changes in the relative proportions of phytoplankton functional groups associated with nutrient enrichment should be easily detected and identified. Each member state has had to develop their own conceptual understanding of normative definitions, and show an understanding of how those broad qualitative definitions can be structured to define knowledge of the phytoplankton communities within UK and ROI marine waters. The structures of these normative definitions are the basis for the development of the UK and ROI classification tools. Assessment tools are linked the understanding of driver and impact as defined by these normative definition (Table 5-1).

Table 5-1: Normative definitions as listed by Water Framework Directive and UK interpretation based on characteristics of UK coastal and transitional waters.

Normative definition	UK interpretation and assessment tool
The composition & abundance of phytoplanktonic taxa are consistent with undisturbed conditions.	Seasonal succession of phytoplankton through the measurement of monthly taxa counts against a reference growth envelope of taxa counts. The growth envelope based on monthly counts of taxonomic groups. Growth envelopes are established through the use of long term phytoplankton data from reference waterbodies. Abundance of phytoplankton taxa are also measured by a single species count against a reference threshold and a total taxa count against a reference threshold.
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.	**For** coastal waters, average biomass conditions are measured by the 90th percentile value of chlorophyll data collected over the growth period. For transitional waters, average phytoplankton biomass conditions are measured by multiple metrics of statistical measurements, including mean and median and % exceedances. Type specific transparency conditions are identified in the nutrient assessment based on the annual SPM measurements and modelled primary production values.
Planktonic blooms occur at a frequency and intensity which is consistent with type-specific physico-chemical conditions.	Elevated chlorophyll concentrations are identified by exceedance of the chlorophyll concentration above a threshold level. This measurement is calculated within the elevated count tool. Type specific physico-chemical conditions are identified by geographical boundaries, separated into north and south marine areas.

The normative definitions should be used as a basis for defining classes of ecological status and therefore the high/good and good/moderate boundaries for each of the biological quality elements. However the normative definitions contain qualitative descriptive terms to which values quantitative terms need to be assigned. Discussion in various expert groups (MPTT, UK TAG) have defined qualitative wording for phytoplankton reference conditions (Table 5-2) with agreement on seasonality of the measures associated with biomass, abundance and species richness. Table 5-3 and Table 5-4 list the normative definitions as prescribed by the WFD with the UK interpretation of the qualitative descriptions for each boundary condition.

UK classification based on phytoplankton information encompasses a number of diagnostic tools measuring the potential consequences of marine eutrophication and link to the Directive's normative definitions. Monitoring tools should be able to discriminate between the five WFD quality classes, measuring anthropogenically induced deviation from reference conditions. "High", "Good" and "moderate" deviations from reference conditions are defined qualitatively by the WFD policy document (CEC, 2000)Conceptual modelling of the cause and effect drivers within UK marine waters has been put forward as a useful guide to development of the quantitative definitions for transitional and coastal waters (Figure 5-1).

Table 5-2: Qualitative reference conditions subscribed to transitional and coastal marine waters. Note the conditions listed are not exhaustive and are intended for use as a guideline.

Reference conditions for Transitional marine waters	Reference conditions for Coastal marine waters
➢ Seasonal peaks in in production but tempered by turbidity, salinity and hydrological effects ➢ Patterns of seasonal growth & succession mirror coastal dynamics, but demonstrate greater variability, in peak, duration & composition.	➢ Species richness high. ➢ Normal pattern of seasonal growth & succession; characterised by low numbers over winter and high levels of growth during spring and autumn periods. ➢ Distinct spring bloom crash due to nutrient depletion and grazing pressure. No summer or persistent bloom. Persistence characterised by the lack of a define inter-bloom period

Table 5-3: Normative definitions for phytoplankton in coastal waters with UK interpretation of the phytoplankton composition and behaviour that links with each definition.

Bio Element	Our Interpretation Structural & functional relevance	Reference Conditions	High	Good	Moderate
Phyto-plankton	**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.** **Nutrient ratios (N/P, N/Si, and P/Si) follow natural seasonal fluctuations.** **Chl-a may be used as a proxy for phytoplankton bloom biomass and is controlled by type-specific physico-chemical conditions.** **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 The composition & abundance of phytoplanktonic taxa show slight signs of disturbance.** **1.2 There are slight changes in biomass compared to type-specific conditions. Such changes do not indicate any accelerated growth of algae resulting in an undersirable disturbance to the balance of organisms present in the water body or to the physico-chemical quality of the water.** **1.3 A slight increase in the frequency and intensity of type-specific planktonic blooms may occur.**	**1.1The composition & abundance of planktonic taxa show signs of moderate disturbance. 1.2 Algal biomass is substantially outside the range associated with type-specific reference conditions and is such as to impact on 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.**

		Species richness high. Normal patterns of seasonal growth, biomass & succession; characterised by low numbers over winter and high levels of growth during spring and autumn periods. Distinct decline in spring bloom biomass due to nutrient depletion and grazing pressures. No summer bloom. Natural nuisance/toxic algal blooms may occur under specific local conditions, e.g. offshore advection of established populations.	**Species richness high. Spring bloom; diatom domination. Diatoms persist throughout growth-period. Increasing numbers of dinoflagellates from late spring. Transition from heterotrophic to autotrophic dinoflagellates from summer to autumn. Autumnal bloom dominated by diatoms or autotrophic dinoflagellates. Nuisance/toxic species @ persistently low levels compared with local background levels. Peaks in chlorophyll infrequent & inter-bloom periods low cf. local background.**	**Slight decline in spp. richness due to modified nutrient ratios. Evidence of minor disturbance from High status.**	**Moderate decline in spp. richness due to modified nutrient ratios. Prolongation of spring bloom with elevated chl-a above background. Disturbance of natural diatom-dinoflagellate succession. Increasing presence of nuisance /toxic species.**

Table 5-4: Normative definitions for phytoplankton in transitional waters with UK interpretation of the phytoplankton composition and behaviour that links with each definition.

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. Patterns of seasonal growth & succession mirror coastal dynamics, but demonstrate greater variability, in peak, duration & composition.	Diatom domination persists throughout growth-period. Nuisance/toxic species @ persistently low levels compared with local background levels. Peaks in chlorophyll infrequent & inter-bloom periods low cf. local background.	Evidence of minor disturbance from High status.	Prolongation of spring bloom with elevated chl-a above background. Elevated numbers of flagellates. Increasing presence of nuisance /toxic species.

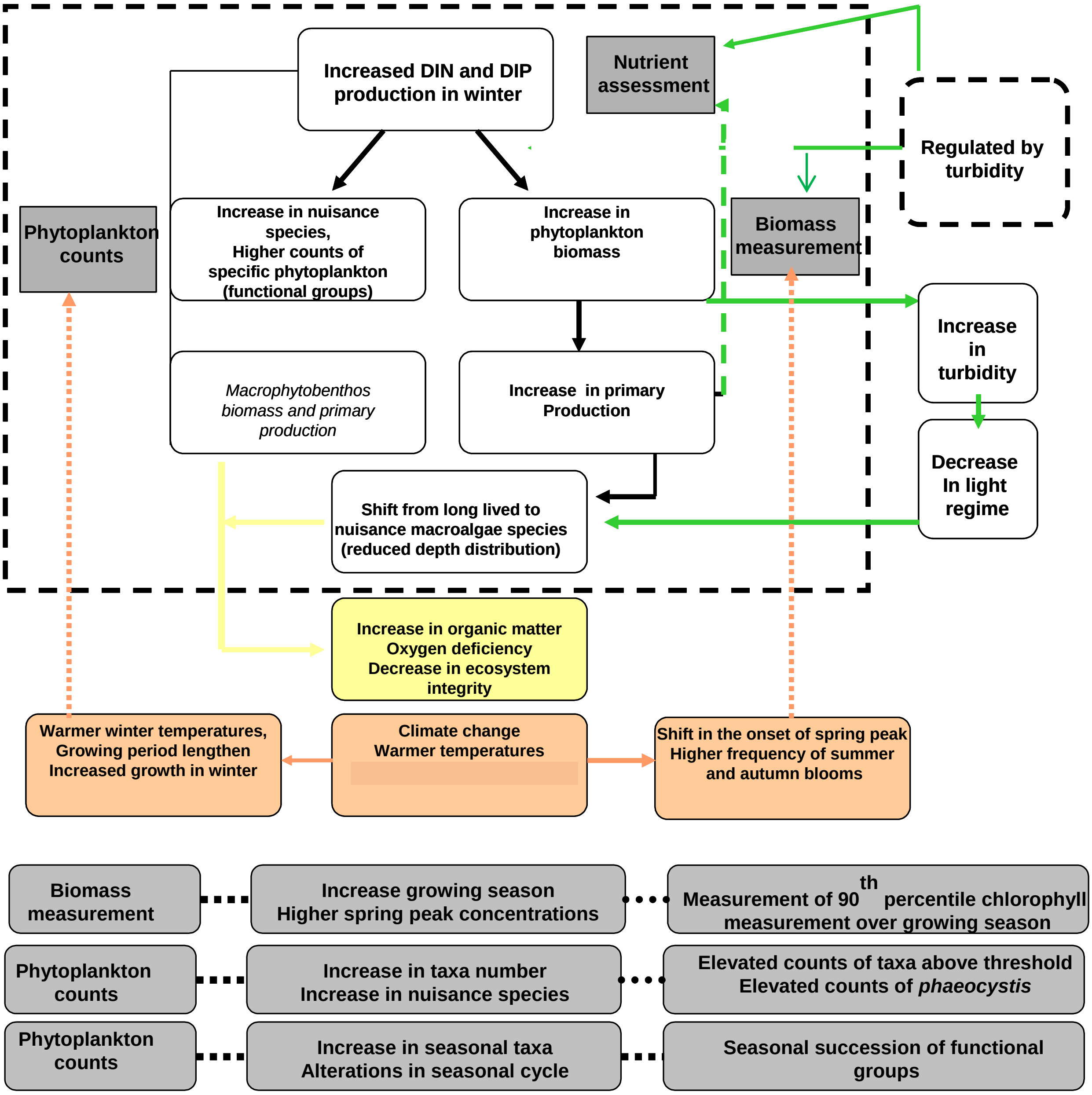


Figure 5-1: Conceptual diagram of the phytoplankton responses to changing nutrient and environmental conditions. The grey boxes outline the normative definitions and the corresponding ecosystem measurement.

6. Assessment of phytoplankton measures in UK waters

6.1 Transitional and Coastal Monitoring (TraC monitoring)

The biological monitoring of transitional and coastal water bodies is focussed in key areas within the river basin areas. Two or more of the biological elements are measured within each waterbody, depending on the pollutant risk identified for each water body (Table 6-1). Classification based on marine plants consists of three elements, including the (i) measurement of phytoplankton in the water column, (ii) the measurement of macroalgae species including both opportunistic blooming species and intertidal rocky shore species and (iii) angiosperms, including both seagrasses and saltmarsh communities. Additional guidance documents on the macroalgae and angiosperms can be found on the UK TAG website (www.wfduk.org).

Table 6-1: Transitional and coastal water biological quality elements applied under the Water Framework Directive in accordance with the requirements of Article 8; Section 1.3 of Annex II and Annex V of the WFD (2000/60/EC).

Quality element	Description
Phytoplankton	Free Floating microscopic plants
Macroalgae	Seaweeds visible to the naked eye
Angiosperms	Sea grasses and saltmarsh plants
Benthic Invertebrates	Worms, molluscs and crustaceans etc living in or on the bed of the estuary or sea
Fish (transitional only)	Fish which spend all or part of their lives in transitional waters

6.2 Assessment of nutrient pressure through phytoplankton measurements

Phytoplankton acts as a direct and indirect measurement to the enrichment of nutrients in the appropriate hydrological conditions. The primary biological response to nutrient enrichment in aquatic environments, given suitable environmental conditions (such as light availability), is the growth of phytoplankton and higher plants (Figure 6-1). However, climate can also be a driver. Known consequences of nutrient enrichment include increased biomass of primary producers such as phytoplankton (indicated by concentrations of chlorophyll a), increased primary production, and increased removal of oxygen from the water in enclosed or semi-enclosed systems due to extra biochemical oxygen demand (BOD) and decomposition of accumulated biomass, resulting in local hypoxic (and anoxic) conditions. Other consequences which can be measured by phytoplankton attributes include shifts in species composition, from diatoms to flagellates, blooms of nuisance and toxic algae, red tides, and the presence of toxic phytoplankton species (Bricker et al., 1999; Bricker et al., 2003b; Painting et al., 2005; Tett et al., 2007; Bricker et al., 2008; Devlin et al., 2009).

Assessments relating to phytoplankton are required to encompass taxonomic composition, abundance, biomass and plankton blooms for the ecological classification of transitional and coastal waters (CEC, 2000). More specifically the wording of the directive state that if a water body is to attain “high” ecological status “the composition and abundance of phytoplanktonic taxa are to be consistent with undisturbed conditions”.

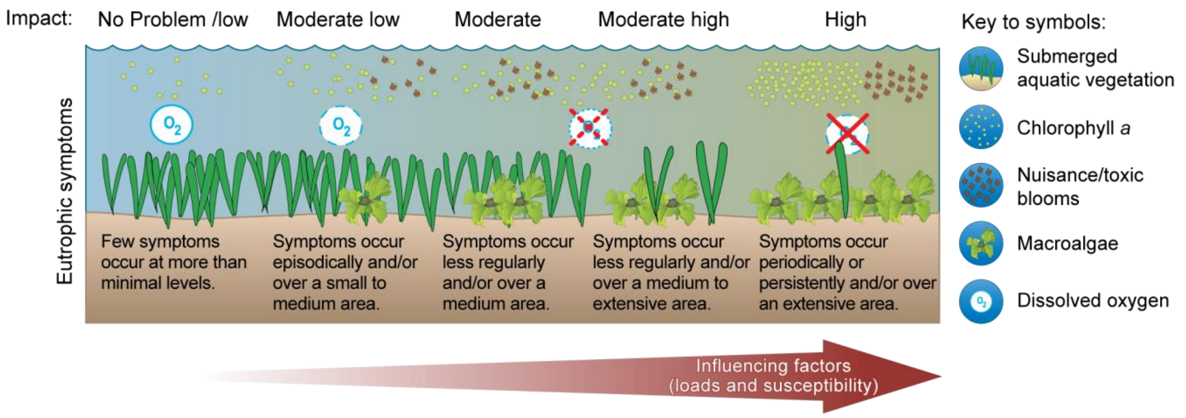


Figure 6-1: Potential impacts of anthropogenic nutrient enrichment of coastal and marine waters. (a) Summary of primary and secondary impacts, and consequences of symptoms. Secondary impacts indicate undesirable disturbance to the system. (b) Gradient of secondary impacts, ranging from low to high in response to influencing factors. N = nitrogen, P = phosphorus, SAV = submerged aquatic vegetation, DO = dissolved oxygen (Devlin et al., 2011)

6.3 Phytoplankton assessment – coastal waters

Three phytoplankton coastal metrics are currently being used in the assessment of marine waters in the UK. The phytoplankton coastal index is designed for consideration at the spatial scale of the waterbody and can be used on all CW where phytoplankton has been collected. For coastal waters, the phytoplankton measures include measurement of chlorophyll biomass, detection of elevated counts of taxa and the occurrence of monthly taxa counts within a seasonal envelope for specific taxonomic groupings. A short summary of the phytoplankton metrics which make up the overall assessment process is shown in Table 6-2. The overall classification process from sampling to classification is outlined in Figure 6-2.

Table 6-2: Brief description of the phytoplankton index for classification of coastal waters.

Metric	Description
Chlorophyll biomass	• Measurements of phytoplankton biomass are measured by the concentration of chlorophyll *a*. Coastal waters are assessed by a single chlorophyll biomass value calculated as the 90th percentile value of chlorophyll biomass over growing season (Mar to Oct). • The final score is a normalised equidistant value between 0 – 1 (= EQR)
Elevated counts of taxa	• The count of taxa that are exceeding thresholds related to disturbed physic-chemical conditions. • CW - final metric assessment is based on the outcomes of three metrics 1 Counts of individual taxa above a threshold 2 Counts of total taxa (sum) above a threshold 3 Counts of chlorophyll biomass above a threshold • The metrics are combined as an average of the % exceedance from three metrics. The final score is a normalised equidistant value between 0 – 1 (= EQR)
Seasonal succession of phytoplankton	Counts of two taxa groups (diatoms and dinoflagellates) The final score is a normalised equidistant value between 0 – 1 (= EQR)

Note: The 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 more WFD compliant monitoring data has become available, as well as recent comprehensive reviews about the relationship between

Phaeocystis and anthropogenic nutrient enrichment (Gowen et al., 2000; Gowen et al., 2008; Davidson et al., 2012; Gowen et al., 2012b), the phytoplankton tool has been modified to better reflect the response of the phytoplankton community to anthropogenic impact.

There were 3 Specific changes since the first River Basin Management Plans:

i) removal of *Phaeocystis* as a separate metric in the elevated count index (see the justification in Appendix 1)
ii) elevated count thresholds set for different biogeographical regions (see the justification in Appendix 2)
iii) Streamlining of counting and identification of phytoplankton (see justification in appendix 3 and below).

A revised taxa list has been generated which is used by all UK laboratories analysing samples for WFD purposes within the UK. Identification categories have been consolidated to account for the routine analysis of samples preserved Lugol's iodine and examined using light microscopy. 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. Similarly some taxa are difficult to identify to species (or in some cases genus) using light microscopy, so identification may be only expected to higher taxonomic levels. This revised taxa list reflects this and also uses size groupings to differentiate some of the higher taxa levels.

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 habitat/aim of survey
Sample collection	• Use of standardised methods (EN15972:2011) • Collect chlorophyll and phytoplankton samples • Collect supporting information e.g. turbidity, temperature
Sample analysis	• Use of standard laboratory methods (EN15204:2006; ISO10260:1992) • Full enumeration of phytoplankton • Taxa identified to the Revised Phytoplankton list • Quality assurance procedures

Calculation: Face value	• 90th percentile chlorophyll – across growing season (Mar - Oct)
	• Elevated cell counts – Average combined % exceedance of three metrics
	  ○ (i) count (%) of Chl 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 scores (ideally of the three scores if all indices used)
Water body classification	• Derive WB average EQR • Assign Class Status (use defined class boundaries) • Calculate Confidence of Class and Risk of Misclassification

Figure 6-2: Outline of the classification process in coastal waters utilising phytoplankton measurements.

6.4 Phytoplankton assessment – transitional waters

Two phytoplankton multimetrics are currently being used in the assessment of transitional waters in the UK. The tools are designed for consideration at the spatial scale of the waterbody and can be used on all TW types where phytoplankton has been collected. For transitional waters, these include the multimetric measurement of a number of statistical values related to chlorophyll measurements and detection of elevated counts of phytoplankton taxa. A short summary of the phytoplankton index for transitional waters which make up the overall assessment process is shown in Table 6-3 with a more detailed outline of the phytoplankton index for transitional waters in Figure 6-3. More detailed descriptions of the metrics are described in Section 7.

Table 6-3: Description of the phytoplankton metrics for the assessment of transitional waters.

Metric	Description
Compliance of chlorophyll measures	Transitional waters are measured by a combination of statistical measurements of chlorophyll biomass that represent various parts of the phytoplankton growth period over the whole year. • **TW** – Five statistical measurements of chlorophyll biomass in transitional waters including mean, median, % values below thresholds - 10 and 20 µg l-1 and % exceedance above 50 µg l-1. Statistical measures taken in two salinity zones • The final score is a normalised equidistant value between 0 – 1 (= EQR)
Elevated counts of taxa	• The count of taxa that are exceeding thresholds related to disturbed physic-chemical conditions. • **CW** - final metric assessment is based on the outcomes of three metrics 1 Counts of individual taxa above a threshold 2 Counts of total taxa (sum) above a threshold 3 Counts of chlorophyll biomass above a threshold • The metrics are combined as an average of the % exceedance from three metrics. The final score is a normalised equidistant value between 0 – 1 (= EQR)

WORK AREA	Considerations
Monitoring Design	• Is TW 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 habitat/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
Sample analysis	• Use of standard laboratory methods (ISO10260:1992; EN 15204:2006) • Full enumeration of phytoplankton • Taxa identified to the Revised Taxa 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 less than 10 µg l^{-1}Chl (70%, 75%) ○ % samples less than µg l^{-1}Chl (80%, 85%) ○ % samples over 50 µg l^{-1}Chl (5%, 5%) • Elevated cell counts – Average combined exceedance of ○ (i) individual taxa exceeding 500,000 cells l^{-1} ○ (ii) count (as %) of total taxa exceeding 10^6 cells l^{-1}
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 (ideally of the two multimetrics if both chlorophyll and phytoplankton are measured
Water body classification	• Derive WB average EQR • Assign Class Status (use defined class boundaries) • Calculate Confidence of Class and Risk of Misclassification

Figure 6-3: Outline of the classification process in transitional waters utilising phytoplankton measurements

6.5 Reference conditions

6.5.1 Chlorophyll – coastal waters

In previous directive, the boundaries for chlorophyll have been based on a justified area-specific % deviation from background chlorophyll concentrations. Background conditions are based on a deviation from Atlantic background concentrations (OSPAR, 2002, 2003, 2005; Painting et al., 2005). For the OSPAR Comprehensive Procedure, appropriate standards for assessing chlorophyll concentration were derived from the background nutrient concentrations by making some reasonable assumptions about nutrient conversion to plant biomass (Malcolm et al., 2002; Painting et al., 2005; Foden et al., 2010; Gowen et al., 2012a) (Malcolm et al, 2002; Painting et al., 2005). There was considerable uncertainty in the calculated background level due to the wide range of factors that could be used to convert carbon to chlorophyll. An appropriate standard for assessing chlorophyll a concentration can be derived from the background nutrient concentrations by making some reasonable assumptions about nutrient conversion to plant biomass.

Algal C:N ratios remain relatively consistent with the Redfield ratio of 6.6:1 (Geider and La Roche, 2002). In contrast, variability of the Chl:C ratio owing to physiological acclimation, light environment and taxonomic composition has been recognised as a major source of uncertainty (Geider et al., 1998). The Chl:C ratio is inversely correlated with irradiance and positively related to growth rate, contributing to the variability found in the Chl:C ratio. Chl:C ratios have been found to range from < 0.01 to 0.06 (g g^{-1})(Geider et al., 1998). Box 1 provides a calculated example of converting the Chl:C ratio to a reference chlorophyll concentration.

Despite the considerable uncertainty in the calculated background level due to the wide range of factors that could be used to convert carbon to chlorophyll, The UK has adopted 10 µg l^{-1} chlorophyll *a* as a guide for assessment. It is therefore proposed that for

- offshore waters **10 µg l^{-1} chlorophyll *a*** is adopted as the reference value (implying a background value of 6.7 µg l^{-1} and a reasonable C:Chl factor of 0.012) for North Sea
- nearshore waters, where the level of production may be expected to be higher, **15 µg l^{-1} chlorophyll *a*** is adopted as the reference value (implying a background value of 10 µg l^{-1} chlorophyll *a* and a C:Chl factor of 0.02).

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 this deviation from offshore Atlantic shelf break background concentrations (Malcolm et al., 2002; OSPAR, 2002, 2003). 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 (Painting et al., 2005; Foden et al., 2010)

These calculations (Box 1) 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}.

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 (Carletti and Heiskanen, 2009) (see Table 6-4).

BOX 1. Calculation of offshore (reference) standard chlorophyll concentrations.

Conversion of µM N to mg C l^{-1}

1. Shelf edge winter N = 7.2 µM: C:N atom ratio = 6.6:1

Therefore = 7.2 x 6.6 = 48 µM carbon

or 48 x 12 = 575 µg C l^{-1} (0.58 mgC l^{-1})

2. To convert 0.58 mgC l^{-1} to equivalent chlorophyll a, a chl:C ratio must be used.

Chl:C ratios can range from 0.01 (g g^{-1}) to 0.06 (ref). Assuming 1:100 as the most conservative Chl:C ratio, a background chlorophyll *a* concentration can be calculated, assuming that all N is assimilated during primary production.

0.58 mg C l^{-1} x 0.01 x 1000 = **5.8 µg Chl l^{-1}**.

With a nutrient elevation threshold of +50% chl a concentration = **8.7 µg Chl l^{-1}**

3. The least conservative estimation of chlorophyll *a* concentration can be calculated using the Chl:C ratio of 0.06.

(0.58 mg C x 0.06 x 1000 = **34.8 µg Chl l^{-1}**.

A 50% increase in conservative chl concentration = **52.2 µg Chl l^{-1}**

Table 6-4: Chlorophyll concentrations (µg l^{-1}) for face value class boundaries for Atlantic and North Sea waters.

	"Atlantic" Waters (µg l^{-1})	"North Sea" Waters (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

Adjustments of the reference thresholds are taken over two different geographical areas reflecting the differences in the adjacent marine waters (Atlantic and North Sea). Thresholds for the metrics differ between two latitudinal areas separated into Northern and Southern hydrobiogeographics zones. These geographical zones are defined with a line joining 55^0 North on the West Coast of Scotland to the Flamborough Front (approximately at Flamborough Head).

These boundaries were tested under the NEA GIG Intercalibration process. The two zones are

- Atlantic Scotland and some E&W waterbodies (St Austell, Fal/ Helford, Cornwall South, Lands' End to Trevose Head, Cornwall North) which have a chlorophyll reference value of 3.33 µg/l
- North Sea (all other English and Welsh waterbodies) which have reference value of 6.67µg/l

The geographical range of Atlantic and North Sea Waters in UK waters is shown in Figure 6-4.

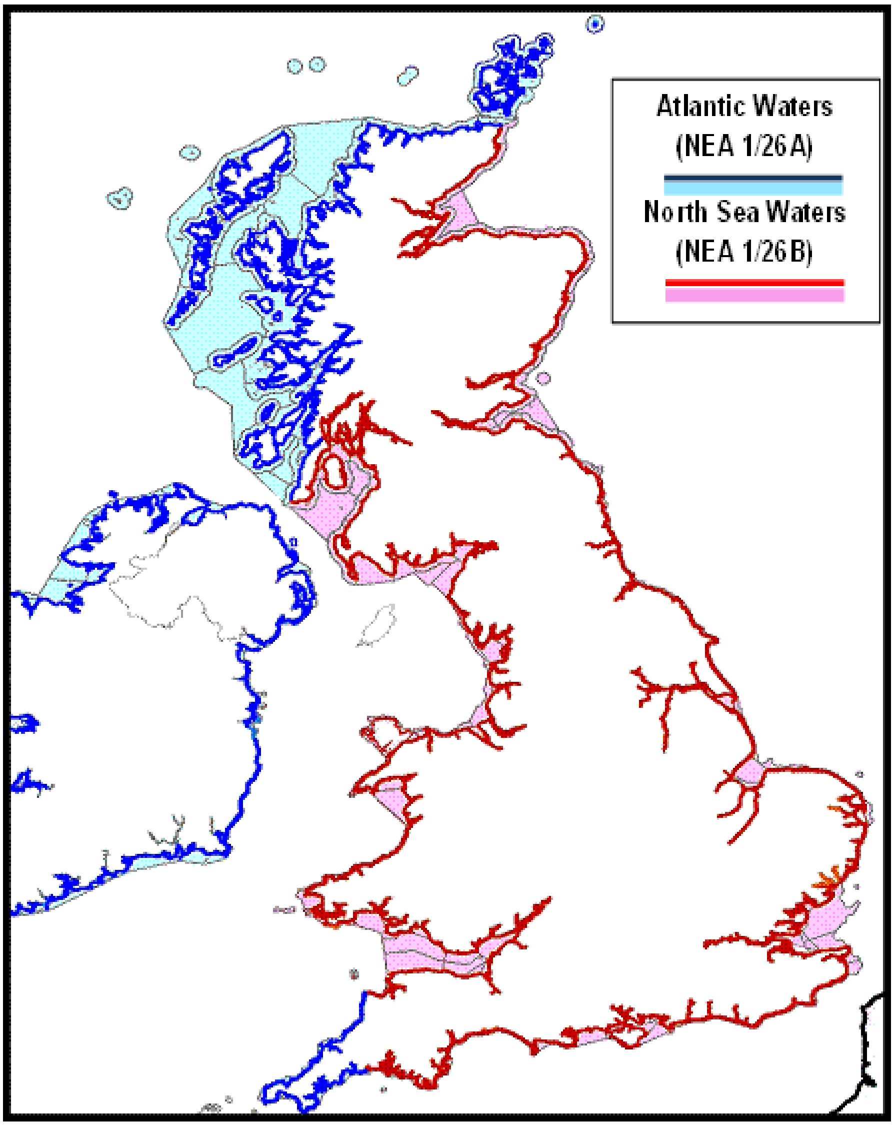


Figure 6-4: Map to show the range of the defined Atlantic and North Sea waters for use with the chlorophyll 90th percentile metric.

6.5.2 Chlorophyll – transitional waters

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 (Devlin et al., 2007a) 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 6-5.

Table 6-5: 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%

6.5.3 Phytoplankton

Reference conditions and boundary thresholds for each metric were constructed based on a combination of scientific review (Belin et al., 1995; Beliaeff et al., 2001; Borja et al., 2004; Borja, 2005; Borja and Heinrich, 2005; Devlin et al., 2007a), thresholds accepted in previous directives (CEC, 1991b; CSTT, 1994, 1997)expert knowledge (Marine Phytoplankton Task Team) and comparison of classification outputs between low and high risk waterbodies in UK waters (Devlin et al., 2007a)

A shift in functional groups may affect ecosystem function in terms of the carbon available to higher trophic levels or settling to the sediments. It isn't clear how such shifts affect, or are affected by, diversity and ecosystem function, however succession of functional groups can potentially provide an metric that represents a healthy planktonic system, with a natural progression of dominant functional groups throughout the seasonal cycle. In addition, testing the baseline conditions associated with the seasonal succession tool every 6 years should encompass changes associated with climate variability. The structure of the seasonal succession involves the measurement of the two main taxonomic groupings (diatoms and dinoflagellates), as cell counts. In England and Wales, generic reference curves were established for coastal waterbodies using long term data from the L4 site. Station L4, the subject of this collection of papers, is one of the well-established European coastal time-series stations. The station is located in the Western English Channel some 10 km south of Plymouth Breakwater with a nominal depth of water of 51 m. Intermittent observations have been made at L4 site by the laboratories in Plymouth for more than 100 years, particularly by the Marine Biological Association (MBA) who founded the station in the latter part of the nineteenth century (Harris, 2010). This site is slightly offshore with monthly data from 1991 (Devlin et al., 2007a). In UK waters, low or no growth conditions are not seen as an issue for the phytoplankton tools and the reference curve for both diatoms and dinoflagellates is now only one sided and the tool is based on the number of exceedances of the upper growth curve.

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 from previous research (Beliaeff et al., 2001; Gailhard et al., 2002)and tested through the outcomes of low, moderate and high risk water bodies (Devlin et al., 2007a; Devlin et al., 2007b)

6.5.4 Elevated Counts of phytoplankton taxa

There are two biogeographical regions for phytoplankton cell counts; Southern and Northern (Figure 6-5. Note that these boundaries are different to the defined waters used for the chlorophyll 90th 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.

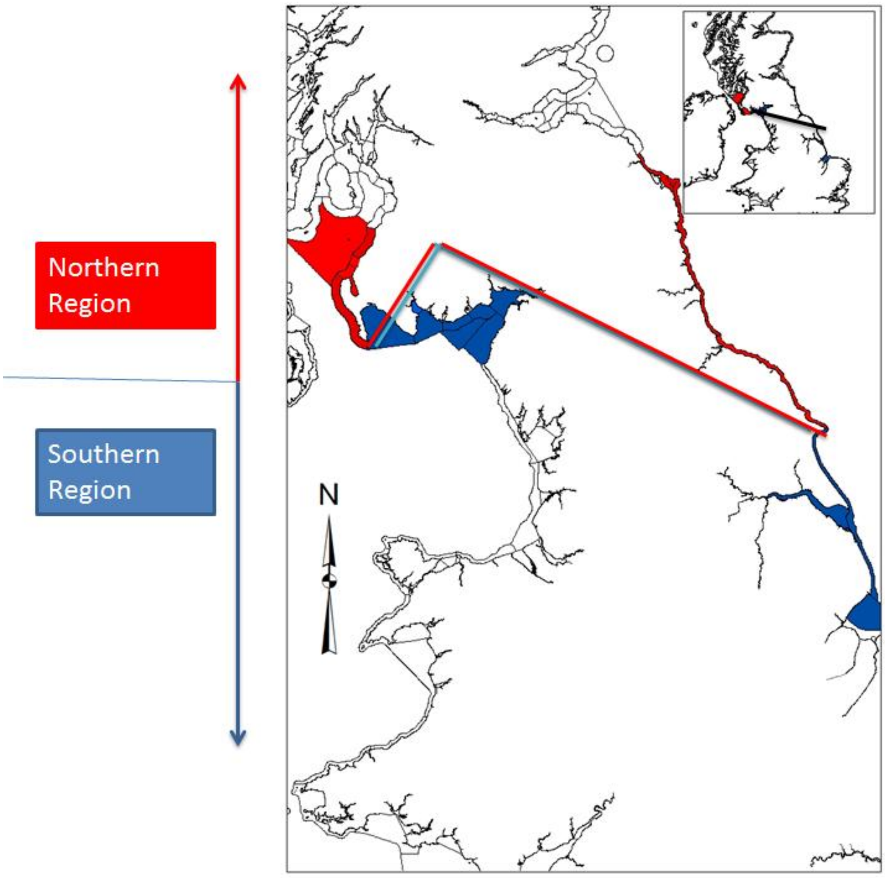


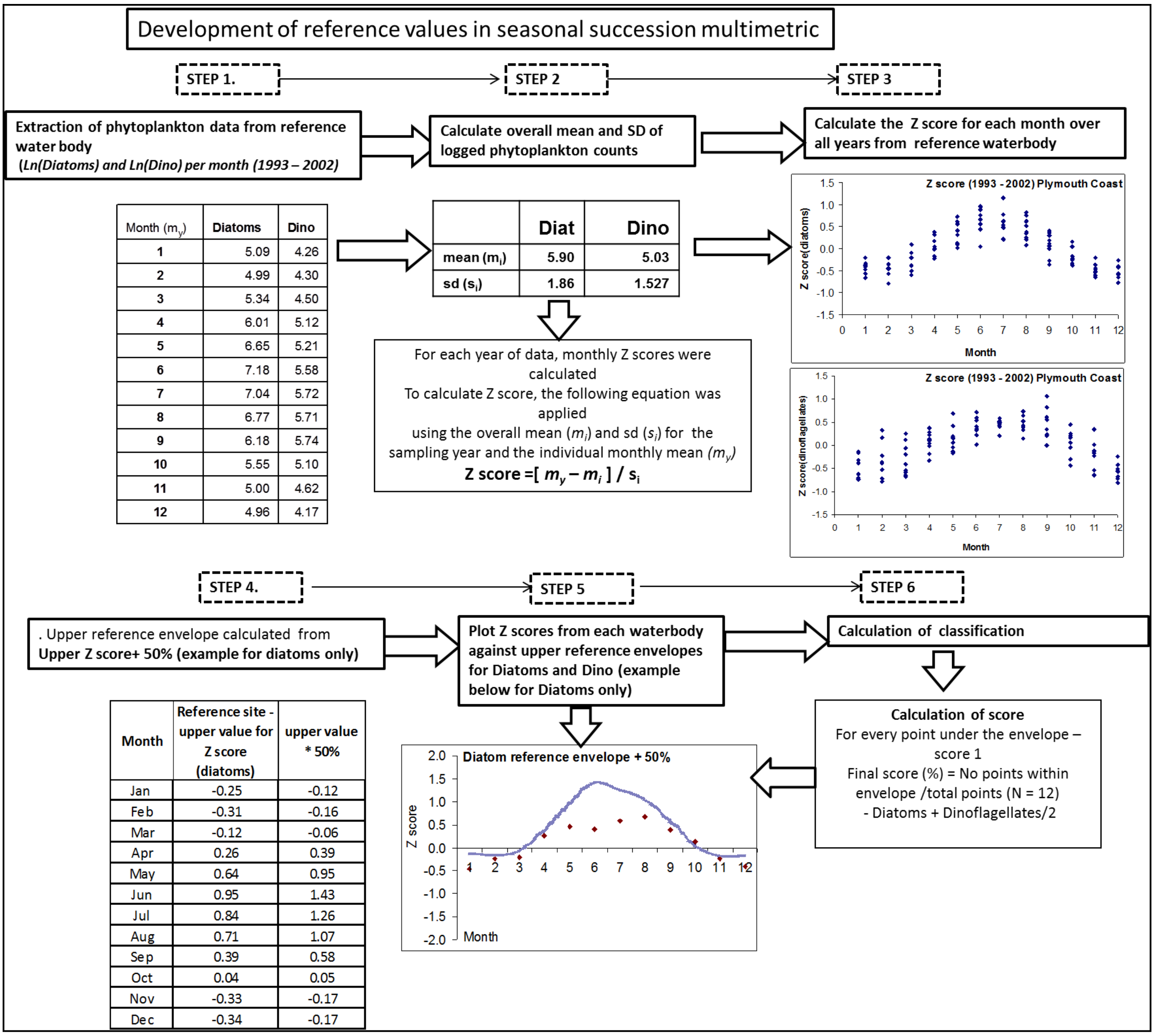
Figure 6-5: Delineation of Southern and Northern coastal biogeographical regions for phytoplankton cell count thresholds. West coast split has Mull of Galloway to Corsewall Point to the north and Luce Bay to the south. The East coast split has North Yorkshire to the north and South Yorkshire/Lincolnshire to the south.

Exceedances are counted in response to the following thresholds:

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

6.5.5 Seasonal Succession

This 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 within geographical regions. 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., 2007a; Harris, 2010; Highfield et al., 2010). 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. In UK waters, low or no growth conditions are not seen as an issue so the reference curve for both diatoms and dinoflagellates has only an upper growth curve and the tool is based on the number of exceedances of this upper line.



Month (m_y)	Diatoms	Dino
1	5.09	4.26
2	4.99	4.30
3	5.34	4.50
4	6.01	5.12
5	6.65	5.21
6	7.18	5.58
7	7.04	5.72
8	6.77	5.71
9	6.18	5.74
10	5.55	5.10
11	5.00	4.62
12	4.96	4.17

Month	Reference site - upper value for Z score (diatoms)	upper value * 50%
Jan	-0.25	-0.12
Feb	-0.31	-0.16
Mar	-0.12	-0.06
Apr	0.26	0.39
May	0.64	0.95
Jun	0.95	1.43
Jul	0.84	1.26
Aug	0.71	1.07
Sep	0.39	0.58
Oct	0.04	0.05
Nov	-0.33	-0.17
Dec	-0.34	-0.17

Figure 6-6: An example (for English waters) showing the process of deriving the seasonal succession reference curve, shown here for diatoms.

The reference condition is 0% exceedance of curve (i.e. 100% compliance). The process of deriving the reference curve for diatoms is illustrated in Figure 6-6 and described fully in (Devlin et al., 2007a). The reference curves are constructed using the values listed in Table 6-6.

Table 6-6: Values used for creating the seasonal succession reference curves for diatoms and dinoflagellates for England and Wales and Scotland.

Diatoms				Dinoflagellates			
	England and Wales	**Scotland Stonehaven**	**Scotland Loch Ewe**		**England and Wales**	**Scotland Stonehaven**	**Scotland Loch Ewe**
Month	**upper bound**	**upper bound**		**Month**	**upper bound**	**upper bound**	
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

7. Phytoplankton metrics for UK waters

7.1 Description of phytoplankton metrics in coastal waters

For coastal waters, the phytoplankton index include measurement of chlorophyll biomass, detection of elevated counts of taxa and the occurrence of reference points within a seasonal envelope for specific taxonomic groupings. Table 7-1 describes these three measurements of phytoplankton community, and lists previous directives in which similar indices have been utilised, with type specific reference conditions that have been developed for each index. A description of the indices and literature reviewed thresholds are presented for chlorophyll concentrations and phytoplankton counts. Each index has been tested against reference phytoplankton data extracted from WFD coastal waterbodies (Devlin et al., 2007a), MPTT, pers comm)

Table 7-1: Description of each phytoplankton metric/measurement currently in place for coastal water assessment.

Phytoplankton measurement	Increases in chlorophyll biomass	Elevated counts of phytoplankton taxa	Alterations in the seasonal succession
Historical directives	CSTT (CSTT, 1994, 1997)OSPAR (Malcolm et al., 2002)ECOQO's (Painting et al, 2005) (Painting et al., 2005)		not previously used in UK assessments
Description of previous assessment criteria	Maximum and mean chlorophyll concentrations during the growing season should remain below elevated levels, defined as concentrations >50% above the spatial (offshore) and/or historical background concentration	Region/area-specific phytoplankton eutrophication indicator species should remain below elevated levels.	n/a
Reference conditions	Generic reference concentration developed for all coastal water bodies (CW's)	Generic reference concentration developed for all coastal water bodies (CW's)	Reference conditions developed for one type specific waterbody Further work needed to identify a generic reference type or further data collection to identify type specific references for other all coastal waters
Normative definition (WFD)	Phytoplankton biomass	Phytoplankton composition and frequency of planktonic blooms	Phytoplankton composition and abundance
WFD Assessment description	Chlorophyll concentration calculated by the 90th percentile [X90th] of all chlorophyll data	Sum of the occurrence of any single species (> 250,000) plus total cell counts (>10^6) and counts of chlorophyll > 10ug/l over a six year period	Sum of the occurrences of calculated Z score (for each functional groups) that falls outside predefined growth envelope

7.2 90th percentile measurement of Chlorophyll biomass (μg l-1)

Phytoplankton biomass is a direct measurement of the amount of phytoplankton material in the water column and in UK waters; it should reflect low numbers in the winter, high spring concentrations, and variable, periodic summer and autumnal blooms. Persistent, elevated chlorophyll concentrations can represent a very simple and integrative measure of the phytoplankton community response to increased nutrient enrichment. In most cases, an increase in phytoplankton biomass can be measured as an increase in the chlorophyll a concentrations. Chlorophyll is a useful expression of phytoplankton biomass and is arguably the single most responsive indicator of N and P enrichment in the marine system (Harding, 1994). Chlorophyll biomass is seen as an easily measurable, repeatable metric that can indicate the excessive growth of phytoplankton species. As defined by the normative definitions (Table 5.2), the appropriate reference condition for the measurement of phytoplankton biomass to represent are planktonic blooms occurring at a frequency and intensity which is consistent with the type-specific physico-chemical conditions.

Environmental data such as phytoplankton chlorophyll exhibits periodicity and episodic change and as a result tends to be asymmetrically distributed with few high values (outliers or spikes) and many low values. A recognised statistical approach is to derive 90th percentile values as a bulk measurement of the data (Aitchison, 1989; Clarke and Warwick, 1994). The 90th percentiles represented a statistical method encompassing the spread of data for chlorophyll biomass omitting highly skewed values, which can be present during bloom periods. Phytoplankton biomass index can be measured as chlorophyll concentration and is calculated as a 90th percentile of all chlorophyll data collected over the growing season (March to October). The 90th percentile value is compared with the threshold value derived from appropriate reference conditions. Thus the metric can be summarised as:

- The observed value of the parameter, phytoplankton biomass during the growing season, is the 90th percentile of the phytoplankton biomass. This should be estimated as the chlorophyll-a concentration in micrograms per litre ($\mu g.l^{-1}$) below which lie ninety percent (90 %) of the measured concentrations of chlorophyll-a made during the phytoplankton growing season from March to October inclusive. 90th percentile chlorophyll concentrations during the growing season (March to October) should remain below thresholds set for the high/good and good/moderate boundaries for type specific conditions.
- The measurement of the 90th percentile of the chlorophyll biomass is taken from a waterbody over the growing season (Mar-Oct). Adjustments of the reference thresholds are taken over two different geographical areas reflecting the differences in the adjacent marine waters (Atlantic and North Sea). The two zones are
    - Atlantic (St Austell, Fal/ Helford, Cornwall South, Lands End to Trevose Head, Cornwall North) which have a chlorophyll reference value of 3.33 ug/l
    - North Sea (all other English and Welsh waterbodies) which have reference value of 6.67 ug/l

A summary of the metric, with sampling requirements, accepted methods, reference and boundary conditions are presented in Figure 7-1.

Element: Chlorophyll
Metric: 90th percentile value of chlorophyll biomass over growing season

Sampling requirements

Sampling period is set for a period of 6 years concurrent with the WFD reporting time frame. Sampling frequency is recommended to be monthly over the growing season (*set as March to October for UK waters*).

Methods

Measurements of chlorophyll biomass are taken from EA monitoring programs through surface sampling
Chlorophyll analysis: Acetone fixed samples - Fluourometry

Tool summary

Tool is based on the calculation of the 90th percentile value of chlorophyll biomass.
Boundary conditions set against the reference value established for Atlantic and North Sea waters
The value of the 90th percentile is compared against boundary conditions to establish classification

Reference conditions

ATLANTIC SEA
The reference condition is defined as the 90th percentile value of all WFD compliant chlorophyll data over the required time frames to be no more than 3.33µg/L
NORTH SEA
The reference condition is defined as the 90th percentile value of all WFD complaint chlorophyll data over the required time frames to be no more than 6.67µg/L

Boundary conditions

	90^{th} %ile value	**Metric range**	**Interim Class**	**EQR**
North Sea waters				
Reference: 6.67	**0--10**	**0.671-1.0**	**High**	**0.8 - 1.0**
	10--15	**0.441-0.67**	**Good**	**0.6 - 0.8**
	15--20	**0.341 - 0.44**	**Mod**	**0.4 - 0.6**
	20--25	**0.271-0.34**	**Poor**	**0.2-0.4**
	>25	**0 - 0.27**	**Bad**	**0 - 0.2**
Atlantic waters				
Reference: 3.33	**0--5**	**0.671 - 1.0**	**High**	**0.8 - 1.0**
	5--10	**0.33 - 0.67**	**Good**	**0.6 - 0.8**
	10--15	**0.22 - 0.33**	**Mod**	**0.4 - 0.6**
	15--20	**0.17 - 0.22**	**Poor**	**0.2-0.4**
	>20	**0 - 0.17**	**Bad**	**0 - 0.2**

Figure 7-1: The structure and application of the 90th percentile chlorophyll metric for assessment of coastal waters.

7.3 Elevated Counts of phytoplankton (CW).

Persistent and high counts of selected phytoplankton taxa are also seen as a measurement of disturbance in the phytoplankton community. Normative definitions describe the reference condition as the abundance of phytoplanktonic taxa being consistent with undisturbed conditions and planktonic blooms occurring at a frequency and intensity which is consistent with type-specific physico-chemical conditions.

To measure this, this multi-metric comprises measurements of phytoplankton species counts to identify the number of occurrences, if any, of high numbers of any single taxa and/or high total taxa (as defined by the revised phytoplankton lists) counts. The phytoplankton multimetric is composed of three metrics, one which is a measure of the frequency that elevated biomass exceeds a reference threshold and two which 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 metric measurements within this multimetric include:

- The number of times that Chlorophyll exceeds 10ug l^{-1}.
- The number of times a single phytoplankton taxa (as defined by the revised phytoplankton taxa list) is over 250,000 cell per litre (threshold for Southern waters) or over 500,000 cells l^{-1} (threshold for Northern waters).
- The number of times the total cell count of a sample is over 10^6 cells l^{-1} (threshold for southern region) or 10^7 cells l^{-1} (threshold for northern regions).
- Reference value for this average is no higher than 5% exceedances for all sub-metrics as a proportion of the total number of sampling times.

The multimetric records the number of events, defined by sampling occasions, where each metric exceeds the biomass or taxa count threshold. The multimetric is then calculated as a proportion of the number of times that the each metric exceeds a threshold *against* the total number of sampling times over a 6 year reporting period. The proportion of exceedances is calculated as an average percentage of the exceedance values give a final classification score.
The observed value of the parameter, bloom frequency of phytoplankton groups, should be calculated according to the following steps:

A summary of the metric, with sampling requirements, accepted methods, reference and boundary conditions are presented in Figure 7.2.

Element: Phytoplankton
Multimetric: Elevated counts of phytoplankton in coastal waters

Sampling requirements

Sampling period is set for a period of 6 years concurrent with the WFD reporting time frame. Sampling frequency is recommended to be monthly over the whole year, with a recommended minimum of 9 months

Methods

Phytoplankton samples collect from surface (bucket)). Samples are fix with Lugols Iodine. Enumeration is through light microscopy. Taxa are defined against a Revised phytoplankton taxa list.

Tool summary

- Tool records the number of sampling dates when chlorophyll biomass or the phytoplankton taxa count exceeds a predefined threshold for each metric.
- The value of each metric is a percentage calculated from the number of exceedances over the total number of sampling times.
- Final classification score for the multimetric is calculated from the average of all the three metrics (scored a as a average %).

Reference conditions

Exceedance of the combined metrics (measured as a mean %) being less than or equal to 5%.
The combined indices include
Sub-metric 1. *% times that chlorophyll exceeds the value of 10µg/L*
Sub-metric 2. % *times that any single species of phytoplankton exceeds a count of*

- 250,000 cells l^{-1} [E&W] or
- 500,000 cells l^{-1} [Sc]

Sub-metric 3. % *of times that the total taxa count of phytoplankton exceeds a count of*

- 1,000,000 (10^6) l^{-1} [E&W] or
- 1,000,0000 (10^7 l^{-1} [S]

Boundary conditions

	% exceedance	Metric range	Interim Class	EQR
Reference: Exceedance ≤5%	0 < x < 10	0.5 < x < 1.0	High	0.8 - 1.0
	10 ≤ x < 20	0.25 ≤ x < 0.5	Good	0.6 - 0.8
	20 ≤ x < 40	0.13 ≤ x < 0.25	Mod	0.4 - 0.6
	40 ≤ x < 60	0.08 ≤ x < 0.13	Poor	0.2-0.4
	60 ≤ x ≤ 100	0.0 ≤ x ≤ 0.13	Bad	0 - 0.2

Figure 7-2: The structure and application of the elevated taxa multimetric for phytoplankton assessment in coastal waters.

7.4 Seasonal Succession of Functional Groups

A shift in functional groups may affect ecosystem function in terms of the carbon available to higher trophic levels or settling to the sediments. It isn't clear how such shifts affect, or are affected by, diversity and ecosystem function, however succession of functional groups can potentially provide an multimetric that represents a healthy planktonic system, with a natural progression of dominant functional groups throughout the seasonal cycle. Counts of two major functional groups, diatoms and dinoflagellates, are averaged over each month over the 6 year reporting cycle. Skewed data is accounted for by the transformation of phytoplankton counts on a natural log scale. This measurement links to the normative definition of both composition and abundance being consistent with undisturbed conditions. This tool requires testing of baseline conditions every 6 years to account for sensitivities to climate change.

The structure of the seasonal succession multimetric involves the measurement of the two main taxonomic groupings (diatoms and dinoflagellates), taken as cell counts and compared to the total phytoplankton count. This multimetric is calculated from the proportion of time that the monthly sum of diatoms and dinoflagellates are under an upper reference threshold. This multimetric also has regional variations, with the reference envelopes being specific to the geographical locations. The metric requires monthly counts for diatoms and dinoflagellates (log means) and the calculation of a z score using the waterbody mean against a reference mean and standard deviation. The final classification is the average value of two metric measurements including

- % frequency of monthly Z score falling within the reference envelope constructed for diatoms and dinoflagellates (for English and Welsh waters) and the % frequency of monthly Z score falling under the upper growth envelope constructed for diatoms and dinoflagellates.
- Final score calculated by the number of points under the upper growth envelope compared to the total number of sampling points. Note the reference envelope will differ between geographical regions.

Figure 6-6 describes the process of establishing reference growth curves for WFD waterbodies. The process entails the extraction of phytoplankton data, identified to one of the functional groups from a reference site. Logged phytoplankton counts are averaged over months, and monthly mean (x_i) and standard deviations calculated for each functional group. Calculation of a monthly Z score establishes comparable seasonal distributions for each functional group for a sampling year. A positive *Z*-score indicates that the observation is greater than the mean and a negative score indicates the observation is less than the mean. Z scores of zero illustrate the monthly sample approaches the overall mean for that sampling period. Generic reference curves were established for coastal waterbodies using long term data from the L4 site. This is identified as a low risk waterbody and has been designated as a non-problem area under the OSPAR comprehensive procedure (Devlin et al., 2007a; Devlin et al., 2009). This tool had originally defined a reference growth envelope for the minimum and maximum Z score values. However, a number of waterbodies have Z scores falling below the minimum Z score which resulted in downgrading of that waterbody. In UK waters, low or no growth conditions are not seen as an issue for the phytoplankton tools and the reference curve for both diatoms and dinoflagellates is now only one sided and the tool is based on the number of exceedances of the maximum (reference plus 50%) value. The observed value of the parameter, seasonal succession of phytoplankton functional groups, should be calculated according to the steps listed in Figure 6-6. A summary of the metric, with sampling requirements, accepted methods, reference and boundary conditions are presented in Figure 7-3.

Element: Phytoplankton
Multimetric: Seasonal succession of phytoplankton functional groups.

Sampling requirements

Sampling period is set for a period of 6 years concurrent with the WFD reporting time frame. Sampling frequency is recommended to be monthly over the whole year, with a recommended minimum of 9 months

Methods

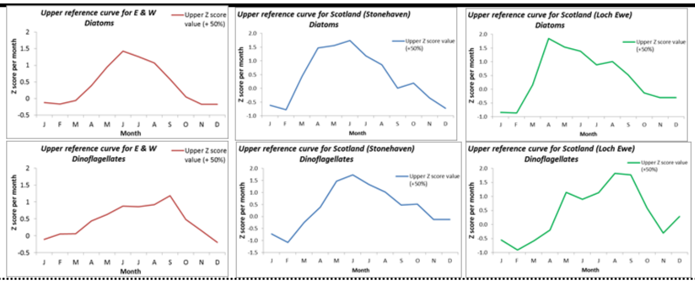
Phytoplankton samples collect from surface. Samples are fix with Lugols Iodine. Enumeration is through light microscopy. Taxa are defined against a Revised phytoplankton taxa list.

Tool summary

The tool records "compliance" of the diatom and dinoflagellates count being within the upper bound value of a reference envelope for every month. Growth envelopes vary over regional conditions.
Monthly scores are calculated for diatoms and dinoflagellates from the log mean of taxa counts against the mean (± SD) of the reference taxa counts (+50%).
Final calculation is the number of times the monthly score falls within the upper bound values for each month averaged over the two functional groups (diatoms and dinoflagellates)

Reference conditions

The value of the Z score will be less or equal to the upper bound value for the reference growth envelope. Upper values are based on a regional reference condition of upper growth limits for each taxa group over each month.
Reference condition for the tool is set at 100% compliance of all sampling points of all sampling points falling below the upper bound value of the reference envelope



Boundary conditions

	% compliance	Metric range	Interim Class	EQR
Reference 100%	80 ≤ x ≤ 100	**0.80 ≤ x ≤ 1**	**High**	**0.8 - 1.0**
	60 ≤ x < 80	**0.6 ≤ x < 0.80**	**Good**	**0.6 - 0.8**
	40 ≤ x < 60	**0.4 ≤ x < 0.60**	**Mod**	**0.4 - 0.6**
	20 ≤ x < 40	**0.2 ≤ x < 0.40**	**Poor**	**0.2-0.4**
	0 ≤ x < 20	**0 ≤ x < 0.2**	**Bad**	**0 - 0.2**

Figure 7-3: The structure and application of the seasonal succession multimetric for phytoplankton assessment in coastal waters.

7.5 Integration of coastal phytoplankton metrics

Figure 7-4 describes the process of classification from data analysis to the integration of phytoplankton metrics. In order to classify the condition of the quality element, phytoplankton, an ecological quality ratio for the phytoplankton index is calculated. Numerical outputs from each tool will be transformed, by linear interpolation to a common ecological ratio scale. The range of each index-specific ecological quality ratio scale will be between the values of 0 to 1. Note further information of the EQR process is discussed in Section 9.

In order to classify the condition of the quality element, phytoplankton, an ecological quality ratio (EQR) for each phytoplankton metric is calculated. In order to combine the different metrics, the face values first need to be normalised into dimension-free scores (Metric score). The WFD prescribes this step by requiring Ecological Quality Ratios (EQRs) to be transformed from the biological measurement. EQRs are the observed value divided by the reference value, the latter being the value expected under undisturbed conditions.) The EQR value ranges between a common range of values (0 to 1), where 1 = High Quality Status and 0 = Bad Quality Status. The multimetric values are reported as equal distant EQR scale, in order to ensure compatibility at the Quality Element EQR stage. (See Figure 7-4-coastal waters and Figure 7-7-transitional waters).

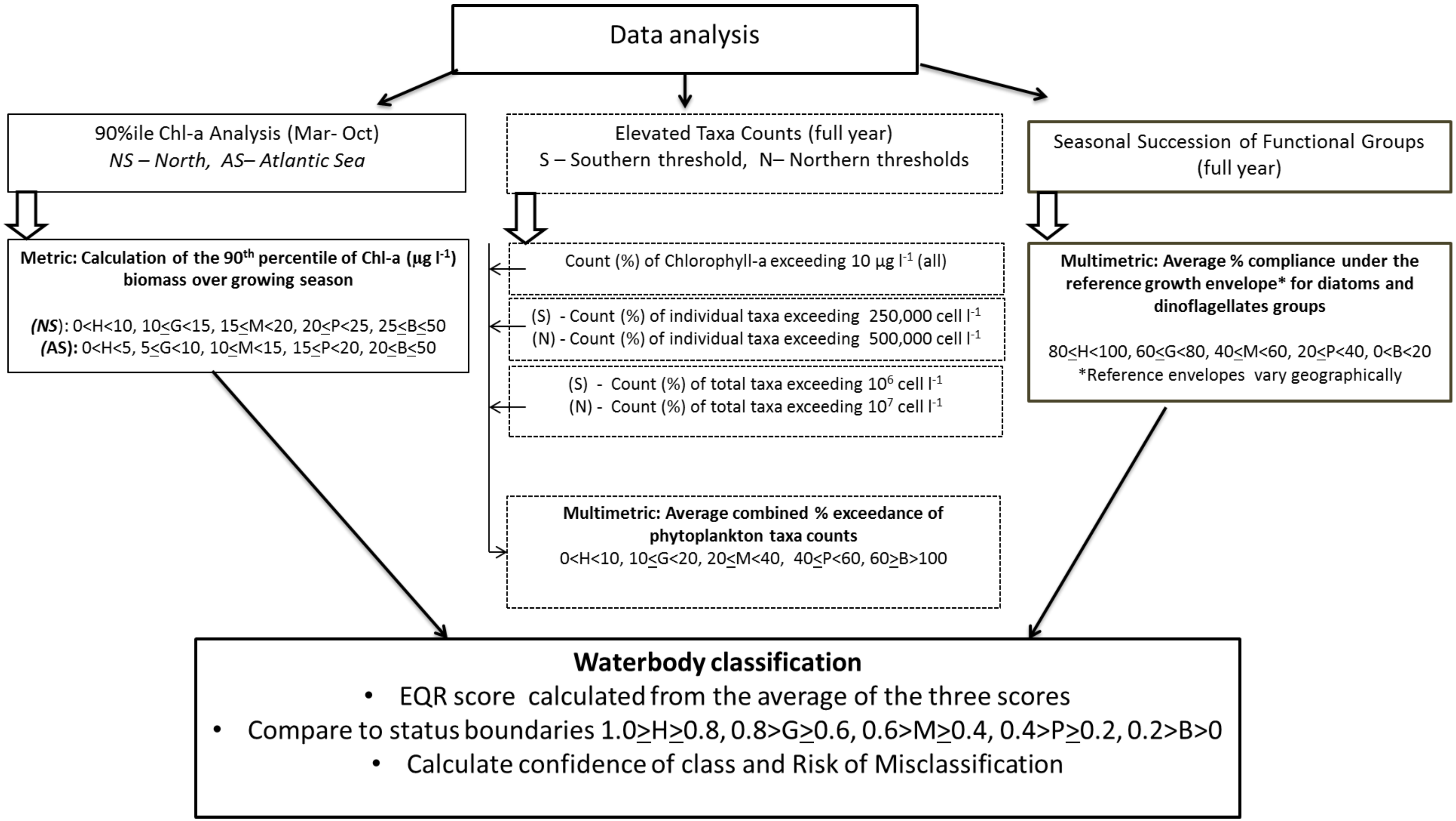


Figure 7-4: Process of classification of coastal waters through sample analysis to final results.

7.6 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., 2007a)Boundaries for the chlorophyll index and some aspects of the elevated count index were tested and agreed through the Phase 1 Intercalibration process for the NEAGIG (Commission Decision 2008/915/EC).The overall class boundaries for the phytoplankton tool are shown in Table 7-2.

Table 7-2: Overall ecological status boundaries for the CW and 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 indices are shown below for chlorophyll (Table 7-3), elevated counts (Table 7-4) and seasonal succession (Table 7-5) for the face value and normalised indices.

Table 7-3: Class status boundaries for the 90^{th} percentile chlorophyll metric for coastal waters. Face value and metric (0-1) ranges are shown.

Metric: 90^{th} percentile measure of chlorophyll biomass	90^{th} percentile chlorophyll ($\mu g\ l^{-1}$) (Face value range)	Metric range (0-1)	Class
North Sea waters Reference: 6.67 $\mu g\ l^{-1}$	>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 $\mu g\ l^{-1}$	>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 7-4: Class status boundaries for the elevated count multimetric for coastal waters. Face value and multimetric (0-1) 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

Table 7-5: Class status boundaries for the seasonal succession multimetric for coastal waters. 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

7.7 Description of phytoplankton metrics in transitional waters

Assessment of phytoplankton in transitional waters consists of two multimetric measurements of chlorophyll and elevated counts of taxa. Table 7-6 describes these two multimetrics, and lists previous directives in which similar indices have been utilised, and describes the type specific reference conditions that have been developed for each index. A description of the indices and literature reviewed thresholds are presented for chlorophyll concentrations and phytoplankton counts.

7.8 Multimetric measures of transitional chlorophyll biomass (µg l-1)

Chlorophyll biomass is recognised as an easily measurable, repeatable metric that can indicate the excessive growth of undesirable phytoplankton species. As defined by the normative definitions (Table 5-4), the appropriate reference condition for the measurement of phytoplankton biomass to represent are planktonic blooms occurring at a frequency and intensity which is consistent with the type-specific physico-chemical conditions. The use of statistical descriptors has been used in many ecological assessments, including the recent OSPAR comprehensive procedure and in 2008 reporting for the Water Framework Directive. In coastal waters, the use of the 90th percentile for the assessment of chlorophyll has been an accepted technique and agreed within the European Intercalibration process. The 90th percentiles represented a statistical method encompassing the spread of data for chlorophyll biomass omitting highly skewed values, which can be present during bloom periods. However, in transitional (estuarine) waters, the higher bloom values, particularly those at the freshwater end, can overly influence the 90th percentile, with high proportion of exceedances against the coastal thresholds. To counter this, this multimetric uses a series of statistical measurements to more fully describe the variability that exists in the transitional waters.

Table 7-6: Description of each phytoplankton multimetric currently in place for transitional water assessment.

Phytoplankton measurement	Statistical measurements of Chlorophyll	Elevated counts of phytoplankton taxa
Historical directives	CSTT (CSTT, 1994; 1997) OSPAR (Malcolm et al, 2002), ECOQO's (Painting et al, 2005)	
Description of previous assessment criteria	Maximum and mean chlorophyll concentrations during the growing season should remain below elevated levels, defined as concentrations >50% above the spatial (offshore) and/or historical background concentration	Region/area-specific phytoplankton eutrophication indicator species should remain below respective nuisance and/or toxic elevated levels (and increased duration)
Reference conditions	Generic reference concentration developed for all coastal water bodies (CW's)	Generic reference concentration developed for all coastal water bodies (CW's)
Normative definition (WFD)	Phytoplankton biomass	Phytoplankton composition and frequency of planktonic blooms
WFD Assessment description	Measurement of statistical values calculated from the chlorophyll data collected over the salinity gradient.	Sum of the occurrence of any single species (> 250,000) plus total cell counts (>10^6)

This multimetric calculates a series of statistical values from the chlorophyll data collected from two salinity zones within the waterbody. The two salinity zones include the mixing zone (5 – 25ppt) and the coastal zone (25 – 35ppt). The statistical measurements are calculated from the chlorophyll data within each zone, including mean, median (50%ile) and the % of values less than 10µg/L, less than 20µg/L and values greater than 50µg/L. Final classification is based on a score out of 10 for the two combined salinity zones.

For chlorophyll measurements taken in the mixing zone (salinity 1-25ppt), the thresholds are set as

- Mean $\leq$ 15µg/L
- Median $\leq$ 12µg/L
- % samples under 10µg/L is less or equal to 70 % (measured as a proportion of the total sample count)

- % samples under 20µg/L is less or equal to 80 % (measured as a proportion of the total sample count)
- % samples over 50µg/L is no more than 5 % (measured as a proportion of the total sample count)

For chlorophyll measurements taken in the coastal zone (salinity > 25pptppt), the thresholds are set as

- Mean ≤ 10µg/L
- Median ≤ 8µg/L
- % samples under 10µg/L is less or equal to 75 % (measured as a proportion of the total sample count)
- % samples under 20µg/L is less or equal to 85 % (measured as a proportion of the total sample count)
- % samples over 50µg/L is no more than 5 % (measured as a proportion of the total sample count)

Details of assessment process, thresholds and outcomes of the application of the tool to UK waters for the monitoring period 2004 to 2009 can be found in Figure 7-5

7.9 Elevated counts of phytoplankton

Persistent and high counts of phytoplankton taxa are also seen as a measurement of disturbance in the phytoplankton community. Normative definitions describe the reference condition as the abundance of phytoplanktonic taxa being consistent with undisturbed conditions and planktonic blooms occurring at a frequency and intensity which is consistent with type-specific physico-chemical conditions. This reference condition will include high species richness presence of a spring bloom with high numbers of diatom species in the bloom period and increasing numbers of dinoflagellates from late spring.

This multimetric is based on the same premise as the coastal phytoplankton metric, designed to assess if the presence, abundance and frequency of occurrence of elevated counts of algal species correspond to undisturbed conditions. However in contrast to the coastal multimetric the estuarine multimetric is composed of just two metrics, both which focus on counts of algae that may result in the decline of ecosystem health or result in an undesirable disturbance. The multimetric comprises of two metrics reporting the number of occurrences, if any, of high numbers of single species and/or high total taxa counts. Note that the transitional elevated count metric does not have any measurement of chlorophyll biomass.

The multimetric works by recording the number of events, defined by sampling occasions, when the sum of ONLY two attributes (measured as a %) exceeds a predefined thresholds over the period of the monitoring program. Each attribute is calculated from the number of times that the sub-metric exceeds the threshold as a proportion of the total number of sampling times and calculated over a 6 year mean. The thresholds identified for transitional waters in England and Wales are

- Index 1 – Count (%) of individual taxa exceeding 500,000 cells l^{-1}
- Index 2 – Count (%) of total taxa exceeding 10^6 cells l^{-1}

7.10 Integration of transitional phytoplankton metrics

Figure 7-7 describes the process of classification from data analysis to the integration of phytoplankton metrics. In order to classify the condition of the quality element, phytoplankton, an ecological quality ratio for the phytoplankton index is calculated. Numerical outputs from each tool will be transformed, by linear interpolation to a common ecological ratio scale. The range of each index-specific ecological quality ratio scale will be between the values of 0 to 1. Note further information of the EQR process is discussed in Section 9.

In order to classify the condition of the quality element, phytoplankton, an ecological quality ratio (EQR) for each phytoplankton metric is calculated. In order to combine the different metrics, the face values first need to be normalised into dimension-free scores (Metric score). The WFD prescribes this step by requiring Ecological Quality Ratios (EQRs) to be transformed from the biological measurement. EQRs are the observed value divided by the reference value, the latter being the value expected under undisturbed conditions.) The EQR value ranges between a common range of values (0 to 1), where 1 = High Quality Status and 0 = Bad Quality Status. The multimetric values are reported as equal distant EQR scale, in order to ensure compatibility at the Quality Element EQR stage. (see Figure 7-7- transitional waters).

Element: Chlorophyll
Multimetric - incorporates compliance assessment of five statistical measures of chlorophyll biomass:

Tool description
➢Tool is based on the calculation of the five statistical measurements of chlorophyll biomass.
Statistical measurements are calculated from the chlorophyll data within two salinity zones, including

- •mean,
- •median,
- •% under 10ug/L,
- •% under 20ug/L
- •% over 50ug/L.

MEAN	MEDIAN	%under 10	%under 20	% over 50
Thresholds for mixing zone metrics				
15	12	70	80	5
Thresholds for coastal zone metrics				
10	8	75	85	5

Sampling requirements.
Sampling period is set for a period of 6 years (WFD reporting time frame).
Sampling frequency is monthly for the full year
Methods
Measurements of chlorophyll biomass are taken from EA monitoring programs through surface sampling. Chlorophyll analysis: Acetone fixed samples - Fluourometry

Box 1: Reference conditions
Each statistical measurement has a threshold associated for each zone and a 0 is awarded for exceedance and 1 for compliance
All 10 statistical measurements of the chlorophyll data, including mean, median, % under 10µg/L, % under 20µg/L and % over 50 µg/L are less or equal to the threshold for that statistical measure. The reference value would be 10.

Element: chlorophyll µg L^{-1}	Position of the % exceedance	Metric range	Class	EQR
ALL E&W transitional waters				
Reference: 10	9--10	0.90-1.0	High	0.8 - 1.0
H/G boundary: 7	7--8	0.70-0.899	Good	0.6 - 0.8
	5--6	0.50 -0.699	Mod	0.4 - 0.6
	3--5	0.30 -0.499	Poor	0.2-0.4
	<3	0-0.299	Bad	0 - 0.2

Table 1: Scoring of the multi metric measurements to a common EQR.

Figure 7-5: The structure and conditions innthe application of the chlorophyll multimetric in transitional waters.

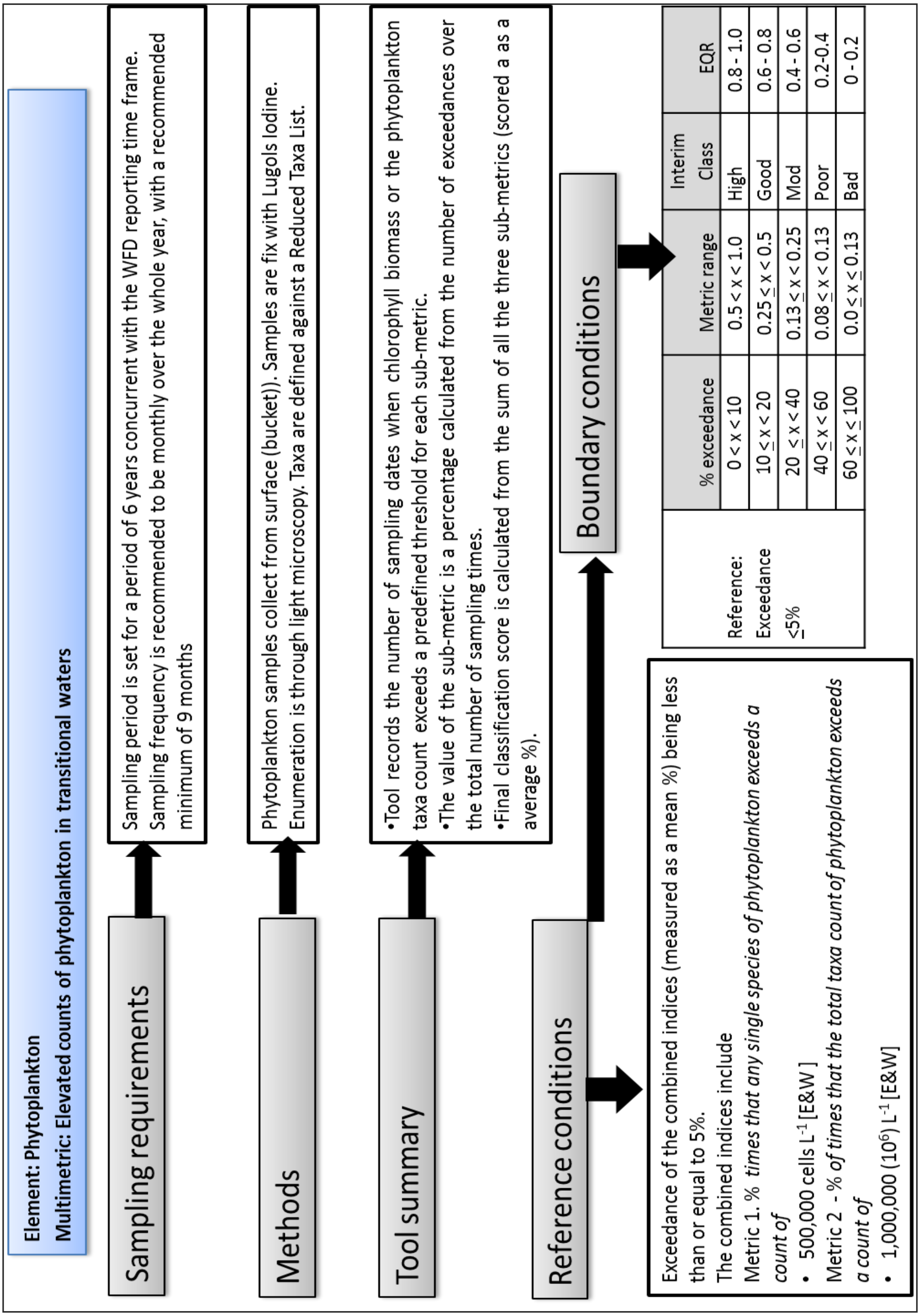


Figure 7-6: The structure and conditions in the application of the elevated count assessment multimetric for transitional waters.

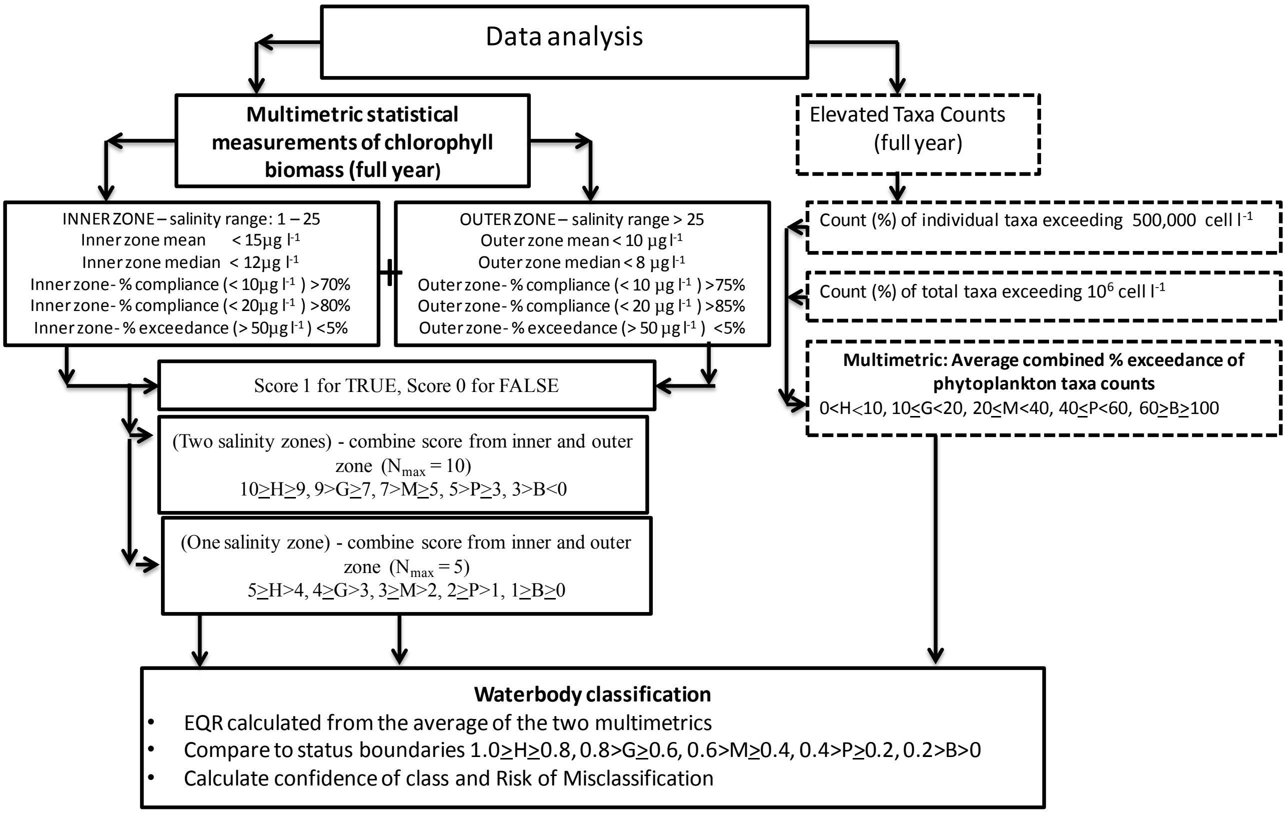


Figure 7-7: Process of classification of transitional waters through data analysis to final equidistant scores.

7.11 Class boundaries

Class boundaries have been defined through a UK process by testing of the phytoplankton historical data held by UK monitoring agencies. Boundaries for some aspects of the elevated count index were tested and agreed through the Phase 1 Intercalibration process for the NEAGIG (Commission Decision 2008/915/EC).The overall class boundaries for the phytoplankton tool are shown in Table 7-7.

Table 7-7: Overall ecological status boundaries for the CW and 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 indices are shown below for chlorophyll (Table 7-8), and elevated counts (Table 7-9) for the face value and normalised indices.

Table 7-8: Class status boundaries for the chlorophyll multimetric for transitional waters. Face value and metric (0-1) ranges are shown.

	Number of compliances (Face Value Range)	Metric Range (0 - 1)	Class	Equidistant class range
Multimetric - 2 salinity zones (10 statistical measurements)				
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 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 zones (5 statistical measurements)				
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 one salinity zone:** **5**(ie all statistics 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
	≥2 - < 3	≥0.2 - < 0.4	Poor	0.2
	≥0 - < 2	0 - < 0.2	Bad	0.2

Table 7-9: Class status boundaries for the elevated count multimetric for coastal waters. Face value and multimetric (0-1) 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

8. Combining the WFD phytoplankton metrics

8.1 Calculation of ecological quality ratios (EQR's) in the phytoplankton assessment

The reporting of any WFD tool requires the calculation of an ecological quality ratio (EQR) number from the actual measurement. In order to combine different metrics and measurements, their values first need to be normalised into dimension-free scores. The WFD prescribes this step by requiring Ecological Quality Ratios (EQRs). EQRs are the observed value divided by the reference value, the latter being the value expected under undisturbed conditions (Figure 8-1).

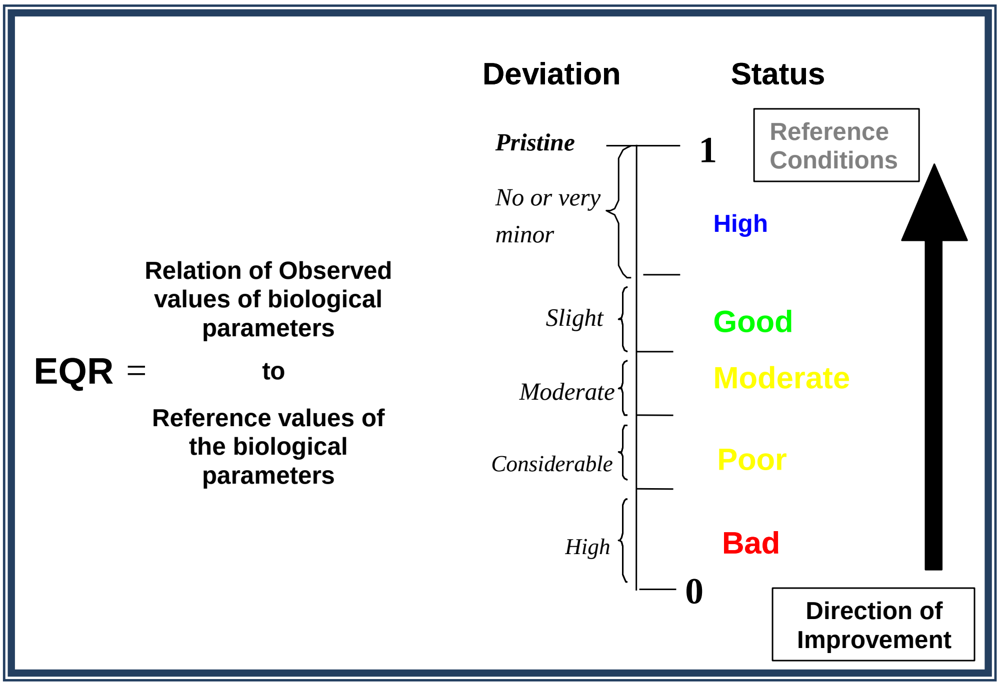


Figure 8-1: Diagram illustrating the principle of the Ecological Quality Ratio and Ecological Quality Range

Face value data is normalised for the phytoplankton tools to ensure compatibility at the final EQR calculation. Each metric or multimetric value is normalised to a EQR based on the observed classification boundaries divided by the reference value for that particular parameter. For example, the 90th percentile chlorophyll metric has two reference values. Therefore the EQR calculated for each regional area will be adjusted against the reference value associated with that regional area.

The second step is to take each of the metrics associated with coastal or transitional waters and normalised to a comparable 0 – 1 scale. Thus the final score can then be combined to calculate the mean EQR for the biological quality element. Note that the phytoplankton EQR will be combined with the other biological quality elements with the coastal or transitional waterbodies. Biological and physicochemical quality elements are aggregated using the 'one out – all out' principle to determine the ecological status (WFD Annex V 1.4.2). In other words, the ecological status defaults to the quality element with the worst classification. For further elucidation of the reporting process for the water body, please refer to documentation from UK TAG (www.ukwfd.org).

8.2 Normalisation process

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 index scores.

8.3 Calculation of the face value.

- The face value for the ***chlorophyll 90th percentile*** is calculated from chlorophyll 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 samples where measured chlorophyll a concentration exceeds the threshold
  - ii) the fraction of all samples where measured individual taxa exceeds the threshold
  - iii) the fraction of all samples 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. (C1, C2 … C12)
  - ii) Converting each monthly value (Ci) 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 8-1).

(i) Comparing each monthly value (Z_i) is to the upper boundary reference value (Figure 8-2, Table 8-1).

(ii) Counting the number of points which fall below the reference curve and calculating the percentage value of compliant data points against all data points.

Table 8-1: 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

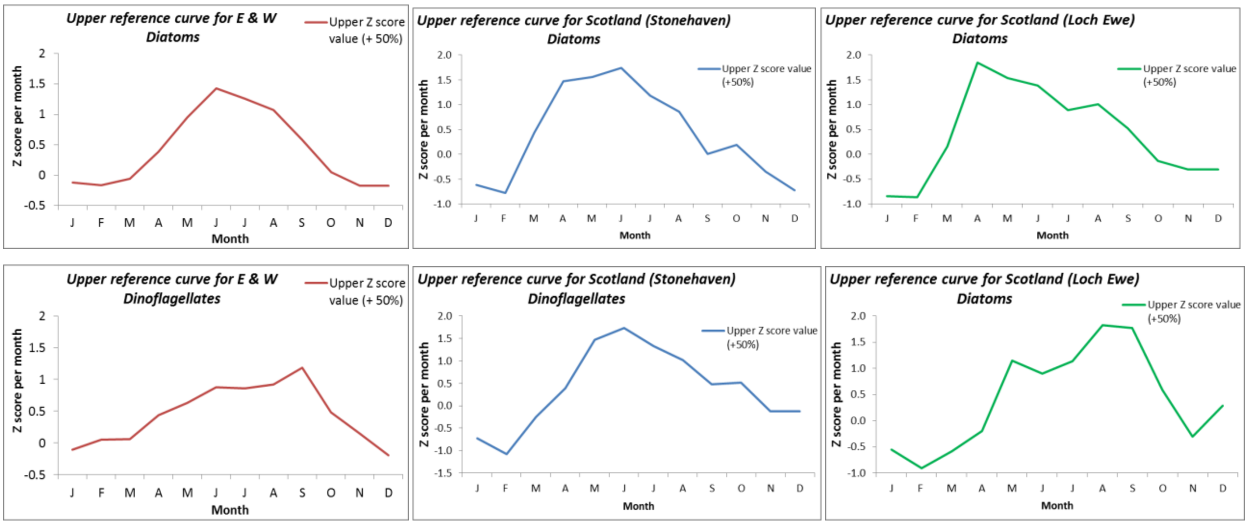


Figure 8-2: Upper reference growth envelopes for England and Wales and Scotland.

8.4 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, 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 8-2 gives the critical values at each class range required for the above equation for coastal waters. Table 8-3 gives the critical values at each class range required for the above equation for transitional waters. 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 tables are “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 8-2: Each metric and the boundary EQR values associated with three metric measurements required for phytoplankton classification of coastal waterbodies.

		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
90%ile Chlorophyll		**µg/l Chlorophyll**	µg/l Chlorophyll	µg/l Chl			
"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

Table 8-3: Each metric and the boundary EQR values associated with two multimetric measurements required for phytoplankton classification of transitional waterbodies.

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 µg l^{-1}Chl 4. % Samples <20 µg l^{-1} Chl 5. % Samples >50 µg l^{-1} Chl Reference for **two** salinity zones: **10** (ie all statistics in both zones compliant 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	% Exceed	% Exceed			
	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

9. Regional differences in boundary conditions

9.1 Background

Reference values and geographical boundaries vary across the waterbodies within the UK. Differences in reference or baseline values can influence the boundary values within the phytoplankton tool assessment (for example, Table 9-1). A difference in biogeographical conditions affects the value of the threshold, such as the count of phytoplankton taxa or can affect the seasonal variation, such as differences in phytoplankton growth envelopes under the seasonal succession metric.

- Variation in reference conditions for coastal waters must reflect a measurable difference in the geographically distinct marine areas.
- Variation in thresholds for coastal waters must reflect a measurable difference in the environmental conditions that influence the behaviour of phytoplankton in geographically distinct areas.

9.2 Description of geographical variation

Latitudinal and geographical differences exist between the different UK marine areas, with northern areas (North East England and Scotland) having colder and longer winter periods with short long day length summers. The longer winter season in the north supports much lower counts of both taxa and biomass over the winter period, whereas the short summer season with longer day length encourages the rapid growth of smaller phytoplankton cells.

Water types are separated into two sea zones (Figure 10-1). Sea Region A is part of the Atlantic sea zone, which is more open and exposed, traps less runoff from the land, low nutrient availability and low measurements of chlorophyll biomass. The chlorophyll reference value for Sea Region A is taken as a maximum value of 3.33µg/L in the growing season (calculated as a 90th percentile measurement). Sea Region B is part of North Sea zone, where the more sheltered water masses can accumulate the more available nutrient rich water. The reference value for the 90th percentile value of chlorophyll biomass during the growing season in the Atlantic Sea zone is established at 6.67µg/L. The reference value for the 90th percentile value of chlorophyll biomass during the growing season in the North Sea zone is established at 6.67µg/L. Boundary values associated with "high" classification is calculated up to 50% of the reference value. Anthropogenic additions would be measured as deviations away from the high/good boundary for both sea zones. These regional differences in the chlorophyll baseline give different boundary values for the 90th percentile chlorophyll metric. These values have also been incorporated into the North East Atlantic Intercalibration, where Sea Region A is characterised at NEA 1/26A and Sea Region B is characterised as NEA 1/26B. (Table 9-1).

	90th ile value	Metric range	Intermim Class	I EQR
North Sea zone				
Reference: 6.67	0 < x < 10	**0.671-1.0**	**High**	**0.8 - 1.0**
	10 ≤ x < 15	**0.441-0.67**	**Good**	**0.6 - 0.8**
	15 ≤ x < 20	**0.341 - 0.44**	**Mod**	**0.4 - 0.6**
	20 ≤ x < 25	**0.271-0.34**	**Poor**	**0.2-0.4**
	25 ≤ x < 50	**0 - 0.27**	**Bad**	**0 - 0.2**
Atlantic sea zone				
Reference: 3.33	0 < x < 5	**0.671 - 1.0**	**High**	**0.8 - 1.0**
	5 ≤ x < 10	**0.33 - 0.67**	**Good**	**0.6 - 0.8**
	10 ≤ x < 15	**0.22 - 0.33**	**Mod**	**0.4 - 0.6**
	15 ≤ x < 20	**0.17 - 0.22**	**Poor**	**0.2-0.4**
	20 ≤ x < 50	**0 - 0.17**	**Bad**	**0 - 0.2**

Table 9-1: Variation in boundary classes for the coastal chlorophyll metric.

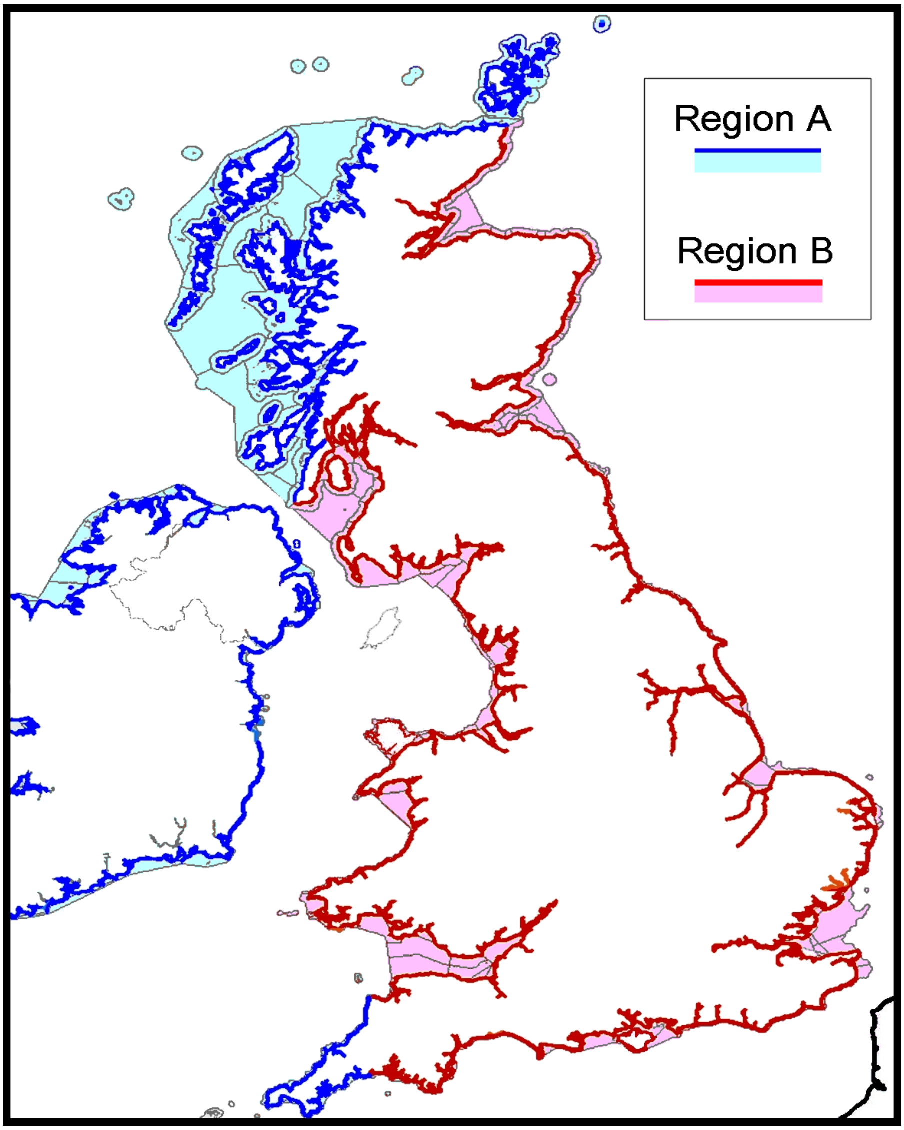


Figure 9-1: Delineation of regions for the reference and associated boundary conditions of chlorophyll biomass

For the UK, phytoplankton communities are divided into northern and southern zones, with geographically distinct phytoplankton communities. For the phytoplankton conditions, slight differences in thresholds and tool structure exists between these two geographical zones These geographical zones are defined with a line joining 55^0 N on the West Coast of Scotland as outlined in Figure 9-2

The geographical conditions have given rise to different regional variations for phytoplankton communities. This is seen in the different thresholds of the elevated count metric for coastal waters, where the southern (England and Wales) waters have lower thresholds for single taxa counts (250,000 cells L^{-1}) and total taxa counts (10^6 cells L^{-1}) compared to Scotland and Northern Ireland which have single taxa exceedance threshold of 500,000 cells L^{-1} and total taxa exceedance of 10^7 cells L^{-1}. Regional variations also exist between the reference values used to define the minimum and maximum values of the seasonal growth envelope. The regional variations are summarised in Table 9-2

Note that at this stage, regional variations in thresholds have been shown for coastal waterbodies only. The thresholds applied for the transitional elevated count tool are the same across UK marine waters and the transitional chlorophyll tool has only been used by England and Wales at this time. Scotland is currently testing the applicability of the transitional chlorophyll metric in Scottish transitional waters.

Figure 9-2: Delineation of Southern and Northern coastal biogeographical regions for phytoplankton cell count thresholds. West coast split has Mull of Galloway to Corsewall Point to the north and Luce Bay to the south. The East coast split has North Yorkshire to the north and South Yorkshire/Lincolnshire to the south

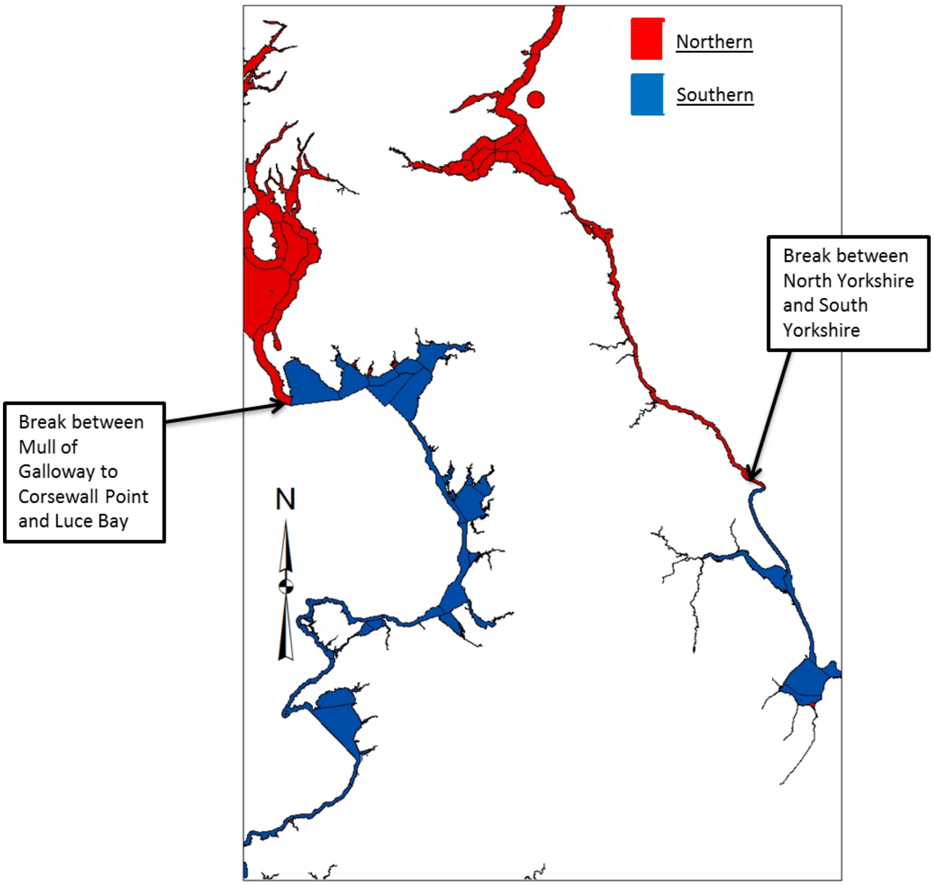


Table 9-2: Description of the regional variations within the chlorophyll and phytoplankton assessment tools.

Measurement	metric	Region	Description of variation	Reasoning
Chlorophyll	90th percentile of chlorophyll biomass	Atlantic seazone	Reference value for the 90th percentile measurement applied to Atlantic waters = 3.33ug/L	
Chlorophyll	90th percentile of chlorophyll biomass	North Sea Zone	Reference value for the 90th percentile measurement applied to North Sea waters = 6.66ug//L	
Phytoplankton	Elevated count of taxonomic groupings	Scotland	Taxonomic groupings include 3 metrics including exceedances of chlorophyll counts (10µg/L), single taxa counts (500,000cells L^{-1}), total taxa counts (10^7cells L^{-1})	Long day length, short summer
Phytoplankton	Elevated count of taxonomic groupings	England, Wales	Taxonomic groupings include 3 metrics including exceedance of chlorophyll counts (10µg/L), single taxa counts (250,000cells L^{-1}), total taxa counts (10^6cells L^{-1})	
Phytoplankton	Seasonal succession of taxonomic groupings.	England and Wales	Seasonal succession of two distinct taxonomic groups including diatoms and dinoflagellates. Reference curves are one sided (maximum Z score only). Reference curve based on E&W reference site – Plymouth outer coast	One sided - Lower envelope exceedance does not signify nutrient enrichment. Reference envelope reflects the seasonal pattern of diatoms and dinoflagellates based on EW long term reference site.
Phytoplankton	Seasonal succession of taxonomic groupings.	Scotland	Seasonal succession of three distinct taxonomic groups including diatoms, dinoflagellates. Reference curves are two sided (minimum and maximum Z score). 2 Reference curves based on Scottish reference sites	One sided - Lower envelope exceedance does not signify nutrient enrichment. Reference envelope reflects the seasonal pattern of diatoms and dinoflagellates based on Scottish long term reference sites.
Phytoplankton	Seasonal succession of taxonomic groupings.	Northern Ireland	NIEA have **no** reference sites so are **not** using the tool	

10. Combining the biological and chemical measurements for a waterbody assessment

This part of the classification process is prescribed by the WFD. The quality element with the lowest (worst) status for a water body determines the overall ecological status. Biology plays a central role in determining poor and bad status and hydromorphology a central role in deciding high status:

In combination with biological classifications, supporting physico-chemical elements including specific pollutants (Annex viii substances) can result in high, good or moderate class but do not determine poor or bad status.

When combined with biological quality elements, hydrology and morphology assessments determine high status only. They do not determine good, moderate, poor and bad status. This is because the Environment Agency is following Common Implementation Strategy guidance which indicates that biological evidence is required to determine poor and bad status. This process is summarised in Figure 10-1.

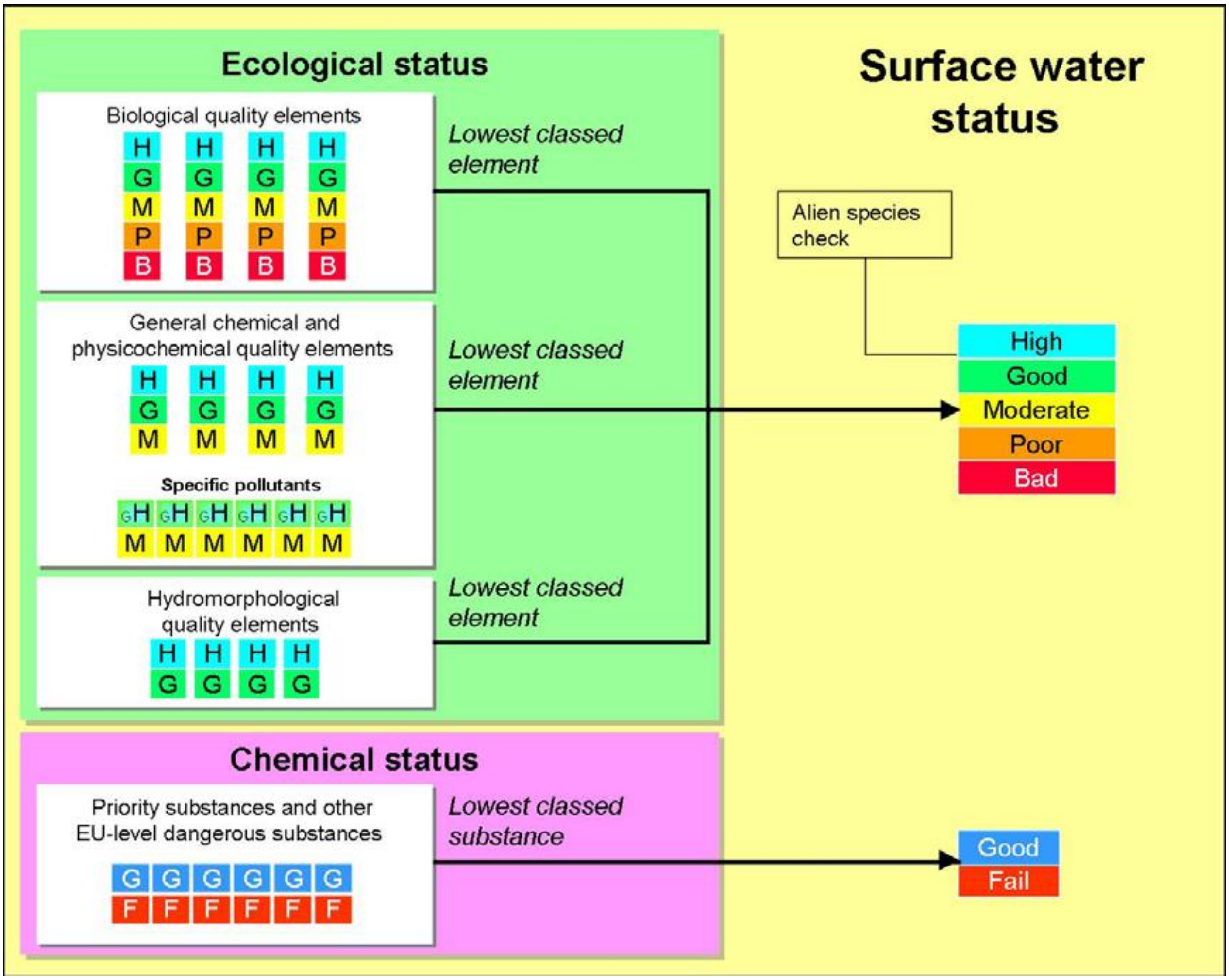


Figure 10-1: Process of combining all the strand of the WFD classification together.

11. Data and monitoring requirements

Each biological quality element is capable of responding to many of the pressures acting on the environment, but classification tools have been developed to indicate a particular pressure when possible (Table 11.1). This has been successful for organic pollution, nutrient enrichment and acidification, but some tools such as the fish-based tools respond to many pressures in combination and can be thought of as indicators of general disturbance.

Table 11-1: Pressures indicated by quality elements

Quality element	Primary Pressures indicated
Transitional and coastal waters	
Phytoplankton	Nutrient enrichment
Macroalgae	Nutrient enrichment, habitat modification
Angiosperms (sea grasses)	Habitat modification, Nutrient enrichment
Benthic invertebrates	Respond equally to organic pollution and toxic chemicals
Fish (transitional only)	Organic enrichment, habitat destruction

Using the relationship described in the above table, the operational monitoring (see UKTAG monitoring guidance) was designed to gather data on the quality element that is most sensitive to the pressure(s) acting on a water body. This is called 'risk-based monitoring'. Data from other monitoring programmes have been and will be used to provide additional data. Examples of other monitoring programmes include the Environmental Change Network, the National Fisheries Monitoring Programme and the Dangerous Substance Directive network.

At water bodies chosen for the surveillance monitoring network, data is collected for all quality elements. The main aim of surveillance monitoring is to look for signs of impact from any pressure in order to validate the risk assessment. So in many water bodies, there will be evidence from several biological quality elements.

A seven-year cut-off period has been used as the criterion for using historic data. Any suitable data from 2001 to 2007 (inclusive) has been made available for draft classifications, however final classifications were made on data from 2004-2009. Many of the new classification tools, particularly in transitional and coastal waters, require up to five years of data to produce reliable classifications, so these first draft and final classifications must be used with caution.

Some quality elements are monitored nearly everywhere. These are particularly powerful for showing the national picture and trends. For other elements, monitoring is targeted according to risk. If there has been no reason to monitor for a particular chemical or quality element, the water body is assumed to be in good status for that item. This means it is important to assess risks when priorities are decided for monitoring. Otherwise, our reports will be biased to indicate good quality to the extent that we have underestimated risk.

On the other hand we need to recognise that the use of many quality elements, and to assign class by the worst of these, will bias the overall picture towards bad quality unless all those elements are measured with 100 per cent precision. This factor needs to be considered when looking at trends. A move to include more quality elements will lead to more reported failure, even if the true picture has remained unchanged for all quality elements. This risk is countered by looking at the individual elements, one at a time.

In the recent reporting round (2004- 2007) the EA identified a data confidence process. This was a qualitative summary of the spatial and temporal frequency of data available for each reporting outcomes.

The data confidence is divided into three classes ("very certain", "certain" and "uncertain") and was assigned through a qualitative process (Table 11.2).

For our classifications of coasts and estuaries data confidence is described in terms of the amount and quality of data available for the classifications. This is not confidence of class in the statistical sense. The techniques for doing this for each tool are discussed in the next chapter.

Table 11-2: Data quantify associated with optimal and minimal sampling numbers for the three phytoplankton metrics.

Tool	Chl 90%		Elevated Counts		Seasonal Succession	
	Optimal	**Minimal**	**Optimal**	**Minimal**	**Optimal**	**Minimal**
Years	**5**	**2**	**5**	**2**	**5**	**2**
months	**Mar-Oct**	**Apr-Sep**	**Jan-Dec**	**9 months**	**Jan-Dec**	**10 Months**
Sites *	**10**	**3**	**10**	**3**	**10**	**3**
* relative to size & variability of waterbody						

12. Confidence of class

12.1 Introduction

The need for a robust assessment of risk from eutrophication to the marine environment is discussed in (Davey, 2009). This is because the vulnerability of marine areas having the potential to suffer from eutrophication depends not only on nutrient loads, but also upon the physical characteristics and hydrodynamics of the receiving waters (such as light regimes and water exchange). Nutrient concentrations are only considered as screening indicators for eutrophication risk in the marine environment (Malcolm et al., 2002; OSPAR, 2002, 2003, 2005; Painting et al., 2005)and will not indicate the risk of eutrophication per se. They will however, indicate pressures from point and diffuse nutrients sources.

In an ideal world with comprehensive monitoring data containing no errors, water bodies would always be assigned correctly to their true class with 100 per cent confidence. But estimates of the truth based on monitoring data 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 is important because of the potential to fail to act because a water body has been wrongly reported as better than it is or to waste resources on water bodies that have been wrongly classed as worse than good.

The Water Framework Directive requires the Environment Agency to classify all surface waterbodies into one of five status classes: High, Good, Moderate, Poor or Bad. In addition, the Agency is required to report the level of confidence associated with waterbody classifications.

In an ideal world of comprehensive monitoring data containing no errors, waterbodies would always be assigned to their true class with 100% confidence, but in reality estimates of the truth based on monitoring are always subject to error. 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 waterbody 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

12.2 Confidence in assessment of ecological status

The ecological status of transitional (estuarine) and coastal waterbodies is assessed using a variety of biological quality elements, including macroalgae and phytoplankton.

For each quality element, the ecological status of the waterbody is measured by an Ecological Quality Ratio (EQR), which comprises one or more sub-metrics that measure different aspects of biological community. For example, the phytoplankton assessment tool has three sub-metrics: chlorophyll-a concentration, elevated counts and seasonal succession. EQRs take a value between 0 and 1, and this range is split into five status classes (Bad, Poor, Moderate, Good and High).

For classification purposes, the estimated EQR is translated directly into a face value class. However, because it is not possible to survey biological community across whole waterbody 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 waterbody 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 (Ellis and Adriaenssens, 2006).

The spreadsheet developed that relates to phytoplankton assessment is Phytoplankton Uncertainty Gets Worked out And Statistically Handled to calculate CofC for the phytoplankton assessment tool; Note that the following section is extracted from the WRc reported commissioned by the Environment Agency (Davey, 2009).

12.3 Overview - 'Phytoplankton Uncertainty Gets Worked out And Statistically Handled'

'Phytoplankton Uncertainty Gets Worked out And Statistically Handled' calculates confidence of class for the WFD TraC Phytoplankton Tool. It performs calculations for multiple waterbodies simultaneously and gives the confidence of class over the whole reporting period (Ellis, 2006).

The phytoplankton tool comprises three sub-metrics.

1. Elevated counts multi-metric - this is the average of three attributes, which each measure the proportion of sampling occasions on which a particular threshold level is exceeded. Those attributes are: phytoplankton biomass (mean chlorophyll), counts of any single taxa, and counts of the total taxa.

2. Seasonal succession multi-metric - calculates the proportion of months in which community composition (as measured by a z-score) falls within a reference envelop for two major functional groups: diatoms and dinoflagellates. The multi-metric is the average of these two proportions.

3. Chlorophyll 90th percentile metric - the 90th percentile of all chlorophyll concentrations during the growing season (March to October inclusive) is taken as a measure of phytoplankton biomass.

Each multi-metric is computed using data for the waterbody as a whole over a six year reporting period. Each multi-metric score is converted into an EQR via a two-step normalisation process. The first step converts the sub-metric score to an EQR scale between 0 and 1, where the status class boundaries are not equidistant (for example, Bad = 0.0 – 0.27, Poor = 0.27 – 0.34, Moderate = 0.34 – 0.44 etc). The second step transforms these EQR values onto an equal-width class scale (Bad = 0.0 – 0.20, Poor = 0.20 – 0.40, Moderate = 0.40 – 0.60 etc). For simplicity, "Phytoplankton Uncertainty Gets Worked out And Statistically Handled" combines these two normalisation steps into one, as illustrated in Appendix C. The three sub-metric EQRs are then averaged to give a Final EQR between 0 and 1 (Figure 12-1).

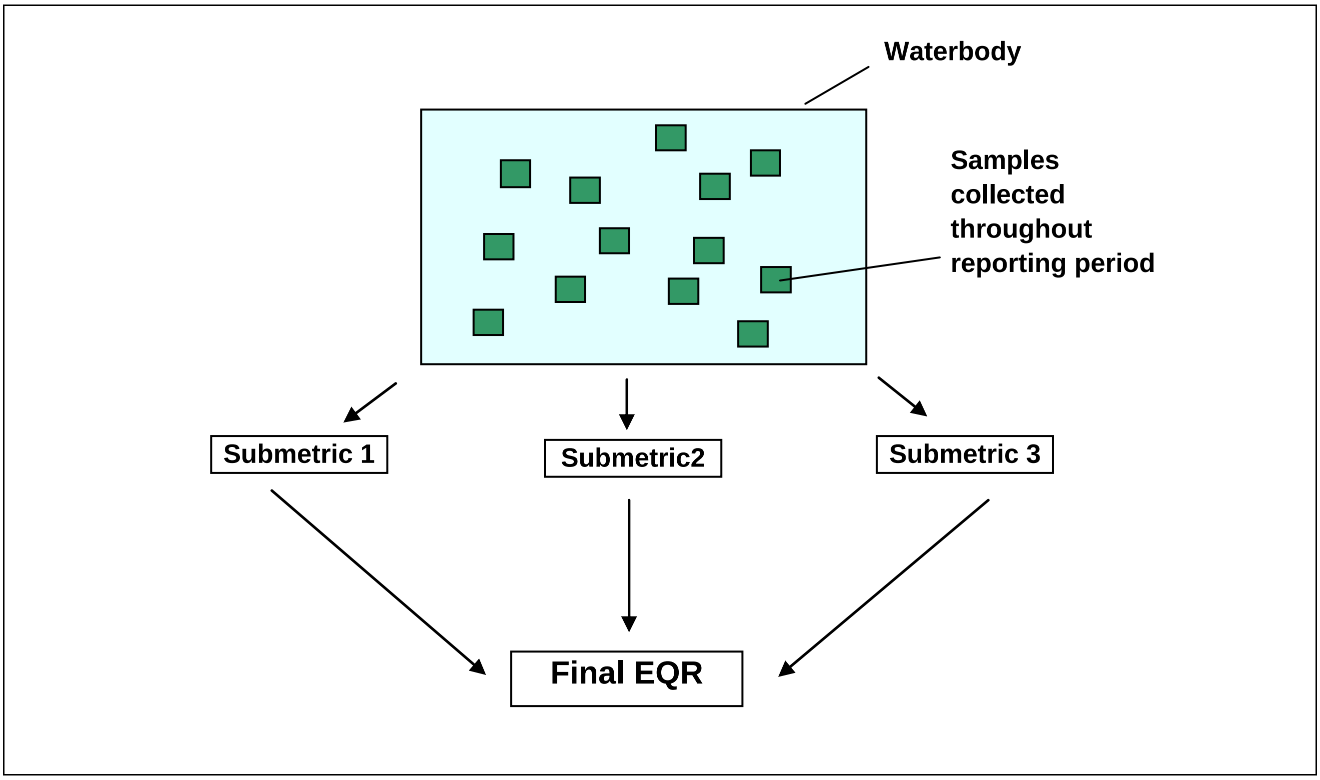


Figure 12-1: Sampling scheme for phytoplankton tool

12.4 Methodology

As each sub-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 sub-metric EQR. Briefly, "Phytoplankton Uncertainty Gets Worked out And Statistically Handled" adopts a bottom-up approach whereby each sub-metric score and its corresponding standard error are first used to compute the confidence of class for each sub-metric. Next, the three sub-metric scores are normalised to produce sub-metric EQRs between 0 and 1. Finally, the sub-metric EQRs are combined to give a Final EQR, and their standard errors are also combined to produce a confidence of class for the Final EQR result.

CofC for Elevated Counts sub-metric

Let n_1*,* n_2*, …,* n_k *represent the number of samples taken of each of the* $k = 4$ *attributes (phytoplankton biomass, counts of any single species, counts of Phaeocystis, and counts of the total taxa) during the reporting period, and let* r_1*,* r_2*,…,* r_k *represent the corresponding number of samples that exceeded the specified threshold values.*

The proportion of exceedances for the i^{th} *attribute is given by:*

$$p_i = \frac{r_i}{n_i} \qquad (1)$$

The sub-metric score (Q*) is calculated as the mean proportion of exceedances across the* k *attributes:*

$$Q = \overline{p} = \frac{\sum_{i=1}^{k} p_i}{k} \qquad (2)$$

Two alternative approaches were considered for calculating the standard error of Q*. The original approach used a Normal approximation to estimate the standard error of each attribute proportion:*

$$SE(p_i) = \frac{p_i(1-p_i)}{n_i} = \frac{r_i(n_i - r_i)}{n_i^3} \qquad (3)$$

This formulation has the benefit that the standard errors of the four attributes can be easily combined to give the standard error of $\overline{p}$*, but it gives unreliable results when the sample sizes are less than 30, and when the proportions are close to 0 or 1. Specifically, it was found that an attribute with very few samples and no exceedances would yield* $p_i = 0$ *and* $SE(p_i) = 0$*, giving the impression of no uncertainty when the paucity of replicate samples means that the uncertainty is actually very high.*
As a result of these problems, Phytoplankton Uncertainty Gets Worked out And Statistically Handled *utilises a less direct but more accurate method for calculating the standard error of* Q*, which proceeds as follows. First, a 95% confidence interval is constructed around each attribute proportion using the Wilson Score approach:*

$$CI(p_i) = \frac{p_i + \frac{1}{2n_i}z^2 \pm z\sqrt{\frac{p_i(1-p_i)}{n_i} + \frac{z^2}{4n_i^2}}}{1 + \frac{z^2}{n_i}} \qquad (4)$$

where z *is the 97.5th percentile of a standard normal distribution, and takes a value of 1.96.*
Second, the upper (UCL) and lower (LCL) 95% confidence limits are converted into an approximate standard error:

$$SE(p_i) \approx \frac{UCL(p_i) - LCL(p_i)}{2 \times 1.96} \qquad (5)$$

Finally, the standard errors for each of the k *attributes are combined to give a standard error for* Q*:*

$$SE(Q) = \sqrt{\frac{\sum SE(p_i)^2}{k^2}} \qquad (6)$$

The sub-metric score Q and its standard error are converted to a sub-metric confidence of class following the Normal distribution approach set out in Appendix B.

CofC for Seasonal Succession sub-metric

As the Seasonal Succession metric (Q) is the average proportion of two attributes (diatoms and dinoflagellates), the standard error of Q is computed in exactly the same way as for the Elevated Counts sub-metric (with $k = 2$ attributes).

CofC for Chlorophyll-a 90th Percentile sub-metric

The chlorophyll 90th percentile sub-metric (Q) is evaluated as the 90th percentile of all chlorophyll concentrations during the growing season (March to September inclusive).
Phytoplankton Uncertainty Gets Worked out And Statistically Handled estimates the 90th percentile non-parametrically as the mth ordered value, where:

$$m = n \times \frac{90}{100} \qquad (7)$$

and n equals the number of chlorophyll-a samples. So for $n = 50$ samples, the 90th percentile is given by the 45th smallest concentration (i.e. the 5 largest). A minimum of nine samples are required to compute the 90th percentile.
(Note: this formulation is preferred to the more conventional Weibull approach, which gives $m = (n-1) * (90/100)$. The Weibull approach assumes (correctly) that the chlorophyll-a measurements are a sample from a wider population, but it occasionally produces a contradiction between the face-value

class and the most probable class in the CofC assessment because of the way that the CofC calculations work (see below). It is important to be aware that the use of equation 34 changes subtly the definition of the chlorophyll sub-metric: strictly, a waterbody has to have a 90th percentile ≤ a threshold to be in the higher status class (i.e. 90% of the population of possible measurements have to be ≤ the threshold), whereas P Phytoplankton Uncertainty Gets Worked out And Statistically Handled assesses whether a waterbody has 90% of sampled measurements ≤ the threshold. In most cases, this makes no appreciable difference to the results.)

Q is converted to a confidence of class using a binomial model. Let the four intermediate sub-metric score boundaries be denoted by L_5, L_4, L_3 and L_2 (in the order Bad/Poor → Good/High). The aim is to determine the levels of confidence we have that the true quality is respectively in Class 5, 4, 3, 2 and 1. To do this, we first do four calculations. For each class boundary 'i' in turn, we determine the number of samples (r_i) that fall below L_i, and ask: What is the probability p_i of observing r_i or fewer out of n samples if the probability of each individual sample being below L_i is exactly 0.9 (i.e. the 90th percentile is on the L_i boundary)?

p_i is calculated using the cumulative probability function of a binomial distribution with $n = n$ and $p = 0.9$.

This enables us to make the following five statements:

- Confidence of class 5 (Bad) = 100(1-p5).
- Confidence of class 4 (Poor) = 100(p5 – p4).
- Confidence of class 3 (Moderate) = 100(p4 – p3).
- Confidence of class 2 (Good) = 100(p3 – p2).
- Confidence of class 1 (High) = 100p2.

Note that these five quantities sum to 100%.

The standard error of Q is approximated by first constructing a 90% confidence interval around the percentile estimate. The lower confidence limit is taken to be the q^{th} smallest value in the dataset, where: q is the largest integer for which the cumulative binomial distribution (n = n; p = 0.9) is ≤ 0.05. Similarly, the upper confidence limit is taken to be the r^{th} smallest value in the dataset, where r is the smallest integer for which the cumulative binomial distribution (n = n; p = 0.9) is ≥ 0.95.

For example, if n = 49 samples, the lower 90% confidence limit is given by the 39th smallest value (i.e. 11th largest) because the probability of getting 39 or fewer 'successes' out of 49 when p = 0.9 is 0.021. Similarly, the upper 90% confidence limit is given by the 47th smallest value (i.e. 3rd largest) because the probability of getting 47 or fewer 'successes' out of 49 when p = 0.9 is 0.963.

The upper (UCL) and lower (LCL) 90% confidence limits are converted into an approximate standard error:

$$SE(Q) = \frac{UCL(Q) - LCL(Q)}{2 \times 1.65} \quad (8)$$

CofC for Final EQR

Before computing the Final EQR, the three sub-metric scores Q and their standard errors are normalised onto an equal class-width scale running from 0 to 1 as described in Appendix C.

The $a = 3$ sub-metric EQRs (Q^*) are averaged to give a Final EQR between 0 and 1 ($\overline{Q}^*$).

The standard error of the Final EQR is computed from the standard errors of the sub-metric EQRs, assuming that the three sub-metrics are independent:

$$SE(\overline{Q}^*) = \sqrt{\frac{\sum_{i=1}^{a} SE(Q^*)^2}{a^2}} \qquad (9)$$

The Final EQR and its standard error are then converted to a confidence of class following the Normal distribution approach(Davey, 2009).

12.5 CUTLASS: Chlorophyll Uncertainty Tool - Likelihood Analysis of Salinity Sub-metrics

WRc was commissioned by the Environment Agency to develop a spreadsheet tool to calculate the confidence of classification associated with assessment of the status of transitional waters. The tool, called CUTLASS (Chlorophyll Uncertainty Tool - Likelihood Analysis of Salinity Sub-metrics), calculates the face value EQR and confidence of class for two components - the Transitional Water Chlorophyll-a Tool and the Transitional Water Elevated Counts Tool. For both tools, calculations are performed for multiple water bodies simultaneously and give the confidence of class over the whole reporting period. The results from the two tools are then combined to give an overall status assessment (Ellis and Adriaenssens, 2006).

12.6 Chlorophyll-a Tool

The chlorophyll-a tool comprises ten sub-metrics (Table 12-1). Each estuarine water body is divided into two salinity bands – low and high. Within each salinity band, chlorophyll-a samples are collected across the water body and throughout the reporting period to give a representative measure of spatial and temporal variability in chlorophyll-a concentrations.

Each sub-metric is computed for the water body as a whole over a reporting period and yields a score of 0 or 1 depending on whether or not it meets a prescribed threshold standard listed in Table 12.1. These 10 scores are summed to give a Total Score between 0 and 10, which is converted into an EQR and then a face value status class (Table 12-2).

If a water body only has data in one salinity band, the sub-metric scores are summed to give a Total Score between 0 and 5, which is then doubled before converting to an EQR and status class using Table 12-2. CUTLASS gives a warning flag when the classification is based only on a single salinity band.

Table 12-1: Chlorophyll-a sub-metrics

No.	Salinity band	Sub-metric description	Threshold
1	Low	Average chlorophyll-a concentration	≤15µg/l
2	Low	Median chlorophyll-a concentration	≤12µg/l
3	Low	% of chlorophyll-a measurements less than 10 µg/l	≥70%
4	Low	% of chlorophyll-a measurements less than 20 µg/l	≥80%
5	Low	% of chlorophyll-a measurements greater than 50 µg/l	≤5%
6	High	Average chlorophyll-a concentration	≤10µg/l
7	High	Median chlorophyll-a concentration	≤8µg/l
8	High	% of chlorophyll-a measurements less than 10 µg/l	≥75%
9	High	% of chlorophyll-a measurements less than 20 µg/l	≥85%
10	High	% of chlorophyll-a measurements greater than 50 µg/l	≤5%

Table 12-2 Total Score and EQR status class boundaries

Total Score	EQR	Status Class
0	0.000	Bad
1	0.067	Bad
2	0.133	Bad
3	0.200	Poor
4	0.300	Poor
5	0.400	Moderate
6	0.500	Moderate
7	0.600	Good
8	0.700	Good
9	0.800	High
10	1.000	High

An absolute minimum of two samples per salinity band is required in order to calculate the sub-metrics. The minimum number of samples required to calculate the sub-metrics can be altered by the user via the Settings worksheet; the default is 10. If there are too few samples for one salinity band, it will be omitted from the calculations and a warning flag will be produced. Similarly, if one or more sub-metrics have not been entered into CUTLASS, that salinity band will not contribute to the EQR result.

12.7 Confidence of class

To measure the uncertainty in the status assessment, the variability in the data is first used to assess the probability that each sub-metric yields a score of 1. The method used to calculate these probabilities depends on the type of sub-metric.

For average chlorophyll-a concentration (sub-metrics 1 and 6), a t-value is calculated to measure how far the observed average ($\bar{x}$) lies from the threshold (X):

$$t = \frac{X - \bar{x}}{s / \sqrt{n}} \qquad (1)$$

where:

s = the standard deviation of the chlorophyll-a measurements;

n = the number of samples.

A positive t-value indicates that the observed average concentration is below the threshold. The probability that the sub-metric yields a score of 1 is given by the cumulative probability of that t-value taken from a t-distribution with n-1 degrees of freedom.

For the median chlorophyll-a concentration (sub-metrics 2 and 7), the probability of the sub-metric yielding a score of 1 is calculated by testing whether the proportion (x) of samples less than or equal to the threshold is less then 0.5. To do this, a z-score is calculated to measure how different the observed proportion is from 0.5:

$$z = \frac{x - 0.5}{\sqrt{\frac{0.5*(1-0.5)}{n}}} \quad (2)$$

A positive z-value indicates that more than half of samples are equal to or less than the threshold standard. The probability that the sub-metric yields a score of 1 is given by the cumulative probability of that z-value taken from a standard normal distribution.

For the % of chlorophyll-a samples less than 10 µg/l (sub-metrics 3 and 8), the probability of the sub-metric yielding a score of 1 is calculated by testing whether the observed proportion (x) of samples less than 10 µg/l is greater than the threshold (X) of 70% or 75% (**Error! Reference source not found.**). To do this, a z-core is calculated to measure how different the observed proportion is from the threshold:

$$z = \frac{x - X}{\sqrt{\frac{X*(1-X)}{n}}} \quad (3)$$

A positive z-value indicates that the proportion of samples less than 10ug/l is greater than the threshold standard. The probability that the sub-metric yields a score of 1 is given by the cumulative probability of that z-value taken from a standard normal distribution.

An identical approach is used for the % of chlorophyll-a samples less than 20 µg/l (sub-metrics 4 and 9).

The same approach is used for the % of chlorophyll-a samples greater than 50 µg/l (sub-metrics 5 and 10), except that the z-score is calculated as:

$$z = \frac{X - x}{\sqrt{\frac{X*(1-X)}{n}}} \quad (4)$$

These 10 sub-metric probabilities are then combined to work out the long-run probability of observing a Total Score of 0, 1, 2 and so on up to 10. As a very simple example, if the probabilities of sub-metrics 1 and 2 yielding a score of 1 are 0.8 and 0.6, respectively, then:

the probability of getting a Total Score of 2 is 0.8 x 0.6 = 0.48;

the probability of getting a Total Score of 1 is 0.8 x (1 – 0.6) + (1 – 0.8) x 0.6 = 0.32 + 0.12 = 0.44; and,

the probability of getting a Total Score of 0 is (1 – 0.8) x (1 – 0.6) = 0.08.

CUTLASS extended this logic to consider all ten sub-metrics and assumes that the uncertainties of the ten sub-metrics are uncorrelated (i.e. the score yielded by one sub-metric has no influence on the scores yielded by the other sub-metrics).

Having determined the long-run probability of each Total Score between 0 and 10, these results are then translated into status classes to give the confidence that the water body is truly in each of the five status classes (this is termed the 'confidence of class').

CUTLASS calculates both a face value class (based on the observed Total Score and EQR) and the confidence of the water body being in each of the five status classes. Occasionally the face value class may not be the same as the most probable class given by the confidence of class assessment, particularly when the EQR is close to a boundary between two classes. This is perfectly correct, and arises because the face value EQR is based on a limited set of monitoring data. For example, consider a water body with an EQR of 0.70: the face value class will be Good, but the confidence of class may say 50% High, 40% Good and 10% Moderate, which 'averages out' at Good. Thus, there is no contradiction between the face value result, which relates to the EQR result produced by the observed monitoring data and the confidence of class, which presents the distribution of outcomes that are expected to arise due to random variation.

To allow the results for the Chlorophyll-a Tool to be combined with the results for the Elevated Counts Tool, an approximate standard error of the Chlorophyll-a EQR was calculated as follows:

$$SE = \sqrt{\sum_{i=0}^{10} p_i \left[\left(\sum_{i=0}^{10} EQR_i \, p_i \right) - EQR_i \right]^2} \qquad (5)$$

where:

EQR_i = an EQR value corresponding to a Total Score of *i*,

p_i = the probability that the water body takes that particular EQR value, and so

$\sum EQR_i \, p_i$ = the expected EQR of the water body.

Elevated Counts Tool

The Elevated Counts tool comprises two sub-metrics:

- The proportion of samples in which the count any single taxon exceeds a pre-determined threshold.
- The proportion of samples in which the count of all taxa exceeded a pre-determined threshold.

Each sub-metric is computed for the water body as a whole over a the reporting period. The two sub-metrics are averaged and then normalised to give an EQR between 0 (Bad status) and 1 (High Status).

Confidence of class

The confidence of class methodology follows that used for the elevated counts part of the phytoplankton classification tool (WRc 2009).

Let n_1 and n_2 represent the number of samples taken for the two sub-metrics (single taxon, all taxa) during the reporting period, and let r_1 and r_2 represent the corresponding number of samples that exceeded the specified threshold.

The proportion of exceedances for the i^{th} sub-metric is given by:

$$p_i = \frac{r_i}{n_i} \qquad (6)$$

The sub-metric score (Q) is calculated as the mean proportion of exceedances across the two sub-metrics:

$$Q = \overline{p} = \frac{\sum_{i=1}^{2} p_i}{2} \qquad (7)$$

Like "Phytoplankton Uncertainty Gets Worked out And Statistically Handled" CUTLASS utilises a less direct but more accurate method for calculating the standard error of Q, which proceeds as follows. First, a 95% confidence interval is constructed around each sub-metric proportion using the Wilson Score approach:

$$CI(p_i) = \frac{p_i + \frac{1}{2n_i} z^2 \pm z\sqrt{\frac{p_i(1-p_i)}{n_i} + \frac{z^2}{4n_i^2}}}{1 + \frac{z^2}{n_i}} \qquad (8)$$

where z is the 97.5th percentile of a standard normal distribution, and takes a value of 1.96.

Second, the upper (UCL) and lower (LCL) 95% confidence limits are converted into an approximate standard error:

$$SE(p_i) \approx \frac{UCL(p_i) - LCL(p_i)}{2 \times 1.96} \qquad (9)$$

Finally, the standard errors for each of the two sub-metrics are combined to give a standard error for Q:

$$SE(Q) = \sqrt{\frac{\sum SE(p_i)^2}{2^2}} \qquad (10)$$

The sub-metric score Q and its standard error are converted to a confidence of class following the Normal distribution approach set out in Appendix B of (Davey, 2009).

The overall EQR is the average of the Chlorophyll-a and Elevated Counts EQRs. If an EQR cannot be produced for one of the tools, then the EQR for the other tool is used for the overall status assessment and CUTLASS produces a warning flag to advise the user that this is the case.

The confidence of class associated with the overall EQR is calculated by first combining the standard errors of the EQRs from the two component tools:

$$SE = \sqrt{\frac{SE_1^2 + SE_2^2}{2^2}} \qquad (11)$$

The overall EQR and its standard error are then converted to a confidence of class following the Normal distribution approach set out in Appendix B of WRc (2009).

This method assumes that the errors of the two tools are uncorrelated.

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Appendix 1:

Water Framework Directive: *Proposed* Metric Change for Coastal Water Phytoplankton classification (submitted to MPTT and MTT August 2012)

Biological Quality Element: Phytoplankton

Water Category: Coastal waters

Classification Tool name: Coastal water phytoplankton toolkit (multimetric)

Metric to be altered: Elevated cell count for coastal waters (*Phaeocystis* submetric).

Geographical zone: England and Wales

Background: For the first River Basin Management Plans (RBMPs) in 2009, coastal water phytoplankton status was assessed using the metrics (i) Chlorophyll 90%th percentile, (ii) elevated cell counts and (iii) seasonal succession.

The 'elevated cell count' metric was based on the integration of the exceedances of four sub-metrics

(i) Exceedances of chlorophyll biomass. Threshold set at 10ug/l
(ii) Exceedances of single taxa (Thresholds set at 250,000 cells/l for Southern waters)
(iii) Exceedances of total taxa (Thresholds set at 1,000,000 cells/l fo Southern waters)
(iv) Exceedances of *Phaeocystis sp.*(1,000,000 cells/l)

Final 'elevated cell count' metric assessment was based on the percentage value against the five class status boundaries, with greater than 20% exceedances equating to moderate or less status. (NB the overall status assessment for a waterbody through the calculation of an Ecological Quality Ratio requires the outputs of all three phytoplankton metrics).

Proposed Change to the Elevated Cell Count Metric:

- The sub-metric for *Phaeocystis sp.* cell taxa is to be omitted from the elevated count tool for the 2nd RBMP
- The final metric will be a combination of three sub-metrics (i) exceedances of elevated chlorophyll biomass, (ii) exceedances of single taxa and (iii) exceedances of total taxa counts.

Justification for Change:

(i) Phaeocystis sp. is only measured sporadically in UK waters
(ii) Phaeocystis sp. is difficult to measure and count under current microscopy methods, adding uncertainty to the reliability of these data.

There have only been 164 sampling occasions over 47 waterbodies (25 England and Wales and 21 Scotland) when *Phaeocystis sp*. has been identified and counted(2000 – 2010), with only 13 waterbodies have reported counts of *Phaeocystis sp*. exceeding the threshold (1,000,000 cells/l). Eleven waterbodies have only ever had one sampling date in which exceedances of the threshold has occurred, while where there have only been multiple exceedances in three waterbodies (see Annex). Thus current data suggests that *Phaeocystis sp.* is not a frequent taxa measured in UK coastal waters.

The use of this sub-metric in the elevated cell count metric may therefore increase the likelihood of falsely inflating the elevated count assessment and consequently the overall EQR for phytoplankton.

Possible issues: *Phaeocystis sp.* is not being counted (measurement issues) but is still present in high numbers in coastal waters. However, elevated counts of any measured taxa, including *Phaeocystis*, are still captured by the remaining single and total cell exceedance metrics.

There may be a research need to identify if this species is ecologically relevant to, and if it is a reliable indicator of eutrophication, in UK coastal waters.

Elevated Count Metric for 2nd RBMP:

It is advised that that elevated cell count metric is composed of three sub-metrics for future assessments - (i) exceedances of 10ug/l for chlorophyll biomass, (ii) exceedances of 250,000 cell/l l for any single taxa; (3) exceedances 1,000,000 cells/l of total taxa. The metric final assessment is based on the mean value of exceedance for the three sub-metrics.

ANNEX (England and Wales Only)

EVIDENCE BASE OF PHAEOCYSTIS EXCEEDANCE (2000-2010)

Waterbodies which have exceeded the Phaeocystis threshold of the 2009 elevated count metric over the period 2000 to 2010. (NB Low Sample numbers in some waterbodies)

Waterbody Name	Sample Number	Phaeocystis > 10^6	Exceedance (%)
Anglesey North	6	1	16.7
Conwy Bay	62	3	4.83
Cumbria	18	1	5.56
Langstone Harbour	9	1	11.1
Mersey Mouth	72	6	8.33
Norfolk East	5	1	20
Norfolk North	3	1	33.3
North Wales	26	5	19.2
Portsmouth Harbour	42	1	2.38
Salcombe Harbour	5	1	20
Wash Outer	86	1	1.16
Whitstable Bay	47	1	2.13
Yorkshire South / Lincolnshire	98	1	1.02

IMPACT ON ELEVATED COUNT METRIC OUTCOME

NB Sub -metric (Sm) scores 1, 2 and 4 are unaffected. Metric outcome is illustrated with and without Sm 3 *(Phaeocystis)*

Sample number	Waterbody Name	Sm1	Sm2	Sm3	Sm4	Final score (4 Sms)	Metric Assessment using 4 Sms	Final score (3 Sms)	Metric Assessment using 3 Sms
5	Salcombe Harbour		40.0	20.0	40.0	25.0	MOD	26.7	MOD
3	Norfolk North	17.9	66.7	33.3	33.3	37.82	MOD	39.32	MOD
5	Norfolk East	34.4	40.0	20.0	40.0	33.59	MOD	38.13	MOD
9	Langstone Harbour	9.5	55.6	11.1	44.4	30.16	MOD	36.51	MOD
18	Cumbria	1.8	11.1	5.6	5.6	6.00	HIGH	**6.14**	HIGH
42	Portsmouth Harbour	5.2	23.8	2.4	16.7	12.02	GOOD	**15.23**	GOOD
62	Conwy Bay	7.8	25.8	4.8	11.3	12.42	GOOD	**14.95**	GOOD
98	Yorkshire South	26.1	10.2	1.0	4.1	10.36	GOOD	**13.48**	GOOD
6	Anglesey North	1.5	33.3	16.7	16.7	17.1	GOOD	17.2	GOOD

26	*North Wales*	*10.0*	*26.9*	*19.2*	*19.2*	*18.8*	*GOOD*	***18.7***	*GOOD*
47	*Whitstable Bay*	*39.9*	*17.0*	*2.1*	*14.9*	*18.5*	*GOOD*	***23.9***	*MOD*
72	*Mersey Mouth*	*69.7*	*26.4*	*8.3*	*13.9*	*29.6*	*MOD*	***36.7***	*MOD*
86	*Wash Outer*	*32.6*	*23.3*	*1.2*	*11.6*	*17.2*	*GOOD*	***22.5***	*MOD*

In the last ten years, there have been **139** sampling occasions in English and Welsh coastal waterbodies where *Phaeocystis* has been identified. Of these *Phaeocystis* has exceeded the threshold (10^6cells/l) on 24 sampling occasions with exceedances identified in 13 waterbodies.

Examples below:.

- Salcombe Harbour – 5 sampling occasions. One *Phaeocystis* exceedance in July 2004
- Anglesey North – 6 sampling occasions. One *Phaeocystis* exceedance in July 2004
- North Wales – 26 sampling occasions. Seven *Phaeocystis* exceedances during period May 2000 to May 2002 (all between March and May). Note that the periods where *Phaeocystis* exceeded 10^6 are the only periods where total taxa exceeded 10^6.
- Whitstable Bay – 47 sampling occasions. One *Phaeocystis* exceedance in May 2001
- Mersey Mouth – 72 sampling occasions. Six occasions of *Phaeocystis* exceedance during period May 2000 to May 2002 (May and July). No exceedances in samples collected since 2002
- Wash Outer - 86 sampling occasions. One occasion of *Phaeocystis* exceedance in June 2001

Appendix 2:

Water Framework Directive: Variable biogeographical conditions across Coastal and Transitional Waters for the assessment of Phytoplankton ((submitted to MPTT and MTT August 2012).

Biological Quality Element: Phytoplankton and nutrients

Classification Tool name: Coastal Waters - phytoplankton toolkit (multimetric)

Metric to be altered: Biogeographical variation and changes in boundary conditions

Background: For the first River Basin Management Plans (RBMPs) in 2009, coastal water phytoplankton status was assessed using the metrics (i) Chlorophyll 90%ile, (ii) Elevated cell counts and (iii) Seasonal succession of phytoplankton functional groups (diatoms and dinoflagellates). The phytoplankton data for the testing of the original boundaries was taken from the existing Environment Agency database. For the continuing purposes of WFD assessment and over the first four years, particularly in England and Wales, phytoplankton was collected at monthly intervals in operational waterbodies. Reference values and geographical boundaries vary across the waterbodies within the UK. Differences in reference or baseline values can influence the boundary values within the phytoplankton tool assessment. A difference in biogeographical conditions affects the value of the threshold, such as the count of phytoplankton taxa or can affect the seasonal variation, such as differences in phytoplankton growth envelopes under the seasonal succession metric.

Description of geographical variation

Latitudinal and geographical differences exist between the different UK marine areas, with northern areas (North East England and Scotland) having colder and longer winter periods with short long day length summers. The longer winter season in the north supports much lower counts of both taxa and biomass over the winter period, whereas the short summer season with longer day length encourages the rapid growth of smaller phytoplankton cells (Gowen et al., 2000; Gowen and Stewart, 2005; Painting et al., 2005; Gowen et al., 2008; Foden et al., 2010).

Water types are separated into two sea zones (Figure 1). Sea Region A is part of the Atlantic sea zone, which is more open and exposed, traps less runoff from the land, low nutrient availability and low measurements of chlorophyll biomass. The chlorophyll reference value for Sea Region A is taken as a maximum value of 3.33µg/L in the growing season (calculated as a 90th percentile measurement). Sea Region B is part of North Sea zone, where the more sheltered water masses can accumulate the more available nutrient rich water. The reference value for the 90th percentile value of chlorophyll biomass during the growing season in the North Sea zone is established at 6.67µg/L. Anthropogenic additions are measured as deviations away from the high/good boundary for both sea zones. These regional differences results in variable boundary values for the 90th percentile chlorophyll metric (Table 1). These geographical differences have also been incorporated into the North East Atlantic Intercalibration, where Sea Region A is characterised at NEA 1/26A and Sea Region B is characterised as NEA 1/26B.

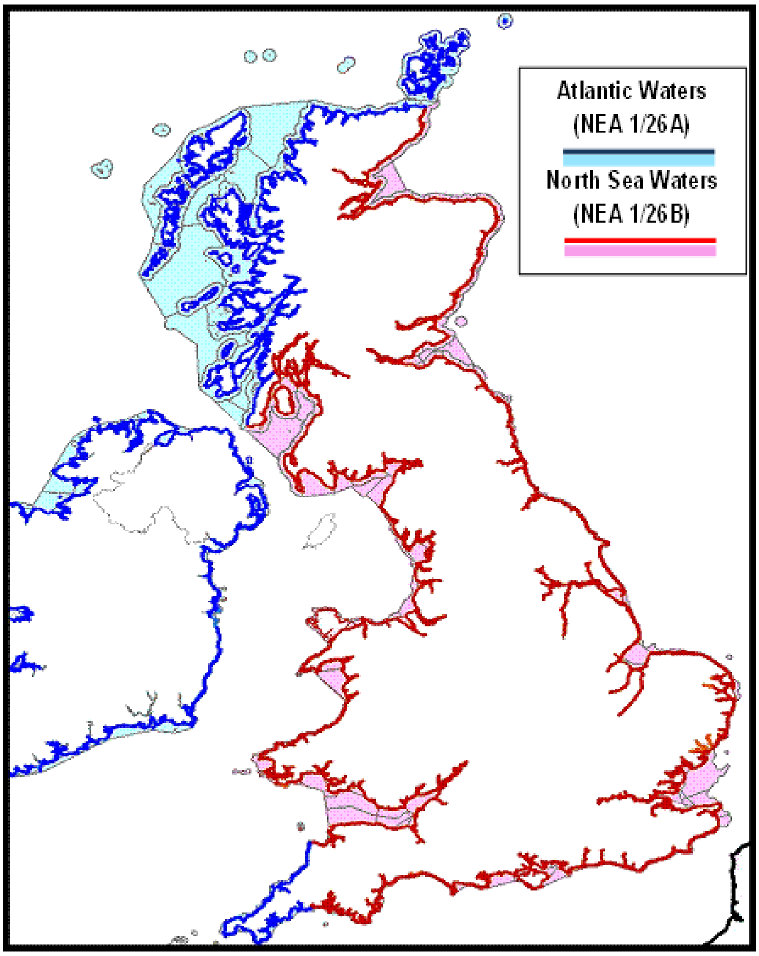

Figure 1: The geographical areas associated with North Sea and Atlantic waters for the chlorophyll metric in coastal waters

	90th ile value	Metric range	Interim Class	EQR
North Sea waters				
Reference: 6.67	0--10	0.671-1.0	High	0.8 - 1.0
	10--15	0.441-0.67	Good	0.6 - 0.8
	15--20	0.341 - 0.44	Mod	0.4 - 0.6
	20--25	0.271-0.34	Poor	0.2-0.4
	>25	0 - 0.27	Bad	0 - 0.2
Atlantic waters				
Reference: 3.33	0--5	0.671 - 1.0	High	0.8 - 1.0
	5--10	0.33 - 0.67	Good	0.6 - 0.8
	10--15	0.22 - 0.33	Mod	0.4 - 0.6
	15--20	0.17 - 0.22	Poor	0.2-0.4
	>20	0 - 0.17	Bad	0 - 0.2

Table 1: Reference conditions and boundary values associated with North Sea and Atlantic waters for the chlorophyll metric in coastal waters

For the UK, phytoplankton communities are divided into northern and southern zones, with geographically distinct phytoplankton communities. These geographical zones are defined with a line joining 55^0 N on the West Coast of Scotland to the Flambourough Front (approximately located at Flamborough Head) as outlined on Figure 2. The geographical conditions have given rise to different regional variations for phytoplankton communities.

This is seen in the different thresholds of the elevated count metric for coastal waters, where the southern (England and Wales) waters have lower thresholds for single taxa counts (250,000 cells L^{-1}) and total taxa counts (10^6 cells L^{-1}) compared to Scotland which has single taxa exceedance threshold of 500,000 cells L^{-1} and total taxa exceedance of 10^7 cells L^{-1}. Regional variations also exist between the maximum reference values used to define the upper contours of the seasonal growth envelope. Variations across all phytoplankton tools are summarised in Table 2.

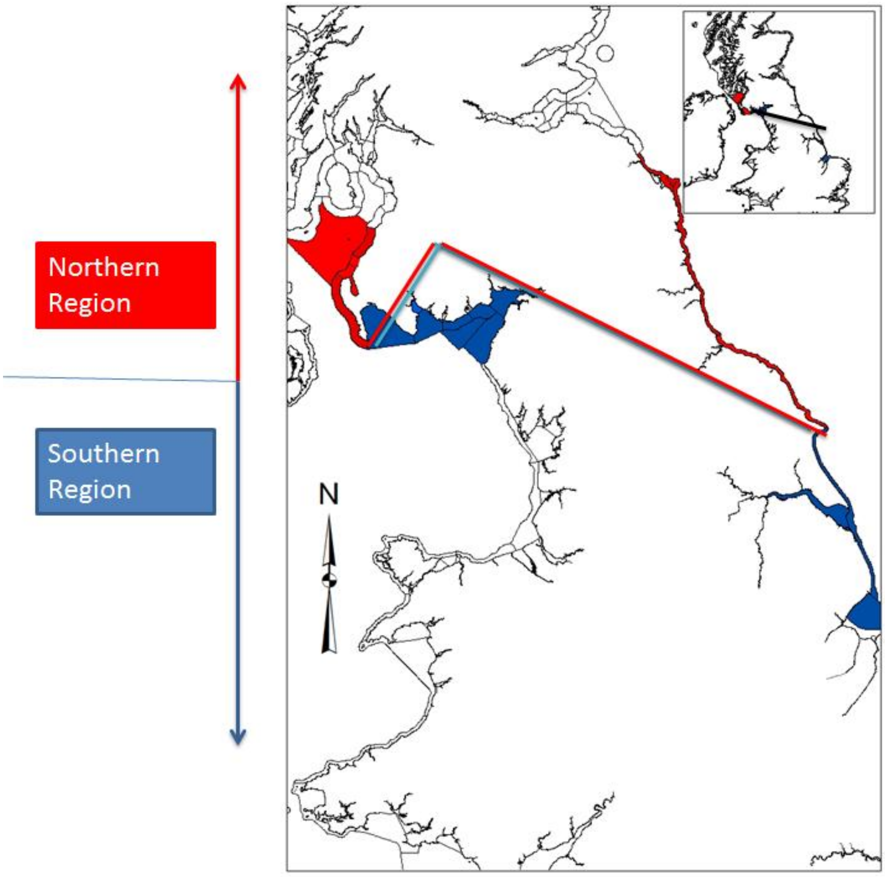

Figure 2: The latitudinal split between northern and southern waters.

Justification for Differences

See Table 2 – Reasoning

Note that at this stage, regional variations in thresholds have been shown for *coastal waterbodies only*. The thresholds applied for the elevated count tool in transitional waters are the same across UK marine waters and the chlorophyll tool for transitional waters has only been used by England and Wales at this time. Scotland is currently testing the applicability of the transitional chlorophyll metric in Scottish transitional waters.

Concerns

- Variation in reference conditions for coastal waters must reflect a measurable difference in the geographically distinct marine areas.
- Variation in thresholds for coastal waters must reflect a measurable difference in the environmental conditions that influence the behavior of phytoplankton in geographically distinct areas.

Table 2: Description of the regional variations within the chlorophyll and phytoplankton assessment tools.

Measurement	metric	Region	Description of variation	Reasoning
Chlorophyll	90^{th} percentile of chlorophyll biomass	Atlantic sea zone	Reference value for the 90^{th} percentile measurement applied to Atlantic waters = 3.33ug/L	Open and exposed, traps less runoff from the land, low nutrient availability and low measurements of chlorophyll biomass (Gowen et al., 2000; Foden et al., 2010).
Chlorophyll	90^{th} percentile of chlorophyll biomass	North Sea Zone	Reference value for the 90^{th} percentile measurement applied to North Sea waters = 6.66ug//L	More sheltered water masses can accumulate the more available nutrient rich water.
Phytoplankton	Elevated count of taxonomic groupings	Scotland	Taxonomic groupings include 3 metrics including exceedances of chlorophyll counts (10µg/L), single taxa counts (500,000cells L^{-1}), total taxa counts (10^7cells L^{-1})	Long day length, short summer promoting higher abundance of smaller phytoplankton (Gowen and Stewart, 2005).
Phytoplankton	Elevated count of taxonomic groupings	England, Wales and **NI**	Taxonomic groupings include 3 metrics including exceedance of chlorophyll counts (10µg/L), single taxa counts (250,000cells L^{-1}), total taxa counts (10^6cells L^{-1})	Shorter day length.
Phytoplankton	Seasonal succession of taxonomic groupings.	England and Wales	Seasonal succession of two distinct taxonomic groups including diatoms and dinoflagellates. Reference curves are one sided (maximum Z score only). Reference curve based on E&W reference site – Plymouth outer coast	Upper envelope only - Lower envelope (minimum value) exceedance does not signify nutrient enrichment (Devlin et al., 2007a) Reference envelope reflects the seasonal pattern of diatoms and dinoflagellates based on EW long term reference site.
Phytoplankton	Seasonal succession of taxonomic groupings.	Scotland	Seasonal succession of three distinct taxonomic groups including diatoms, dinoflagellates. Reference curves are two sided (minimum and maximum Z score). 2 Reference curves based on Scottish reference sites	Upper envelope only - Lower envelope (minimum value) exceedance does not signify nutrient enrichment. Reference envelope reflects the seasonal pattern of diatoms and dinoflagellates based on Scottish long term reference site.
Phytoplankton	Seasonal succession of taxonomic groupings.	Northern Ireland	NIEA have no reference sites so are not using the tool	

Outcomes

Differences in regional outcomes were analysed using the variations in reference values and thresholds by applying the different thresholds to all waterbodies within UK marine waters. The outcomes of this analysis are shown in Annex 1. Regional variations in thresholds have been shown for coastal waterbodies only. The thresholds applied for the transitional elevated count tool are the same across UK marine waters and the transitional chlorophyll tool has only been used by England and Wales at this time.).

Altering the boundary values for the 90^{th} percentile tool would move just under 50% of E&W waterbodies from high to lower classes, including 19 (up from 9) falling moderate and below. The Scottish assessment shows more of the good classification moving up to high classification. This supports the chlorophyll reference value being lower in the Northern regions (5μg/L) and higher in the Southern regions (10μg/L).

For phytoplankton counts, the regional differences are a doubling of the single taxa threshold from 250,000 to 500,000 cells/L from southern to northern waters and a order of magnitude difference between the total taxa threshold (10^6 cells/L to 10^7cells/L). E&W also count the occurrence of high numbers of *Phaeocystis* sp. Variations of the thresholds applied to E&W and Scottish waterbodies show that the E&W outcomes do not change substantively in any of the variations. The difference between using the lower thresholds of 250,000 cells/L and 10^6cells/L to the higher thresholds of 500,000 cells/L and 10^7 cells/L affects only 3 waterbodies moving from above moderate to below. The use of the fourth index (Phaeocystis sp) only moves waterbodies from a good to high status. This reflects that the majority of E&W waterbodies have low counts of phytoplankton taxa, and the lower thresholds are recommended for the southern waters. In contrast, the Scottish waters, using recommended thresholds of 500,000 cells/L and 10^7 cells/L for single and total taxa counts, have 39 waterbodies classifying above moderate, which falls to only 18 when using the lower thresholds of 250,000 cells/L and 10^6 cells/L. Thus it is important for Scottish waters to apply the regional threshold to avoid an overestimate of waterbodies failing.

Outcomes of the seasonal succession tool show clearly that the shape of the seasonal growth curve is regionally influenced with high numbers of failures for waterbodies when using other regional growth curves (Table 3). The growth curve is defined by the minimum (for Scottish waters) and maximum monthly z scores for the taxonomic groupings and have been constructed by the use of reference phytoplankton data.

Table 3: Outcomes of the different permutations of the WFD phytoplankton classification metric applied to England and Wales waterbodies.

England and Wales waterbodies								
		Variation		Classification count				
	Index		**No. WB**	**High**	**Good**	**Mod**	**Poor**	**Bad**
Chlorophyll	**90th percentile**	H/G threshold set at 5ug/L	65	27	19	10	4	5
		H/g threshold set at 10ug/L	65	45	11	4	1	4
Phytoplankton	**Elevated Taxa Count**	4 indices, chlorophyll (10), TT threshold (10^6), SS threshold (250,000) and Phaeo sp (10^6)	38	28	8	1	0	0
		3 indices, chlorophyll (10), TT threshold (10^6)and SS threshold (250,000)	38	25	9	4	0	0
		3 indices, chlorophyll (10), TT threshold (10^7), SS threshold (250,000)	38	27	9	2	0	0
		3 indices, chlorophyll (10), TT threshold (10^6), SS threshold (500,000)	38	28	8	2	0	0
		3 indices, chlorophyll (10), TT threshold (10^7), SS threshold (500,000)	38	31	6	1	0	0
	Seasonal Succession	E&W reference curve. No Microflagellates. Upper thresholds	38	11	12	15	0	0
		Scottish reference curve. No Microflagellates. Upper thresholds	38	1	3	21	12	1
		NI reference curve. No Microflagellates. Uppe thresholds	38	1	9	27	1	0

Scottish waterbodies								
		Variation		Classification count				
	Index		**No. WB**	**High**	**Good**	**Mod**	**Poor**	**Bad**
Chlorophyll	**90th percentile**	H/G threshold set at 5ug/L	52	34	8	5	2	1
		H/g threshold set at 10ug/L	52	42	5	2		1
Phytoplankton	**Elevated Taxa Count**	4 indices, chlorophyll (10), TT threshold (10^6), SS threshold (250,000) and Phaeo sp (10^6)	46	7	24	14	1	0
		3 indices, chlorophyll (10), TT threshold (10^6)and SS threshold (250,000)	46	3	15	23	5	
		3 indices, chlorophyll (10), TT threshold (10^7), SS threshold (250,000)	46	9	16	17	4	1
		3 indices, chlorophyll (10), TT threshold (10^6), SS threshold (500,000)	46	9	23	14	0	0
		3 indices, chlorophyll (10), TT threshold (10^7), SS threshold (500,000)	46	20	19	7	0	0
	Seasonal Succession	E&W reference curve. No Microflagellates. Upper and lower thresholds	48	1	5	23	19	5
		Scottish reference curve. Includes Microflagellates. Upper and lower thresholds	46	2		11	37	3
		NI reference curve. Includes Microflagellates. Upper and lower thresholds	46	1	3	26	20	3

Appendix 3:

Water Framework Directive: Proposed Metric Change for Coastal and Transitional Water Phytoplankton classification (submitted to MPTT and MTT August 2012)

Biological Quality Element: Phytoplankton

Water Category: Coastal and transitional waters

Classification Tool name: Coastal and Transitional waters phytoplankton toolkit (multimetric)

Metric to be altered: Elevated cell count for TW and CW waters and seasonal succession for CW waters

Background: For the first River Basin Management Plans (RBMPs) in 2009, coastal water phytoplankton status was assessed using the metrics (i) Chlorophyll 90%ile, (ii) Elevated cell counts and (iii) Seasonal succession of phytoplankton functional groups (diatoms and dinoflagellates). The phytoplankton data for the testing of the original boundaries was taken from the existing Environment Agency database. For the continuing purposes of WFD assessment and over the first four years, particularly in England and Wales, phytoplankton was collected at monthly intervals in operational waterbodies. The data was analysed, where possible using a full taxa list. The total number of taxa identified is 449 taxa, either at a species or genera grouping. The phytoplankton enumeration is carried out by number of different agencies with variations in methods and expertise and could potentially lead to differences in outcomes.

A Reduced Taxa List (RTL) was put forward as a set list of phytoplankton taxa which would be used by all UK agencies in in classifying phytoplankton community. The RTL was developed by FRS in consultation with the Marine Plant Task Team. The current version of RSL has 194 genera/groupings , of which a number of groupings are defined by size. If size is unknown, then the species will fall under a general genera descriptor.

Note that there are a number of records which have now been deleted and are not included in any ongoing tool analysis. For example, *Mallomonas*, *Meringosphaera*, *Parapedinella*, *Pediastrum* and *Plagioselmi*s. A full list of deleted species is included within Appendix 1.

Proposed Change to the Elevated Cell Count Metric for CW and TW and Seasonal succession metric for CW:

- Move from Full Taxa list to a Reduced Taxa List. The number of species or genera groupings accounted for in the RTL is 250 generic groupings. The RTL data would contribute to the phytoplankton metrics in coastal and transitional waters.

Justification for Change:

(i) Difficulty in sampling and identifying the smaller taxa.
(ii) Consistency between analysts
(iii) Increased costing for analyst to identify smaller or more difficult taxa

Concerns

- The outputs of the current phytoplankton metrics would change using the different taxa groupings within the calculation of the phytoplankton metrics.
- The genetic diversity of the phytoplankton community information would be reduced and may potentially impact on our understanding of undesirable disturbance as measured by the phytoplankton data.

Outcomes

The outputs of the current phytoplankton metrics would change using the different taxa groupings within the calculation of the phytoplankton metrics.

- Use of the RTL in the seasonal succession metrics does NOT have an impact on the final classification boundaries. The seasonal succession metric calculates the number of diatoms and dinoflagellates per month over the sampling period. Counts within these functional groups are the same using both FTL and RTL. Classification down to individual species is not relevant for this metric.
- The use of the RSL in the elevated count tool does have a slight impact on the outcomes of the individual sub-metrics. It does not affect the total cell count sub-metric as all taxa are summed over each sampling occasion and classification to individual species is not relevant. However it does have slight impact on the individual species index as there are a small number of occasions where single cell counts are increased through the grouping of taxa within the RTL.
- NOTE that many of the taxa counted in the FTL are identified to genera only (17 out of the top 30 occurrences) and link to a similar generic grouping under the RTL. Thus little or no information is lost for that taxa grouping.
- However, the overall outcomes are not changed between FTL and RTL due to the very small number of occasions in which the single species counts are altered. Thus the exceedances (expressed as a %) are only affected minimally and no change related to a classification boundary change.
- The difficulties in identification to full species when using the FTL. The use of lugols in sample collection and the turbidity issues in UK waters can make identification to species difficult, particularly for the smaller genera. Thus there are many occasions where analysts have only identified to the broader genera grouping.
- Low counts of taxa. A large number of species in UK waters are counted at low numbers outside of the growing season and increasing the taxa counts through larger groupings still does not significantly increase taxa counts as to fail the threshold (250, 000 and 500,000 cells per litre for the single species index for E&W and Scotland respectively). High counts of smaller taxa, which can typically occur during the bloom periods or through the growing season, can be dominated by small, less easily recognised taxa and thus they have already been grouped to a very generic grouping or listed as "other".
- Thus in investigating the number of occurrences for each species or grouping under the RTL does not affect the outcomes of the current phytoplankton metrics.
- The genetic diversity of the phytoplankton community information would be reduced and may potentially impact on our understanding of undesirable disturbance as measured by the phytoplankton data.
- Counts of abundance (N) and counts of phytoplankton taxa (S) differ when applying the RTL as opposed to the FTL. As expected, the number of species counted per month are higher for the FTL (Fig. 1)

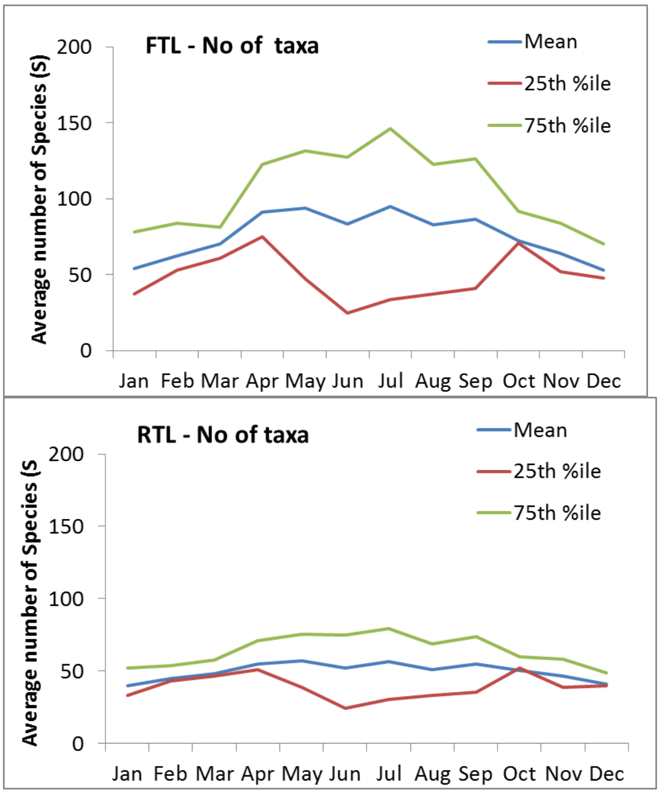

Figure 1: The average number of species counted per month over all waterbodies. The 25th and 75th percentiles over each month are also calculated for both taxa lists

- Species counts are similar over the mean values, however there are higher values associated with the 75th percentiles, representing the higher occurrence of unknown or smaller taxa which have been grouped into a generic description within the RTL. There are taxa groups which link to multiple species within the FTL and are incorporated into one or two genera groupings under the RTL. Thus if you did have high numbers of individual species in this grouping, it would be expected that species counts would be reduced as shown in Figure 1.
- The higher variability may also be influenced by the rare species, which affect the species count but do not influence the phytoplankton metric outcome.
- The variation in species count can be clearly seen through each water type over each month (Fig. 2). The highest level of similarity occurs in the winter months, where number of species and abundance is low. In contrast, the dissimilarity between numbers of species is highest over the growing season. There are also differences in water types, with the largest differences occurring in the CW5 and TW3 waterbodies. This indicates that the grouping of taxa could be type specific and related to ecological and physical functioning.
- Further work is required on the ecological functioning of the taxa groupings and if the RTL affects the assessment of diversity and functioning with the UK phytoplankton community.

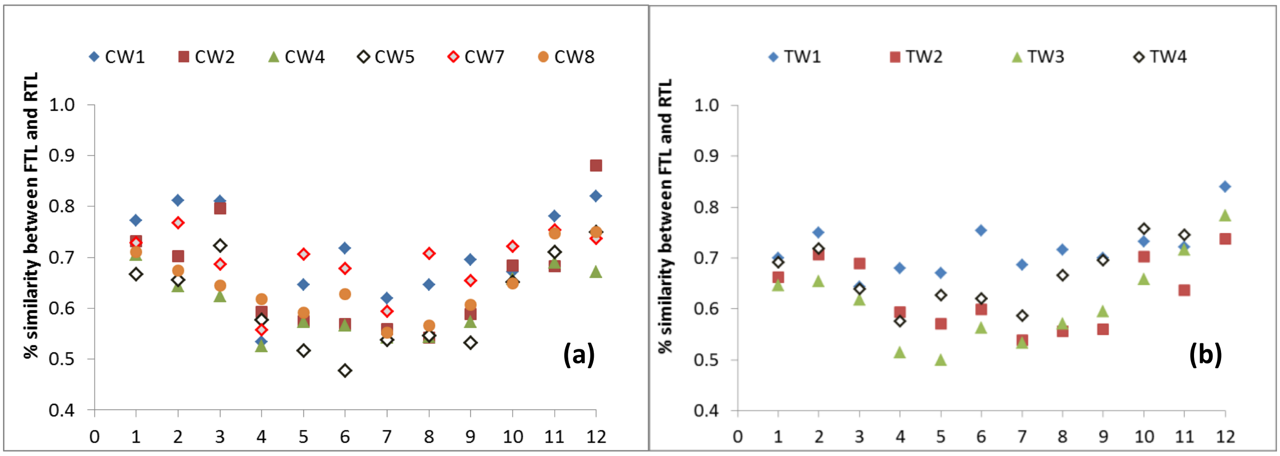

Figure 2: The difference in species number for each water type. Data is averaged over each month(1 – 12) and presented for coastal waters (a) and transitional waters (b).

Elevated Count and Seasonal succession Metric for 2nd RBMP:

- Use of the RTL in the current phytoplankton metrics does not influence the outcomes of the metrics or impact on the overall assessment of UK waters using the phytoplankton biological quality element.
- The RTL has no impact on outcomes for the CW and TW elevated count metric, or in the seasonal succession metric for coastal waters.
- Thus the RTL is a useful, cost and time saving method that allows faster and more homogenous identification of UK phytoplankton community and can be applied to the current WFD process.
- However some caution needs to be applied if further work on diversity or functioning is required in ongoing assessments, both for the WFD and potentially the Marine Strategy Directive.