Guidance on the quantitative analysis of phytoplankton in freshwater samples

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1. Introduction

Phytoplankton are increasingly being used to monitor the ecological quality and health of the water environment and also to measure the effectiveness of management or restoration programmes or regulatory actions.

The European Water Framework Directive (2000/60/EC) requires member states to monitor phytoplankton abundance and composition and a uniform procedure has been developed by CEN.

The following guidance has been developed with reference to the CEN standard "Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)" (CEN 2004), Test Methods and Procedures: Freshwater Phytoplankton NRA (1995) and "PL100 Quantitative and qualitative phytoplankton analysis" (SYKE) as well as reference texts such as Utermöhl (1958) and Lund , Kipling and LeCren (1958).

Analysis should be carried out using sedimentation chambers with an inverted microscope (Utermöhl technique).

This method is suitable for studies investigating the abundance, composition and biovolume of phytoplankton in rivers and lakes.

2. Terms and definitions

The terms and definitions used are as those as described in "Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)" CEN 2004.

3. Principles

The quantitative analysis described here includes the identification, enumeration and calculation of biovolumes of Lugol's iodine preserved water samples.

The preserved sample is thoroughly mixed and a sub-sample of known volume is placed in a sedimentation chamber. When the algae have settled to the bottom of the chamber, they are counted and identified using an inverted microscope.

The statistical reliability of the analysis depends upon the distribution of algal units/cells within the sedimentation chamber and assumes that the algae are randomly distributed within the chamber. If the algae are randomly distributed (and comply with a Poisson distribution) then a 95% confidence limit of +_ 20% can be achieved by counting about 100 algal units (Lund, Kipling and LeCren, 1958). Note that random distributions are not always achieved in sedimentation chambers so alternative protocols or methods may have to be used.

The counts for individual taxa are converted to algal biomass by using the cell/unit volume of the count units. The volumes are based on measurements made during counting.

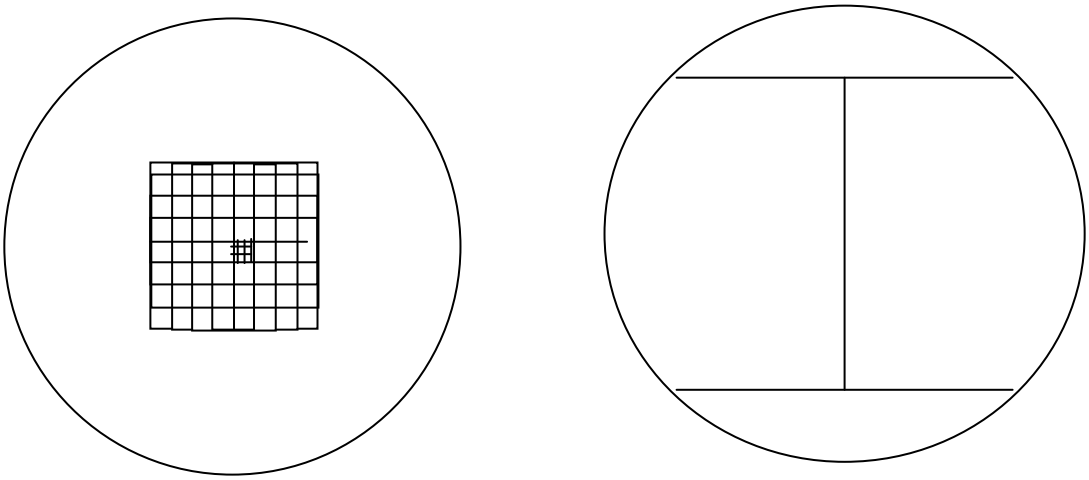
4. Equipment

- Sedimentation chambers of 5 to100ml capacity (Hydro-Bios plankton chambers or similar are recommended). Sedimentation chambers with volumes greater than 10mls are usually combination chambers and consist of a base plate and upper removable column which is slid aside once the algae have settled.

- Inverted microscope with phase contrast (and/or DIC/Normarski) including:
    - long working distance condenser with numerical aperture of >0.5
    - 10x or 12.5x binocular eyepieces, one with a square grid e.g. Whipple eyepiece graticule, Miller Square or similar, and another with a cross-hair graticule (Figure 4.1)
    - low power objective (5x or 10x)
    - 10x, 20x, 40x and 100x oil immersion, phase &/or DIC objectives
    - ideally the microscope should be fitted with a (digital) camera
    - a mechanical stage

Figure 4.1 Eyepiece graticules

(a) Whipple graticule **(b) cross-hair graticule**



- Variety of pipettes with wide bore tips

- Glass cylinders for initial sedimentation

- Supply of ultra high purity or membrane filtered water is required for topping up, diluting and general cleaning.
- Supply of acidified Lugol's iodine. Make up by dissolving 100g of KI (potassium iodide) in 1 l of distilled water then adding 50g I (iodine). Shake until all dissolved and add 100g of glacial acetic acid. Store in dark bottle.

- Computer with algal counting spreadsheet.

Calibration of equipment

- Each counting chamber should be marked with a unique mark or number and a note made of the counting chamber area. This is calculated by measuring the cover slip aperture (rather than the chamber itself) using either a vernier gauge or

the microscope stage vernier if one is present. The mean of 5 diameters should be taken and the area of the chamber calculated using the formula πr^2. Both the measurements of the diameters and the chamber volume should be recorded against the individual counting chamber in a log book.

- All eyepiece/graticule and objective combinations should be calibrated with a stage micrometer (e.g. 100µm x 10µm divisions) and the dimensions and areas of counting fields, transects and the whole chamber area should be calculated for each of the magnifications used and recorded in a log book.

5. Preparation of samples

5.1 Acclimatisation.

Stored and preserved samples, sedimentation chambers and all equipment used should be allowed to acclimatise to the same (room) temperature for at least 12 hours (preferably 24 hours). This has been found to be one of the most important factors in achieving a random distribution of algal cells in the chambers.

5.2 Sample mixing.

Just before taking a sub-sample to fill the sedimentation chamber, the sample must be thoroughly mixed. It is recommended that the mixing is done manually and that this is standardised; the sample should be mixed using a combination of alternating horizontal rolling and vertical tumbling (turning upside down) of the sample bottle for 2 minutes. These actions should be **gentle** and not involve any vigorous shaking.

5.3 Sub-sample preparation and setting up chambers.

After thorough mixing, a known volume of sample is used to fill the sedimentation chamber. The method and care taken to fill the chambers is crucial as it determines the final distribution of settled algae in the chamber. If care is taken then a random distribution allows uniform counting strategies and statistical methods to be used. If a random distribution is not achieved then alternative and often more complex methods must be employed.

The exact volume of sample used to fill the chamber depends on the phytoplankton density. Large volumes of up to 100 ml may be required for oligotrophic waters whilst at high phytoplankton densities dilution may be required.

Ideally, enough sample should be taken to completely fill the chamber in one addition, either directly pouring from the sample bottle or using a wide-bore pipette. Fill a little more than needed and allow a little to over-spill the chamber when you slide the lid across.

This recommendation, to fill the chamber in one addition, raises a number of difficulties for samples with either very low or very high phytoplankton densities. A number of options are available for dealing with varying densities of phytoplankton:

1) Use a sedimentation chamber of an appropriate size depending on how abundant the algae are (chlorophyll concentrations may be used as a guide if available). For example use a 2.5 ml chamber if densities are high or a 10 ml chamber if densities are low.

2) For very low densities, a pre-concentration step may be necessary. Let sample settle in a measuring cylinder - usually 250 ml is sufficient. Leave for 3 days, then draw off top water leaving 25 ml at bottom of cylinder (i.e. x10 concentration). If needed this can be repeated with up to 4 250 ml cylinders and the 4 lots of 25 ml then poured into a 100 ml measuring cylinder for a second pre-concentration to 10 ml (i.e. x100 concentration).

3) For very high densities, where 2.5 or 5 ml of sample is too much it may be necessary to add a smaller measured volume. Use an accurate wide-bore pipette and add 0.5 or 1 ml of sample to the chamber, then top up with distilled water. You must be very careful not to add too much water - so none spills over. The alternative is to count fields at x100 magnification.

A general rule is to aim for about four counting units per field of view at high (×400) magnification.

The following points should be noted:

- ensure all equipment and sample are acclimatised to room temperature and be as constant as possible.

- place the sedimentation chamber on a horizontal flat surface – a perspex or thin acrylic board (which is a poor heat conductor) is ideal – and it should be placed away from strong heat, light and vibration sources.

- take enough sample, either directly from the bottle or with a pipette, to completely fill the chamber in one addition.

- close the chamber with a thick cover slip, making sure air bubbles are avoided.

- make a note of the sample volume, sample site and date next to the chamber or label the flat sedimentation board.

- chamber volumes should be measured accurately as their volume rating is only a guide (5 ml chambers can range from 4.7 - 5.2 ml). To measure chamber volume, weigh the chamber and lid whilst empty, then fill with distilled water and re-weigh. The weight in grammes is equivalent to volume in ml. Repeat three times and record the average.

- allow contents to settle, undisturbed, for at least 4 hours per cm height of chamber. For 10 ml HydroBios chambers settle for at least 12 hours and for 50 ml chambers at least 48 hours settling time is recommended.

- if there are large numbers of buoyant cyanobacteria present you can add either a drop of diluted detergent or glacial acetic acid to the chamber before closing the chamber with the cover slide.

- after sedimentation if combination chambers are used, then slide the chamber column aside and replace it with a thick cover slide. With both combination chambers and 5 or 10 ml HydroBios type chambers, check for and try to avoid introducing any air bubbles at this stage. This can be eliminated by carefully topping up with UHP or membrane filtered water from a dropper pipette whilst sliding the cover slide back into place.

- the sedimentation chamber should be gently moved to the microscope stage. Open chambers should not be moved as the settled algae will be easily disturbed.

After the appropriate settlement period and before counting two checks need to be made:

1. the overall distribution pattern of particles should be checked using a stereo zoom or inverted microscope at very low power (4x or 10x objectives). A random (Poisson) distribution is required and this is recognised by the irregular pattern, often with open spaces. If particles are not randomly distributed and for example are concentrated in one area of the chamber or found in concentric rings towards the edge of the chamber then a new sample should be set up. The distribution of particles/algal cells or units should be checked from time to time and this can be done using the methods outlined in Annex F of the CEN method. The simplest of these being to undertake a count of one taxa and calculate the variance to mean ratio – this approximates the Chi squared distribution for n-1 df – the result is then checked using a goodness to fit test for Chi squared.

2. If the algal density is too low or too high then another sample should be set up and the volume adjusted accordingly. It can sometimes be extremely difficult to judge the correct volume but the general advice is

   - if there are too many particles then they may not settle independently and pile up, also it can be very difficult to count and can lead to inaccuracies from "fatigue"
   - if there are too few particles, the errors increase especially when counting random fields or transects and large areas of the chamber need to be observed. The density of detritus or non-algal particles is also important especially if algal densities are low, and skill is needed to judge the ideal volume to sediment.

6. Counting

6.1 General

The counting procedure involves recording the taxa observed and the number of algal units (objects) for each taxon in a known area of the counting chamber. As the volume of sample added and area of the whole chamber observed is recorded, the concentration of each individual taxon can then be calculated.

The observed taxa are identified to the required taxonomic level (see section 6.3). **It is very important to remember that it is better to correctly identify algae to lower taxonomic level than misidentify to a higher level**.

It is useful to scan the sample at a variety of magnifications before the quantitative analysis is undertaken and to compile a taxa list before beginning the count.

If there is evidence of significant benthic contamination or littoral taxa present (eg periphyton) such that the open water taxa are obscured, then it may not be worth undertaking a full count.

Where small numbers of littoral or benthic taxa such as *Surirella* and *Nostoc*, are present, they should not be counted.

6.2 Counting procedure

The count should be carried out in the following manner;

- a low magnification (e.g. x 40 or x100), whole chamber count to pick up large taxa, followed by;

- transect counts at an intermediate magnification (x250), which are helpful to enumerate “intermediate-sized” taxa that are too small for the low-magnification count but too large to be reasonably counted using fields of view at high magnification, followed by;

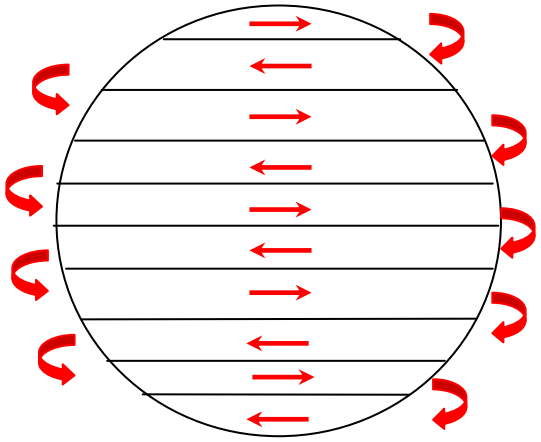
- a high magnification count (x400 or greater) using fields of view. This picks up the small taxa. Aim to count 100 fields of view (i.e. about 400 units assuming the recommended sample concentration)

Details are provided in sections 6.2.1 to 6.2.3 below.

6.2.1 Counting the whole chamber at low magnification for large taxa.

Working at low power (x40 to x100) the whole chamber should be scanned in a series of horizontal transects (figure 6.1) and the larger taxa (e.g. *Ceratium*), large colonial or filamentous forms (e.g. *Microcystis*, *Fragilaria*) counted. A cross-hair graticule eyepiece (figure 4.1) is used when counting the whole chamber. Algae that lie between the two horizontal lines are counted as they pass the horizontal line. Algal objects that cross the top line are included whilst those crossing the bottom line are not and will be counted on the next transect (or vice versa).

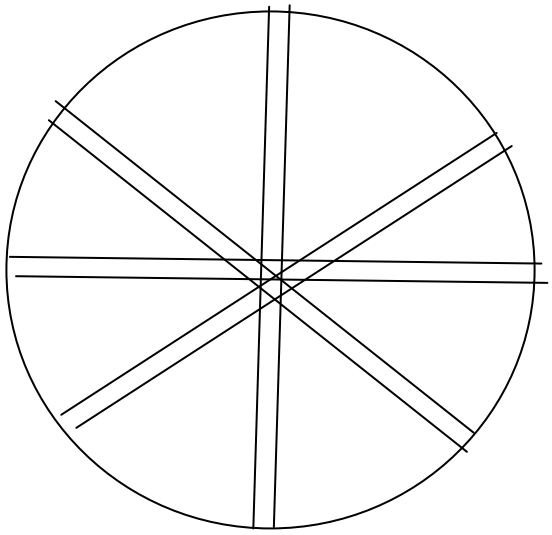
Figure 6.1 Counting method for whole chamber.



6.2.2 Counting transects.

Algal objects larger than approximately 20 µm (small *Cryptomonas*) can be counted at a magnification of approximately x200 in 3 - 5 randomly chosen diameter transects of the counting chamber (figure 6.2). The cross-hair eyepiece and method for counting algal objects described in the section above is used also. The chamber is rotated between transect to randomly chosen positions.

Figure 6.2 Counting method for diameter transects.



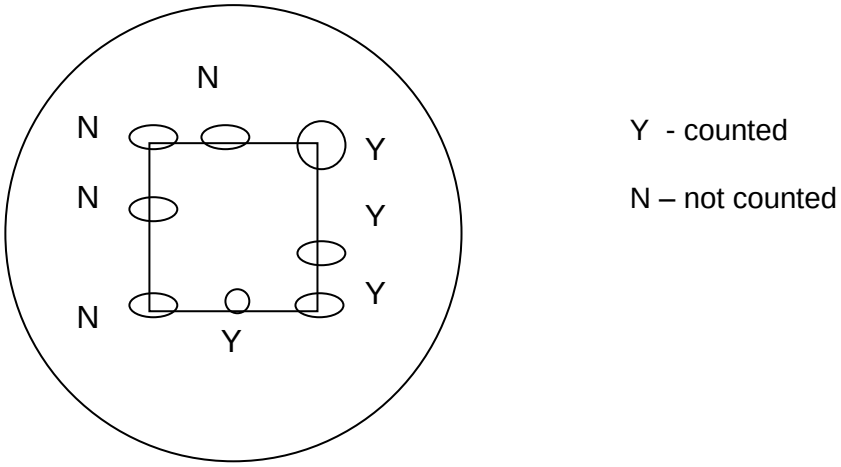
6.2.3 Counting randomly selected fields.

Small algae, less than about 20 µm (e.g. *Rhodomonas*, small centric diatoms), should be counted in 100 (or more) randomly selected fields at x400 magnification (or greater) using a square or Whipple graticule, Miller Square or similar in the ocular eyepiece to delineate the counting area. Fields can be selected either in a pseudo-random way by the counter or using a mechanical stage with a vernier that allows random positions to be found from random number coordinates or using an electronic stage with built in random position control.

A tally of the number of fields counted is required as well as the counts of individual identified algal units (cells, colonies or filaments).

When counting random fields it is important to take a consistent approach to decide whether algal objects lying across the grid lines are counted in or out. A simple rule should be adopted as described in the CEN method (2004) e.g. algal objects (cells, colonies or filaments) crossing both the top and the left hand side of the grid are not counted whilst those crossing the bottom or right hand side of the grid are counted (see Figure 6.4).

Figure 6.3 Example of rule for counting cells on edge of field



6.2.4 Point to consider when counting

- **Algal objects and counting units**: Algal objects or counting units are independent algal cells, colonies or filaments/trichomes. One species or taxa may be present in the sample as different counting units and may be counted at different magnifications. For example, *Microcystis* colonies will probably be counted in the whole-chamber or transect but individual *Microcystis* cells (which may be present if colonies are disintegrating) will be counted in random fields. Similarly *Dinobryon* colonies are most likely to be counted in diameter transects and single *Dinobryon* cells will be counted in random fields.

Other examples of counting/algal units include:

- Colonies e.g. *Aphanothece, Coelosphaerium, Sphaerocystis*
- Algal cells which can occur as single cells but also form colonies, e.g. *Aulacoseira, Dinobryon, Melosira*.
- Colonies which have more or less permanent cell numbers, e.g. *Desmodesmus/Scenedesmus* (2, 4 or 8 cells), *Pandorina* (16 cells) *Crucigenia* (4 cells)
- Filaments or trichomes e.g. *Anabaena, Aphanizomenon, Oscillatoria, Planktothrix*
- Colonies where the size and shape vary e.g. *Microcystis*

- **Calculating cells per colony/filament** – it may be necessary to estimate the numbers of cells per colony or filament and if this is the case then the colonies or filaments should be treated as individual algal objects or units as described above. For some taxa the cell numbers per colony may be consistent or have several modes as illustrated above whilst for others the cell numbers do not have a

consistent distribution e.g. *Microcystis* where the number of cells per colony can vary from a few cells to several million cells.

For estimating biovolumes of colonies or coenobia:-

- Using cell volumes – make direct counts of cells in 'sub-colonies' or small areas. These can then be multiplied up by number of 'sub-colonies' or the ratio of small area to whole colony to get the total cell numbers, e.g. *Microcystis, Woronichinia*, etc.
- Using colony/coenobium measurements – measure colony width and depth e.g. *Pediatrum*, *Microcystis* (using Reynolds & Jaworski's formula embedded in counter spreadsheet)

For estimating biovolumes of filaments:-

- Using filament measurements – calculate mean dimensions by measuring the length and diameter of at least 30 filaments. For high-magnification random field of view counts, only the lengths of the filaments lying within the grid should be measured. For whole chamber or transect counts at low or intermediate magnification whole filament lengths can be measured.
- Using cell volumes – combine counting of filaments with the mean numbers of cells per filament, e.g. *Aphanizomenon*
  - Count the number of filaments in the normal way (transects or random fields) and measure the length of at least thirty filaments to calculate the average length (see above for difference in measuring filament length between transect and random fields approaches)
  - From up to 10 filaments, calculate the average number of cells per unit length (e.g. 20 µm). This can be measured at a higher magnification if the cells are small or hard to distinguish easily (e.g. some species of *Oscillatoria)*.
  - Then the number of cells per filament is calculated by multiplying up the average filament length by the average number of cells per unit length.
- Where the algae form spiral filaments e.g. *Anabaena circinalis,* the average number of cells per gyre is counted and then the number of gyres per filament is estimated. The two numbers are multiplied together to give the estimated number of cells per filament.

6.3 Identification and coding

Appendix A provides a list of taxa which is to be used to guide the required level of identification. It includes Whitton Codes, accepted names, biovolume formulae and biovolume ranges, where available. If taxa can be identified but are not included within this list, photographs and drawings (including measurements) should be taken and the inclusion of the 'new' taxa to the list should be checked with the Project Manager.

The standard flora for identification is the Freshwater Algal Flora of the British Isles (Whitton et al., 2003) but other identification guides are also available and may be used if they prove more helpful for certain taxonomic groups (see Section 10).

It is very important to remember that it is better to correctly identify algae to lower taxonomic level than misidentify to a higher level.

The following codes and accepted names have been adopted for the purposes of WFD phytoplankton enumeration for 'difficult' taxa following a workshop of many of the UK analysts (Table 6.1). These have been incorporated into the taxa list in Appendix A.

Table 6.1 Codes agreed for taxa of specific size classes or unidentified taxa groups commonly recorded by UK counters

Whitton Code	Accepted name
05040001	*Cryptomonas* sp. (small) Length <20 µm
05040002	*Cryptomonas* sp. (medium) Length 20-30 µm
05040003	*Cryptomonas* sp. (large) Length >30 µm
05100020	*Rhodomonas lens*
09550000	*Pseudopedinella* sp.
12000001	Small centric diatom (5-<10 µm diameter)
12000002	Medium centric diatom (10-20 µm diameter)
12000003	Large centric diatom (>20 µm diameter)
12000004	Very small centric diatom (<5 µm diameter)
13000001	Small pennate diatom (Length <10 µm)
13000002	Medium pennate diatom (Length 11-20 µm)
13000003	Large pennate diatom (Length >21µm)
90000000	Picoplankton - unidentified single cells <2 µm diameter
90000003	Nanoplankton - unidentified non-flagellate cells, 2–20 µm length
90000004	Unidentified cells >20 µm diameter
90000005	Nanoplankton - Unidentified flagellates 2–20 µm length

Verification of species identification should be carried out for any difficult species, especially those of cyanobacteria, chrysophytes or green algae by sending samples with drawings, photographs and measurements to taxonomic experts.

Intra and inter laboratory identification comparisons should be carried out on a regular basis to avoid and minimise identification difficulties. Quality assurance and validation of counts is described in detail in section 8 below.

7. Calculation of phytoplankton biovolume

Biovolumes must be measured for all taxa and is done by assigning simple geometric shapes to each cell, filament or colony, measuring the appropriate dimensions and inputting these into formulae to calculate the cell volume.

The counting spreadsheet which will accompany this guidance includes, for all the taxa listed in Appendix A, a fixed, pre-determined, formula for the biovolume of each taxon. All that is required is for the appropriate average dimensions to be input to the spreadsheet so that the biovolume can be calculated automatically (see points listed below).

Measurements of the required cell dimensions (length, width, diameter) are made at an appropriate magnification using a calibrated ocular eyepiece, e.g. a Whipple Graticule. The eyepiece is rotated so that the scale is put over the required cell dimension and the measurement made by taking the ocular measurement and multiplying by the calibration factor for that magnification and eyepiece combination.

The following points should be noted:

- it is important to measure the linear dimensions of at least ten individual units of all taxa observed in the sample and for taxa of more variable size, at least 20

individuals should be measured to estimate mean dimensions. If the cells are very variable then up to 50 cells should be measured.

- for some species with external skeletons much larger than cell contents, e.g. *Dinobryon*, *Rhizosolenia*, the dimensions of the plasma/organic cell contents should be measured, not the external skeleton dimensions.

- for filamentous taxa, the average biovolume can be estimated using the method described in 6.2.4 for estimating number of cells per filament/colony, except for biovolume it is only necessary to measure average filament length of at least 30 filaments and average diameter of 3 to 5 filaments.

- for colonial taxa count cell numbers and multiply by mean cell dimensions (often single measure of dimensions needed). If the colony is very large or cells are very small, mean cell numbers may have to be estimated. This is best done by estimating cell numbers in a more restricted area of the colony and estimating how many similar areas are contained within the counting field.

A new CEN standard is being prepared currently for calculating cell volumes of phytoplankton (CEN 2007)

8 Data entry

An Excel spreadsheet will be provided for data entry. It contains the fixed taxon list and provides biovolume formulae for each. It also allows the raw data to be summarised. All required details must be recorded on the counting sheet and should be input into the counting spreadsheet according to the accompanying instructions.

Data to be entered will include information on the sample site and date of collection, date of analysis, who carried out the count, information on the chamber and counting areas and the volume of sample used. For each taxa found, the number of units counted, the number of fields of view (or equivalent for whole chamber or diameter transects) in which it was counted and average dimensions of the taxa will be recorded. For taxa which are counted in more than one form, e.g. individual cells and filaments/colonies, it is important to fill in one row for cells counted and the other for filaments or colonies.

Cells/ml and biovolumes for each taxa are automatically calculated.

The ranges of biovolumes for many taxa (from the published literature) are included in the spreadsheet so that calculated biovolumes can be validated against published ranges. If the calculated biovolumes are significantly different to the published ranges then measurements of taxa dimensions and the calibration of eyepiece graticules should be checked.

9 Quality Assurance and validation of counts

Detailed quality assurance methodology and validation of counts are given in CEN (2004), NRA (1995) and Environment Agency (1998).

The following should be noted:

- Details of microscopes, chambers (individually identified and calibrated) and calibration of all ocular/objective combinations should be recorded in a note book and kept for reference. If fixed volume pipettes these should be calibrated annually.

- Checks for random distribution of sample should be done visually at low magnification for each sample, whereas a more detailed check using simple Chi squared test should be done if a sample does not appear to be randomly sedimented or 1 sample every 3 months or so.

- Intra (same chamber and sample) and inter (replicate subsamples from same sample) chamber counts should be carried out at regular intervals by the same analyst and if possible by further analysts.

In addition, it is recommended that

- where ring-tests are undertaken, a staged approach should be adopted:
    1) determining mainly counting errors – group of analysts to count limited number of named taxa (1 to 3) or latex particles/pollen grains in set fields – can be done using photographs or videos
    2) repeat transect or field counts by 2 or more analysts on real sample to check identification and counting errors.
    3) Full count comparisons

- regular workshops should be held (3 - 4 times per annum) to carry out identification and ring tests, possibly combined with ½-1 day taught workshop on difficult groups

10 Acknowledgements

This guidance has been developed with reference to the CEN standard on the enumeration of phytoplankton (CEN 2004), but it has been elaborated with explicit details to encourage standardised phytoplankton counts for the Water Framework Directive by UK and Irish counters. In this respect, the following people have contributed to the development of this guidance: Sarah Pritchard, Jo Girvan, Jane Fisher, Nadia Solovieva, Genevieve Madgwick & Tom Barker

11 Literature

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Appendix A

Whitton Code	Accepted name	Colony biovolume formula	Cell biovolume formula	Minimum Biovolume	Typical Biovolume	Maximum Biovolume
12010010	Acanthoceras zachariasii		Sphere			
17020010	Actinastrum hantzschii		Cone			
01020040	Anabaena catenula	Circle based ellipse	Circle based ellipse			
01020042	Anabaena catenula var. solitaria	Circle based ellipse	Sphere			
01020050	Anabaena circinalis	Circle based ellipse	Sphere			
01020090	Anabaena flos-aquae	Circle based ellipse	Circle based ellipse			
01020000	Anabaena sp.	Circle based ellipse	Circle based ellipse			
01020140	Anabaena spiroides	Circle based ellipse	Sphere			
01020190	Anabaena viguieri	Circle based ellipse	Sphere			
17050030	Ankistrodesmus falcatus		Cone			
17050050	Ankistrodesmus fusiformis		Cone			
17050000	Ankistrodesmus sp.		Cone			
17060020	Ankyra judayi		Cone	234	1021	1299
01040020	Aphanizomenon flos-aquae	Circle based ellipse	Circle based cylinder - long			
01040040	Aphanizomenon issatschenkoi	Circle based ellipse	Circle based cylinder - long		309	
01040000	Aphanizomenon sp.	Circle based ellipse	Circle based cylinder - long			
01050020	Aphanocapsa delicatissima	0.5 sphere	Sphere			
01050030	Aphanocapsa elachista	Sphere	Sphere			
01050000	Aphanocapsa sp.	0.5 sphere	Sphere			
01060020	Aphanothece clathrata	0.5 sphere	Circle based ellipse		105	
01060000	Aphanothece sp.	0.5 sphere	Circle based ellipse			
13080010	Asterionella formosa		Cuboid/rectangle		270	1400
12030060	Aulacoseira granulata	Circle based cylinder - long	Circle based cylinder - long	46		260
12030062	Aulacoseira granulata v. angustissima	Circle based cylinder - long	Circle based cylinder - long			
12030070	Aulacoseira islandica	Circle based cylinder - long	Circle based cylinder - long			

12030080	Aulacoseira italica	Circle based cylinder - long	Circle based cylinder - long			
12030084	Aulacoseira italica v. tenuissima	Circle based cylinder - long	Circle based cylinder - long	80		400
12030000	Aulacoseira sp.	Circle based cylinder - long	Circle based cylinder - long	20		180
09030010	Bitrichia chodatii		Circle based ellipse			
09030020	Bitrichia longispina		Circle based ellipse			
09030000	Bitrichia sp.		Circle based ellipse		2.15	
17080010	Botryococcus braunii	Circle based ellipse	Circle based ellipse			
17080000	Botryococcus sp.	Circle based ellipse	Circle based ellipse	0.4		3.3
16060000	Carteria sp.		Circle based ellipse			
06020020	Ceratium cornutum			31		504
06020030	Ceratium furcoides				30	
06020040	Ceratium hirundinella					
16180000	Chlamydomonas sp.		Circle based ellipse	11	14.3	17
09050030	Chromulina nebulosa		Circle based ellipse			
09050000	Chromulina sp.		Circle based ellipse			
01130020	Chroococcus dispersus		Sphere			
01130060	Chroococcus minutus		Sphere			
01130000	Chroococcus sp.		Sphere	30		280
05020010	Chroomonas acuta		Oval based ellipse	20		70
05020000	Chroomonas sp.		Oval based ellipse	30		180
08010010	Chrysochromulina parva		Oval based ellipse			
09130000	Chrysococcus sp.		Sphere	30		120
09150000	Chrysolykos sp.		Circle based ellipse	100		430
09170000	Chrysopyxis sp.		Circle based ellipse	10	34	70
17170010	Closteriopsis acicularis		Cone	236		860
17170020	Closteriopsis longissima		Cone	10		70
17170000	Closteriopsis sp.		Cone			
27040030	Closterium aciculare		Cone	3		30
27040040	Closterium acutum		Cone			
27040044	Closterium acutum v. variabile		Cone	32		91
27040340	Closterium kuetzingii		Cone			
27040500	Closterium parvulum		Cone			
27040000	Closterium sp.		Cone			

17200010	Coelastrum astroideum	Sphere	Circle based ellipse			
17200020	Coelastrum microporum	Sphere	Sphere		301	
17200000	Coelastrum sp.	Sphere	Sphere			
17200070	Coelastrum sphaericum	Sphere	Sphere			
01150010	Coelosphaerium kuetzingianum	0.2 sphere	Sphere			
01150000	Coelosphaerium sp.	0.2 sphere	Sphere			
17210010	Coenochloris fottii		Circle based ellipse			
17230020	Coenocystis planktonica		Circle based ellipse			
27050000	Cosmarium sp		Oval based ellipse			
17250000	Crucigenia sp.		Oval based ellipse			
17250030	Crucigenia tetrapedia		Cuboid/rectangle	84	128	150
05040030	Cryptomonas erosa		Oval based ellipse	35	105	183
05040040	Cryptomonas marssonii		Oval based ellipse		25560	
05040050	Cryptomonas ovata		Oval based ellipse		18600	
05040000	Cryptomonas sp.		Oval based ellipse		44000	70000
05040003	Cryptomonas sp. (large) Length >30µm		Oval based ellipse			
05040002	Cryptomonas sp. (medium) Length 20-30 µm		Oval based ellipse			
05040001	Cryptomonas sp. (small) L<20µm		Oval based ellipse	9905		25000
12070000	Cyclotella sp.		Circle based cylinder - short			
13260042	Diatoma elongatum		Cuboid/rectangle	21120	57000	99700
13260000	Diatoma sp.		Cuboid/rectangle	41000	64500	103000
13260040	Diatoma tenuis		Cuboid/rectangle		18560	
17330040	Dictyosphaerium pulchellum	0.25 sphere	Sphere	40	75	125
17340000	Didymocystis sp		Circle based ellipse			
17350020	Didymogenes palatina		Circle based ellipse	467	887	970
09230030	Dinbryon crenulatum		Circle based ellipse	99	112	158
09230010	Dinobryon bavaricum		Circle based ellipse			
09230050	Dinobryon divergens		Circle based ellipse		183	
09230070	Dinobryon sertularia		Circle based ellipse			
09230080	Dinobryon sociale		Circle based ellipse			
09230000	Dinobryon sp.		Circle based ellipse			
09230090	Dinobryon suecicum		Circle based ellipse			

25010010	Elakatothrix gelatinosa		Cone + hemisphere			
09250000	Epipyxsis sp.		Circle based ellipse			
27110000	Euastrum sp.		Oval based ellipse			
16260010	Eudorina elegans	0.25 sphere	Sphere			
04020000	Euglena sp.		Oval based ellipse			
13370030	Fragilaria capucina		Cuboid/rectangle			
13370040	Fragilaria crotonensis		Cuboid/rectangle			
13370000	Fragilaria sp.		Cuboid/rectangle			
06050000	Glenodinium sp		Oval based ellipse			
17420000	Gloeocystis sp.		Sphere	22		1000
17430020	Golenkinia radiata		Sphere	1000		20000
17430000	Golenkinia sp.		Sphere			
17440020	Golenkiniopsis longispina		Sphere			
01320010	Gomphosphaeria aponina	0.75 * sphere	Circle based ellipse			
01320000	Gomphosphaeria sp.	0.75 * sphere	Circle based ellipse			
27130000	Gonatozygon sp.		Circle based cylinder - long			
07010010	Gonyostomum semen		Cone + hemisphere			
06070110	Gymnodinium helveticum		Oval based ellipse			
06070000	Gymnodinium sp.		Oval based ellipse	329	580	? 2200
09290000	Kephyrion sp.		Circle based ellipse			
25030010	Koliella longiseta		Cone	424	597	3816
25030000	Koliella sp.		Cone		377	575
17540040	Lagerheimia genevensis		Circle based cylinder - long	254	487	? 3185
17540000	Lagerheimia sp.		Circle based cylinder - long	35	540	5828
12000003	Large centric diatom (>20 µm diam.)		Circle based cylinder - short	377		615
13000003	Large pennate diatom >20 µm		Cuboid/rectangle			
09310030	Mallomonas akrokomos		Cone + hemisphere			
09310080	Mallomonas caudata		Cone + hemisphere	1501	4671	14223
09310000	Mallomonas sp.		Circle based ellipse			
12000002	Medium centric diatom 10-20µm diam.		Circle based cylinder - short			

13000002	Medium pennate diatom 10-20 µm		Cuboid/rectangle	141		1884
12110000	Melosira sp.	Circle based cylinder - long	Circle based cylinder - long	1207		3418
12110080	Melosira varians	Circle based cylinder - long	Circle based cylinder - long	114	480	983
01460000	Merismopedia sp.	Cuboid/rectangle	Circle based ellipse			
17570010	Micractinium pusillum		Sphere	60	204	2993
17570000	Micractinium sp		Sphere	320	550	1482
01490010	Microcystis aeruginosa		Sphere			
01490020	Microcystis flos-aquae		Sphere	81	388	1011
01490000	Microcystis sp.		Sphere	169	640	2228
01490030	Microcystis wesenbergii		Sphere			
17580010	Monoraphidium arcuatum		Cone			
17580020	Monoraphidium contortum		Cone	37	200	912
17580030	Monoraphidium convolutum		Cone			
17580040	Monoraphidium griffithii		Cone	544	880	2700
17580050	Monoraphidium irregulare		Cone		158	
17580070	Monoraphidium komarkovae		Cone	920	1600	9800
17580080	Monoraphidium minutum		Cone			
17580110	Monoraphidium pusillum		Cone	828		2185
17580000	Monoraphidium sp.		Cone	440	1185	7349
17580120	Monoraphidium tortile		Cone		2402	
90000003	Nanoplankton - unidentified single cells, 2–20 µm diam.		Sphere			
13520000	Navicula sp.		Cuboid/rectangle			
13540020	Nitzschia acicularis		Cuboid/rectangle * 0.5			
13540000	Nitzschia sp.		Cuboid/rectangle			
09350000	Ochromonas sp.		Circle based ellipse	49		2078
17640130	Oocystis borgei		Circle based ellipse		509	
17640050	Oocystis lacustris		Circle based ellipse	31		205
17640000	Oocystis sp.		Circle based ellipse			
01530010	Oscillatoria agardhii	Circle based cylinder - long	Circle based cylinder - long	17		181
01530012	Oscillatoria agardhii var. isothrix	Circle based cylinder - long	Circle based cylinder - long	16		132

01530160	Oscillatoria limnetica	Circle based cylinder - long	Circle based cylinder - long	3		71
01530170	Oscillatoria limosa	Circle based cylinder - long	Circle based cylinder - long			
01530230	Oscillatoria redekei	Circle based cylinder - long	Circle based cylinder - long		52	
01530000	Oscillatoria sp.	Circle based cylinder - long	Circle based cylinder - long			
16470010	Pandorina morum	Sphere	Sphere			
16470000	Pandorina sp.	Sphere	Sphere			
17680020	Pediastrum biradiatum	Circle based cylinder - short				
17680030	Pediastrum boryanum	Circle based cylinder - short		31	258	716
17680050	Pediastrum duplex	Circle based cylinder - short		19		293
17680080	Pediastrum simplex	Circle based cylinder - short		58	95	130
17680000	Pediastrum sp.	Circle based cylinder - short				
17680090	Pediastrum tetras	Circle based cylinder - short		4000		32226
09360000	Pedinella sp.		Oval based ellipse			
06110050	Peridinium cinctum		Oval based ellipse	15		189
06110000	Peridinium sp.		Oval based ellipse	15		167
06110100	Peridinium willei		Oval based ellipse			
04070000	Phacus sp.		Oval based ellipse	19	21	24
90000000	Picoplankton - unidentified single cells <2 µm diam.		Sphere			
17690010	Planktosphaeria gelatinosa		Sphere			
09430000	Pseudokephyrion sp.		Circle based ellipse	448		1732
09550000	Pseudopedinella sp.		Sphere		523	
17780000	Quadrigula sp.		Cone			
05100010	Rhodomonas lacustris		Cone + hemisphere	12	35	144
05100012	Rhodomonas lacustris var nannoplanctica		Cone + hemisphere			

05100020	Rhodomonas lens		Cone + hemisphere	31		485
05100000	Rhodomonas sp		Cone + hemisphere			
17810030	Scenedesmus acuminatus		Circle based ellipse	26		121
17810080	Scenedesmus armatus		Circle based ellipse	4		68
07810160	Scenedesmus communis		Circle based ellipse		353	
17810220	Scenedesmus falcatus		Circle based ellipse	44		107
17810340	Scenedesmus opoliensis		Circle based ellipse	31		421
17810000	Scenedesmus sp.		Circle based ellipse	44		283
17830030	Schroederia setigera		Cone	32		103
17830000	Schroederia sp.		Cone		124	
12000004	Very small centric diatom (<5 µm diam.)		Circle based cylinder - short	3		102
12000001	Small centric diatom (5-<10 µm diam.)		Circle based cylinder - short			
13000001	Small pennate diatom <10 µm		Cuboid/rectangle	73	110	411
01750010	Snowella lacustris	0.75 * sphere	Circle based ellipse		1415	
01750000	Snowella sp.	Sphere	Sphere			
17910020	Sphaerocystis schroeteri		Sphere			
17910000	Sphaerocystis sp.		Sphere			
09450000	Spinifertomonas sp.		Sphere			
27360040	Spondylosium planum		Oval based ellipse			
27380330	Staurastrum cingulum			187	643	8181
27380840	Staurastrum longipes					
27380860	Staurastrum lunatum					
27380000	Staurastrum ophiura			11		394
27381120	Staurastrum planctonicum			19		289
27370000	Staurastrum sp.					
27381460	Staurastrum tetracerum			28		430
27390190	Staurodesmus incus					
27390000	Staurodesmus sp.			36	147	793
12180000	Stephanodiscus sp.		Circle based cylinder - short			
09480000	Stichoglea sp.		Circle based ellipse		69	
13810010	Synedra acus		Cuboid/rectangle			
13810120	Synedra nana		Cuboid/rectangle	139		905
13810000	Synedra sp.		Cuboid/rectangle	30		387

13810180	Synedra ulna		Cuboid/rectangle			
09530000	Synura sp.		Circle based ellipse	9		113
13820010	Tabellaria fenestrata		Cuboid/rectangle	29		157
13820020	Tabellaria flocculosa		Cuboid/rectangle			
13820022	Tabellaria flocculosa var. asterionelloides		Cuboid/rectangle	41	218	247
13820000	Tabellaria sp.		Cuboid/rectangle			
17960010	Tetraedron caudatum		Cuboid/rectangle	11	45	130
17960030	Tetraedron minimum		Cuboid/rectangle		570	916
17960000	Tetraedron sp.		Cuboid/rectangle		377	
17970000	Tetrastrum sp.		Cone + hemisphere	21436		95529
17970050	Tetrastrum staurogeniaeforme		Cone + hemisphere	8150		33809
17970060	Tetrastrum triangulare		Cone + hemisphere			
04100000	Trachelomonas sp.		Circle based ellipse			
18010010	Treubaria setigera		Circle based cylinder - short			
90000004	Unidentified cells >20 µm diam.		Sphere	129	154	262
17000001	Unidentified colonial greens.		Sphere		22763	
01000000	Unidentified cyanophytes - colonial algae <2 µm diameter.		Sphere	1916		15215
90000005	Unidentified flagellates 2 – 20 µm diam.		Sphere		1767	
17000000	Unidentified small green round cells (sgrt)		Sphere		7503	
09540000	Uroglena sp.		Circle based ellipse		3031	
12200000	Urosolenia		Cone		1608	
12200010	Urosolenia eriensis		Cone			
12200020	Urosolenia longiseta		Cone		48444	
16770010	Volvox aureus		Circle based ellipse			
16770010	Volvox sp.		Circle based ellipse			
01780010	Woronichinia naegeliana	0.2 sphere	Circle based ellipse			
01780000	Woronichinia sp.	0.2 sphere	Circle based ellipse			
27430020	Xanthidium antilopaeum		Oval based ellipse	163	323	696
27430000	Xanthidium sp.		Oval based ellipse			

Algal Biovolume formula and names:

Biovolume shape	Formula	Taxon examples
CIRCLE BASED CYLINDER - LONG	3.141592654*L*D*D/4	*Aphanizomenon, Aulocolsaera*
CIRCLE BASED CYLINDER - SHORT	3.141592654*H*D*D/4	Centric diatoms,
CIRCLE BASED ELLIPSE	3.141592654*L*D*D/6	
OVAL BASED CYLINDER	3.141592654*L*D*H/4	
OVAL BASED ELLIPSE	3.141592654*L*D*H/6	
CONE	3.141592654*L*D*D/12	*Mallamonas akrokomos*, horn of *Staurastrum*
CONE + HEMISPHERE	(3.141592654*D*D)12*(D/2+ L)	*Rhodomonas, Mallamonas caudata*
DOUBLE CONE	3.141592654*L*D*D/12	*Ankistrodesmus, Closterium*
CUBOID/RECTANGLE	L*D*H	*Tabellaria*, pennate diatoms, *Merismopedia*
CUBOID/RECTANGLE * 0.5	0.5*L*D*H	*Nitzschia acicularis*
SPHERE	3.141592654*D*D*D/6	*Microcystis, Sphaerocytis*, picoplankton cells
0.2 SPHERE	0.2*3.141592654*D*D*D/6	*Woronichinia*
0.25 SPHERE	0.25*3.141592654*D*D*D/6	*Eudorina*
0.5 SPHERE	0.5*3.141592654*D*D*D/6	*Aphanothece, Aphanocapsa*
0.75 * SPHERE	0.75*3.141592654*D*D*D/6	*Snowella, Gomphosphaeria*

L = length (µm)
D = Diameter or width (µm)
H = Depth or height (µm)
P = Numbers of arms/branches in *Staurastrum* half cell