Quality Assurance for Phytoplankton Data

Bowburn Consultancy

Thames Region Operational Investigation

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This report summarises the need and means of phytoplankton quality assurance within the Environment Agency, Thames Region. The information within this document is for use by the Agency staff and others interested in the quality assurance of phytoplankton data.

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LIST OF ABBREVIATIONS AND ACRONYMS

AQL	Acceptable Quality Level
BMWP	Biological Monitoring Working Party
CCA	Constrained canonical analysis
DI	Dominance Identity
DQO	Data Quality Objective
GLM	Generalized Linear Modelling
ISO	International Standards Organization
QA	Quality Assurance
QC	Quality Control
RIVPACS	River Invertebrate Prediction and Classification System
SCD	Squared chi-squared distance
UWWTD	Urban Wastewater Treatment Directive

SUMMARY

1. Phytoplankton analyses have several roles within the Environment Agency's Thames Region, some of which are likely to influence major investment projects and the enforcement of legislation. There is, therefore, a *prima facie* case for ensuring that results from these analyses are of a known and defensible quality.

2. Statistical errors can be introduced into a phytoplantkon analysis at several stages. The process of enumeration is subject to various errors associated with non-random distributions of cells within counting chambers, but the scale of these errors needs to be assessed relative to that of natural variation within the river and of errors introduced at the sampling and sub-sampling stages.

3. Quality assurance strategies used by Anglian Region and Thames Region are compared and recommendations on a new strategy for Thames Region, based on the best elements of existing approaches, is described.

4. Several areas where further research is required are highlighted. In particular, these include development of a taxonomic data quality objective and further development of methods for the objective evaluation of replicate samples analyzed by two or more analysts.

KEYWORDS

Phytoplankton, Rivers, Quality Assurance

1. INTRODUCTION:

1.1 Quality Assurance (QA) in Perspective

The need for explicit quality standards in applied ecology arises today, paradoxically, from its success. As our understanding of ecosystems increases, so the opportunities to use ecological data to influence decision-making and investment programs have increased. This in turn has placed a responsibility upon the scientists performing the work to produce data that can be subjected to close scrutiny, sometimes in a court of law. One recent consequence of this is the adoption of quality management systems by organisations responsible for such data. Chemical analyses were amongst the earliest environmental measurements to be treated in this way (Cheeseman & Wilson, 1978), but similar systems for ecological analyses are also being developed (Environment Agency, 1996). In this report, the application of QA systems to analyses of phytoplankton from the River Thames will be discussed. The objective is to describe methods that will give users and managers confidence that data produced within Thames Region are adequate for the uses to which they are put.

The International Standards Organisation (ISO) defines "quality" as:

> "The totality of features and characteristics of a product or service that bear on its ability to satisfy stated or implied needs" (ISO 8402)"

This is often paraphrased as "conformance to requirements" and consists of processes to detect and avoid both poor-quality work and situations where an over-zealous operative is spending too much time on a sample. A manager is less interested in data than in the information that is extracted from this data. Whilst poor quality data will always lead to poor quality information, there are also situations where a decision can be based upon less data than a biologist could potentially "mine" from the sample. This is illustrated well by the BMWP score system and RIVPACS, where identification of benthic invertebrates to family or order is sufficient for most general water quality assessment. Although there are situations where finer levels of taxonomy are useful, it is important to distinguish situations where such approaches are valuable. This is based on the assumption that a finite time is available to biologists and that productivity will be maximized by adjusting the level of an analysis, depending upon the anticipated uses of the data.

Recognition of this in the USA has led to the development of "Data Quality Objectives" (DQOs: Environmental Protection Agency, 1988) which define the quality of data required to make decisions, balanced against the time and resources available. Where the intention of a survey is to analyze data using an existing index , this index creates a *de facto* DQO for that method (Kelly, 1998); however, such indices are not generally used in phytoplankton analyses and this creates some problems in establishing suitable targets for data collection. A potential risk of this approach is to reduce the value of the data for long term monitoring for reasons of short-term expediency. In the case of benthic diatoms, the use of a relatively simple index (Trophic Diatom Index) as a DQO is justified by the ability to store samples indefinitely in herbaria (Kelly, 1997). However,

the long-term value of data is itself dependent upon factors that may not be easy to predict (e.g. changing regulatory requirements).

A further problem in the definition of DQOs is the need to distinguish what is possible with existing personnel and resources from what is actually required from the data. The situation in Thames Region in 1997 may permit a sophisticated level of taxonomy because staff have several years experience of working on the river and have spent time consulting experts on particular groups. Under such conditions, there is a temptation to set the DQO at a level achievable under these conditions. Were such highly-trained staff to leave, a considerable investment would be required to train a successor to perform the same task. For this reason, it is important to be clear about the difference between "data" (i.e. the end-product of a series of analyses) and "information" (i.e. that part of the data that is actually necessary to reach a decision).

This report is primarily concerned with procedures used to ensure the quality of phytoplankton data from the River Thames. However, this question of appropriate DQOs is of critical importance and it is worth considering briefly the ways in which phytoplankton analyses are used throughout the Agency. Three uses of such data have been identified:

1. reactive monitoring of blue-green algal "incidents". If "scums" are present, quantification is not required and identification of the species is sufficient. Where blue-green algae form greater than 5% of a bloom, then enumeration is required. If numbers of a notifiable species exceed a "threshold value" specific to that species then relevant people and organisations are informed, according to the policy on Blue-green Algal Monitoring and Management of Incidents (Environment Agency, 1997);

2. studies where the primary interest is in modelling phytoplankton dynamics with a view to predicting the impact of new developments. At present, such studies have mainly relied upon estimates of the total algal biomass present, but this may change in the future (see below); and

3. detailed studies where the dynamics of particular taxa is of interest, along with fundamental research on algal ecology. Likely situations include monitoring and managing blooms of "nuisance" algae.

1.2 The Role of Phytoplankton Analyses in Thames Region

The present objectives of phytoplankton analyses in the River Thames are:

1. to perceive and describe long-term trends and patterns in phytoplankton in the river;

2. to relate these to other environmental measurements collected by the Agency; and

3. to predict the influence of new developments within the Thames Basin. These predictions can then be used to set environmentally sensitive abstraction/discharge consents and operating agreements. Planned and proposed developments include:

i. The Thames Water Abingdon Reservoir Proposal, an off-line reservoir which will draw water from the Thames near Abingdon. This water will be used both as a potable supply and to augment flow in the Thames.

ii. A proposal by Thames Water to transfer water from the lower Severn to the upper Thames to augment discharge at times of low flow

iii. -Various proposals for river regulation and abstraction by Thames Water, which are monitored by the Teddington Low Flow survey to ensure no deleterious effects in the lower freshwater and upper estuarine Thames

iv. Maidenhead Flood Alleviation Scheme; a scheme for a second channel to relieve pressure on the main river at times of high flow

v. Water quality improvements required subsequent to designation of the river as a "eutrophic sensitive area" under the terms of the Urban Wastewater Treatment Directive (UWWTD). Studies will be required to assess the role of nutrient stripping from major sewage treatment works on the biota;

Analyses of blue-green algal samples using the "threshold level" are also performed in the Region, but are primarily concerned with standing waters and are not considered further in this Report.

The European Union's directive establishing a framework for Community action in the field of water policy ("Framework Directive", presently in draft) will require (Article 4) an evaluation of "good ecological status" of surface waters, one criterion of which (Annex 5) is the composition and abundance of the aquatic flora. A future role of phytoplankton analyses within Thames Region might therefore include the implementation of this directive.

A major role of phytoplankton data is to predict the impact of new developments using mathematical models (Whitehead & Hornberger, 1984; Whitehead & Williams, 1984). As such, the primary role will be to detect relatively large-scale changes in total algal biomass and species composition, caused by factors such as the removal of limiting nutrients from certain sewage works (possibly required under the UWWTD) or the input of algae-laden to the river from the proposed reservoir. Until recently, such modelling was relatively crude, with the dynamics of all individual taxa within a phytoplankton community replaced by a single measure of biomass (usually chlorophyll$_a$). Recent developments in ecological modelling (e.g. the RIVERSTRAHLER model: Billen *et al.*, 1994; Garnier *et al.*, 1995) permit the dynamics of individual groups of algae (e.g. diatoms, Chlorophyta etc.) to be modelled separately and open the way for more sophisticated models in the future. For these, estimates of cell numbers replace chlorophyll determinations as the key measurements. Although at present the RIVERSTRAHLER model is only concerned with relatively broad categories, information on the composition of communities clearly provides valuable supporting evidence and may permit use of more sophisticated models in the future. Another approach that has been explored for the River Thames is to model the response of individual species using Generalized Linear Models (GLM; Ruse & Love, 1997)

For the purpose of this report it will be assumed that a model such as the RIVERSTRAHLER model will become available for the River Thames in the next few years, thus creating an imperative for accurate and precise estimates of total cell numbers belonging to major taxonomic groups. Although not required by RIVERSTRAHLER at present, the identity of all common taxa found in a sample from the Thames is also desirable if the use of GLM is to be continued. This is a verbal formulation of a DQO for phytoplankton analyses from Thames Region and the remainder of this report will establish the work required to quantify this DQO, and the broad framework of a QA system to achieve it.

2. SOURCES OF ERROR IN PHYTOPLANKTON ENUMERATION

The total error associated with a measurement is the sum of the error components of all the stages which contributed to that measurement. In practice, the errors associated with ecological assessments are associated with two types of variable (Cairns & Smith, 1994):

1. **noise variables** - environmental variables which can be controlled in a laboratory setting but not in most field situations; and,

2. **control variables** - those variables which can potentially be controlled by the experimenter.

Although errors associated with noise variables cannot be controlled, it is important to understand the contribution that these make to the total variance as this provides an indication of the representativeness of a particular sample. Moreover, the scale of the variance associated with noise variables, relative to that for the control variables, provides an indication of the likely benefits to the process of a reduction in the variance of control variables. In the case of phytoplankton analyses, the noise variables are all the processes which affect the spatial and temporal heterogeneity of phytoplankton in a river (2.1), whilst the control variables are all the processes which occur subsequent to the sample's removal from the river (2.2 - 2.5). A formal analysis of errors associated with these processes will identify those stages which make the greatest contribution to the total error. If the error associated with a measurement is to be reduced, then it is to these stages that attention needs to be focused.

2.1 Natural Dispersion Patterns of Phytoplankton in Rivers

That phytoplankton is variable in space and time is axiomatic. The key to successful long-term monitoring, however, is to separate significant longitudinal and spatial trends from the background "noise". In long-term studies such "noise" may include "unusual" years, caused, for example, by freak weather patterns. Whilst gross patterns of within-year variation at any one site are well understood (see, for example, Swale, 1964; Lack, 1971; Lack & Berrie, 1975, for data on Thames and important tributaries), finer scale patterns and their relevance to monitoring are less so.

Academic studies on river phytoplankton, such as those listed above, are often based upon weekly or fortnightly sample collection: a luxury that may not be afforded to an Agency biologist for routine monitoring. Kiss *et al.* (1996) examined the consequences of a reduced sampling intensity on a detailed dataset from the River Danube at Göd. In 1979, a year with many floods, reducing sampling frequency from weekly to fortnightly missed three "peak" values of phytoplankton density, whilst sampling less frequently led to losses of the main characteristics of the seasonal changes. By contrast, in 1986, when there were few large floods, a reduction in sampling frequency had a less pronounced effect although monthly sampling still missed the peak of the spring "bloom" of diatoms.

Such fluctuations in cell numbers also occur over shorter periods of time, with diurnal variations in biomass of phytoplankton observed by Kiss *et al.* (1996) in the River Danube at Göd, with minima observed in the mornings and maxima in the afternoons. The scale of these changes during a period of low flows in July 1992 was up to 40% from morning to evening. Diurnal variations in phytoplankton abundance have also been observed for the River Great Ouse (D. Balbi, pers. comm.).

A further assumption implicit in much work on river phytoplankton is that there is sufficient turbulent mixing that a single sample from the main flow of the river can be assumed to be "typical". Alongside a long-held view that side arms and submerged vegetation may be important sources of inocula for river phytoplankton (Hynes, 1970; Whitton, 1975), recent work has now demonstrated the presence of "fluid dead zones" within the main body of a river where algae may be trapped and gradually released (Reynolds, 1994; Reynolds *et al.*, 1991).

A consequence of this finding is to raise doubts about the validity of the assumption regarding the representativeness of high quality estimates of cell numbers based on a single spot water sample. Under some conditions this assumption has been tested and shown to be valid (Kowalczewski & Lack, 1971); under others, it is not (Kiss, *et al.*, 1996). Although Kowalczewski and Lack (1971)'s study was performed on the River Thames, later studies have shown that the river is only well mixed under high flows (A. Love, pers. comm.). Given the early stage of studies into the relationship between fluid dynamics and phytoplankton composition, it is probably impossible to generalize about sampling strategies. Whilst steps can be taken to minimise the problems posed by such situations (such as avoiding areas close to low-head navigation dams - Wehr & Thorp, 1997) this short-scale spatial variation represents, alongside the temporal variation discussed earlier, one more source of inherent error in all estimates of phytoplankton abundance.

2.2 Relative Contribution of Different Stages to Total Error

Further sources of random and systematic errors are introduced into phytoplankton enumeration at the following stages (Table 2.1):

Sampling - removal of water sample and measurement of known volume into a container;

Sub-sampling - removal of a measured part of this sample, followed by concentration, if appropriate. This may take place in two or more stages;

Enumeration - transfer of a portion of sub-sample ("sub-sub-sample") to counting chamber, identification of taxa, strategies for counting all or part of sample in chamber, conventions for handling filamentous and colonial taxa (see 2.4 & 2.5); and,

Table 2.1. Sources of error in estimates of phytoplankton numbers.

Stage	Issues
Sampling	• choice of strategy in relation to spatial and temporal variation in phytoplankton community structure
	• measurement of sample into container
Sub-sampling and Pre-treatment	• homogenisation of sample
	• choice of method (sedimentation or centrifugation
	• measurement of sub-sample into measuring cylinder
	• size of measuring cylinder
	• quantity of Lugol's iodine used
	• use of appropriate time for sedimentation of cells
	• risk of cross-contamination between cylinders
Enumeration	• precision of apparatus used to fill counting chambers
	• estimation of chamber volume (Lund cells only)
	• consistent taxonomy
	• clear specification of appropriate size ranges
	• magnification used
	• problems with identifying some taxa (e.g. centric diatoms) to species from live or preserved material
	• "edge" effects etc. in counting chambers
Data handling	• conventions used to convert from counted units (e.g. coenobia, filaments, colonies) to cell numbers
	• conversion from cell numbers to cell volume

Data handling - all processes occurring subsequent to enumeration.

Most of these sources of error are quantitative, with the important exception of sample identification which is a qualitative judgement which influences the measurement process. The choice of sampling strategy is another qualitative judgement, although it can be quantified to some extent by stratified sampling (e.g. Irish & Clarke, 1984). This

involves dividing the sample area into several homogenous strata of equal size in order to increase the accuracy of population estimates and ensure that sub-divisions of the population are adequately represented. Mistakes constitute a further source of error. Some (such as transcription errors) can be detected by checking procedures whilst others are more difficult to discover.

This issue was studied for experimental enclosures in Blelham Tarn by Irish and Clarke (1984) who showed sub-sample and enumeration effects to be minor, compared to spatial variation between the four quadrants of the experimental enclosures that were sampled. Under these conditions effort is better spent improving experimental design to minimise these sources of error rather than improving enumeration techniques. Errors introduced after the enumeration process (e.g. associated with conversion from number of filaments to number of cells) were not included.

Although Irish and Clarke (1984)'s study relates to lakes, where spatial heterogeneity is well-known, the authors noted surprise at the magnitude of variation, even during winter months, when it is generally assumed that turbulent mixing processes predominate and that phytoplankton are uniformly distributed. Such is also generally assumed to be the case for rivers and further work of this nature is required for the River Thames. If Irish and Clarke (1984)'s findings are substantiated for this system, then it is possible that improvements to the enumeration procedure alone will not lead to improvements in data quality.

2.3 Comparison between Lund cells and sedimentation chambers

Various techniques have been proposed for phytoplankton enumeration of which enumeration of cell numbers using one of several designs of counting chamber is most common. Other important factors include optical quality and the importance of avoiding non-random distributions of cells.

For the purposes of this document, counting chambers are divided into two groups:

1. haemocytometers, Sedgwick-Rafter chambers (American Public Health Association, 1992), "Lund cells" (Lund, 1959) and other designs of counting chamber, which are designed for use with conventional (i.e. non-inverted) microscopes; and

2. Sedimentation chambers (Utermöhl, 1958: see Lund *et al.*, 1958), designed for use with inverted microscopes.

In the first group of chambers, the correct volume of sample is carefully introduced into the chamber by capillary action, taking care to avoid air bubbles. If concentration is required, this needs to be done in advance. The sample is then counted using a conventional microscope. Sedimentation chambers, by contrast, allow a greater volume of sample to be introduced. This is then allowed to settle (effectively concentrating the algae) before enumeration. Use of very fine glass ("coverslip" quality) at the base of the chamber, coupled with an inverted microscope, means that the algae are very close to the

objectives, ensuring high optical quality and even, if necessary, the use of oil-immersion objectives.

Each type of chamber has various advantages and disadvantages (Table 2.2). A key aspect in the evaluation of counting chambers is the statistical distribution of counting units on the chamber. Both types of chamber are used in the Agency at present and it is important to establish the extent to which data from such chambers are comparable. Data on this are highly variable and may reflect conditions under which the work takes place. Lund (1959) found no significant difference between counts of *Ankistrodesmus falcatus* var. *spirilliformis* made by "Lund cells" and counts of the same sample made in haemocytometers and sedimentation chambers. A later study by Anglian Water Authority (1979) looked at seven different taxa in samples from various water bodies in Anglian Region. Two-way analysis of variance was used to examine differences between analysts and between 15 replicate counts made by the same analyst. In no case was there any significant difference between analyst for a particular taxon, but there was considerable inter-analyst variation for both types of cell. In total, 57% of analyses using Lund cells were significantly different, compared to 43% for sedimentation chambers; however, Lund cells gave lower and more consistent coefficients of variation (Anglian Water Authority, 1979). Lund (1959) found no "edge" effects when counting *Lycopodium* spores and *Chlorella* in Lund cells; however, significant edge effects have been found in most types of chamber at different times (e.g. Sandgren & Robinson, 1984) and it is probably safer to assume that edge effects are a persistent problem facing phytoplankton enumeration, whatever the type of counting chamber used. Studies such as these are difficult to evaluate so long after the tests were performed, but it is clear that all methods for phytoplankton enumeration can potentially lead to significant inter-operator differences. Evaluation must therefore be extended to include non-quantitative aspects as well (Table 2.2).

So long as a laboratory possesses an inverted microscope and is prepared to invest in sedimentation chambers and associated equipment, there are few practical problems associated with this technique. Use of Lund cells is potentially risky in environments dominated by larger taxa and colonial and filamentous forms; however, river phytoplankton in general is dominated by small-celled organisms so there are unlikely to be significant problems caused by these being trapped or entangled by the bore of the pipette. However, identification of smaller taxa may be aided by the greater optical quality possible using sedimentation chambers.

2.4 Phytoplankton Enumeration: Evaluation of Sampling Strategies

2.4.1 Background

Except for the largest phytoplankton taxa (which tend to be uncommon in rivers), it is not possible to count all the cells of a species in a counting chamber. Instead the counting chamber is subdivided into fields, and a full count is undertaken only for a fraction of all the fields in the counting chamber. It is essential that, within the context of the chosen strategy, the fields chosen for sampling be chosen at random.

Table 2.2. Comparison of "Lund cells" and sedimentation chambers: practical aspects

	"Lund cells" & relatives	Sedimentation chambers
initial purchase cost	✓ low	✗ high[1]
ease of filling chambers	✗ tricky	✓ easy
time required for sedimentation	✓ low	✗ high[2]
time required for count	no difference between approaches[3]	
suitable for very small taxa	✓ yes	✓ yes
suitable for very large taxa	✗ no[4]	✓ yes
optical quality	✗ low	✓ high

[1] assumes laboratory already possesses inverted microscope
[2] however, use of sedimentation chamber may circumvent need for preliminary concentration stage
[3] data from Anglian Water Authority (1979)
[4] bore of pipette and depth of cell may affect their introduction

Sandgren and Robinson (1984) note a consistent non-random settling of phytoplankton cells in standard counting chambers. They observed an edge effect which may result in a sizeable error in cell density estimates. Moreover, this error would be undetectable using the traditional technique of counting replicate transects across the counting chambers. For two dominant species they found 1.49 to 1.65 more cells in edge samples than from central samples. These effects could not be attributed either to population density or to species cell size.

Anglian Region (National Rivers Authority, 1995) recommend taking diameter transect counts to eliminate transverse contagiousness within the counting chamber. However, they also note that these tend to under sample chamber edges and over sample the middle of the chamber, a flaw which is particularly serious when the distributions display aggregation (see 2.5).

2.4.2 How many fields should be counted?

Suppose that a number n of chambers are filled, and m fields in each chamber are counted. Two possible sources of variation are the variation due to differences between counts on the same chamber, and the variation due to differences between chambers. It is in general necessary to estimate these sources of variation by, for example, Analysis of

Variance techniques for particular choices of m, n. The methodology is summarised in Venrick (1971; 1978b) and Woelkerling *et al.* (1976). These estimates can be used to guide appropriate choices for m and n, taking into account the different costs involved and additional caveats described in 2.5.3. One difficulty involves the assumption of underlying Normality for the various tests and estimation procedures. As far as contagious distributions are concerned, further work needs to be done to examine the effect of contagion on these sampling strategies (see 2.5.3). Alternatively, Woelkerling *et al.* (1976) suggested that nonparametric procedures were nearly as efficient as parametric procedures in this context.

Sandgren and Robinson (1984) recommend a stratified sampling approach to minimise the source of error due to bias from edge effects. Consequently, it is necessary to deal with at least three sources of variation:

- differences between edge and central fields;
- differences between fields on the same chamber; and,
- differences between chambers.

This has apparently not been considered in the literature.

2.4.3 How many cells should be counted?

Woelkerling *et al.* (1976) summarise recommendations on the number of cells that should be counted to arrive at a prescribed degree of precision made in the literature before 1974. These range from counting a minimum of 10 fields (American Public Health Association) to 10 fields for each of two counting chambers, to 30 fields in each of three replicate counting chambers (McAlice, 1971), to 10 fields in each of four chambers. Woelkerling *et al.* (1976) conclude from their experiments that it is in general better to count fewer fields over more chambers, whilst Venrick (1978a) suggests that it is often preferable to make several imprecise estimates of counts from many fields rather than to make a single precise count from a single field. She goes on to summarise the methodology for determining count sizes to achieve prescribed counting precision, assuming Poisson and other distributions for taxa. However, the methodology for clumped distributions is crude and dated. For contagious distributions, it is in general not possible to advise on the number of chambers and number of fields needed to achieve a particular precision, as this will require making assumptions about the form of the underlying distribution. If we make assumptions which lead to the use of the negative binomial distribution, as is recommended below, it will be almost certainly necessary to carry out the calculations using a computer program.

A further issue is the relationship between number of fields counted and number of taxa detected. McAlice (1971) claims that a count of 25 fields can be expected to reveal 80-90% of species present, whilst one of 35 fields should reveal 90-95% of species. Such a generalisation can, however, ignores the species richness of the sample. Pappas and Stoermer (1996) describe a means of computing an appropriate count size to achieve a "representative" count (i.e. one that records all the taxa present); however, the practical

utility of this, given the objectives of Thames Region's monitoring programme (1.2), is dubious.

2.5 Phytoplankton enumeration: alternative statistical methods for clumped distributions

2.5.1 Introduction and review

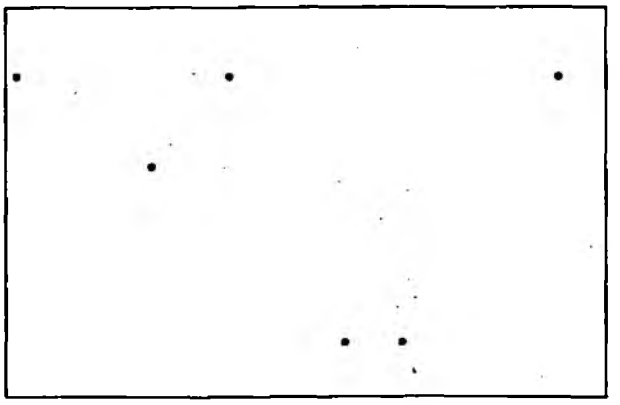
Lund *et al.* (1958)'s advocation of Poisson-based estimates of phytoplankton abundance from cell counts continues to be widely accepted, despite considerable evidence (e.g. Edgar & Laird, 1993) that a random spatial dispersion of cells in the counting chamber is rarely, if ever, appropriate (see Fig. 2.1 for explanation of terms). In particular, for water samples taken from the River Thames, aggregations of cells are routinely observed. The presence of such aggregations violates the assumption on which Poisson interval estimates are based and, moreover, the Poisson distribution is not particularly robust to violations of its underlying assumptions. In practice, such violations can be detected by testing the goodness of fit of the Poisson distribution to counts: over dispersion indicates some degree of aggregation or *contagion*. Appropriate methods can be found in most statistical textbooks (e.g. Elliott, 1977)

Several papers, in different fields of application, deal with the deficiencies of the Poisson model in cases where some degree of aggregation is present (e.g. Eduard and Aalan (1988) for mold spores). Rott (1981) carried out a series of intercalibration tests using phytoplankton samples taken from a number of lakes in Scandinavia and central Europe and noted that the most frequently found type of phytoplankton distribution indicated contagion. McAlice (1971) showed that overdispersion tends to be general for counts with means greater than 10. As estimates of total cell numbers are invariably based upon sums of numbers of all the taxa present, there can be a substantial propagation of errors (Duarte *et al.*, 1990).

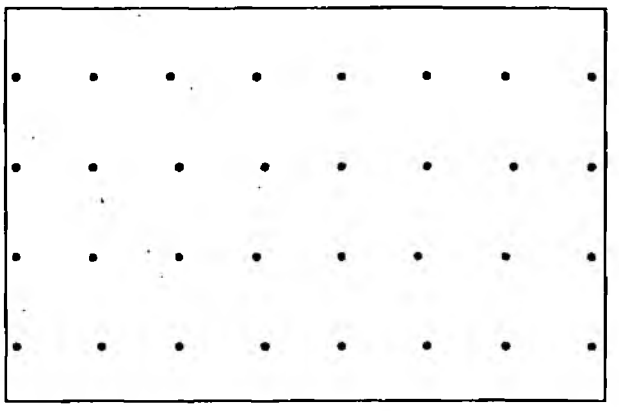
Methods do exist which attempt to disaggregate the cells prior to counting. These methods include alkaline hydrolysis, sonication and heating (e.g. Box, 1981) but none are particularly effective and can damage the cells to be counted. Alternatively, methods have been devised for estimating the number of cells in *Microcystis* colonies from colony diameter (Reynolds, 1973) but has several drawbacks: colonies are not always spherical, the spacing of cells within colonies is highly variable and the relationship does not hold when there is wide variation in colony diameter (Reynolds & Jaworski, 1978).

There is a long history in statistics and statistical ecology of fitting contagion models to counts. Contagion models are appropriate when clusters (e.g. colonies) of objects (e.g. cells) have been observed, with each cluster containing one or more objects (Cliff & Ord, 1981). A number of contagion models have been suggested, of which the most common are the *negative binomial* and *Neyman Type A* distributions. The former has been applied to a very wide range of distributions occurring in ecology, entomology and parasitology (Ludwig & Reynolds, 1988; Seber, 1982; Elliott, 1977; Binns, 1986).

a. random distribution. (variance = mean)



b. regular distribution (idealized form) (variance < mean)



c. contagious distribution (variance > mean)

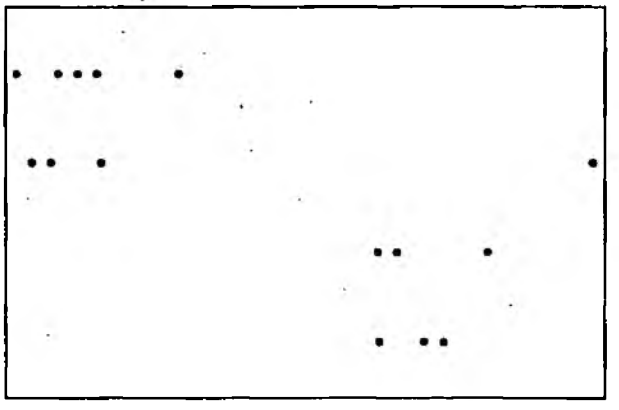


Figure 2.1 The three types of spatial distributon.

Theoretically, a negative binomial distribution can arise in a number of ways. One biologically meaningful justification is if colonies of a phytoplankton taxon are distributed spatially at random, but that the number of cells per colony follows a logarithmic distribution (i.e. due to cell division within the colony). Other assumptions that are necessary are:

- that there are no inhibitory or attractional interactions between colonies;
- that there are no overlapping colonies; and,
- that there is no tendency for neighbouring areas to show similar characteristics (which would result in a regular, rather than random, distribution).

A critical discussion of modelling aggregation using the negative binomial distribution is given by Taylor *et al.* (1979). They point out that it is not universally appropriate, and does suffer from drawbacks varying from difficulties estimating the parameters to difficulties in interpreting the aggregation parameter. However, they concede its basic flexibility and simplicity and that it often fits well enough for practical purposes, provided that results are not extrapolated beyond the bounds of actual data. One of their major criticisms of the use of the negative binomial distribution, namely that its clumping parameter has no universal biological meaning, is irrelevant in this context as we are concerned with accurate counts as opposed to explanation of biological phenomena.

The clusters typically encountered during phytoplankton enumeration are either units of colonial algae (e.g. *Scenedesmus, Pediastrum*) or filaments (e.g. *Oscillatoria, Aulacoseira*). Consequently, for the negative binomial distribution to be a realistic model in this case it is necessary to assume that the distribution of cells in the cluster is logarithmic at least to a first order approximation. McAlice (1971) notes as effective a logarithmic transformation to Normalise counts of cells of chain-forming species, and prefers this to fitting a negative binomial distribution, which he regards as time consuming (as it was, before the advent of calculators, etc.). The Anglian Region Procedures Manual (National Rivers Authority, 1995) also recommends applying logarithmic transformations to counts in order to Normalise them, and to reduce overdispersion.

An alternative option is to assign confidence limits to units rather than cells, as it should be easier to demonstrate a random distribution of these. However, as cell numbers are the standard unit of expressing phytoplankton counts in many studies, this may create difficulties in comparing data over time.

2.5.2 A general strategy

Suppose that for a given species there are counts x_1, x_2, x_3 ..., x_n from n sampling units. The sample size must be larger than one, otherwise it will not be possible to test for overdispersion. Next, the sample mean and variance are calculated, using standard formulae.

Table 2.3. Selected data for phytoplankton sample collected from River Thames at Littleton on 20 August 1997 and analyzed by Alison Love.

	field									
taxon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**mean**	**variance**
Chlorella	0	2	0	2	0	2	0	1	0.88	0.98
centric diatoms	12	16	10	13	12	21	20	15	14.88	15.55

A test for overdispersion is then performed. If the test indicates the presence of overdispersion to a significant degree, a negative binomial distribution is fitted to the data. This strategy does not allow explicitly for underdispersion (which would occur if the samples were regularly spaced in the sampling unit, rather than randomly distributed), but in this context serious underdispersion is hardly ever encountered, and the Poisson fit will be assumed to give a reasonable answer should such a situation arise.

A standard test for overdispersion is based on the variance to mean ratio, sometimes called the index of dispersion: $I = s^2/n$. The quantity $(n - 1)I$ approximates to a chi-squared distribution with n-1 degrees of freedom. An example of its use is given below.

Data for two taxa from a sample collected from the River Thames, Littleton, 20 August 1997 are given in Table 2.3. The calculations are as follows:

For *Chlorella*: $I = s^2/n$ $= 0.98 / 0.88$ $= 1.12$

$\chi^2 = (n - 1) I$ $= 7 \times 1.12$ $= 7.85$

The critical value for χ^2 with 7 degrees of freedom is 14.07, so the null hypothesis that *Chlorella* cells are randomly distributed can be retained.

For centric diatoms: $I = s^2/n$ $= 15.55 / 14.88$ $= 1.04$

$\chi^2 = (n - 1) I$ $= 7 \times 1.04$ $= 7.32$

As for *Chlorella*, the computed value of χ^2 is less than the critical value, so the null hypothesis that centric diatoms are randomly distributed can be retained.

2.5.3 Using the negative binomial distribution

Descriptions of the distribution and techniques to fit the distribution to data are widely available in the ecological literature (Elliott, 1977; Ludwig & Reynolds, 1988; Seber, 1982) and comprehensive details are given in Johnson *et al.* (1992).

The mean count of a binomial distribution is μ and the variance is $\mu + \mu^2/k$. k is sometimes referred to as the contagion parameter: high (low) values of k are associated

with low (high) dispersion. Notice that as k becomes very large, the variance tends to μ and the negative binomial distribution tends to the Poisson distribution, with mean and variance both equal to μ.

The parameters μ and k must usually be estimated from the data and this is where the main difficulties lie. We are only interested with the case $s^2 > \bar{x}$, as otherwise we would be fitting a Poisson distribution to the counts. Initial estimates of k and μ are given by the method of moments; however, these estimates are not satisfactory if k and $\bar{x}$ are less than 4 (Elliott, 1977; Ludwig & Reynolds, 1988). More efficient estimates may be obtained by the method of maximum likelihood. This is an iterative ("trial and error") procedure which depends upon there being at least one sampling unit with a zero count. Further, more complicated methods have also been proposed. In practice, it should be possible to use a computer program to automate the procedure. A summary of the estimation methods and further references is given in Johnson *et al.* (1992).

Whatever the estimation procedure, the larger the sample size, the more reliable the estimates. It has been suggested that precise estimates of the k parameter are possible only for a sample size of $n \geq 5$ (Bowman, 1984). Consequently, estimates carried out using smaller sample sizes must be treated with caution.

Once estimates of k and μ have been obtained, it is necessary to test or verify for goodness-of-fit of the hypothesised distribution. Methods for testing negative binomial goodness of fit are presented in Elliott (1977). It is not clear what course to recommend in case the negative binomial fit is rejected, as by this stage both the negative binomial and Poisson fits would have been rejected. Finally, confidence intervals can be calculated directly using the negative binomial distribution, or using a Normal approximation. A number of Normal approximations are possible, depending upon the magnitude of the parameter k. The possible transformations are listed in Elliott (1977: Table 4) and Johnson *et al.* (1992: Section 5.7) and their use in calculating confidence limits illustrated in Elliott (1977: Section 6.2). Small samples will give very wide confidence intervals and a sample size of five should be thought of as a bare minimum. As above, because of the complexity of the calculations and the number of different practices which will depend upon the value of the parameter k, the calculations should be performed by computer.

Using the same basic approach, different confidence intervals could be calculated for different levels of taxonomic resolution (species-level identification, genus-level identification, total cell numbers etc.) using the same basic data, so long as the limitations of each of these estimates was properly understood.

2.5.4 Other approaches to estimating density for clumped distributions

There is a growing body of literature dealing with the use of distance-based measures of abundance, rather than count-based measures. Ludwig and Reynolds (1988) discuss this from an ecological viewpoint whilst Diggle (1979) and other contributors to Cormack and Ord (1979) provide a useful review. Distance-based methods use the distances between cells to arrive at estimates of population density. As such, they are based on (x, y) coordinates locating individual cells or colonies in the counting chamber. In view of the difficulty and time taken to establish these measurements under typical laboratory

conditions, and taking into account that most phytoplankton counting chambers display edge effects (see 2.4 and Sandgren & Robinson, 1984), there seems little point in pursuing distance-based methods as far as phytoplankton counting is concerned.

Other measures of contagion are also possible. For example, Taylor *et al.* (1979) compare the use of the negative binomial distribution unfavourably with transformations based on a power law which has been developed in a series of papers beginning with Taylor (1961).

2.5.5 Building a database of information

The problem described above is made considerably harder than it should be by the continual re-estimation of contagion parameters from small but expensive samples. It would seem better to establish and maintain a database of counts so that clumping distributions of individual species become better known. This requires that at the counting stage, not only individual cell counts are made, but also that separate colonies, aggregates or filaments are listed separately, with the cell numbers counted for each. If frequency distributions are relatively stable over time then it should be possible to take smaller sample sizes and use the contagion parameters for the various species from the database for estimating densities, rather than re-estimating them from the small samples. Much of the effort here can be computerised. If such a scheme is desirable and feasible, regular checking and calibration should be performed to ensure that the contagion parameters for different species remain appropriate. In time it may become possible simply to count colonies of a species, (perhaps including a rough categorisation into size for taxa such as *Microcystis*) and then to appeal to the database for the standard contagious distribution for the species.

2.6 Conclusions and Recommendations

This section has emphasised the problems encountered in producing reproducible data on a single sampling occasion. Two general points emerge:

1. The representativeness of high quality estimates of cell numbers based on a single spot water sample needs to be tested for the River Thames. If this cannot be demonstrated, then there may be little justification for increasing effort spent on the enumeration stages.

2. Assigning confidence limits to counts of total cell numbers or dominant taxa is likely to involve a statistical model such as the negative binomial which can handle contagion. This has two serious implications:

    i. Counting procedure will have to be modified. Ideally, at least five replicates per sample are required. This may not necessarily involve a substantially greater effort as the number of fields counted at present could be simply allocated between an appropriate number of chambers. Some preliminary experiments would be required to determine the appropriate number of cells and chambers (2.4.2).

ii. The mechanics of using such a model are such that a computer program will almost certainly be required. However, such a program will, in turn, allow a considerable dataset to be accumulated which may, in time, allow standard contagion parameters to be computed for different taxa.

With some modification to counting and recording conventions, along with programs to perform the calculations described above, this system could be incorporated into an existing database. However, a further possibility might be to develop a system for the direct entry of phytoplankton count data. Such systems have been proposed before (e.g. Cunningham & Purewal, 1984), though early designs were restricted by the limited processing power available. The potential for such a system today, given facilities such as graphic user interfaces, database searches based on "fuzzy" logic and touch-sensitive screens, might have benefits that extend beyond data entry and the computation of confidence limits to providing taxonomic assistance and ecological information.

3. OPTIONS FOR FULL QA OF PHYTOPLANKTON DATA

3.1 QA Procedures Presently Used in Anglian Region

Different approaches to QA of phytoplankton data have evolved in the two regions of the Agency most actively involved in the collection of phytoplankton data. In Anglian Region, a considerable body of expertise in phytoplankton methods has developed, with several staff with specialist skills spread through the Region's laboratories. This contrasts with the situation in Thames Region where most analyses have been performed by a single analyst. Anglian Region's interest has been primarily focused on standing waters (lakes, reservoirs and broads) although some rivers are also studied, in contrast to Thames Region's focus on River Thames. The development of QA methods reflects these conditions.

Anglian Region have produced a procedures manual for phytoplankton methodologies, detailing all aspects from sample collection to reporting. There is also a separate section on quality audits. Present policies on QA, along with their present state of implementation, are described in Table 3.1.

Table 3.1 Quality Assurance procedures used for phytoplankton analyses in Anglian Region.

Stated in procedure manual (section)	State of implementation
regular enumeration spot checks and inter-laboratory calibrations (8.11)	enumeration spot checks are used in some laboratories, but not others. Inter-laboratory calibrations have been tried, but problems have been encountered (see below)
regular visits by quality manager or deputy to ensure adherence to standardized methodologies (8.11)	no quality manager appointed. Northern Region organise these checks internally
Quality checks on data entry and database computations (10.4)	Not enforced
Internal and external taxonomic audits (12.9).	These are used, although a strict "audit" format is not necessarily followed in all cases
Regular performance checks on all items of equipment (15)	No formal checks; down to individual diligence. Northern Area have informal system to ensure that equipment is checked at least once per year.

In essence, the Anglian Region philosophy regarding QA of phytoplankton analyses is that samples are not amenable to "traditional" quality control (QC) and that effort is better applied and results most effectively assured by a combination of documentation, training (formal and informal), regular review and discussion of methods plus some formal QA exercises. Plans to appoint a quality manager to oversee all aspects of biological QA have not materialised and this may have delayed the uptake of all the methods outlined in the procedure manual.

Four methods of taxonomic "audit" are described in section 12.9 of the Procedure Manual. These are not "audits" in the formal sense of the word and "check" is perhaps a more appropriate term. The methods are:

On-site taxonomic audits / checks: an individual operator and either a quality manager or an operator from another laboratory within the Region examine a routine sample together. In addition to general discussions, Whipple field comparisons are carried out using a Whipple graticule and a purpose-designed recording sheet. The first operator identifies the taxa present and their locations on the Whipple field and then folds the sheet down the middle to obscure the first operator's identification and the second operator repeats the procedure. Differences in identification are then discussed.

This method, which was developed in-house, is a useful qualitative method for identifying weak areas (in both operators), as well as for taxonomic training and improving overall taxonomic quality.

Circular / inter-laboratory taxonomic/enumeration audits/checks ("ring tests"): Either an actual sample or a culture is circulated to all participating laboratories and the algae present identified to the lowest taxonomic level within the capabilities of the individual operator. In the case of "mixed" samples each taxon identified is accompanied by a labelled drawing or photograph so that the audit co-ordinator was sure which names were being applied to each taxon.

Results from such audits allow weaknesses to be identified and allow early remedial action (including, if necessary, consultation with external specialists). Such inter-laboratory comparisons have been widely used (e.g. Hobro & Willen, 1977; Rott, 1981) but problems have been encountered in Anglian Region (thought to be caused by leaky sample tubes - a similar problem was found by Rott, 1981)

Spot-check taxonomic / enumeration audits / checks: samples are chosen at random are sent to a checking laboratory along with labelled drawings or photographs of the dominant taxa found.

This technique is the closest of the four to an "audit". As described in the Procedure Manual it is a qualitative method, but it is similar to the methods being developed in Thames Region for quantitative or semi-quantitative QC. No formal system for evaluating "performance" has yet been devised.

External audits / checks: samples are sent to an appropriate authority, along with labelled drawings or photographs and taxonomic notes. These are a necessary

component of overall taxonomic quality, effectively verifying identifications made during the internal procedures described above.

Details of regular performance checks for individual items of equipment are listed in Table 3.2. At present this system is recommended, but is not enforced.

Table 3.2. Types and frequency of quality audits of equipment used for phytoplankton enumeration in Anglian Region. For details see National Rivers Authority (1995)

Audit type	Recommended frequency
sampling equipment and method (check precision)	annual per equipment type / sample type
filtered water (check for absence of contaminating algae)	two months
siphoning efficiency (resuspend collected supernatant and examine for presence of algae)	100 siphons
automatic pipettes (accuracy and precision)	100 deliveries
microscope checks (centering pin, phase rings etc.)	two months
intra-chamber transect counts	four months
intra-chamber field counts	two months
randomness movements	two months
chamber replication (if not done routinely)	two months
data computation system (if applicable)	twice per year
enumeration / taxonomy spot checks	four per year per analyst
enumeration / taxonomy circulations	four per year
enumeration / taxonomy site checks	four per year
database / raw data comparisons	annually
external taxonomic checks	every spot check and circulation and when necessary

3.2 QA Procedures Presently Used in Thames Region

The system that has evolved in Thames Region is different from that used in Anglian Region as there are fewer trained staff and, as a consequence, less potential for intra-regional comparisons. Instead, effort has gone into the development of objective methods for external audits. Early attempts were dogged by problems caused by violations of the assumption of random distributions (2.5.1) and the present version of the audit is semi-quantitative, rather than fully quantitative.

Samples for auditing are selected at random from those examined as part of routine monitoring programs. The prime objective of the audit count is to validate the taxonomy and the secondary objective is to evaluate the relative numbers of each taxon in the sample. For this reason, wet mounts are used to maximise the optical quality of the microscopic image. The number of units counted is the same as in the primary count. Once these have been counted, the slide is scanned and any other taxa encountered are listed as "present". A qualitative audit is performed on all taxa present in the primary or audit counts in numbers greater than or equal to 4. The lower 95% confidence limit (based on the Poisson distribution) of this value is 1. If the detection limit were set any lower, then there is a statistically significant likelihood of a taxon found in the primary count not being found in the audit count. (It should be noted that the audit is performed on the numbers of units counted, rather than the numbers of cells).

Results of the audit are analyzed using an Excel spreadsheet. The purpose of this spreadsheet is simply to draw attention to taxa that deviate markedly from expectation. For statistical reasons (discussed below), this is performed using abundance classes, rather than absolute numbers. Five abundance classes are used, as follows:

1	rare	$< 1\ \%$
2	occasional	$> 1 \leq 5\ \%$
3	frequent	$> 5 \leq 10\ \%$
4	common	$> 10 \leq 50\ \%$
5	abundant	$> 50\ \%$

Both the primary and audit counts are converted to abundance classes. If there is a difference of more than one class for any taxon, then this is brought to the attention of the primary analyst. In most cases, the auditor should be able to suggest a cause for these deviations. No formal analysis of the performance of an analyst takes place and the auditor makes direct telephone contact with the appropriate analyst if there are any concerns regarding the analysis.

The audit focuses on the identification and enumeration stage and deals with the number of phytoplankton units counted rather than the final concentration of cells in the water. If 120 units of a randomly-distributed taxon are counted, then approximate 95% confidence limits will be 98 - 142. To calculate the final concentration of cells, the number of units counted has to be multiplied by other values (e.g. cell volume, pipetting accuracy, conversion factors for filaments and colonies) which also introduce errors. If the objective of the audit is to check enumeration and taxonomic skill, then these

additional errors may confuse interpretation of an audit and should be assessed and controlled by separate means.

This system was used throughout 1997 and proved useful for detecting deviations in performance particularly for the Thames Low Flow survey which was performed by less highly trained staff. However, these deviations should also be detected using methods employed in Anglian Region. Some elements of the Anglian Region procedure would be difficult to apply in Thames Region as there are fewer trained staff. Such problems may be overcome either by joining forces with other regions, or by delegating the role of "quality manager" to an external source. This latter option may even have the advantage of ensuring that the task does not get pushed to one side by other duties.

Similarly, the system used in Thames Region might have some application within Anglian Region, particularly if an objective basis can be found for the evaluation of "performance".

3.3 Options for measurements of operator "performance"

An important aspect recognised by both Anglian and Thames Region is the lack of rigorous measures of overall performance that can be applied to phytoplankton identifications and enumerations. Whilst enumerations of single taxa from replicate samples can, with appropriate transformation, be compared using conventional statistical techniques, "real" samples always contain a number of taxa, thus necessitating several such comparisons.

Repeating the same statistical test several times increases the risk of a "Type I error" (erroneous rejection of the null hypothesis) unless the critical probability is decreased. Assume that four samples are subjected to audit and that comparisons are made between numbers of the five most dominant taxa in each recorded in the primary and audit counts. If a critical probability of $p = 0.05$ is chosen, then one of the twenty comparisons made might be expected to show a significant difference between primary and audit counts. If the units of any particular taxon are unusually clumped, then this failure rate may be higher. Thus, a quantitative audit cannot be based solely on a lack of significant differences.

Furthermore, results from such a comparison are difficult to distil into a single measure of "performance" that can be used to measure an individual's ability to meet a required standard or demonstrate improvement over time. Such performance standards are now accepted for routine invertebrate analyses and, presumably, the same arguments can be applied to phytoplankton analyses. The problem until now has been in finding suitable objective tools for such a procedure.

One approach that is being explored for benthic diatom samples is the use of measures of similarity and dissimilarity (Kelly, 1998). Such measures are already widely used in ecology, both in classification techniques ("cluster analysis") and to quantify differences between fossil assemblages and modern analogues (Overpeck *et al.*, 1985; Huntley, 1990; Flower *et al.*, 1997). To envisage how such a measure might be applied in taxonomic QA consider a single phytoplankton sample that is analysed by several

taxonomists of widely differing abilities, including one acknowledged expert. Were a dendrogram to be constructed from their analyses, then those samples which were clustered closest to that of the "expert" might be judged to be of higher quality than those that were further away on the dendrogram. The dendrogram itself is constructed from a matrix of similarity (or dissimilarity) measures calculated between all pairs of samples and it is possible that such measures may permit a single integrated measure of "performance" to be calculated for a sample. As a database of such comparisons built up, so it may be possible to determine an "acceptable quality level" (AQL: see 4.2.5) for phytoplankton analyses be determined. Ultimately, it may even allow quality management techniques such as control charts to be employed, although the throughput of samples in any one Agency laboratory is probably not sufficient to permit this. Other possible uses include setting performance thresholds for work sent to outside contractors and for evaluating results of "ring tests" and other inter-laboratory comparisons.

A variety of dissimilarity coefficients were applied to fossil pollen spectra and all gave broadly similar results (Overpeck *et al.*, 1985). On the basis of this, one (squared χ^2 distance; SCD) was applied to data collected as part of an inter-laboratory comparison (Kelly, 1997a). However, considerable variation was observed between values of some dissimilarity measures such as the with higher values corresponding to greater diversity and more complex communities (Kelly, 1998). Although such properties are clearly useful when interpreting ecological processes, if dissimilarity values are to be used for biological QA, then such variation can potentially disguise differences between replicate counts. A simpler measure that is less influenced by the structure of the community is the "dominance identity" (DI, Engelberg, 1987):

$$D_{1,2} = \Sigma\, q_i$$

where $D_{1,2}$ is the dominance identity between samples 1 and 2 and q_i is the smaller of the two relative abundances of species i. D can vary between 0% and 100% with Engelberg (1987) regarding values of > 60% as indicating a high degree of structural identity between the two samples. dominance identity is influenced by the complexity of the diatom community only insofar as this challenges the analyst's taxonomic ability. Preliminary results suggest that this property makes DI, although simpler than many other similarity/dissimilarity measures, a better option for comparisons of operator efficiency.

The following example illustrates how DI works. Consider two replicate counts, as follows:

	Sample 1	Sample 2
Taxon 1	**40**	55
Taxon 2	32	**20**
Taxon 3	18	**14**
Taxon 4	**5**	8
Taxon 5	5	**3**

The Dominance Identity in this example is 82 (i.e. the sum of the bold values in the table), whereas in the following example, it is only 55.

	Sample 1	Sample 2
Taxon 1	40	**2**
Taxon 2	**32**	65
Taxon 3	**18**	30
Taxon 4	5	**3**
Taxon 5	5	**0**

If the two counts were identical it would be 100 and if they contained no species in common it would be zero. In the first example, we could consider the two counts to be replicates of each other, whilst in the latter example we would have grounds for suspecting that one of the analysts was at fault.

A further option that may be worth exploring is to use Monte Carlo simulations to predict the expected range of variation of individual taxa in a sample prior to a comparison and establish the likely range of a similarity or dissimilarity measure based on this. Results could then be expressed as the difference between actual and predicted similarity. Such an approach, although technically more sophisticated relies upon a knowledge of expected error rates for different taxa.

Work on such measures is still at a very early stage and more evaluation is required to see how they might usefully fit into a practical QA system. However, if these, or similar approaches, are successful, then the prospect will be opened for objective evaluation of performance in the future.

3.4 Options for Development of Taxonomic Standards

There are authenticated records of 5003 species (and infraspecific taxa) of freshwater alga in the UK (Whitton *et al.*, 1998), most of which might, in principle, be found suspended in a river such as the Thames. At present, guidelines on appropriate levels of taxonomy for routine counting have been vague, typically to identify to the lowest taxonomic level possible, especially for the dominant taxa. There are, however, a number of problems with such a policy:

1. The taxonomic level that is "possible" increases as the range of specialist floras in a laboratory increases. However, as a general rule, identification of a "new" species is a time consuming process, particularly as many of the relevant floras are not written in English.

2. An individual's taxonomic skills change over time and the level of identification "possible" for a beginner may result in data that are not comparable with other data collected. A lack of clear written guidelines may not be a problem where several phytoplankton specialists share resources and can oversee the work of a beginner, but may cause problems for a Region reliant upon a single specialist, particularly if, for any reason, that person changes job without an opportunity to brief a successor. Such situations, rather than the steady-state, are the real test of a QA system.

3. The relationship between "data" and "information" is rarely straightforward, particularly for situations involving complicated mixes of the "qualitative" (what taxon?) and "quantitative" (how many?). Whilst increased effort at counting and identifying may result in "better" data from an academic perspective (e.g. Pappas & Stoermer, 1996), it is erroneous to assume that this automatically translates into better environmental information, leading to more robust decisions.

4. The greater the complexity of data, the more difficult it will be to verify by any of the methods described in 3.1 or 3.2.

5. The lack of an agreed taxonomic standard can lead to substantial nomenclatural problems as different floras are used by different people over a period of time to name essentially the same organism. This was a major problem for Kelly and Whitton (1994) when examining data on benthic algae from streams in Northern England collected over a twenty year period. The accumulated effect of a number of small shifts in convention may not be apparent over short time periods (e.g. 2 - 3 years) but may greatly reduce the value of specific identifications over longer periods.

Whilst all of these create a strong case for one or more agreed taxonomic standards for phytoplankton work, development of such standards will not be easy. It will, however, be easier for a single, well-studied river such as the Thames than for a wider geographical region. It should also be viewed within the framework of a wider DQO for a particular analysis, and related to the information needs of that analysis.

In essence, a taxonomic standard has to balance ease of identification against the extra environmental information gained from that identification. Such a task could be

performed intuitively, or semi-quantitatively (i.e. abundant taxa only are identified to species; generic identification is sufficient for others) but a third, and potentially more useful approach is to use an ordination technique such as Constrained Canonical Analysis (CCA: ter Braak & Prentice, 1988) to produce plots of taxa superimposed upon the principle axes of variation (Noppe *et al.*, 1998). Where species of a particular genus are clustered closely together, then there is no *a priori* case for specific identification. When they occupy quite different positions on the CCA plot, then they are conveying different types of environmental information and specific level determination is justified.

This can be illustrated by consideration of Fig. 3.1 in which phytoplankton taxa collected from the River Thames in the winter are plotted on the two principal axes of variation revealed by CCA (Ruse and Love (1997). *Monoraphidium minutum* and *M. contortum* are widely separated on this plot, suggesting that identification to species does contribute extra environmental information, whilst medium- and large-sized oval *Chlorella* occur very close to each other suggesting that this distinction is not necessary (and possibly taxonomically invalid). Although the CCA procedure itself is objective, decisions about what constitutes a "significant" distance may have to be

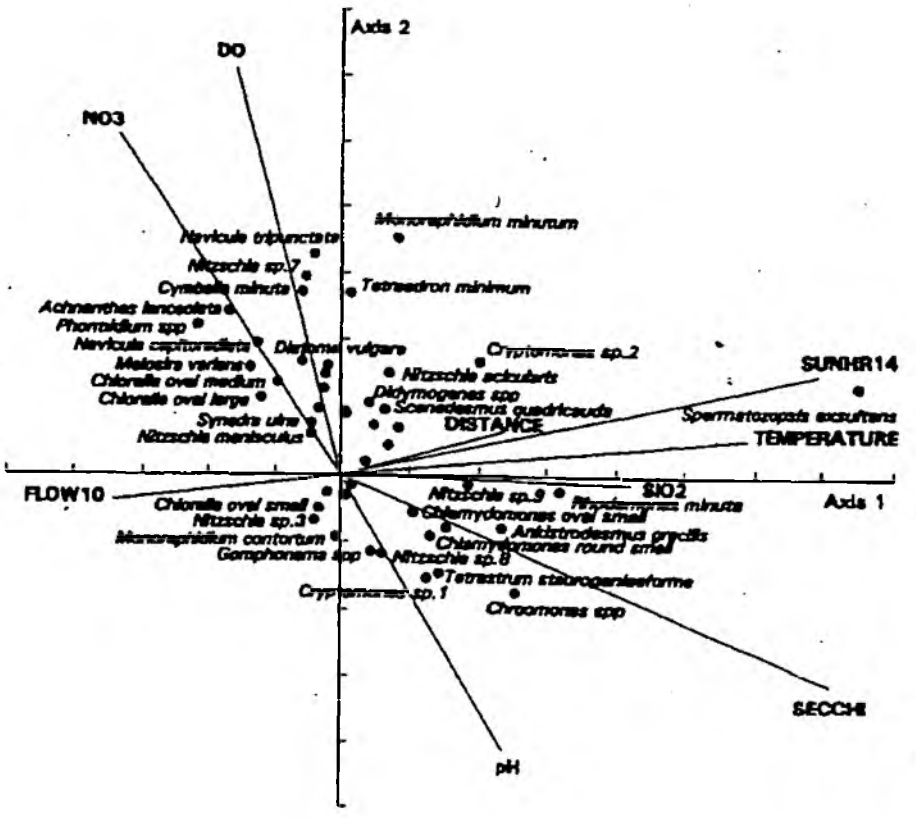


Fig. 3.1. Plot of first two axes of variation from Canonical Correspondence Analysis (CCA) of phytoplankton taxa and environmental data from the River Thames during Winter months between August 1992 and April 1995 (from Ruse & Love, 1997)

subjective (and will vary depending upon the amount of variation explained by the axis in question. However, such an approach may underpin the process of development of a taxonomic standard relevant to a particular DQO.A conclusion leading on from this is

that species level information is required in the first instance in order to make decisions about appropriate DQOs. Moreover, we cannot assume the same species will behave similarly and give similar information in different rivers.

4. BEST PRACTICABLE QA PROCEDURES FOR THAMES REGION

It should be clear from this report that a number of different approaches to QA of phytoplankton analyses are possible. Three possible options for Thames Region are:

1. **Do nothing - i.e. continue with methods outlined in 3.2.** The audit in use at present is adequate for detecting major taxonomic problems but is semi-quantitative so provides little reassurance about the final concentrations of algae in the River Thames.

2. **Adopt Anglian Region's methods.** These, if fully implemented, would provide a high level of basic QA, although there are some problems:

   i. Some elements will be difficult to run in Thames Region, which has fewer trained phycologists than Anglian Region.

   ii. The QA methods stops short of formal tests of reproducibility of samples collected as part of routine surveys (due to practical problems associated with measuring "reproducibility").

   iii. Elements of the Phytoplankton Procedures Manual are vague on some details of methodology, e.g. appropriate taxonomic levels.

3. **Define new suite of QA procedures appropriate to the situation in Thames Region.** This is the favoured option, as it allows the best elements of current practice in both Regions to be adopted, and for new ideas, such as DQOs as well as approaches described in 2.5 and 3.2 to be explored.

4.1 Are Existing Enumeration Methods Adequate for Producing Reproducible Data?

The method for phytoplankton enumeration and identification used in Thames Region used at present is as follows:

1. Use of Lund cell for enumeration.

2. Fields are selected at random, avoiding the edges of the chamber.

3. Ientification of all taxa to the finest level that is practicable using light microscopy. Most centric diatoms are "lumped" together into a single class, and broad categories (based on size and class) are used to classify *Chlorella* and *Chlamydomonas*-type cells. (Note: some data on composition of centric diatom communities during the spring bloom are available but have not yet been analyzed.).

4. Approximately 1 in 5 samples sent for audit.

Although sedimentation chambers are more widely used in the water industry than Lund cells for phytoplankton enumeration, there not enough conclusive data to justify a change of practice in Thames Region on statistical grounds alone. The small size of most characteristic river phytoplankton taxa may make the type of chamber less of an issue than for laboratories examining mainly lake and reservoir samples. The main advantage of switching to sedimentation chambers would be an improvement in the optical quality of samples. However, if it can be shown that errors introduced during enumeration are less than the sum of errors introduced during sampling and concentration, then there may be little practical benefit in improving enumeration procedures until these have been addressed.

Recommendation:

1. A formal evaluation of errors introduced at all stages (including sampling and concentration) community composition, total cell/unit numbers and biomass (as chlorophyll) should all be considered.

2. A comparison between Lund cells and sedimentation chambers for enumerating typical river phytoplankton samples should be considered. If there is a significant improvement in accuracy and precision of data through using sedimentation chambers, then a change in practice should be considered.

3. Whatever type of chamber is adopted, a further test needs to be performed to decide whether randomised or stratified counts are required in light of potential 'edge' effects.

Taxonomic standards that are achievable by Thames Region personnel at present are generally high, although the failure to distinguish many centric diatoms, especially *Cyclotella* and *Stephanodiscus* spp., may lose some important ecological information. This capacity represents a considerable investment in training and the practical justification for such training needs to be examined, before new staff are trained. The publication of a checklist of freshwater algae from the British Isles (Whitton *et al.*, 1998) is an opportunity to harmonize nomenclature.

Recommendations:

1. Develop one or more taxonomic DQOs for the River Thames, using a combination of methods, including CCA. Use these DQOs to define training requirements for new staff. Separate DQOs might be developed for blue-green algal events, routine river samples, routine lake samples etc.

2. Harmonise all nomenclature in line with the new British Isles checklist of freshwater algae (Whitton *et al.*, 1998). Consider preparing a brief handbook to the 'state of the art' taxonomic literature as an interim measure before publication of the British Freshwater Algal Flora.

Recommendations (cont.)

3 Evaluate the benefits of improving taxonomic standards for groups such as centric diatoms which are presently "lumped" together.

4.2 Appropriate QA procedures for River Thames phytoplankton studies

4.2.1 DQOs

The DQO concept offers several practical benefits for routine studies, particularly by relating the effort expended upon a sample to the type of information that is required from that sample. At present, samples from the River Thames are processed to one of two standards ("Teddington Low Flow Survey" and "Thames Water Abingdon Reservoir Project").

Recommendation:

The rationale behind analyzing samples from the River Thames to two different taxonomic standards should be examined. Depending upon the outcome, one or more DQOs for River Thames phytoplankton studies should be established.

4.2.2 Manuals

The methodology for phytoplankton sampling and enumeration is described briefly at present. By contrast, Anglian Region have a much longer manual (approximately 90 pages). There are also plans for a national series of methodology manuals for biology. Whilst there would be benefits from improving the manual used at present in Thames Region, it would be wise to avoid simply repeating the contents of the Anglian Region manual. However, if the DQO concept is adopted, then there are clearly grounds for Anglian Region and Thames Region methods to coexist and to complement each other, and for both to lay the groundwork for parts of a national manual.

Recommendation:

The Thames manual should be developed and refined to provide a comprehensive guide to data collection to the standards described at the end of section 4.2. This should be done in consultation with staff in Anglian Region to ensure that there is minimal overlap.

4.2.3 Training

Guidelines for training are outlined in the Anglian Region Phytoplankton Manual, and these should be suitable for staff in Thames Region as well. The level of identification used for the River Thames surveys is such that a relatively high level of taxonomic training is required. This is not a task for an absolute beginner and some prerequisites should be specified for anyone who is to be involved in this work. As an absolute

minimum these should include a professionally-run algal identification course (see Anglian Region Phytoplankton Manual for details). An appropriate DQO will also provide a framework for evaluating the suitability of these courses.

As phytoplankton work in Thames Region has tended to be assigned to a single biologist, options for in-house training are limited at present. As a matter of policy, one extra biologist should be trained in basic phytoplankton identification as a "reserve" in case of job changes, unforeseen illness etc. As considerable investment is required to train an individual for phytoplankton enumeration, effort should also be made to minimise staff turnover in these posts, perhaps by recognising the specialist nature of the work and rewarding it appropriately.

Recommendation:

Stages of training for staff involved in phytoplankton enumeration should be specified in the Manual.

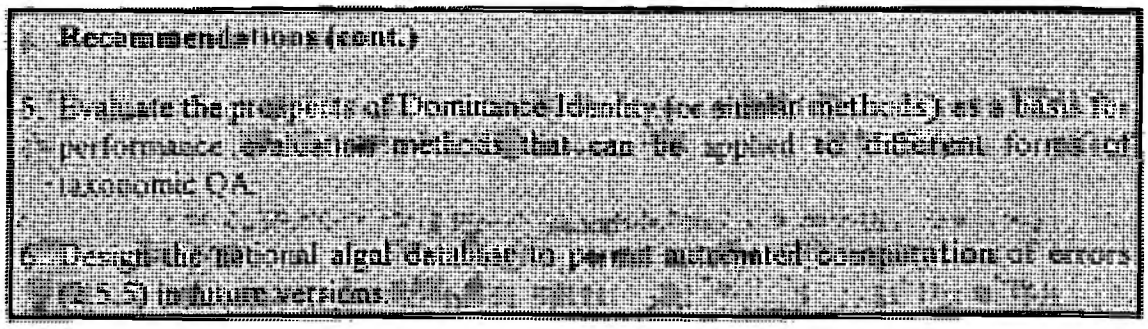
4.2.4 Audits and other checking/testing procedures

Despite the many difficulties associated with objective evaluation of such procedures, there is clearly a role for these in a QA system. Even if throughput of samples is insufficient to justify formal QC procedures, other types of checks are useful adjuncts to on-going training and, even if objective evaluation is impracticable, provide a basis for discussion and learning.

The low number of staff involved in phytotplankton identification in Thames Region creates difficulties in adopting some of the methods used in Anglian Region. However, there may be options for collaboration between regions, or by assigning the role of "quality manager" to an outsider.

Recommendations:

1. Consider joining Anglian Region's inter-laboratory taxonomic/enumeration ring test.
2. If Anglian Region's exercises are not appropriate, consider developing an alternative inter-Regional (or inter-organisational) ring test concentrating on river phytoplankton.
3. Assign the role of "Quality Manager" to an outsider and specify a certain number of on-site taxonomic checks per year, along with a basis for external audits.
4. Institute regular checks of all equipment used in phytoplankton sampling, concentration and enumeration and a system of documentation for ensuring that these tests are kept up to date.



4.2.5 Appropriate quality targets for phytoplankton data from Thames

Underlying all efforts to quantify errors at different stages in the sampling and enumeration processes is the principle of reproducibility: that another individual, following identical methods, will obtain results that fall within the confidence limits calculated for the sample by the original analyst. However, calculation of these confidence limits is not straightforward (2.5) and will require modifications to Thames Region's enumeration protocols.

Were this achieved, then it would be theoretically possible to define an AQL for phytoplankton enumeration, and to use this as a basis for QC using established procedures such as control charts. Such a process is theoretically possible but is not an appropriate short-term objective and would require substantial investment in time and effort to develop procedures.

An alternative approach, which is feasible in the medium-term, is further development and testing of the dominance identity concept (3.3). Based on data already collected, and being collected as part of Thames Region's ongoing programme of external audits, the scale of variation in DI expected under "normal" conditions could be calculated and used as the basis for the establishment of an AQL. Such an AQL would be limited in scope, as DI is concerned only with the proportions of taxa in a sample and would have to be supported by a programme testing other stages of the enumeration process (e.g. accuracy of pipettes) as well as discussions and comparisons amongst the participants. It would, however, enable major swings in performance (e.g. due to change of analysts) to be detected and provide a baseline against which new staff could test their abilities.

5. CONCLUSIONS AND OPTIONS FOR FUTURE RESEARCH

It was clear from the literature searches involved in preparing this report that the development of formal methods for QA of phytoplankton data are still in their infancy. One suspects that other organisations have addressed this issue, but their reports are largely unpublished. It was, however, clear from discussions with phycologists from elsewhere in Europe that many organisations "trust" their analysts to produce robust data and have not addressed the issue of QA at all.

QA methods are already used successfully for environmental analyses concerned only with quantities (e.g. chemical analyses: Cheeseman & Wilson, 1978) or qualities (e.g. invertebrate samples for BMWP / RIVPACS: Environment Agency, 1997). Phytoplankton enumeration, however, involves assessments of both quantity and quality and this creates a number of problems for developing methods. Quantitative aspects are complicated further by the clumped distributions that are typically encountered. The combined effect of these two factors is to make traditional approaches to QA difficult to implement without considerably more research.

The basis of the approach proposed in this report is the definition of a DQO for phytoplankton analyses in the River Thames (1.2) in order to define the needs to which the data will be put. This DQO in turn defines methodology, level of taxonomy required and training requirements and sets a standard against which evaluations of all these aspects can be assessed. The suite of QA procedures adopted draws on approaches used in Anglian Region (3.1) but with modifications to suit the circumstances of Thames Region. Several ideas highlighted in this report will require further research and development before they can be employed.

A number of issues have been raised in this report which need to be addressed by further research. However, the nature of this work means that it needs to be performed in house. Therefore, the main recommendation from this report is that time is set aside for staff within Thames Region to gather basic measurements required on which decisions regarding data quality can be based.

Ideas developed in this report will hopefully generate discussion both within Thames Region and in other Regions of the Agency. If this is the case, then it may also be appropriate to organise a workshop for Agency staff, along with staff from SEPA, water companies and others with an interest in phytoplankton enumeration to discuss and develop these ideas further.

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APPENDIX 1: GLOSSARY OF TERMS USED IN ECOLOGICAL QA

Acceptable quality level (AQL)	The limiting process average (i.e. the worst value of the underlying average quality) that is still acceptable for AQC purposes. If the process average is less than the AQL then corrective action is required.
Analytical Quality Control (AQC)	Procedures to control errors in laboratory analyses within specified limits. Often referred to simply as quality control
Data Quality Objectives	Qualitative and quantitative expressions that define requirements for data precision, bias, method sensitivity and range of conditions over which a method yields satisfactory data (Environmental Protection Agency, 1996)
Performance characteristics	Qualitative and quantitative expressions that define performance of a method
Primary analysis	The main analysis of the sample. In the case of phytoplankton analysis, this includes the identification and enumeration of the taxa present in the sample.
Process average	The quality of the primary analysis during a given period.
Quality Assurance	(1) A set of operating principles that, if strictly followed during sample collection and analysis, will produce data of known and defensible quality (APHA, 1989) (2) Procedures to quantify and control or reduce errors (Environment Agency, 1996)
Quality Audits	An independent measurement of the quality of the laboratory analysis of samples.
Quality Control	see Analytical Quality Control
Quality Management	Collective term for the procedures required for QA
Traceability	The ability to trace the history, application or location of an item or activity, or similar items or activities, by means of recorded identification.