1

Introduction to Freshwater Algae

1.1 General introduction

Algae are widely present in freshwater environments, such as lakes and rivers, where they are typically present as micro-organisms – visible only with the aid of a light microscope. Although relatively inconspicuous, they have a major importance in the freshwater environment, both in terms of fundamental ecology and in relation to human use of natural resources.

This book considers the diversity of algae in freshwater environments and gives a general overview of the major groups of these organisms (Chapter 1), methods of collection and enumeration (Chapter 2) and keys to algal groups and major genera (Chapter 4). Algae are considered as indicators of environmental conditions (bioindicators) in terms of individual species (Chapter 1) and as communities (Chapter 3).

1.1.1 Algae – an overview

The word 'algae' originates from the Latin word for seaweed and is now applied to a broad assemblage of organisms that can be defined both in terms of morphology and general physiology. They are simple organisms, without differentiation into roots, stems and leaves – and their sexual organs are not enclosed within protective coverings. In terms of physiology, they are fundamentally autotrophic (obtaining all their materials from inorganic sources) and photosynthetic – generating complex carbon compounds from carbon dioxide and light energy. Some algae have become secondarily heterotrophic, taking up complex organic molecules by organotrophy or heterotrophy (Tuchman, 1996), but still retaining fundamental genetic affinities with their photosynthetic relatives (Pfandl *et al.*, 2009).

The term 'algae' (singular alga) is not strictly a taxonomic term but is used as an inclusive label for a number of different phyla that fit the broad description noted above. These organisms include both prokaryotes (Section 1.3, cells lacking a membrane-bound nucleus) and eukaryotes (cells with a nucleus plus typical membrane-bound organelles).

Humans have long made use of algal species, both living and dead. Fossil algal diatomite deposits, for example, in the form of light but strong rocks, have been used as building materials and filtration media in water purification and swimming pools. Some fossil algae, such as *Botryococcus,* can give rise to oil-rich deposits. Certain species of green algae are cultivated for the purpose of extracting key biochemicals for use in medicine and cosmetics. Even blue-green algae, often regarded as nuisance organisms, may have beneficial uses. This is particularly the case for *Spirulina,* which was harvested by the Aztecs of Mexico and is still used by the people around Lake Chad as a dietary supplement. *Spirulina* tablets may still be obtained in some health food shops. Blue-green algae are, however, are better known in the freshwater environment as nuisance organisms, forming dense blooms having adverse effects on human activities

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by producing toxins, clogging water courses and impairing recreational activities.

1.1.2 Algae as primary producers

As fixers of carbon and generators of biomass, algae are one of three major groups of photosynthetic organism within the freshwater environment. They are distinguished from higher plants (macrophytes) in terms of size and taxonomy, and from photosynthetic bacteria in terms of their biochemistry. Unlike algae, photosynthetic bacteria are strict anaerobes and do not evolve oxygen as part of the photosynthetic process.

The level of primary production by algae in freshwater bodies can be measured as fixed carbon per unit area with time (mg C m^{-3} h^{-1}), and varies greatly from one environment to another. This is seen, for example, in different lakes – where primary production varies with trophic status and with depth in the water column (Fig. 1.1). Eutrophic lakes, containing high levels of available nitrogen and phosphorus, have very high levels of productivity in surface waters, decreasing rapidly with depth due to light absorption by algal biomass. In contrast, mesotrophic and oligotrophic lakes have lower overall productivity – but this extends deep into the water column due to greater light penetration.

Although algae are fundamentally autotrophic (photosynthetic), some species have become secondarily heterotrophic – obtaining complex organic compounds by absorption over their outer surface or by active ingestion of particulate material. Although

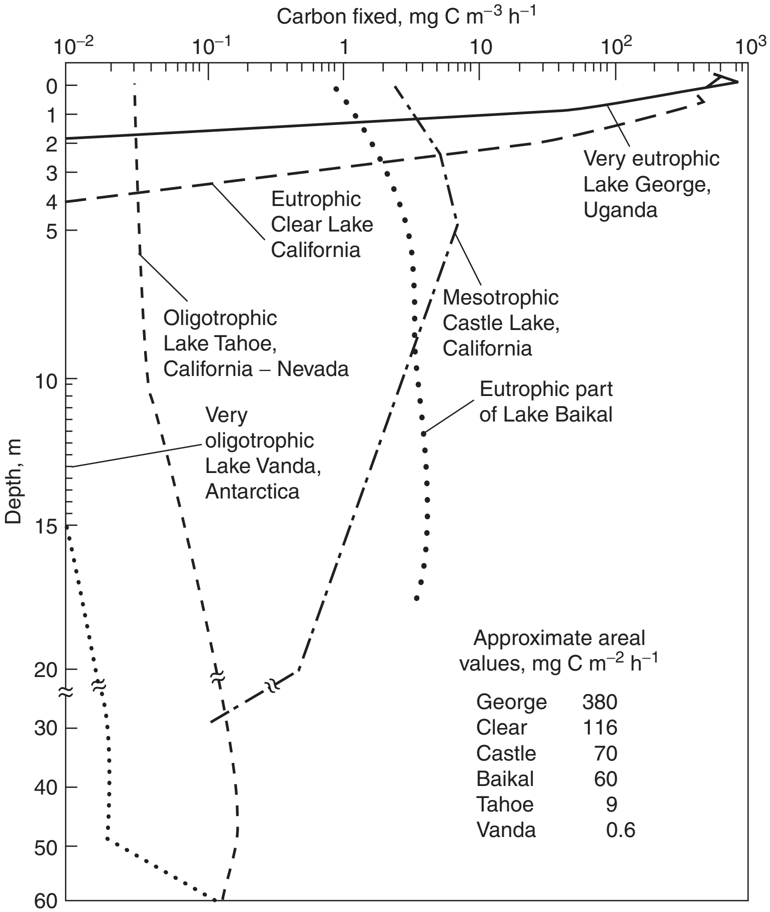

Figure 1.1 Algal photosynthesis: primary production in six contrasting lakes. Showing the rate of photosynthesis (carbon fixation per unit area) with depth in lakes of varying nutrient availability. These range from very eutrophic (Lake George, Uganda) to eutrophic (Clear Lake, California; part of Lake Baikal), mesotrophic (Castle Lake, California), oligotrophic (Lake Tahoe, California–Nevada) and very oligotrophic (Lake Vanda, Antarctica). Reproduced, with permission, from Horne and Goldman (1994).

such organisms often superficially resemble protozoa in terms of their lack of chlorophyll, vigorous motility and active ingestion of organic material, they may still be regarded as algae due to their phylogenetic affinities.

1.1.3 Freshwater environments

Aquatic biology can be divided into two major disciplines – limnology (water bodies within continental boundaries) and oceanography (dealing with oceans and seas, occurring between continents). This book focuses on aquatic algae present within continental boundaries, where water is typically fresh (non-saline) and where water bodies are of two main types:

- standing (lentic) waters – particularly lakes and wetlands
- running (lotic) waters – including streams and rivers.

The distinction between lentic and lotic systems is not absolute, since many 'standing waters' such as lakes have a small but continuous flow-through of water, and many large rivers have a relatively low rate of flow at certain times of year. Although the difference between standing and running waters is not absolute, it is an important distinction in relation to the algae present, since lentic systems are typically dominated by planktonic algae and lotic systems by benthic organisms.

Although this volume deals primarily with algae present within 'conventional freshwater systems' such as lakes and rivers, it also considers algae present within more extreme freshwater environments such as hot springs, algae present in semi-saline (brackish) and saline conditions (e.g. estuaries and saline lakes) and algae present within snow (where the water is in a frozen state for most of the year).

1.1.4 Planktonic and benthic algae

Within freshwater ecosystems, algae occur either as free-floating (planktonic) or substrate-associated (largely benthic) organisms. Planktonic algae drift freely within the main body of water (with some species able to regulate their position within the water column), while substrate-associated organisms are either fixed in position (attached) or have limited movement in relation to their substrate. These substrate-associated algae are in dynamic equilibrium with planktonic organisms (see Fig. 2.1), with the balance depending on two main factors – the depth of water and the rate of water flow. Build-up of phytoplankton populations requires a low rate of flow (otherwise they flush out of the system) and adequate light levels, so they tend to predominate at the surface of lakes and slow moving rivers. Benthic algae require adequate light (shallow waters) and can tolerate high rates of water flow, so predominate over phytoplankton in fast flowing rivers and streams. Benthic algae also require adequate attachment sites – which include inorganic substrate, submerged water plants and emergent water plants at the edge of the water body. The distinction between planktonic and non-planktonic algae is ecologically important and is also relevant to algal sampling and enumeration procedures (Chapter 2).

Planktonic algae

Planktonic algae dominate the main water body of standing waters, occurring as a defined seasonal succession of species in temperate lakes. The temporal sequence depends on lake trophic status (Section 3.2.3; Table 3.3) with algae forming dense blooms (see Glossary) in eutrophic lakes of diatoms (Fig. 1.16), colonial blue-green algae (Fig. 1.5) and late populations of dinoflagellates (Fig. 1.10). During the annual cycle, phytoplankton blooms correspond to peaks in algal biovolume and chlorophyll-*a* concentration, and troughs in turbidity (see Fig. 2.8).

Benthic algae

Benthic algae occur at the bottom of the water column in lakes and rivers, and are directly associated with sediments – including rocks, mud and organic

Table 1.1 Size Range of Phytoplankton

Category	Linear Size (Cell or Colony Diameter, µm)	Biovolume* (µm^3)	Unicellular Organisms	Colonial Organisms
Picoplankton	0.2–2	4.2×10^{-3}–4.2	Photosynthetic bacteria Blue-green algae *Synechococcus* *Synechocystis*	–
Nanoplankton	2–20	4.2–4.2×10^3	Blue-green algae Cryptophytes *Cryptomonas* *Rhodomonas*	
Microplankton	20–200	4.2×10^3–4.2×10^6	Dinoflagellates *Ceratium* *Peridinium*	Diatoms *Asterionella*
Macroplankton	>200	$>4.2 \times 10^6$	–	Blue-green algae *Anabaena* *Microcystis*

Biovolume values are based on a sphere (volume = $^4/_3\pi r^3$).
Table reproduced from Sigee, 2004.

debris. These attached algae may form major growths on inorganic surfaces or on organic debris, where they are frequently present in mixed biofilms (with bacteria, fungi and invertebrates also present). Under high light conditions, the biofilm may become dominated by extensive growths of filamentous algae – forming a periphyton community (Fig. 2.23). Attached algae may also be fixed to living organisms as epiphytes – including higher plants (Fig. 2.29), larger attached algae (Fig. 2.28) and large planktonic colonial algae. Some substrate-associated algae are not attached, but are able to move across substrate surfaces (e.g. pennate diatoms), are loosely retained with gelatinous biofilms or are held within the tangled filamentous threads of mature periphyton biofilms.

Many algal species have both planktonic and benthic stages in their life cycle. In some cases they develop as actively photosynthetic benthic organisms, which subsequently detach and become planktonic. In other cases the alga spends most of its actively photosynthetic growth phase in the planktonic environment, but overwinters as a dormant metabolically inactive phase. Light micrographs of the distinctive overwintering phases of two major bloom-forming algae (*Ceratium* and *Anabaena*) are shown in Fig. 2.7.

1.1.5 Size and shape

Size range

The microscopic nature of freshwater algae tends to give the impression that they all occur within a broadly similar size range. This is not the case with either free floating or attached algae.

In the planktonic environment (Table 1.1), algae range from small prokaryotic unicells (diameter <1 µm) to large globular colonies of blue-green algae such as *Microcystis* (diameter reaching 2000 µm) – just visible to the naked eye. This enormous size range represents four orders of magnitude on a linear basis (×12 as volume) and is similar to that seen for higher plants in terrestrial ecosystems such as tropical rainforest.

Planktonic algae are frequently characterized in relation to discrete size bands – picoplankton (<2 µm), nanoplankton (2–20 µm), microplankton (20–200 µm) and macroplankton (>200 µm). Each size band is characterized by particular groups of algae (Table 1.1).

In the benthic environment, the size range of attached algae is even greater – ranging from small unicells (which colonize freshly exposed surfaces) to extended filamentous algae of the mature periphyton community. Filaments of attached algae such as *Cladophora*, for example, can extend several centimetres into the surrounding aquatic medium. These macroscopic algae frequently have small colonial algae and unicells attached as epiphytes (Fig. 2.28), so there is a wide spectrum of sizes within the localized microenvironment.

Diversity of shape

The shape of algal cells ranges from simple single non-motile spheres to complex multicellular structures (Fig. 1.2). The simplest structure is a unicellular non-motile sphere (Fig. 1.2b), which may become elaborated by the acquisition of flagella (Fig. 1.2c), by a change of body shape (Fig. 1.2a) or by the development of elongate spines and processes (Fig. 1.2d). Cells may come together in groups without defined number or shape (Fig. 1.2e) or may form globular colonies that have a defined morphology (Fig. 1.2f,g). Cells may also join together to form linear colonies (filaments) which may be unbranched or branched (Fig. 1.2h, i).

Although motility is normally associated with the possession of flagella, some algae (e.g. the diatom *Navicula* and the blue-green *Oscillatoria*) can move without the aid of flagellae by the secretion of surface mucilage. In many algae, the presence of surface mucilage is also important in increasing overall cell/colony size and influencing shape.

Size and shape, along with other major phenotypic characteristics, are clearly important in the classification and identification of algal species. At a functional and ecological level, size and shape are also important in terms of solute and gas exchange, absorption of light, rates of growth and cell division, sedimentation in the water column, cell/colony motility and grazing by zooplankton and (Sigee, 2004).

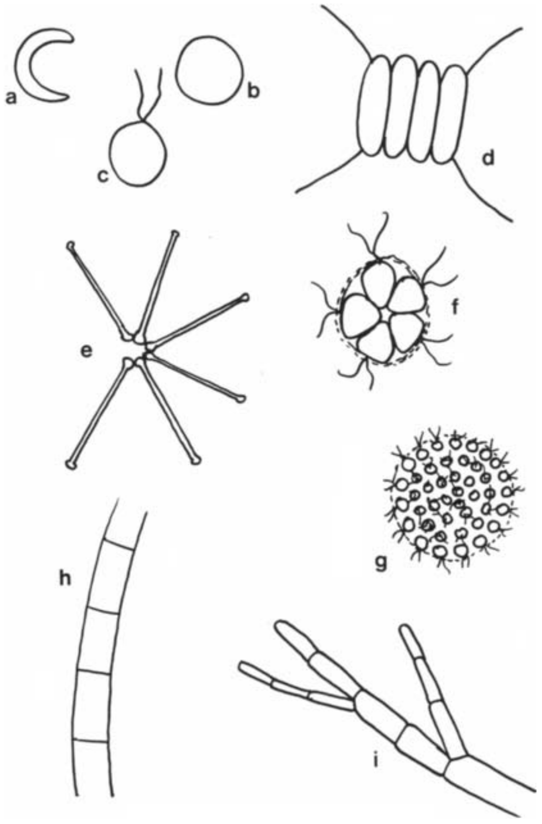

Figure 1.2 General shapes of algae. Non-motile unicells: (a) *Selenastrum*; (b) *Chlorella*. Motile unicell: (c) *Chlamydomonas*. Non-motile colony: (d) *Scenedesmus* (e) *Asterionella*. Motile colony: (f) *Pandorina*; (g) *Volvox*. Unbranched filament (h) *Spirogyra*. Branched filament (i) *Cladophora*. Reproduced, with permission, from Bellinger (1992).

1.2 Taxonomic variation – the major groups of algae

Freshwater algae can be grouped into 10 major divisions (phyla) in relation to microscopical appearance (Table 1.2) and biochemical/cytological characteristics (Table 1.3). Some indication of the ecological and taxonomic diversity of these groups is given by the number of constituent species (Table 1.2) for freshwater and terrestrial algae in the British Isles (taken from John *et al*., 2002), with green algae and diatoms far outnumbering other groups – reflecting their widespread occurrence and ability to live in diverse habitats. Diatoms in particular (over 1600 species) are ecologically successful, both as planktonic and benthic organisms. In addition to the above groups, John *et al*. (2002) also list other phyla – Raphidophyta (two species), Haptophyta (five species), Eustigmatophyta (three species), Prasinophyta (13 species) and Glaucophyta (two species). Although these minor phyla have taxonomic and phylogenetic interest, they have less impact in the freshwater environment.

Table 1.2 Major Divisions of Freshwater Algae: Microscopical Appearance

Algal Division (phylum)	Index of Biodiversity[a]	Typical Colour	Typical Morphology of Freshwater Species	Motility (Vegetative Cells/Colonies)	Typical Examples
1. Blue-green algae *Cyanophyta*	297	blue-green	Microscopic or visible – usually colonial	Buoyancy regulation Some can glide	*Synechocystis* *Microcystis*
2. Green algae *Chlorophyta*	992	grass-green	Microscopic or visible – unicellular or filamentous colonial	Some unicells and colonies with flagella	*Chlamydomonas* *Cladophora*
3. Euglenoids *Euglenophyta*	124	Various colours	Microscopic – unicellular	Mostly with flagella	*Euglena* *Colacium*
4. Yellow-green algae: *Xanthophyta*	73	yellow-green	Microscopic – unicellular or filamentous	Flagellate zoospores and gametes	*Ophiocytium* *Vaucheria*
5. Dinoflagellates *Dinophyta*	54	red-brown	Microscopic – unicellular	All with flagella	*Ceratium* *Peridinium*
6. Cryptomonads *Cryptophyta*	15	various colours	Microscopic – unicellular	Mostly with flagella	*Rhodomonas* *Cryptomonas*
7. Chrysophytes *Chrysophyta*	115	golden brown	Microscopic – unicellular or colonial	Some with flagella	*Mallomonas* *Dinobryon*
8. Diatoms *Bacillariophyta*	1652	golden brown	Microscopic – unicellular or filamentous colonies	Gliding movement on substrate	*Stephanodiscus* *Aulacoseira*
9. Red algae *Rhodophyta*	22	red	Microscopic or visible – unicellular or colonial	Non-motile	*Batrachospermum* *Bangia*
10. Brown algae *Phaeophyta*	2	brown	Visible – multicellular cushions and crustose thalli	Non-motile	*Pleurocladia* *Heribaudiella*

Data from John *et al*., 2002.

[a] Biodiversity: number of species of freshwater and terrestrial algae within the British Isles.

Table adapted from Sigee, 2004.

Table 1.3 Major Divisions of Freshwater Algae: Biochemical and Cytological Characteristics

	Pigmentation#					Chloroplast Fine-Structure		
Algal Division (phylum)	Chlorophylls	Carotenes	Diag.* Carotenoids	Starch-like Reserve	External Covering	Outer Membranes	Thylakoid Groups	Flagella (Vegetative Cells & Gametes)
1. Blue-green algae *Cyanophyta*	$\mathbf{a}$	$\boldsymbol{\beta}$	**zea-**	Cyano-phycean starch$^{\alpha}$	Peptidoglycan matrices or walls	0	0	0
2. Green algae *Chlorophyta*	$\mathbf{a,b}$	$\boldsymbol{\alpha, \beta, \gamma}$	**viola-**	True starch$^{\alpha}$	Cellulose walls, scales	2	2–6	0–many. Similar (isokont)
3. Euglenoids *Euglenophyta*	$\mathbf{a,b}$	$\boldsymbol{\beta, \gamma}$		Paramylon$^{\beta}$	Protein pellicle	3	3	1–2 emergent
4. Yellow-green algae: *Xanthophyta*	$\mathbf{a,c_1,c_2}$	$\boldsymbol{\alpha, \beta}$		Chrysolaminarin$^{\beta}$	Pectin or pectic acid wall	4	3	2 unequal (heterokont)
5. Dinoflagellates *Dinophyta*	$\mathbf{a,c_2}$	$\boldsymbol{\beta}$	**peri-**	True starch$^{\alpha}$	Cellulose theca (or naked)	3	3	2 unequal (heterokont)
6. Cryptomonads *Cryptophyta*	$\mathbf{a,c_2}$	$\boldsymbol{\alpha, \beta}$	**allo-**	True starch$^{\alpha}$	Cellulose periplast	4	2	2 equal (isokont)
7. Chrysophytes *Chrysophyta*	$\mathbf{a,c_1,c_2,c_3}$	$\boldsymbol{\alpha, \beta, \varepsilon}$		Chrysolaminarin$^{\beta}$	Pectin, plus minerals and silica	4	3	2 unequal (heterokont)
8. Diatoms *Bacillario-phyta*	$\mathbf{a,c_1,c_2,c_3}$	$\boldsymbol{\beta, \varepsilon}$	**fuco-**	Chrysolaminarin$^{\beta}$	Opaline silica frustule	4	4	1, reproductive cells only
9. Red algae *Rhodophyta*	$\mathbf{a}$	$\boldsymbol{\alpha, \beta}$		Floridean starch$^{\alpha}$	Walls with galactose polymer matrix	2	0	0
10. Brown algae *Phaeophyta*	$\mathbf{a,c_1,c_2,c_3}$	$\boldsymbol{\beta, \varepsilon}$		Laminarin$^{\beta}$	Walls with alginate matrix	4	3	2 unequal (heterokont) reproductive cells only

Data from Lee (1997), van den Hoek *et al*. (1995), John *et al*. (2002) and Wehr and Sheath (2003).

#Major pigments are shown in bold type.

*Diagnostic carotenoids, used for HPLC analysis (Fig. 2.11): zea- (Zeaxanthin: also present in chlorophytes, cryptophytes), viola- (violaxanthin), peri- (peridinin), allo- (alloxanthin), fuco- (fucoxanthin, also present in chrysophytes).

Starch-like reserves α:α-1,4 glucan; β:β-1,3 glucan.

Table adapted from Sigee, 2004.

In terms of diversity, freshwater algae also have a major division into prokaryotes (blue-green algae) and eukaryotes (remaining groups) based on cell size, ultrastructure, antibiotic resistance and general physiology. Even within the eukaryote groups, fundamental differences in phenotype and molecular characteristics indicate evolutionary derivation from a range of ancestral types (polyphyletic origins).

1.2.1 Microscopical appearance

The colour of freshwater algae is an important aspect their classification (Table 1.2), and ranges from blue-green (Cyanophyta) to grass green (Chlorophyta), golden brown (Chrysophyta, Bacillariophyta), brown (Phaeophyta) and red (Rhodophyta). Variations in colour are shown in Fig. 1.3, and in the colour

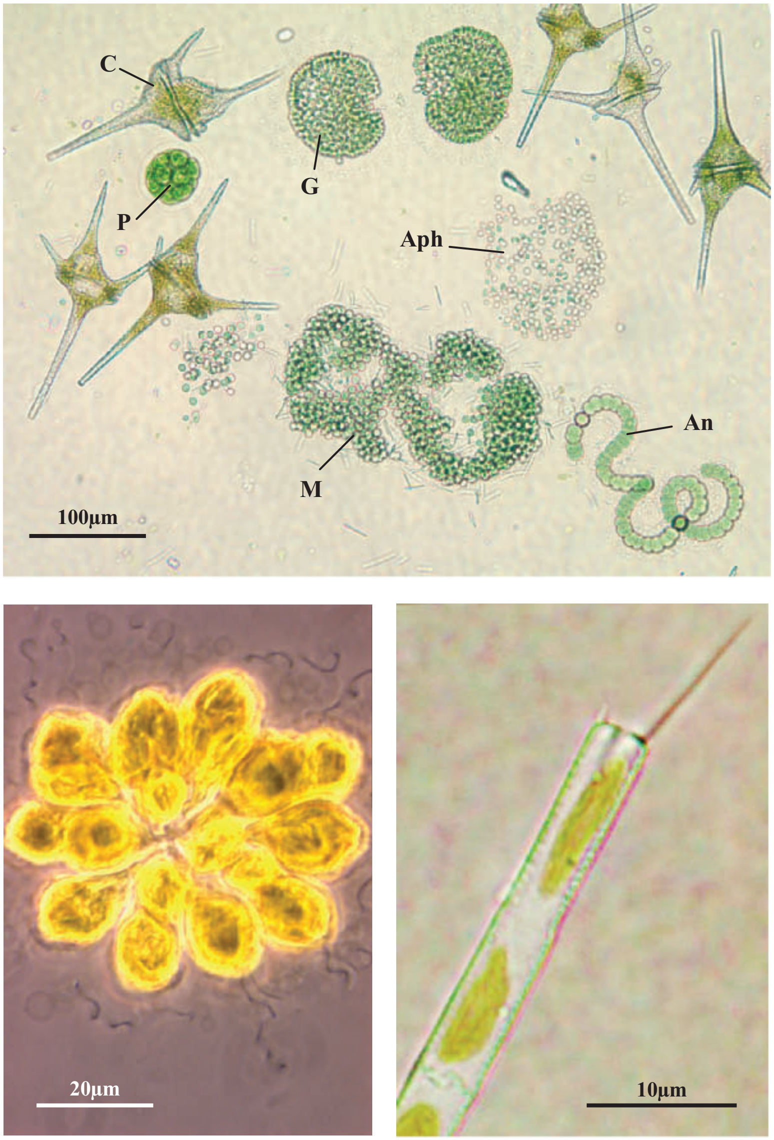

Figure 1.3 Colour characteristics of different algal groups. Top: Fresh lake phytoplankton sample showing colour differences between major algal phyla: Dinophyta (brown: C), Cyanophyta (blue-green: An, Aph, M) and Chlorophyta (grass-green: P). Algal genera: An – *Anabaena*, Aph – *Aphanothece*, C – *Ceratium*, G – *Gomphosphaeria*, M – *Microcystis*, P – *Pandorina*. Bottom left: *Synura* (cultured alga, lightly fixed) showing golden brown colour of Chrysophyta. Bottom right: End of filament of *Aulacoseira. granulata* var. *angustissima* (with terminal spine) from lake phytoplankton showing olive-green chloroplasts (Bacillariophyta).

photographs of Chapter 4. The use of colour as a taxonomic marker can be deceptive, however, since the normal balance of pigments may vary. Green algae living on snow, for example, may have a preponderance of carotenoid pigments – forming a 'red bloom' (Hoham and Duval, 2001). Heterotrophic algae take up complex organic molecules by surface absorption (organotrophy) or ingestion (phagotrophy), and have either retained photosynthetic pigments (photo-organotrophs, mixotrophs) or lost pigmentation completely (obligate heterotrophs) (Sigee, 2004). Even within a 'normal' ecological situation, the colour of a particular alga can show considerable variation (see, for example, *Anabaena*, Fig. 4.24).

Apart from colour, the other obvious characteristics under the light microscope are overall size, whether the organism is unicellular or colonial and whether it is motile (actively moving) or non-motile. Within different groups, algae may be largely unicellular (euglenoids, dinoflagellates, cryptophytes), multicellular (brown algae) or a mixture of the two (other groups). Motility (single cells or entire colonies) is also an important feature, with some algal groups being entirely flagellate (dinoflagellates, cryptophytes) while others are a mixture of flagellate and non-flagellate organisms (green algae, xanthophytes). Other groups of algae are entirely without flagella, but are able to move by buoyancy regulation (blue-greens), gliding movements on substratum (blue-greens, diatoms) or are entirely non-motile (red and brown algae).

1.2.2 Biochemistry and cell structure

Major biochemical features of freshwater algae include pigmentation, food reserves and external covering (Table 1.3). Different groups have distinctive combinations of chlorophylls and carotenes, while only three groups (blue-greens, cryptomonads, red algae) have phycobilins. All pigmented algae have chlorophyll-*a*, which can therefore be used for the estimation of total biomass (Chapter 2). Diagnostic carotenoids have been particularly useful in high-performance liquid chromatography (HPLC) identification and quantitation of major algal groups within mixed phytoplankton samples (Section 2.3.3, Fig 2.11), and have been particularly useful in the analysis of estuarine eutrophication (Section 3.5.2).

Visualization of key differences in cell structure normally requires the higher resolution of oil immersion (light microscopy), transmission or scanning electron microscopy (TEM or SEM) and includes both internal (e.g. chloroplast fine structure) and external (e.g. location/number of flagella, cell surface ornamentation) features. Comparisons of light and electron microscope images are shown in Fig. 1.4 (light/TEM) and Figs. 4.56 and 4.57 (light/SEM).

1.2.3 Molecular characterization and identification

Although identification of algal taxa is normally based on microscopic characteristics (particularly morphology and colour), there are a number of situations in which molecular techniques have taken precedence, such as the following.

- No clear morphological characteristics are available. This has particularly been the case for unicellular blue-green algae.
- Algae are relatively inaccessible and difficult to visualize. This is the case for biofilms, where algae are enclosed in a gelatinous matrix, and in many cases are a relatively small component of a very heterogeneous community of organisms.
- Diversity is being studied within species, where strains are often distinguished in biochemical and genetic terms.

Ultimately, species definition and identification in both prokaryote and eukaryote algae may depend on molecular analysis, with determination of unique and defining DNA sequences followed by development of species-specific nucleotide probes from these (see below) .This approach would be particularly relevant in the case of blue-green algae, but there are a number of problems in relation to species-specificity in this group (Castenholz, 1992):

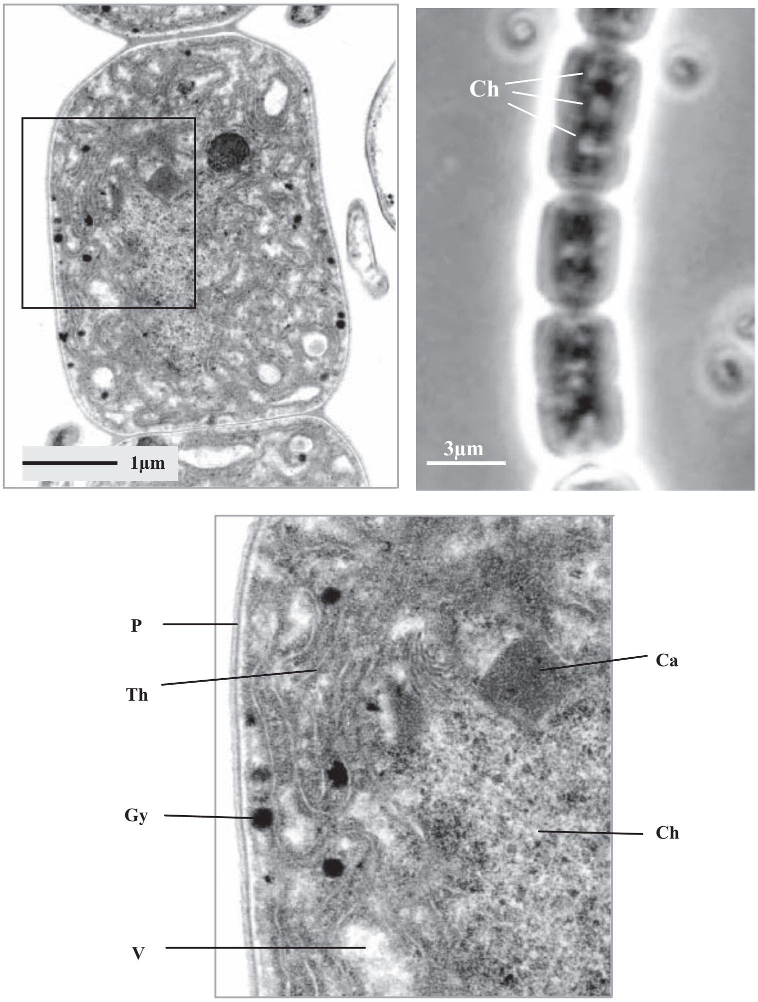

Figure 1.4 Prokaryote features of blue-green algae. Top right: phase contrast (light microscope) image of live filamentous colony of *Anabaena*, showing central patches of pale chromatin (Ch). Top left: transmission electron micrograph of whole cell of *Anabaena* showing central patch of granular chromatin. Bottom: Detail from top left, showing fine-structural detail. Ch – central region of chromatin (no limiting membrane), Ca – carboxysome (polyhedral body), Gy – glycogen granule (cyanophycean starch), Th – peripheral thylakoid membranes, V – vacuole, P – thin peptidoglycan cell wall.

- Polyploidy (multiple genomic copies per cell) may occur, with variation between the multiple genomes. As many as 10 multiple genome copies have been observed in some blue-green algae.

- Horizontal gene transfer means that some DNA fragments are dispersed over a range of species.

DNA/RNA sequence analysis

Analysis of DNA sequences has been widely used for the identification of both blue-green (16S rRNA genes) and eukaryote algae (18S rRNA and chloroplast DNA). This technique has been used by Droppo *et al.* (2007), for example, to determine the taxonomic composition of biofilms – identifying bacteria, blue-green algae and some eukaryote unicellular algae. The technique involves:

- collecting a sample of biomass from the entire microbial community

- obtaining a DNA sample; this may involve extraction from a mixed environmental sample such as biofilm or soil (Zhou *et al.*, 1996)

- polymerase chain reaction (PCR) amplification of a specific nucleotide region – typically 16S or 18S rRNA genes

- separation of the amplified strands by denaturing gradient gel electrophoresis (DGGE), or purification of the PCR products using a rapid purification kit

- sequence analysis with comparison to a standard database; sequence identification is normally based on a match of at least 90%.

The pattern of bands in the DGGE gel gives an estimate of community complexity, and the intensity of individual bands (derived from individual species) a measure of population size. In addition to providing taxonomic information where classical morphological characteristics do not apply, molecular identification also has the advantage that the whole of the microbial community (communal DNA sample) is being analysed in an objective way. Non-photosynthetic bacteria, Archaea, and protozoa are also identified in addition to prokaryote and eukaryote algae (Droppo *et al.*, 2007; Galand *et al.*, 2008).

Potential limitations of molecular analysis are that identification may be tentative (often only to genus level) and taxonomic quantitation (relative numbers of different algae) is difficult. The technique has been particularly useful in relation to the biodiversity of marine blue-green unicellular algae, but has also been used in a number of freshwater systems (Table 1.4) and at the freshwater/marine interface (*nif* H genes).

nif H genes Foster *et al.* (2007) used DNA quantitative PCR (QPCR) technology to amplify and detect the presence of species-specific *nif* H genes in blue-green algae. This gene encodes the iron-containing protein nitrogenase (the key enzyme involved in nitrogen fixation), and provides a marker for nitrogen fixing (diazotrophic) algae in the freshwater environment. The technique was used to demonstrate that the blue-green algal symbiont *Richelia*

Table 1.4 Molecular Identification of Algal Species in Aquatic Environmental Samples

Environmental Sample	Technique	Reference
Picoplankton in Lake Baikal	Direct sequencing	Semenova and Kuznedelov (1998)
Colonial blue-greens: *Anabaena*, *Microcystis*, *Nodularia*	PCR-RFLP analysis of the *cpcB-A* intergenic spacer & flanking regions	Bolch *et al.* (1996)
Flagellate nanoplankton	SSU rRNA probes for *Paraphysomonas* (Chrysophyte)	Caron *et al.* (1999)
Mixed diatom populations and laboratory cultures	Large subunit rRNA probe for *Pseudo-Nitzschia* (Diatom)	Scholin *et al.* (1997)
Estuarine river samples	Use of ITS-specific PCR assays for *Pfiesteria* (Dinoflagellate)	Litaker *et al.* (2003)
Laboratory biofilm samples: blue-greens and unicell eukaryotes	Amplification of 16sRNA genes, with denaturing gel electrophoresis (DGGE)	Droppo *et al.* (2007)
Diazotrophic blue-green algae within the Amazon river freshwater plume	Quantitative PCR (QPCR) analysis of the *nif* H gene (encodes part of the nitrogenize enzyme)	Foster *et al.* (2007)
Arctic ecosystems: Freshwater stamukhi lake and inflow river	Sequencing of 18s rRNA genes to identify major cryptophyte river populations, plus minor lake populations of diatoms	Galand *et al.* (2008)
Lake and river flagellate samples	Population heterogeneity in *Spumella* (Chrysophyte) – SSU rRNA sequences	Pfandl *et al.* (2009)
Pond samples	Population heterogeneity in *Desmodesmus* (Chlorophyte) – ITS2rRNA sequences	Vanormelingen *et al.* (2009)

SSU rRNA – small subunit ribosomal RNA.
ITS – internal transcribed spacer.

(associated with the diatom *Hemiaulus hauckii*) was specifically linked to the Amazon freshwater outflow (river plume) in the western tropical North Atlantic (WTNA) ocean, and that the *H. hauckii–Richelia* complex could be used as a bio-indicator for pockets of freshwater within the WTNA ocean.

Molecular probes The development of species-specific oligonucleotide probes from DNA sequence data, followed by *in situ* hybridization, has considerable potential for the identification and counting of algae in environmental samples.

As with direct sequencing, this technique has particular advantages with small unicellular algae, where there are often relatively few morphological features available for identification. Caron *et al*. (1999) sequenced the small-subunit ribosomal genes of four species of the colourless chrysophyte genus *Paraphysomonas*, leading to the development of oligonucleotide probes for *P. imperforata* and *P. bandaiensis*.

Molecular probes have major potential for the detection of nuisance algae, particularly those that produce toxins. They have been used, for example, to distinguish toxic from non-toxic diatom species (Scholin *et al*., 1997), where differentiation would otherwise require the time-consuming application of scanning and transmission electron microscopy. They have also been used for the rapid identification of *Pfiesteria piscicida*, a potentially toxic dinoflagellate that has been the cause of extensive fish mortalities in coastal rivers of the eastern United States. Litaker *et al*. (2003) used unique sequences in the internal transcribed spacer (ITS) regions ITS1 and ITS2 to develop PCR assays capable of detecting *Pfiesteria* in natural river assemblages. These have been successfully used to detect the potentially harmful organism in the St Johns River system, Florida (USA).

1.3 Blue-green algae

Blue-green algae (Cyanophyta) are widely-occurring throughout freshwater environments, ranging in size from unicellar forms such as *Synechococcus* (barely visible under the light microscope – Figs. 2.16 and 4.31) to large colonial algae such as *Microcystis* (Fig. 4.34) and *Anabaena* (Fig. 4.24a). Large colonies of the latter can be readily seen with the naked eye, and show a simple globular or filamentous form with copious mucilage. The balance of photosynthetic pigments present in blue-green algae (Table 1.3) varies with light spectrum and intensity, resulting in a range of colours from brown to blue-green (Fig. 4.24a). The phycobilin pigments are particularly prominent, with phycocyanin tending to predominate over phycoerythrin at low light levels, giving the cells the blue-green colour typical of these algae. It is thus an advantage to observe material from shaded as well as better illuminated situations wherever possible.

1.3.1 Cytology

Prokaryote status

The prokaryote nature of these algae is indicated by the small size of the cells (typically $<10\mu m$ diameter) and by the presence of central regions of nucleoid DNA (not enclosed by a nuclear membrane). These nucleoid regions (Fig. 1.4) can be observed both by light microscopy (as pale central areas within living cells) and by transmission electron microscopy (as granular regions in chemically fixed cells) – where the absence of a limiting membrane can be clearly seen. Although these algae lack the cytological complexity of eukaryote organisms (no membrane-bound organelles such as mitochondria, plastids, microsomes, Golgi bodies) they do have a range of simple granular inclusions such as carboxysomes, cynophycin granules, polyphosphate bodies and glycogen particles (Fig. 1.4). Photosynthetic pigments are associated with thylakoid membranes, which are typically dispersed throughout the peripheral protoplasm.

As with other prokaryotes, cell walls are made up of a peptidoglycan with a lipopolysaccharide layer outside and are generally quite thin. Some species also have a mucilage layer outside the cell wall which may be dense or watery, structured or unstructured. The outside layers of the cell wall can sometimes become stained straw coloured or brownish from iron and other compounds in the surrounding water as in *Scytonema* and *Gloeocapsa*.

The fundamental bacterial nature of these organisms distinguishes them from all other algae, and determines a whole range of features – including molecular biology, physiology, cell size, cell structure and general morphology.

Gas vacuoles

In some species gas vacuoles may be formed, appearing under the light microscope as highly refractive or quite dark structures. Gas vacuoles aid buoyancy in planktonic species allowing the cells to control their position in the water column. Cells may then congregate at a depth of optimal illumination, nutrient concentration or other factor for that species. This is not necessarily at the surface as there the light intensity may be too great and cause photoinhibition or permanent cell damage. Movement up and down in the water column can enhance nutrient uptake as it allows the cells to migrate to depths where essential nutrients, e.g. phosphates, are more abundant and then up towards the surface for light energy absorption. When gas vacuole containing species are present in a sample of water they often tend to float to the surface (this can cause problems when preparing the sample for cell counts; see Chapter 2).

In addition to gas vacuole-mediated movements of planktonic blue-greens within the water column, other types of motility also occur – including gliding movements of filamentous algae (such as *Oscillatoria*) on solid substrata.

1.3.2 Morphological and taxonomic diversity

Blue-green algae are remarkable within the prokaryote kingdom for showing the range of size and form noted above, with some organisms forming quite large, complex, three-dimensional colonies. Limited differentiation (Figs. 4.24a–c) can occur within colonies, with the formation of heterocysts (nitrogen-fixation) and akinetes (thick-walled resistant cells). In filamentous forms the cells may be the same width along the filament or in some genera they may narrow or taper towards the end, even forming a distinct hair-like structure in the case of *Gloeotrichia* (Fig. 4.22). Clear branching occurs in the most complex forms, with a fundamental distinction between 'true branching' as in *Stigonema* (Fig. 4.20), or 'false branching' as in *Tolypothrix* (Fig. 4.21). True branching involves division of a single cell within the filament, giving rise to two or more daughter cells which themselves form branches. In contrast, false branching involves lateral extension of the filament without the single cell division and daughter cell development as above.

Freshwater blue-green algae can be divided into four main groups (Table 1.5) in relation to general morphology, presence/absence of specialized cells and the nature of branching in filamentous forms. These four groups form the basis for current taxonomy of this phylum, as adopted by John *et al*. (2002), Komarek *et al*. (2003a,b).

- Chroococcales. The simplest blue-green algae, occurring essentially as solitary cells (no filamentous forms), typically enclosed by a thin layer of mucilage. The cells may remain as single cells, or be aggregated into plate-like or globular colonies. Typically planktonic with some colonial forms (e.g. species of *Microcystis*) forming massive surface blooms containing individual colonies that are recognizable with the naked eye. They typically lack specialized cells, though one group (typified by *Chaemaesiphon*) forms exospores.

- Oscillatoriales. Filamentous algae, lacking heterocysts and akinetes. These relatively simple algae occur as planktonic (some bloom forming) or benthic aggregations. In some cases they form dense mats on mud or rocky substrata which secondarily detach as metaphyton into to the main body of water (Fig. 2.1).

- Nostocales. A diverse group of filamentous algae, planktonic or benthic, with heterocysts and akinetes but not showing true branching. Filaments may be unbranched or show false branching. These algae are able form large colonies by lateral association of filaments (bundles), 3-D tangles or as radiating filaments from the centre of the sphere.

- Stigonematales. Filamentous algae, with heterocysts and akinetes and showing true branching. Structurally the most complex blue-green algae, with some thalli (e.g. *Fischerella*) differentiated into multiseriate/uniseriate basal filaments and uniseriate erect branches. Largely benthic algae, with genera such as *Stigonema* commonly attached to substrata in standing and flowing waters, detaching to form planktonic masses.

Table 1.5 Taxonomic and Morphological Diversity in Freshwater Blue-Green Algae

Taxonomic Order Major Morphotype	Colony Size and Form	Attached or Planktonic	Example*
Chroococcales			
(a) Unicellular to spheroid colonies, lacking specialized vegetative, resistant or reproductive cells	Single cells	Planktonic or attached to plant and substrate surfaces	*Synechococcus* (4.31)
	Small colonies (4–32 cells)	Free-floating, tangled-up with filamentous algae or attached to substrates	*Chroococcus* (4.33) *Gleocapsa* (4.32)
	Flat plate of cells	Free-floating or sedentary	*Merismopedia*
	Large solid spherical colony	Planktonic	*Aphanocapsa* (4.35) *Microcystis* (4.34)
	Large hollow spherical colony	Planktonic	*Gomphosphaeria* (4.30) *Coelosphaerium*
(b) Unicellular, forming exospores	Cells remain single or form multi-layered colonies	Attached to surfaces of aquatic plants, algae and inorganic substrate	*Chamaesiphon*
Oscillatoriales			
Filamentous algae, lacking heterocysts and akinetes	Elongate straight filaments	Planktonic or benthic	*Oscillatoria* (4.27)[†]
		Benthic mat	*Phormidium* (4.29)
	Elongate spiral filaments	Planktonic or on mud surfaces	*Spirulina* (4.26)
Nostocales			
Filamentous algae forming heterocysts and akinetes, but no true branching	Bundles of Elongate filaments	Planktonic or benthic	*Aphanizomenon* (4.23) *Nostoc* (4.25)
	3-D tangle of filaments	Planktonic	*Anabaena* (4.24)
	Spherical colony of radiating filaments	Planktonic	*Gloeotrichia* (4.22)
Stigonematales			
Filamentous algae forming heterocysts and akinetes, with true branching	Branched mass of filaments	Benthic or planktonic	*Stigonema* (4.20)
	Differentiation into basal filaments and erect branches	Benthic	*Stauromatonema* *Fischerella*

*Figure numbers in brackets; † Planktonic species of *Oscillatoria* are placed in the genera *Planktothrix*, *Pseudanabaena* or *Limnothrix* by some authors.

The range of size and form noted in Table 1.5 indicates a wide morphological diversity, which is useful in taxonomic identification. The relative importance of molecular versus morphological characteristics in relation to taxonomy reflects the debate as to whether these organisms should be treated as bacteria (cyanobacteria – Stanier *et al.*, 1978) or algae (blue-green algae: Lewin, 1976). Although they fundamentally resemble bacteria in their prokaryote features, they also differ from bacteria in carrying out photosynthesis coupled to O_2 evolution, and in the complexity of their morphology. Although current taxonomy is based primarily on phenotypic characters (see above), ultrastructural and molecular data provide useful supplementary information (Komarek *et al.*, 2003a). Current molecular analyses support the separation of non-heterocystous and heterocystous genera (Rudi *et al.*, 2000), and are particularly relevant in the case of unicellular blue-greens, where morphological features are not adequate. The classical (eukaryote) use of morphology to define species is also problematic in this group, since the species concept and definition of species is limited by a complete absence of sexual reproduction.

In the absence of sexual processes, reproduction in this group is by vegetative or specialized asexual means. Asexual spores (akinetes) consist of vegetative cells that are larger than normal. They generally have thickened walls and, in filamentous forms, are often produced next to heterocysts (Fig. 4.24c). Baeocysts (small spherical cells formed by division of a mother cell may) be produced in some coccoid species and are released into the environment. In some filamentous forms deliberate fragmentation of the filament can occur – releasing the fragments (hormogonia) to produce new filaments.

1.3.3 Ecology

Blue green algae are thought to have arisen approximately 3.5 billion years ago (Schopf, 1993) during which time they have been the dominant form of life for about 1.5 billion years. As a result of this long evolutionary history, they have adapted to (and frequently dominate) all types of freshwater environment – including extreme conditions (thermal springs, desiccating conditions), brackish (semisaline) conditions, high and low nutrient environments and planktonic/benthic habitats. At high latitudes, blue-green algae are particularly adapted to low temperatures (Tang & Vincent, 2002) – often dominating the benthic environment by forming dense mats. In milder climates, these organisms frequently dominate surface waters, where they are able to out-compete other phytoplankton under eutrophic conditions (Section 3.2.3).

Algal blooms

In mid to late summer, eutrophic temperate lakes frequently develop massive populations of colonial blue-green algae These may rise to the surface of the lake, forming a thick layer of algal biomass at the top of the water column, out-competing other algae and having major impacts on zooplankton and fish populations (Sigee, 2004). The ability of blue-greens to out-compete other freshwater algae has been attributed (Shapiro 1990) to a range of characteristics, including:

- optimum growth at high temperatures – *summer temperatures*

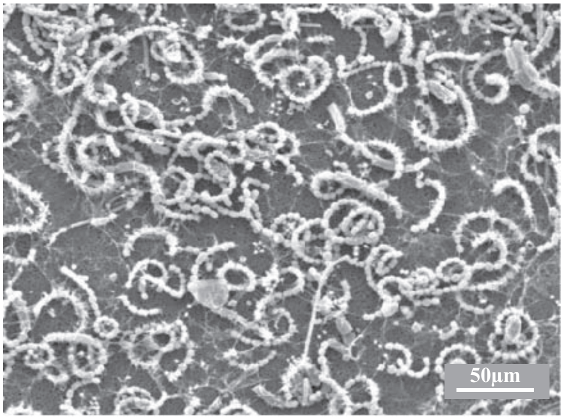

Figure 1.5 Midsummer blue-green algal bloom in a eutrophic lake. SEM view of lake epilimnion phytoplankton sample (August), showing the dense population of filamentous *Anabaena flos-aquae* that totally dominated the algal bloom (chlorophyll-*a* concentration 140 μg ml^{-1}). Copious mucilage associated with this alga is seen as numerous fine strands, formed during the dehydration preparation process. See also Figs 2.8 (seasonal cycle) and 4.24 (live *Anabaena*). Photo: Sigee and Dean.

- low light tolerance – *important within the dense algal bloom, and enabling algae to survive lower in the water column*
- tolerance of low N/P ratios – *allowing continued growth when N becomes limiting*
- depth regulation by buoyancy – *avoiding photoinhibition during the early phase of population increase, and allowing algae to obtain inorganic nutrients from the hypolimnion when the epilimnion becomes depleted in mid to late summer*
- resistance to zooplankton grazing – *limiting the impact of herbivory on algal growth*
- tolerance of high pH/low CO_2 concentrations – *allowing continued growth of blue-greens (but not other algae) at the lake surface during intense bloom formation*
- symbiotic association with aerobic bacteria – *bacterial symbionts at the heterocyst surface maintain*

Table 1.6 Ecological Diversity of Freshwater Blue-Green Algae

Major Ecosystem	Specific Conditions	Benthic Algae	Planktonic Algae
Standing waters – lakes and ponds	Nutrient status: meso- to eutrophic	*Gloeotrichia*: Attached to substrates. Detached colonies planktonic	Colonial blue-greens: *Microcystis*, *Anabaena*
	Oligotrophic		Unicellular blue-greens: *Synechococcus*
	Hard and soft waters		*Aphanothece*
	Soft, acid lakes	*Cylindrospermum*: often forming dark patches on submerged vegetation	*Tolypothrix*: Planktonic or in submerged vegetation
Wetlands	Soft waters – common in bogs	*Chroococcus* – Attached to substrates, mixed with tangles of filamentous algae or free-floating	
		Merismopedia – typical of bog-water communities	
	Open water in bogs		*Aphanocapsa*
Running waters – streams and rivers		Benthic mats: *Oscillatoria tenuis*	*Oscillatoria rubescens*
Extreme environments			
Polar lakes, ponds, streams	Low temperature, low light (ice cover)	Benthic mats: *Oscillatoria* (Tang *et al.* 1997)	
Thermal springs	45 °C or greater	*Mastigocladus laminosus* *Oscillatoria* (pigmented) (Darley, 1982)	*Synechococcus lividus* (Stal, 1995)
Brackish waters – saline lakes	range of salinity		*Aphanothece halophytica* (Yopp *et al.*, 1978)

the local reducing atmosphere required for nitrogen fixation and are also an important source of inorganic nutrients in surface-depleted waters (Sigee, 2004).

Species preferences

Many blue-green algae can tolerate a wide range of environmental conditions, so strict ecological preferences are unusual in this group. *Microcystis aeruginosa* (Fig. 4.34) for example can form massive growths in eutrophic lakes, but low numbers may also occur in oligotrophic waters (Reynolds, 1990). The distinction between planktonic and benthic organisms is also frequently not clear-cut. *Microcystis* is regarded as a typical planktonic alga, but may also occur as granular masses on lake bottoms, and (as with the majority of temperate lake algae) overwinters on lake sediments rather than in the water column. Many benthic algae become detached, and either remain loose on the substratum or rise in the water column and become free-floating. Colonies of *Gloeotrichia*, for example, often detach from substrates and become planktonic (Fig. 4.22), where they grow and may reach bloom proportions.

In spite of these cautions, many blue-green algae can be characterized in relation to particular environments and whether they are typically attached or planktonic. Some examples are shown in Table 1.6, for both ‘normal’ and ‘extreme’ environments. As with other algae, ecological preferences typically relate to multiple (rather than just single) environmental factors. Colonial blue-green algae, which grow particularly well in high-nutrient lakes are also suited to hard waters (high Mg, Ca) and to the alkaline conditions typical of these environments. Conversely, oligotrophic lakes, which support unicellular rather than colonial blue greens, tend to be soft, slightly acid waters.

1.3.4 Blue-green algae as bio-indicators

As with other algal groups, the presence or absence of particular species can be a useful indicator of ecological status (Table 1.6). The dominant presence of colonial blue-greens, forming dense summer blooms (Fig. 1.5) has been particularly useful as an indicator of high nutrient status, and these algae are a key component of various trophic indices (Chapter 3). Conversely, populations of unicellular blue-green algae are indicative of oligotrophic to mesotrophic conditions.

1.4 Green algae

In the freshwater environment, green algae (Chlorophyta) range in size from microscopic unicellular organisms to large globular colonies and extensive filamentous growths. They are characterized by a fresh green coloration due to the presence of chlorophylls-*a* and -*b*, which are not obscured by accessory pigments such as β-carotene and other carotenoids. In exceptional cases the carotenoid pigments may occur in large amounts, obscuring the chlorophylls and giving the alga a bright red colour. This is seen with the flagellate *Haematococcus* (Fig. 4.54), which frequently colours bird baths and other small pools bright red, and the snow alga *Chlamydomonas nivalis* – where one of the red carotenoids has a major photoprotective function.

1.4.1 Cytology

In addition to their characteristic pigmentation and other biochemical features (Table 1.3), green algae also have a number of distinctive cytological aspects.

- Flagella, when present, occur in pairs. These are equal in length without tripartite tubular hairs, and have a similar structure (isokont). Some genera have four or even eight flagella, but this is unusual.

- Chloroplasts vary in shape, size and number. In unicellular species they tend to be cup-shaped (Fig. 4.55), but in filamentous forms may be annular (Fig. 4.19), reticulate (Fig. 4.17), discoid or ribbon-like (Fig. 4.13). They have a double outer membrane, with no enclosing periplastidal endoplasmic reticulum.

- Production and storage of the photosynthetic reserve (starch) occurs inside the plastid, with granules frequently clustered around the pyrenoid. In all other eukaryote algae the storage material occurs mainly in the cytoplasm.

In motile species an eyespot is frequently present, appearing red or orange (Fig. 4.38) in fresh specimens. The cell wall in green algae is made of cellulose.

1.4.2 Morphological diversity

Green algae are the most diverse group of algae, with about 17 000 known species (Graham and Wilcox, 2000). This diversity is reflected by the varied of morphology, with organisms being grouped into a range of growth forms – depending on whether they are unicellular, colonial or filamentous (Table 1.7). The level of greatest morphological and reproductive complexity is represented by Charalean algae (e.g. *Chara*, *Nitella*), which can reach lengths of over a metre, have whorls of branches at nodes along the length of the thallus and have been frequently confused with aquatic higher plants such as *Ceratophyllum*.

In the past, this morphological diversity provided the taxonomic basis for green algal classification (Bold and Wyne, 1985) – with orders primarily being defined largely on a structural basis. These included the orders Volvocales (flagellate unicells and simple colonial forms), Chlorococcales (unicells and non-motile coenobial colonies), Ulotrichales (unbranched filaments) and Chaetophorales (branched filaments). More recently, the application of cytological, comparative biochemical and molecular sequencing techniques has demonstrated the occurrence of extensive parallel evolution. On the basis that classification should reflect phylogeny, these original groupings are thus no longer valid, and a new classification is emerging where individual orders contain a mixture

Table 1.7 Range of Morphologies in Freshwater Green Algae

Major Morphotype	Attached or Planktonic	Example*
Flagellate unicells	Planktonic	*Chlamydomonas* (4.55)
Nonflagellate Unicells	Planktonic or benthic (often associated with periphyton)	Crescent shaped – *Selenastrum* (4.78) Equatorial division – *Micrasterias* (4.81); *Closterium* (4.80)
Colonial	Planktonic	Net – *Hydrodictyon* (4.1) Hollow sphere – *Volvox* (4.40), *Coelastrum*, *Eudorina* (4.39) Solid sphere – *Pandorina* (4.36) Plate of cells – *Gonium* (4.38), *Pediastrum* (4.47) Small linear colonies – *Scenedesmus* (4.50) Branching colonies – *Dictyosphaerium* (4.45)
Disk-shaped plate, one cell thick	Attached to higher plants (epiphyte) or to stones	*Coleochaete*, *Chaetosphaeridium*
Unbranched filaments	Typically attached, but may detach to become planktonic	*Uronema*, *Microspora*, *Oedogonium* (4.17), *Zygnema* (4.15), *Spirogyra* (4.13)
Branched filaments		*Chaetophora*, *Draparnaldia*, *Cladophora* (4.6)
Large complex algae with whorls of branches	Attached	*Chara, Nitella*

*Figure numbers in brackets.

unicells, globular colonial and filamentous forms (Graham and Wilcox, 2000).

1.4.3 Ecology

Green algae are ecologically important as major producers of biomass in freshwater systems, either as planktonic (standing waters) or attached (running waters) organisms – where they respectively may form dense blooms and periphyton growths.

Algal blooms

In mesotrophic and eutrophic lakes, green algae do not normally produce the dense blooms seen with diatoms and blue-green algae – but do become dominant or co-dominant in early summer, between the clearwater phase and midsummer mixed algal bloom. In smaller ponds, filamentous *Spirogyra* and colonial *Hydrodictyon* frequently form surface blooms or scums, while in garden pools and birdbaths *Haematococcus* may form dense populations. When nutrient levels become very high, a switch may occur from colonial blue-green to green algae as major bloom formers. This is can be seen in some managed fishponds, where organic and inorganic nutrients are applied to enhance fish production by increasing carbon flow through the whole food chain. In the Trebon basin biosphere reserve (Czech Republic), application of lime (supply of carbonate and bicarbonate ions), organic fertilizers and manure leads to high pH and hypertrophic nutrient levels. In these conditions, a short diatom bloom (*Stephanodiscus*) is replaced in early summer by populations of rapidly growing unicellular and small colonial green algae (*Scenedesmus* and *Pediastrum*) which continue to dominate for the rest of the annual cycle (Pokorny *et al*., 2002a; Pechar *et al*., 2002). Extensive growth of attached algae (periphyton) may also occur under high nutrient conditions. Branched thalli of *Cladophora* can form extended communities of shallow water vegetation along the shores of eutrophic lakes and streams, breaking off in storms to form mass of loose biomass which can degrade to generate noxious odours. Another filamentous alga, *Mougeotia*, can form large mucilaginous subsurface growths in lakes that have been affected by acid rain.

Table 1.8 Ecological Diversity of Freshwater Green Algae

Major Ecosystem	Specific Conditions	Benthic Algae	Planktonic Algae
Standing waters – lakes and ponds	Wide range of conditions	*Oedogonium*	*Scenedesmus*, *Pediastrum*. *Tetraedron* *Chlamydomonas*
	Eutrophic to hypertrophic	*Cladophora*	*Scenedesmus*, *Pediastrum*.
	Oligotrophic		Desmids
	High (H) and low (L) pH	*Chara*, *Nitella* (H) *Coleochaete* (L)	*Mougeotia* (L)
	Hard water – high (Ca) concentration	*Hydrodictyon*	
	Salt lakes		Small unicells, *Dunaliella*
Standing waters – wetlands	Wide range of conditions		*Selenastrum* *Chlamydomonas*
	Low nutrient, low pH bogs		*Spirogyra*, *Mougeotia*, desmids
Running waters – streams and rivers	Wide range of conditions		*Chlamydomonas*, *Spirogyra*
	Hardness (Ca concentration)	*Hydrodictyon*	
Running waters – Estuaries, brackish water	Wide range of conditions		*Scenedesmus*
	High salinity		*Dunaliella*
Specialized microenvironments	Very high nutrients, e.g. High sewage level	*Prototheca*	*Euglena*
	Endosymbiont in invertebrates	*Chlorella**	
	Associated with Calcareous deposits	*Oocardium stratum*	

*Also widely occurring as a free-living organism.

Species preferences

Individual species of green algae differ considerably in their ecological preferences, ranging from broad spectrum organisms (*Spirogyra*, *Chlamydomonas*) to species with very restricted habitats.

Spirogyra occurs in a wide range of habitats, where it is typically attached to stable substratum (as periphyton) but also occurs as free floating mats (Lembi *et al*., 1988) – derived either by detachment of from periphyton (vegetative propagation) or from benthic zygotes (sexual derivation). In surveys of North American sites, McCourt *et al*. (1986) recorded *Spirogyra* at nearly a third of all locations, and Sheath and Cole (1992) detected *Spirogyra* in streams from a wide range of biomes – including desert chaparral, temperate and tropical rainforests and tundra.

Other green algae show clear preferences for particular environmental conditions (Table 1.8) that include degree of water movement (lentic versus lotic conditions), inorganic nutrient status (oligotrophic to eutrophic), pH, hardness (Ca concentration) and salinity. These conditions frequently occur in combination, with many moorland and mountain water bodies being low nutrient and neutral to acidic, while lowland sites in agricultural areas are typically eutrophic and alkaline.

Nutrient status Many desmids (e.g. *Closterium*) are particularly characteristic of oligotrophic (low nutrient) lakes and ponds – often in conditions that are also slightly acidic (see above) and dystrophic (coloured water). Desmid diversity is particularly high in these conditions, sometimes with hundreds of species occurring together at the same site (Woelkerling, 1976). Desmids are also typical of nutrient-poor streams, where they are permanent residents of periphyton – making up to 10% of total community biomass. These desmids are associated with plants such as the bryophyte *Fontinalis*, achieving concentrations as high as 10^6 cells g^{-1} of substrate (Burkholder and Sheath, 1984). Desmids often constitute a significant proportion of algal biomass in wetlands (bogs and fens) where they are also a major aspect of species diversity.

Some species of desmids – such as *Closterium aciculare*, are more typical of high nutrient, slightly alkaline lakes – and are indicators of eutrophic conditions. Periphytic algae typical of eutrophic waters include *Cladophora.*

Acid lakes Although desmids are typical of neutral to slightly acid lakes, other green algae are adapted to more extreme conditions. These include the filamentous green alga, *Mougeotia*, which can form substantial sub-surface growths in acidified waters – and is widely regarded as an indicator of early environmental change (Turner *et al*., 1991). Whether acidification is the result of acid precipitation, experimentation (Webster *et al.*, 1992) or industrial pollution, the acid conditions also tend to be associated with:

- relatively clear waters, due to low levels of phytoplankton
- increases in the concentration of metals such as Al and Zn
- reduced levels of dissolved organic carbon, with derivation largely from external lake (allochthonous) rather than internal (autochthonous) sources
- food web changes, including a reduction in the numbers of herbivores.

Laboratory studies (Graham *et al.*, 1996a,b) showed that *Mougeotia* was physiologically adapted to such conditions, with the ability to photosynthesis over a wide range of irradiances (300–2300 μmol quanta $m^{-2}\ s^{-1}$) and tolerance to a broad range of both pH (pH 3–9) conditions and metal concentrations. The physiological adaptations of this alga, coupled with reduced grazing from herbivores, probably accounts for the extensive growth and domination of the benthic environment in many acidic waters.

Alkaline and calcium-rich waters Some planktonic (*Closterium aciculare*) and benthic algae (e.g. *Nitella* and *Chara*) are adapted to grow in alkaline habitats, many of which are also rich in calcium (hard waters). *Nitella* produces lush meadows on the bottoms of neutral to high pH lakes, and *Chara* can form dense, lime encrusted lawns in shallow alkaline waters. *Hydrodictyon* is also typical of hard-water conditions, occasionally blanketing the surface of ponds and small lakes, and occurring widely in larger alkaline standing waters and hardwater streams. Extensive growths of this alga can also be observed in agricultural situations such as irrigation ditches, rice paddy fields and fish farms – where there is some degree of eutrophication.

Some calcium-loving algae occur in very restricted environments. The unusual, slow-growing desmid *Oöcardium stratum*, for example, occurs in calcareous streams and waterfalls at the top of branched calcareous tubes, in association with deposits of ‘travertine’ and ‘tufa’.

Saline waters Various green algae are adapted to saline conditions in salt lakes and estuaries, where salt levels range from low (brackish waters) to high levels. With some widely occurring genera such as *Scenedesmus*, brackish conditions are at one end of the continuum of environmental tolerance. Other algae, such as *Dunaliella*, are specifically adapted to a more extreme situation. This organism occurs in the highly saline waters of commercial salt pans (salterns) and natural salt lakes such as the Great Salt Lake of Utah (USA). *Dunaliella* is able to tolerate external salt concentrations of >5 M, by balancing the high external osmotic pressure (OP) with a high internal OP generated by photosynthetically produced glycerol.

Specialized environments In addition to the free-living occurrence of particular algae in lakes and rivers that have a range of characteristics (see above), other green algae are adapted to more specialized conditions. Some of these involve a change in the mode of nutrition from autotrophic to heterotrophic, and include various species of the non-flagellate unicell *Chlorella* – a widespread endosymbiont of freshwater invertebrates. Other green algae retain their free-living existence, but are able to supplement photosynthesis (mixotrophy) by absorption of exogenous dissolved carbon (Tuchman, 1996) such as amino acids and sugars through their cell surface (osmotrophy). The ability to use external organic carbon may be important in two situations – when photosynthesis is limiting, and when external soluble organic material is in excess.

- Limiting photosynthesis. This may occur, for example, under conditions of chronic low light intensity (e.g. arctic lakes). It is also typical of many acid lakes, where low pH reduces the level of dissolved inorganic carbon to levels that are unable to saturate photosynthesis. Laboratory experiments on *Coleochaete*, an acidophilic alga have demonstrated an ability to use dissolved organic carbon in the form of hexose sugars and sucrose (Graham *et al*., 1994)

- High levels of external dissolved organic carbon. Some osmotrophic green algae are photoheterotrophic, only able to use organic carbon when light is present, and when their photosynthesis is inhibited by the high availability of dissolved organic carbon (Lewitus and Kana, 1994). The colourless unicellular alga *Prototheca* has completely lost the ability to photosynthesize and obtains all its carbon from external sources present in soils and freshwater environments that are contaminated by sewage. This evolutionary relationship of this obligate heterotroph to green algae is indicated by the presence of starch-containing plastids.

1.4.4 Green algae as bioindicators

Contemporary algae

Habitat preferences of contemporary green algae (Table 1.8) are frequently useful in providing information on physicochemical characteristics of the aquatic environment. Filamentous green algae, for example, often dominate environments stressed by cultural eutrophication, acidification and metal contamination (Cattaneo *et al*., 1995).

Fossil algae

Green algae do not typically produce resistant walls that persist in aquatic sediments, and are much less useful than diatoms and chrysophytes as bioindicators in terms of the fossil record. There are some exceptions to this, where the cell wall contains fatty acid polymers known as algaenans, that can withstand millions of years of burial (Gelin *et al*., 1997). *Scenedesmus* and *Pediastrum* are among the most common members of green algae that have fossil records, containing algaenans and also some silicon. The desmid *Staurastrum* is also frequently seen as fossil remains, due to the impregnation of cell wall material with polyphenolic compounds which confer resistance to bacterial decay (Gunnison and Alexander, 1975).

1.5 Euglenoids

Euglenoid algae (Euglenophyta) are almost entirely unicellular organisms, with a total of 40 genera worldwide (about 900 species) – most of which are freshwater. Cells are typically motile, either via flagella or (in non-flagellate cells) by the ability of the body to change shape (referred to as ‘metaboly’).

About one-third of the Euglenoids are photosynthetic and classed amongst the algae. The rest are colourless, being either heterotrophic or phagotrophic, and are usually placed in the Protozoa. In photosynthetic organisms, pigmentation is closely similar to the green algae (Table 1.3), but the variable presence of carotenoid pigments means that these organisms can routinely vary in colour from fresh green (Fig. 4.51) to yellow-brown. In some situations, the accumulation of the carotenoid astaxanthin gives cells a bright red coloration. This is seen particularly well in organisms such as *Euglena sanguinea*, which forms localized blooms in ponds and ditches.

1.5.1 Cytology

Euglenoids are typically elongate, spindle shaped organisms (Figs 1.6 and 4.51) and usually contain several chloroplasts per cell, which vary in appearance from discoid to star, plate or ribbon-shaped. Distinguishing cytological features of euglenoids (Table 1.3) include the following.

- The presence of an anterior flask shaped depression or reservoir within which the flagella are inserted. Although two flagella are present only one emerges from the reservoir into the surrounding medium, the second being reduced and contained entirely within the reservoir. An eyespot is often present and is located close to the reservoir.

- The production of paramylon as storage reserve. This β-1,3-linked glucan does not stain blue-black with iodine solution and is found in both green and colourless forms.

- The presence of a surface coat or pellicle, which gives the cell a striated appearance. This occurs just below the plasmalemma and is composed of interlocking protein strips that wind helically around the cell. In some the pellicle is flexible (allowing the cell to change its shape), while in others the pellicle is completely rigid giving a permanent outline to the cell. Genera such as *Trachelomonas* have cells surrounded by a lorica the anterior end of which is a narrow, flask-shaped opening through which the flagellum emerges.

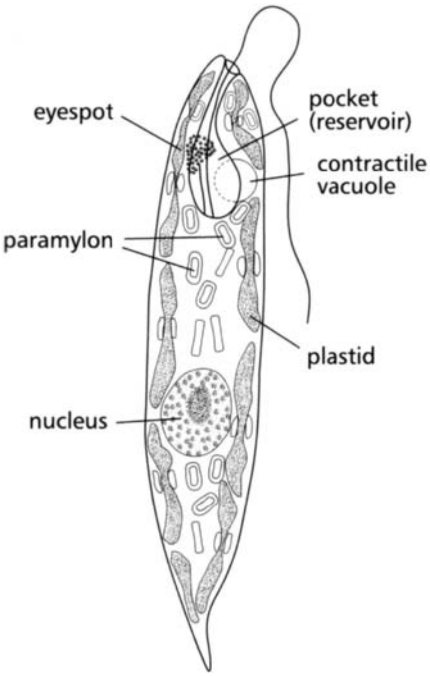

Figure 1.6 Diagrammatic view of *Euglena*, showing major cytological features. Reproduced, with permission, from Graham and Wilcox (2000). See also Fig. 4.51 (live *Euglena*).

Reproduction is asexual by longitudinal division, with sexual reproduction being completely unknown in this group of organisms.

1.5.2 Morphological diversity

Almost all euglenoids are unicellular, with colonial morphology being restricted to just a few organisms where cells are interconnected by mucilaginous strands. One of these is *Colacium*, a stalked euglenoid that is widespread on aquatic substrates – but is particularly common attached to zooplankton such as *Daphnia*. In the sessile state, this organism does not have emergent flagella on the cells – which occur at the end of the branched mucilaginous attachment stalks. Individual cells may produce flagella, however, swim away and form a new colony elsewhere.

With the relative absence of colonial form, diversity in this group is based mainly on variation in ultrastructural features – including feeding apparatus, flagella and pellicle structure (Simpson, 1997). A further aspect of diversity is the distinction between green and colourless (autotrophic/heterotrophic) cells, with approximately two thirds of all species being heterotrophic. Some euglenoids (e.g. strains and species of *Euglena*) are facultative heterotrophs, able to carry out heterotrophic nutrition when photosynthesis is limiting or when surrounding concentrations of soluble organic materials are high. In the heterotrophic state, these organisms retain their plastids as colourless organelles and absorb soluble nutrients over their whole surface (osmotrophy). Other euglenoids (*Petalomonas*, *Astasia*, *Peranema*) are obligate heterotrophs and have lost their plastids completely. Many of these organisms consume particulate organic material (phagocytic) and have evolved a complex feeding apparatus.

1.5.3 Ecology

Euglenoids are generally found in environments where there is an abundance of decaying organic material. This is in line with the heterotrophic nature of many of these organisms and the ability to take up complex organic material either in the soluble or particulate state. Typical habitats include shallow lakes, farm ponds, wetlands, brackish sand and mudflats. Within these environments, euglenoids are particularly associated with interfaces such as sediment-water and air-water boundaries (Walne and Kivic, 1990) and should probably not be regarded as open water truly planktonic algae (Lackey, 1968).

Certain euglenoid algae are able to tolerate extreme environmental conditions. One of these, *Euglena mutabilis*, is able to grow in very low pH waters. This alga has a pH optimum of pH 3.0, can tolerate values below pH 1.0, and is typical of acidic metal-contaminated ponds and streams draining mines. Other euglenoids, found in brackish habitats, are able to tolerate wide ranges in salinity (Walne and Kivic, 1990).

1.5.4 Euglenoids as bioindicators

Euglenoid algae are not particularly useful as environmental bioindicators, either in terms of contemporary populations or fossil records. Although present-day algae show some adaptations to specific environments (see above), there is no established environmental library and species may be difficult to identify. Within lake sediments, the lack of calcified or silicified structures that are resistant to decay means that hardly any remains have survived in the fossil record.

1.6 Yellow-green algae

Yellow-green algae (Xanthophyta) are non-motile, single celled or colonial algae, with a distinctive pigmentation that gives the cells a yellow or fresh green appearance (e.g. Fig. 4.18 – *Tribonema*). Although there is a wide range in morphology (see below), this phylum contains relatively few species (compared to major groups such as green algae) and the algae tend to be ecologically restricted to small water bodies and damp soils.

1.6.1 Cytology

Yellow-green algae are most likely to be confused with green algae when observed in the fresh condition, but differ from them (Table 1.3) in a number of key features.

- Distinctive pigmentation, with chlorophylls (a, c_1 and c_2 – but not b), carotenoids (especially β-carotene) and three xanthophylls (diatoxanthin, vaucheriaxanthin, heteroxanthin).

- Carbohydrate storage as oil droplets or chrysolaminarin (usually referred to as leucosin) granules.

- Walls composed mainly of pectin or pectic acid (sometimes with associated cellulose or siliceous material). The walls often occur as two spliced and overlapping sections, breaking into ‘H’-shaped pieces on dissociation of the filaments.

- When flagella are present they are of unequal length (heterokont) the longer one pointing forward and the shorter (which can be more difficult to see using a light microscope) sometimes pointing forwards or backwards

- Chloroplast fine structure – four outer membranes (incorporating an endoplasmic reticulum) and thylakoids occurring in threes.

Cells contain two or more discoid and green to yellow-green chloroplasts, with associated pyrenoids rarely seen. An eyespot may be present.

1.6.2 Morphological diversity

Yellow-green algae show a range of morphology, from unicellular to colonial and filamentous (Table 1.9). Unicellular forms are non-flagellate in the mature (vegetative) state, with motility being restricted to biflagellate zoospores (asexual) or motile gametes (sexual) reproductive stages. The zoospores have unequal and morphologically dissimilar flagella (heterokont algae – Fig. 1.7). Unicellular algae include simple free-floating forms (*Botrydiopsis*, *Tetraedriella*), or may have associated rhizoidal systems or an attachment stalk. Some yellow-green algae form simple globular colonies, which may be mucilaginous and free floating (e.g. *Gloeobotrys*) or attached via a stalk (*Ophiocytium*). Filamentous forms may be unbranched (*Tribonema* – Fig. 4.18) or branched (*Heterococcus*), and reach their most massive state in the coenocytic siphonaceous genera *Botrydium* and *Vaucheria* (Fig. 4.3).

1.6.3 Ecology

Yellow-green algae are rather limited in their exploitation of aquatic habitats, tending to occur on damp mud (at the edge of ponds) and soil, but not occurring extensively in either lentic or lotic systems. Where planktonic forms do occur, they tend to be present in ditches or small ponds. *Tribonema* species are often found as free-floating filaments in temporary waters, and *Botrydiopsis arrhiza* is found at the edge of ponds and in patches of water in sphagnum bogs, where it may form yellowish water blooms. Even *Gloeobotrys limneticus*, which has widespread occurrence as mucilaginous colonies in lake plankton, never forms extensive or dominant populations (as seen, for example, by blue-green algae). Various yellow-green algae are epiphytic, including *Mischococcus* and *Ophiocytium* (attached to filamentous algae) and *Chlorosaccus* (attached to macrophytes).

As with other algal groups, some yellow-green algae have developed alternative modes of nutrition. *Chlamydomyxa* is an amoeboid, naked form that has retained its photosynthetic capability (still has chloroplasts) but has also become holozoic, ingesting desmids, diatoms and other algae and digesting them within internal food vacuoles. *Chlamydomyxa* is typical of low-nutrient acid bogs, and the holozoic mode of nutrition may be important for supplementing nitrogen uptake in such adverse conditions.

1.6.4 Yellow-green algae as bioindicators

Yellow-green algae have not been widely used as bioindicator organisms, partly because they are

Table 1.9 Range of Morphologies in Yellow-Green Algae

Major Morphotype	Attached or Planktonic	Example*
Unicells		
Coccoid	Planktonic or benthic	*Botrydiopsis*
Pear-shaped, with rhizoidal system	Present on mud surface	*Botrydium*
Ovoid cells on stalk	Epiphytic on attached or planktonic algae	*Characiopsis*
Amoeboid	Benthic, often in hollow cells of *Sphagnum*	*Chlamydomyxa*
Colonial		
Small to large globular colonies	Planktonic	*Gloeobotrys*
Branched colony on stalk	Planktonic or attached	*Ophiocytium*
Filaments		
Unbranched	Typically planktonic, occasionally attached when young	*Tribonema* (4.18)
Branched	Attached, damp soils	*Heterococcus*
Coenocytic, (siphonaceous)	Attached	*Botrydium*, *Vaucheria* (4.3)

*Figure numbers in brackets

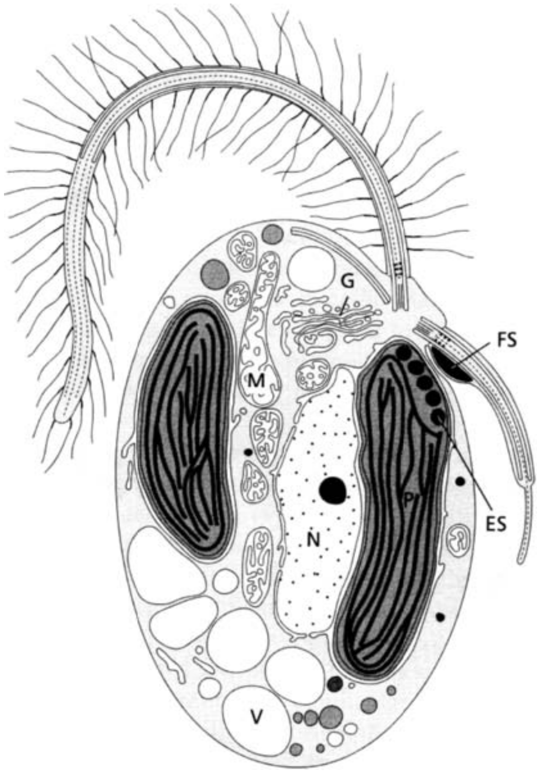

Figure 1.7 Line drawing of heterokont zoospore, typical of xanthophyte algae, with a long anterior flagellum bearing two rows of stiff hairs plus a short posterior flagellum. This is smooth and oftemn bears a swelling (FS) that is part of the light-sensing system. ES – eyespot, G – Golgi body, M – mitochdondrion, N – nucleus, P – plastid. Reproduced, with permission, from Graham and Wilcox (2000).

not a prominent group in the aquatic environment. Different species do have clear environmental preferences, however, that could be used to provide information on ambient conditions. These are discussed by John *et al*. (2002), and include algae that are prevalent in acid bogs (*Botrydiopsis* spp., *Centritractus* spp.), calcareous waters (*Mischococcus* spp., *Ophiocytium* spp.), humic waters (*Botrydiopsis* spp., *Tribonema minus*), organically-rich conditions (*Chlorosaccus* spp.), inorganic nutrient-enriched waters (*Goniochloris fallax*) and brackish (partly marine) environments (*Vaucheria prolifera*, *Tetraedriella* spp.).

1.7 Dinoflagellates

Dinoflagellates (Dinophyta) are mostly biflagellate unicellular algae, although some (e.g. *Stylodinium*) are without flagella and are attached. They are predominantly found in the surface waters of marine systems (about 90% are marine), with only about 220 species present in freshwater environments. Dinoflagellates contain chlorophylls-*a* and -*c*, but are typically golden- or olive-brown (Fig. 1.3) due to the major presence of carotene and the accessory xanthophyll peridinin. The chloroplasts can be plate-like (Fig. 4.57) in some species or elongate in others. Pyrenoids are present, and the main storage product is starch. Lipid droplet reserves may also be found, and some dinoflagellates possess an eyespot.

1.7.1 Cytology

Dinoflagellates have a number of distinctive cytological features.

- A large central nucleus (usually visible under the light microscope) containing chromosomes that are typically condensed throughout the entire cell cycle. These have an unusual structure, with genetically active (transcriptional) DNA on the outside and genetically inactive DNA (structural DNA) forming a central core (Sigee 1984).

- Cell wall material lies beneath the cell membrane, in contrast to many other algae – where the cell wall, scales or extracellular matrix occur on the outside of the plasmalemma. The cell wall in dinoflagellates is composed of cellulose (within subsurface membrane-bound vesicles), forming discrete discs (thecal plates), which give the dinoflagellate a distinctive armoured appearance. The presence of thecal plates is difficult to see in light microscope images of living cells, but is clear when chemically-fixed cells are viewed by scanning electron microscopy (Fig. 4.57). The number, shape and arrangement of thecal plates is taxonomically diagnostic (compare Figs 1.8 and 1.9). A typical arrangement of plates can be seen in *Peridinium* (Fig. 1.8), with a division into two

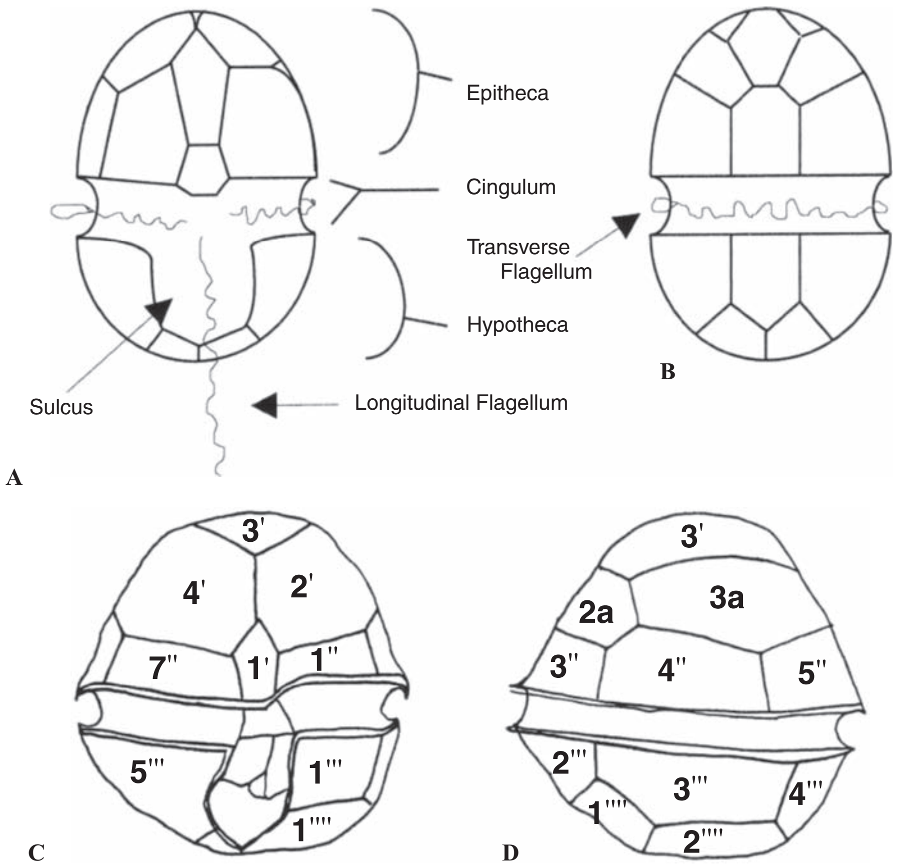

Figure 1.8 Dinoflagellate symmetry and plates. Top: Diagrammatic view of typical dinoflagellate cell showing flagellar insertion and grooves within the plate system. A – ventral view; B – dorsal view. Bottom: Pattern of numbered thecal plates characteristic of the dinoflagellate *Peridinium*. C – Ventral view; D – dorsal view. See also Fig. 4.57. Reproduced, with permission, from Wehr and Sheath (2003).

major groupings, forming an apical epitheca (or epicone) and a posterior hypotheca (or hypocone). Heavily armoured species tend to be more angular in outline, and plates may be extended in some species into elaborate projections. The presence of such projections or horns in *Ceratium*, for example (Fig. 1.9), increases the surface area to volume ratio of the cell and possibly reduces its sinking rate by increasing frictional resistance. Projections may also help in reducing predation. In contrast to armoured dinoflagellates, some species have very thin plates or they may be absent altogether (naked dinoflagellates).

1.7.2 Morphological diversity

These biflagellate unicellular organisms are of two main types, depending on the point of insertion of the flagella.

- Desmokont dinoflagellates, where the two flagella emerge at the cell apex (e.g. *Prorocentrum*). Relatively few species occur in this group, which is also characterized by the presence of two large plates (valves) covering a major portion of the cell.

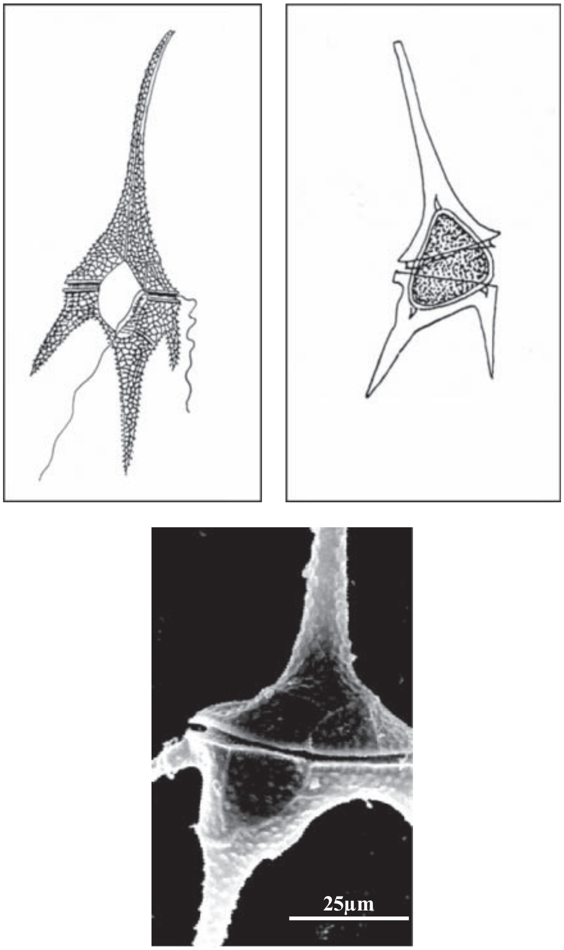

Figure 1.9 Morphology of *Ceratium*. Top: Diagrammatic views of mature cell (left) and cyst formation (right). Bottom: Central region of mature cell (SEM preparation) showing details of equatorial groove and thecal plates with pores. See also Figs 2.7 (cyst) and 4.56 (whole cells).

- Dinokont cells, where the flagella emerge from the middle of the cell (e.g. *Ceratium*, *Peridinium*). Dinokont cells have a distinct symmetry (Fig. 1.8) with dorsoventral division into epitheca and hypotheca (see above). These are separated by an equatorial groove (cingulum), with a further small groove – the sulcus, extending posteriorly within the hypocone. The two are flagella inserted in the ventral region of the cell. Beating of the flagella – one of which is extended while the other is contained in the equatorial groove – gives the cell a distinctive rotatory swimming motion. The term ‘dinoflagellate’ is derived from the Greek word *dineo*, which means ‘to whirl.’

Dinoflagellates do not show the range of morphology, from unicellular to colonial forms, seen in other algal groups. There are some unusual non-flagellate amoeboid, coccoid and filamentous forms, however, which reveal their phylogenetic relationship to mainstream dinoflagellates by the characteristic biflagellate structure of their reproductive cells - known as dinospores (zoospores).

1.7.3 Ecology

In freshwater environments, dinoflagellates are typically large-celled organisms such as *Ceratium*, *Peridinium* and *Peridiniopsis*. Their large size reflects the high nuclear DNA levels (large amounts of genetically inactive chromatin) and correlates with a long cell cycle and low rate of cell division. These features are typical of organisms that are K-strategists, dominating environments that contain high populations of organisms living under intense competition (Sigee, 2004).

Dinoflagellates are meroplanktonic algae, present in the surface waters of lakes and ponds at certain times of year. The annual cycle is characterized by two main phases.

- A midsummer to autumn bloom, when phytoplankton populations are very high and the surface waters are dominated either by dinoflagellates (Fig. 1.10) or colonial blue-green algae. At this point in the seasonal cycle, the surface water concentration of phosphorus is very low and dinoflagellates such as *Ceratium* survive by diurnal migration into the lower part of the lake – where phosphorus levels are higher. Dinoflagellates are adapted to this daily migratory activity by their strong swimming motion – which is coupled to an efficient phototactic capacity.

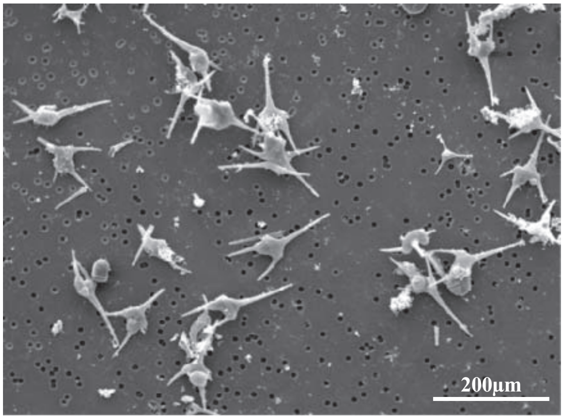

Figure 1.10 Autumn dinoflagellate bloom in a eutrophic lake. SEM view of epilimnion phytoplankton sample (September), showing almost complete dominance by *Ceratium hirundinella*. See also Fig. 2.8 (seasonal cycle) and Fig. 4.56 (live *Ceratium*). Photo: Sigee and Dean.

- An overwintering phase, where dinoflagellates survive on the sediments as resistant cysts. These non-flagellate cells (Fig. 2.7) lack an equatorial groove and initially form in surface waters at the end of the summer/autumn bloom before sinking to the bottom of the lake. Germination of cysts occurs in early summer, either on sediments or recently mixed waters, giving rise to vegetative cells which take up newly available phosphorus.

Ceratium and *Peridinium* are geographically widely dispersed, occurring particularly in waters that have high calcium-ion concentrations (hard waters) and low levels of inorganic nutrients (see above). Although these two organisms are phototrophic, obtaining their nutrients in inorganic form, other dinoflagellates exhibit some degree of heterotrophy. Some are able to ingest food particles by engulfment of whole cells, formation of a feeding veil (pallium) or use of a feeding tube (phagopod). In some cases, these dinoflagellates are pigmented and mixotrophic, combining heterotrophic nutrition with photosynthesis. Other dinoflagellates such as *Peridiniopsis* are obligate heterotrophs and colourless. The common freshwater heterotrophic dinoflagellate *Peridiniopsis berolinensis* uses a fine cytoplasmic filament to make contact with suitable prey such as insect larvae, then ingests the contents.

Heterotrophic dinoflagellates are particularly adapted to conditions where photosynthesis is limiting. A number of colourless species, for example, are found under the ice of frozen lakes during the winter season, where photosynthesis is severely limited by low irradiance levels – which are about 1% of surface insolation.

1.8 Cryptomonads

Cryptomonads (Cryptophyta) are a group of relatively inconspicuous algae that are found in both marine and freshwater environments. They are generally small to medium-sized unicells, and in many standing waters are a relatively minor part of the phytoplankton assemblage – both in terms of cell numbers and biomass.

1.8.1 Cytology

The fine structure of cryptomomads is illustrated by the light microscope images of *Rhodomonas* (Fig. 4.52) and *Cryptomonas* (Fig. 4.53), and by the line drawing shown in Fig. 1.11. Each cell bears two flagella, which are about the same length as the cell and slightly unequal. One of these flagella propels the cell, while the other is stiff and non-motile. The flagella are inserted near to an anterior ventral depression, the vestibulum. The cells tend to be more convex on one side than the other and the front end, where the flagella arise, tends to be obliquely truncated. On this side arises a gullet, which is not always clearly visible using a light microscope. Two elongate chloroplasts are present in *Cryptomonas*, lying along the length of the cell (Fig. 4.53) and on either side of the midline. The colour of cryptomonad chloroplasts may be blue, blue-green, reddish, olive green, brown or yellow-brown depending upon the accessory pigments present. These may include phycocyanin, phycoerythrin, carotene and xanthophylls. A pyrenoid is present and starch

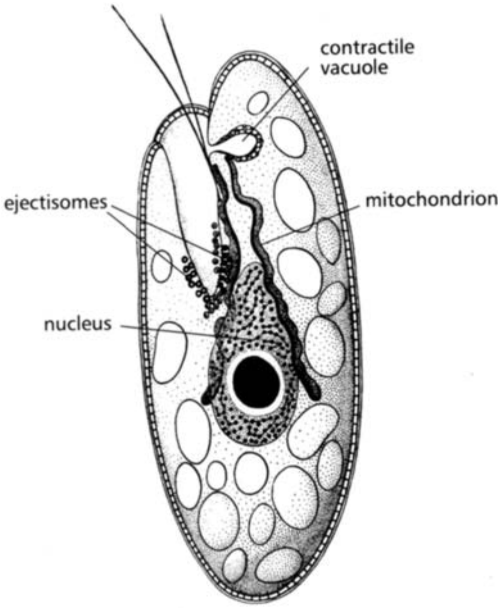

Figure 1.11 Cryptomonad morphology. Diagram of a non-photosynthetic cryptomonad with two flagella emerging from a depression. Reproduced, with permission from Graham and Wilcox (2000).

is stored. Further distinctive cytological features, include:

- a prominent contractile vacuole
- large ejectile organelles (ejectisomes), which discharge their content into the vestibular cavity and probably act as a defence response to herbivorous zooplankton
- the presence of an internal 'cell wall' (the periplast) below the plasmalemma, which is composed of round, oval, rectangular or hexagonal organic plates
- a complex flagellar root apparatus
- distinctive plastids, with the associated presence of a much reduced nucleus – referred to as a nucleomorph; this phylogenetic survival is thought to be derived from the original (red algal) symbiont that lead to the establishment of plastids in cryptomonads.

1.8.2 Comparison with euglenoid algae

Cyptomonads share a number of similarities with euglenoid algae. In both cases:

- algae are fundamentally biflagellate unicells, with flagella emerging from an apical depression
- cells are essentially naked, with a rigid covering of the cell occurring inside the plasmalemma
- species able to produce thick walled cysts that can survive adverse conditions
- different species occur in a variety of aquatic environments, both freshwater and marine
- heterotrophic (colourless) algae are well represented alongside autotrophic (green) species.

In spite of these similarities, there are a number of major differences between the algal groups, indicating a distinct phylogenetic origin: Key biochemical differences include the presence of starch (stains blue-black with iodine) in crypomonads, with photosynthetic pigments which include phycobilins and show some similarity to red algae. Ultrastructural differences also occur in terms of flagellar ornamentation, and flagellar roots are different from euglenoids. The environmental biology of cryptomonads is also quite distinct from euglenoids, indicating a different ecological role for these organisms. Cryptomonads are genuinely planktonic algae, widespread amongst the plankton of a range of water bodies – in contrast to euglenoids, which tend to occupy mud, sand and water interfaces.

1.8.3 Biodiversity

Cryptomonads are a relatively small group of algae, with a total of about 12 genera (van den Hoek *et al.*, 1995) containing approximately 100 known freshwater species and 100 known marine species.

Table 1.10 Variation in Plastid Number and Coloration in Cryptomonads

Trophic State	Plastid Characteristics*
Autotrophic	*Rhodomonas* – a single boat-shaped, red coloured plastid (4.52) *Chroomonas* – a single H-shaped blue-green plastid with a pyrenoid on the bridge. *Cryptomonas* – two plastids, each with a pyrenoid (4.53)
Heterotropohic	*Chilomonas* – a colourless cell that contains a plastid (leucoplast) that lacks pigmentation. No pyrenoid

*Figure numbers in brackets.

The low diversity in terms of number of species is reflected in the uniformity of morphology. There are no clear colonial forms, though some species are able to form irregular masses embedded in mucilage (palmelloid stage) under adverse conditions. Differences do occur in relation to plastids – including presence or absence, number and pigmentation (Table 1.10). Variations also occur in other key cytological features such as the presence or absence of nucleomorphs, occurrence of ejectisomes and structure of the periplast – providing a basis for the classification of these organisms (Novarino and Lucas, 1995).

1.8.4 Ecology

Cryptomonads are typical of temperate, high latitude standing waters that are meso- to oligotrophic. They appear to be particularly prominent in colder waters, becoming abundant in many lakes in early spring, when they may commence growth under ice. Algae such as *Rhodomonas* may form red blooms when the ice melts in early spring. In other (Antarctic) lakes, where ice persists, cryptomonad species such as *Cryptomonas* sp. and *Chroomonas lacustris* may contribute up to 70% of the phytoplankton biomass, dominating the algal flora for the whole of the limited summer period (Spaulding *et al*., 1994).

Cryptomonads are also typical of the surface waters of temperate lakes during the clearwater phase of the seasonal cycle, between the diatom spring bloom and the beginning of the mixed summer bloom. During this period, when algal populations are severely predated by zooplankton populations, the limited competition between algal cells favours rapidly-growing r-selected organisms such as cryptomonads (Sigee, 2004).

In oligotrophic freshwater lakes, cryptomonads often form large populations about 15–20 m deep within the lake, where oxygenated surface waters interface with the anoxic lower part of the water column. In this location, these algae form deep water accumulations of photosynthetic organisms, referred to as 'deep-chlorophyll maxima'. Studies on cultures of *Cryptomonas phaseolus* and *C. undulata* isolated from deep chlorophyll maxima (Gervais, 1997) showed that these organisms had optimal growth under light-limiting conditions, suggesting photosynthetic adaptation to low light intensities. Other factors which may be important in the ability of these organisms to form large depth populations include – the ability for heterotrophic nutrition, relative absence of predation by zooplankton, access to inorganic nutrients regenerated by benthic decomposition and tolerance of sulfide generated in the reducing conditions of the hypolimnion.

The ability of cryptomonads to carry out heterotrophic nutrition, using ammonium and organic sources as a supply of carbon and nitrogen is ecologically important. Evidence for uptake of particulate organic material comes from direct observation of phagocytosis and has been documented in both pigmented (e.g. *Cryptomonas ovata*) and various colourless species (Gillott, 1990). Ultrastructural studies also provide evidence that many pigmented cryptomonads are mixotrophic, capable of both autotrophic and heterotrophic nutrition. The blue-green cryptomonad, *Chroomonas pochmanni*, has a specialized vacuole for capturing and containing bacterial cells. Bacteria are drawn into this through a small pore at the cell surface, and subsequently pass into digestive vesicles within the cytoplasm.

1.8.5 Cryptomonads as bioindicators

Although they are common in oligo- and mesotrophic conditions, cryptomonads also occur in eutrophic lakes (Fig. 2.16) and have limited use as bioindicators. Individual species do not provide the contemporary diagnostic range for different habitats seen in other algal groups, and cryptomonads do not survive as clearly identifiable remains within lake sediments.

1.9 Chrysophytes

Chrysophytes (Chrysophyta), commonly referred to as the 'golden algae', are a group of microscopic algae that are most readily recognized by their golden-brown colour (Figs 1.3 and 4.37). This is due to the presence of the accessory pigment fucoxanthin within the chloroplasts, masking chlorophylls-*a* and -*c* (Table 1.3). There are around 200 genera and 1000 species of chrysophytes, present mainly in freshwater although some may be found in brackish and salt waters.

1.9.1 Cytology

Distinguishing cytological features (Table 1.3) include the presence of two dissimilar (long and short) flagella (heterokont condition) in motile organisms, plastids with four outer membranes plus triplet thylakoids, chrysolaminarin as the main storage product (present in special vacuoles) and cell walls composed of pectin – covered in some species with small silica spines or scales. Lipid droplets also frequently occur, and an eyespot may be visible in some species, associated with the chloroplast. In order to survive adverse conditions round walled cysts (stomatocysts) may be produced, with silica scales present in some species. Although most chrysophyte species are photosynthetic, some may be partially heterotrophic or even fully phagotrophic (Pfandl *et al*., 2009). Many species are small and are important members of the nanoplankton. They are widely distributed throughout freshwater systems, and are of particular interest in relation to their morphological diversity, ecology and as indicator organisms. Details of taxonomy and ultrastructure are given by Kristiansen (2005).

1.9.2 Morphological diversity

Chrysophytes exhibit considerable diversity in their general organization, ranging from unicellular to spherical (e.g. *Synura* – Fig. 4.37) and branching (e.g. *Dinobryon* – Fig. 4.2) colonial types. The great morphological diversity shown by these organisms (Table 1.11) follows a similar diversity seen in other

Table 1.11 Range of Body Structure and Form in Chrysophytes

General Structure	Colony Status	Examples*
Flagellate cells (monads)		
Simple cell wall	Single cells:	*Chromulina*, *Ochromonas*
	Colonies	*Uroglena*
Surrounded by envelope (lorica)	Colony	*Dinobryon* (4.2)
Cells covered in silica scales	Single cells	*Mallomonas*
	Colony	*Synura* (4.37)
Non-flagellate unicells		
Motile by pseudopod	Golden amoebae	*Chrysamoeba*
Non-motile	Cells surrounded by a jelly	*Chrysocapsa*
	Cells surrounded by solid cell wall	*Stichogloea*
Colonies with distinctive morphology		
	Simple branched filaments	*Sphaeridiothrix* *Phaeothamnion*
	Two-dimensional cell plates	*Phaeoplaca*

*Figure numbers in brackets.

algal phyla and includes flagellates, amoeboid forms, cells in jelly (capsal), filamentous algae and plate-like (thalloid) organisms. Each of these types, however, can transform into another phase (e.g. flagellates can become amoeboid) – so that grouping in relation to morphology is dependent on the stage of life cycle. The occurrence of a resistant (stomatocyst) phase (see above) is also a key part of the life cycle.

1.9.3 Ecology

Chrysophytes are ecologically important in a number of ways (Kristiansen, 2005).

- In many ecosystems they play an important role as primary producers. This is particularly the case for adverse conditions – such low nutrient and acid lakes (e.g. Nedbalova *et al*., 2006).
- They have a versatile nutrition, with many members (e.g. *Dinobryon*) being mixotrophic. These organisms are able, in addition to photosynthesis, to obtain their carbon from organic sources – either by surface absorption of organic compounds or by phagocytosis of particulate organic matter.
- They can be nuisance algae, giving a fishy smell to drinking water reservoirs when they reach high population levels.

1.9.4 Chrysophytes as bioindicators

Their diverse ecological preferences (Table 1.12) make chrysophyte species potentially useful as environmental indicator organisms – both in terms of contemporary water assessment and sediment analysis.

Table 1.12 Chrysophyte Species as Bioindicators

Strongly acid, often humic conditions	*Dinobryon pediforme, Synura sphagnicola, Mallomonas paludosa, M. hindonii, M. canina*
Weakly buffered, slightly acid clear water lakes	*Mallomonas hamata, Synura echinulata, Dinobryon bavaricum* var. *vanhoeffenii*
Alkaline conditions	*Mallomonas punctifera, Synura uvella*
Alkaline/saline conditions	*Mallomonas tonsurata, M. tolerans*

Contemporary water quality

Although potentially very useful, chrysophytes are rarely used in monitoring projects. The main reason for this is that the best ecologically-studied species (with silica scales) require electron scanning microscopy for identification – a technique not routinely used as a tool in water quality assessment.

Although chrysophytes have traditionally been held to indicate oligotrophic conditions, the situation is more complex. Studies by Kristiansen (2005) have shown that the presence of a few species such as *Uroglena* or *Dinobryon* may indicate oligotrophy, but greater chrysophyte species diversity at lower overall biomass is more indicative of eutrophic conditions. The value of individual chrysophyte species as ecological indicators varies considerably (Kristiansen, 2005). *Synura petersenii*, with a broad ecological range, is of limited use – but other narrow-range species (Table 1.12) can be used particularly in relation to pH and salinity.

Sediment analysis

The main application of chrysophytes as environmental bioindicators is in paleoecology. Their major advantage for this (as with diatoms) is the presence of non-degradable silica cell wall material, so that they remain undamaged in the lake sediments. The use of these algae in paleoecology depends on accurate means of classification and identification, undisturbed sediment layers and absolute dating of the layers – e.g. by the lead isotope Pb^{210}.

Two main types of chrysophyte remains are useful in freshwater sediments – silica scales (from vegetative planktonic cells) and stomatocysts (resistant spores). Stomatocysts have the advantage of better preservation compared to scales, but the disadvantage that (with a few exceptions) they cannot be referred to particular species. Although stomatocysts cannot be identified in terms of species, they can be recorded as distinct morphological types (morphotypes). These can be correlated with other microfossils (e.g. diatoms) and with pollen to obtain environmental indicator values. Quantitative assessment of past aquatic environments in relation to fossil chrysophytes (stomatocysts) parallels that of diatoms (Section 3.2.2) and can be obtained in relation to species ratios (environmental indices), transfer functions (Facher & Schmidt, 1996) and group analysis.

Species ratios The ratio of chrysophytes to other algal groups may provide a useful index to assess environmental change. Studies by Smol (1985) on the surface sediments of Sunfish Lake (Canada) demonstrated a marked increase in the ratio of diatoms to somatocysts, coincident with the arrival of settlers to the lake catchment area. The diatom/somatocyst ratio is a trophic index, signalling an increase in eutrophic status of the lake at the time of human settlement. The above ratio change resulted from a population decrease in the chrysophyte *Mallomonas* (indicating oligotrophic conditions) and an increase in eutrophic diatoms. The increase in trophic ratio parallels an increase in the pollen count of ragweed, an indicator of forest clearance and farming activity.

Transfer functions The environmental properties of stomatocyst morphotypes can be assigned as numerical descriptors or 'transfer functions.' These define each morphotype in relation to a range of environmental data (pH, conductivity, phosphorus contents and maximal depth of the lake).

The transfer functions have been derived by multivariate analysis of sediment calibration sets, obtained from a series of North American lakes in which both environmental data and stomatocyst assemblages were available (Charles and Smol, 1994).

Group analysis The various environmental factors which influence correlations between morphotypes can be identified by statistical analysis – particularly principal component analysis (PCA). Studies by Duff and Smol (1995) on 181 morphotypes obtained from 71 different lakes (in the United States) showed that differences in pH and lake morphometry had the greatest influence in determining statistical associations. PCA analysis separated morphotypes into three main groups, respectively relating primarily to acid environments, neutral/alkaline environments and lake morphometry (e.g. lake depth).

1.10 Diatoms

Diatoms (Bacillariophyta) are a very distinct group of algae, identifiable under the light microscope by their yellow-brown coloration (Fig. 1.13) and by the presence of a thick silica cell wall. This cell wall typically appears highly refractive under the light microscope, giving the cell a well-defined shape. Removal of surface organic material by chemical oxidation (Section 2.5.4) reveals a complex cell wall ornamentation – as illustrated in the light microscope images comparing living and digested cells of *Stephanodiscus* (Fig. 4.58). Cell wall ornamentation can be seen even more clearly with the higher resolution of the scanning electron microscope and is important in species identification.

Diatoms occur as non-flagellate single cells, simple colonies or chains of cells, and are widely-occurring in both marine and freshwater environments. Their success in colonizing and dominating a wide range of aquatic habitats is matched by their genetic diversity – with a worldwide total of 285 recorded genera, encompassing 10 000–12 000 species (Round *et al.*, 1990; Norton *et al.*, 1996). Diatoms are also very abundant in both planktonic and benthic freshwater environments, where they can form a large part of the algal biomass, and are a major contributor to primary productivity.

1.10.1 Cytology

Distinctive cytological features (Table 1.3) include:

- plastids with periplasmic endoplasmic reticulum and the presence of girdle lamellae
- chrysolaminarin and lipid food reserves, present outside the plastid

- a distinctive cell wall, the frustule, composed of opaline silicon dioxide (silica) together with organic coatings.

Silica cell wall

The presence of silica in the diatom cell wall can be detected by cold digestion in a strongly oxidizing acid to remove organic matter (Section 2.54). If the cell wall resists this treatment it is probably silica. The cell wall of diatoms differs from that of other algae in being almost entirely inorganic in composition. It has evolved as an energy-efficient structure, requiring significantly less energy to manufacture than the cellulose, protein and mucopeptide cell walls of other algae (Falkowski and Raven, 1997). This gives diatoms a major ecological advantage at times when photosynthesis is limited (e.g. early in the seasonal cycle), but has a number of potential disadvantages.

- The cell wall of diatoms is very dense. These organisms are only able to stay in suspension in turbulent conditions, limiting the development of extensive planktonic diatom populations to unstratifiied waters.
- The formation of the diatom cell wall is dependent on an adequate supply of soluble silica (silicic acid) in the surrounding water. The diatom spring bloom of temperate lakes strips out large quantities of silica from the water, reducing concentrations to a level that becomes limiting.
- Unlike other types of cell wall material, silica is rigid and unable to expand. This means that daughter cells are unable to enlarge and progressive cell divisions result in a gradual decrease in cell size. Ultimately this decrease reaches a critical level, at which point sexual reproduction is required to completely shed the original cell wall and form new, large daughter cells.

Cell wall structure

The frustule is composed of two distinct halves – the epitheca and hypotheca – which fit together rather like the lid and base of a pill-box (Fig. 1.12). At cell division, two new walls are formed internally at the cell equator 'back to back', and become the hypothecae of the two daughter cells. The original epitheca and hypotheca of the parent diatom become

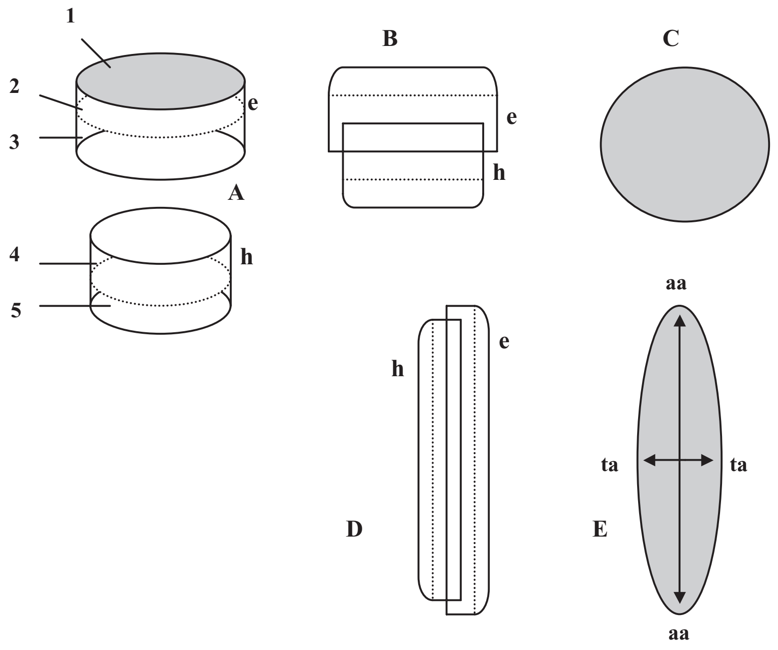

Figure 1.12 Diatom frustule structure – comparison of centric and pennate diatoms. **Centric diatom:** A. Separate views of epitheca (e) 1. Epivalve 2. Mantle of epivalve. 3. Epicingulum and hypotheca (h) 4. Hypocingulum. 5. Hypovalve. B. Complete frustule – girdle or side view (showing overlap of cingula). C. Valve or face view of epivalve. Pennate **diatom:** D. Complete frustule – girdle view. E. Valve view, showing apical or longitudinal axis (aa) and transapical or transverse axis (ta).

the epithecae of the two daughter cells, so the epitheca is always the oldest part of the frustule.

In both centric and pennate diatoms (see below) the epitheca consists of two main parts – a circular disc (the valve) with a rim (the mantle) plus an attached band or girdle (the epicingulum). The hypotheca correspondingly consists of a hypovalve, hypovalve mantle and hypocingulum. When the epitheca and hypotheca fit together to form the complete frustule the hypocingulum fits inside the epicingulum to give an overlap.

Because of frustule morphology (Fig. 1.12), diatoms can be observed from either a girdle (side) view or from a valve (face) aspect – with girdle and valve views of live diatoms being clearly distinguishable under the light microscope (Fig. 1.13). The two possible valve views, of the top (epivalve) or bottom (hypovalve) of the diatom, are not distinguishable in many genera – but do differ in some cases (e.g. *Cocconeis*, *Achnanthes*). Axes of symmetry in pennate diatoms are shown in Figs 1.12 and 1.13.

Frustule markings

A wide range of surface markings can be seen on the face (epivalve and hypovalve) of diatoms. These have been recorded in considerable detail (Barber and Haworth, 1981; Round *et al*., 1990; Wehr and Sheath, 2003) and form the basis for the classification and identification of these organisms (Chapter 4).

Clear visualization of the frustule markings requires the high resolution of either oil immersion (light microscopy) or scanning electron microscopy, and is normally carried out after the removal of surface organic matter by chemical (acid digestion) cleaning. Chemical fixation for scanning electron microscopy may also strip away some of the overlying organic material to reveal frustule surface structure.

The terminology of diatom morphology (see Glossary) includes various descriptors of frustule markings – including eye-shaped structures (ocelli), small pores (punctae) and fine lines (striae). Illustrations of diatom surface markings are shown diagrammatically in Fig. 1.14 and in various figures and plates in Chapter 4. Although the biological significance of much of this surface detail is obscure, the presence of one major surface structure – the raphe – is clearly associated with locomotion. The secretion of mucus from this channel or canal promotes movement on solid surfaces. In some diatoms such as *Nitzschia* (Fig. 4.70a, b) the raphe is elevated from the main diatom surface as a keel, allowing more intimate contact between the raphe and substrata. Such keeled diatoms are able to move particularly well on fine sediments, and reach their maximum abundance in the epipelon of pools and slowly-flowing streams (Lowe, 2003). A raphe is not always present in pennate diatoms, and is never seen in centric diatoms (see below).

1.10.2 Morphological diversity

The morphological diversity of diatoms can be considered in relation to two main aspects – the distinction between centric/pennate diatoms and the range of unicellular to colonial forms.

Centric and pennate diatoms

Diatoms can be separated into two major groupings – centric and pennate diatoms, based primarily on cell shape and frustule morphology (Fig. 1.12). Centric diatoms typically have a discoid or cylindrical shape, with a radial symmetry when seen in face or ‘valve’ view. Pennate diatoms have an elongate bilateral symmetry, with longitudinal and transverse axes, and often have the appearance of a ‘feathery’ (hence ‘pennate’) ornamentation when seen in surface view (Fig. 1.13). Pennate diatoms have a range of shapes (Barber and Haworth, 1981), with broad division (Fig. 1.15) into isopolar (ends of valve similar in size and shape), heteropolar (ends of valve differing in size and shape), asymmetrical (outline dissimilar either side of the longitudinal axis) and symmetrical (outline of valve similar either side of the longitudinal axis).

The fundamental differences between centric and pennate diatoms in relation to structure and symmetry reflect a range of other distinguishing features (Table 1.13) – including motility, number and size of plastids, sexual reproduction and ecology. Centric diatoms are mainly planktonic algae, and the occurrence of oogamy – with the production of a

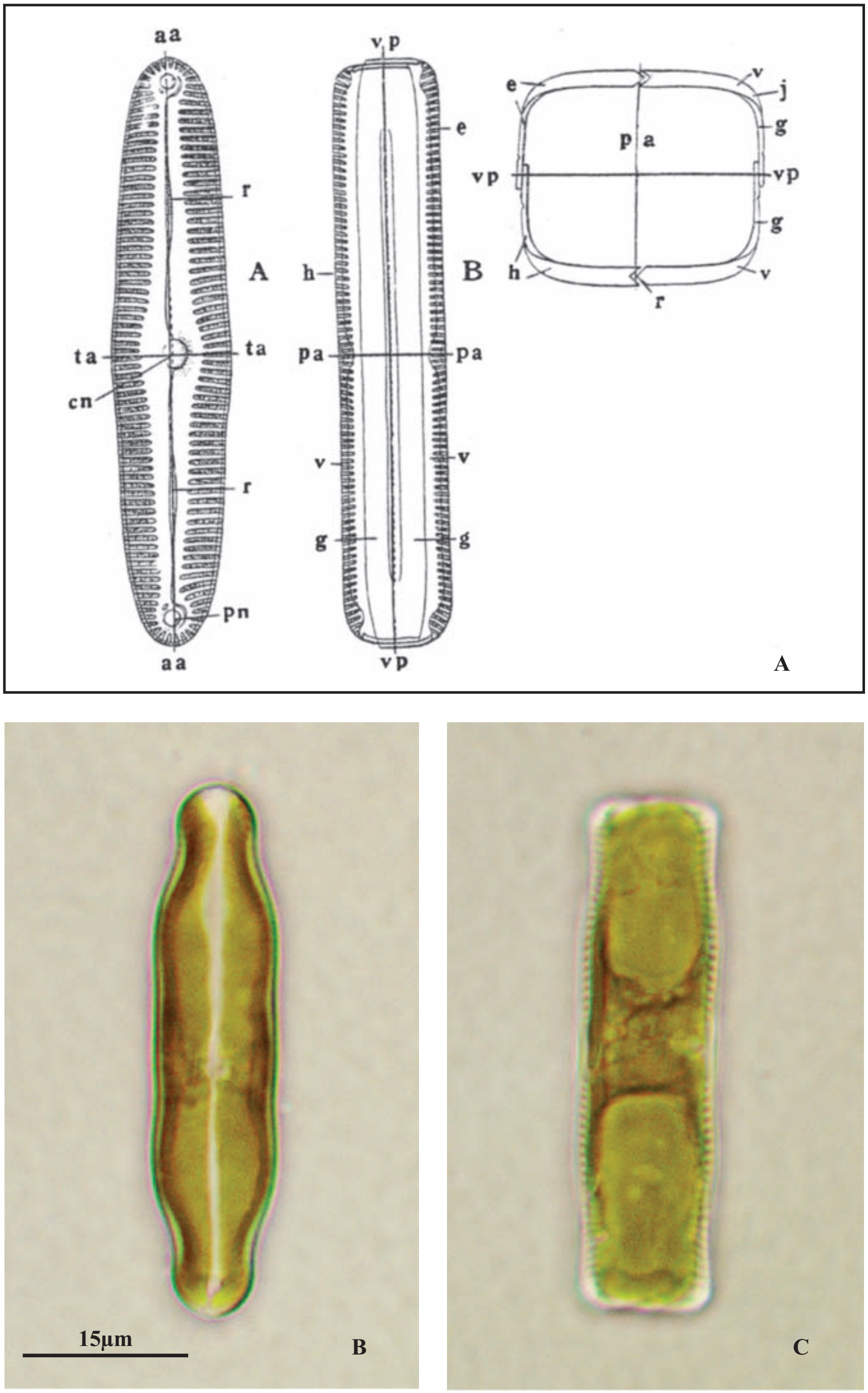

Figure 1.13 Details of frustule structure in a pennate diatom (*Pinnularia*). Top: Diagrams of cell wall structure, with principal axes. Left: Valve view. aa – apical axis, ta – transapical axis, pn – polar nodule, r – raphe. Middle: Girdle view. pa – pervalvular axis, vp – valvar plane, h – hypotheca, e – epitheca, g – girdle. Right: Transverse section. Reproduced, with permission, from Fritsch (1956). Bottom: Light microscope images of fresh (unfixed) cells in valve (B) and girdle (C) view.

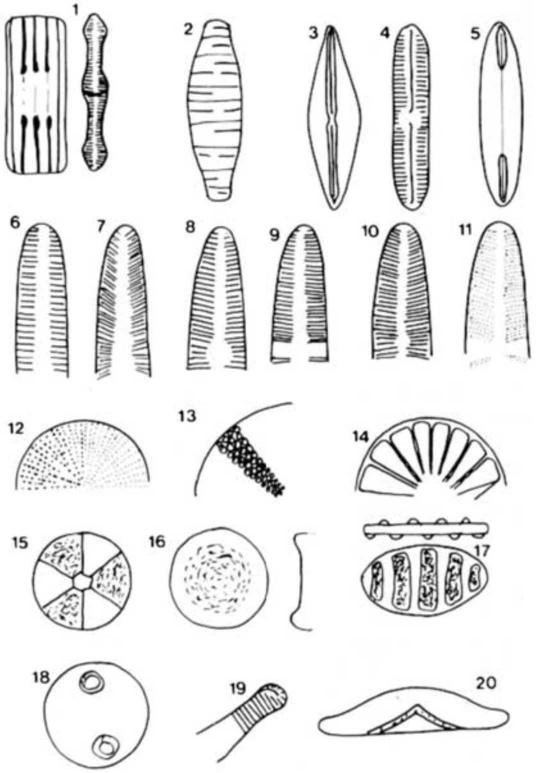

Figure 1.14 Diatom valve markings. 1 internal septa; 2 transapical costae; 3 raphe in thickened ribs; 4 normal raphe; 5 shortened raphe in ribs; 6 parallel striae; 7 radiate striae; 8 central area round; 9 central area transverse; 10 central area small; 11 central area acute angled; 12 punctae in radial rows interspersed with subradial rows; 13 coarse areolae; 14 areolae grouped in segments; 15 sections of valve alternately raised and depressed giving shaded appearance; 16 central region raised or depressed; 17 pennate with raised or depressed areas; 18 ocelli on surface; 19 rudimentary raphe as in *Eunotia*; 20 raphe curved to point at centre as in *Epithemia*. Reproduced, with permission, from Bellinger, 1992.

large number motile sperm, is regarded as a strategy for increasing the efficiency of fertilization in open water environments. Pennate diatoms are almost always isogamous, with equal sized non-flagellate gametes (two per parent cell). In these algae, efficiency of fertilization is promoted by the pairing of gamete parental cells prior to gamete formation, a possible adaptation to more restricted benthic environments. Fossil and molecular evidence suggests that centric diatoms arose prior to pennate ones, implying that in these algae isogamy has been phylogenetically derived from oogamy (Edlund and Stoermer, 1997). This is in contrast to the evolutionary sequence normally accepted for other algal groups - particularly green and brown algae.

Unicellular and colonial diatoms

The genetic diversity of diatoms is not matched by the complexity of their morphological associations – which are limited to small chains and groups of cells. In planktonic diatoms, simple filamentous (e.g. *Aulacoseira* – Fig. 4.9) and radial (e.g. *Asterionella* – Figs 1.16, 4.42) colonies have evolved, but there is no formation of spherical globular colonies that are a key feature of some other algal groups.

Colonial forms of diatoms arise by means of simple linkages, which are of three main types.

- Mucilage pads. These occur at the ends of cells to join diatom cells in various ways. *Asterionella* has elongate cells which are joined at their inner ends to form stellate colonies. In *Tabellaria*, cells are linked valve to valve as short stacks, with connection of the stacks at frustule edges by mucilage pads to form a zig-zag pattern. Mucilage pads are seen particularly clearly in Fig. 2.28, where chains of *Tabellaria* are attached to *Cladophora*.

- Interlinking spines. In diatoms such as *Aulacoseira*, chains of cells are joined valve to valve by spines. The valves are of two main types (Davey and Crawford, 1986)

  - Separation valves, with long tapering spines and straight rows of pores,

  - Linking valves, with short spines and curved rows of pores

  Cells are strongly linked into chains via the short spines on the linking valves, but weakly linked via the long spines of the separation valves – where

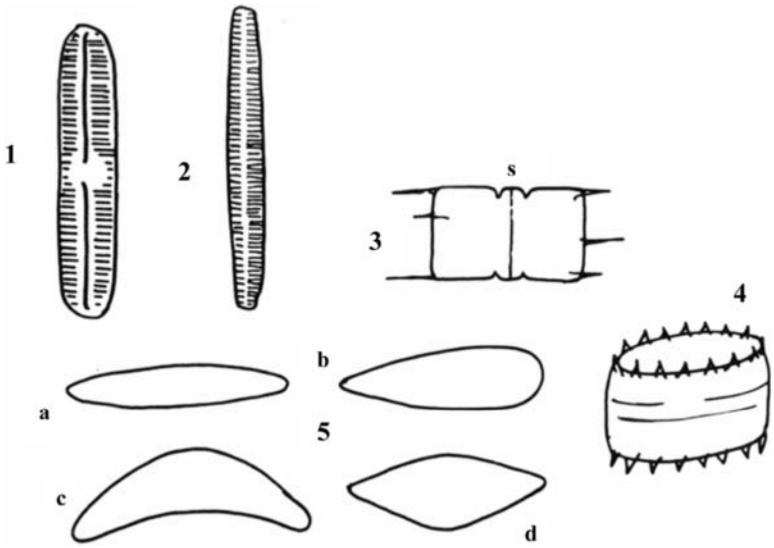

Figure 1.15 Diatom morphology. 1 pennate diatom with raphe; 2 with pseudoraphe; 3 sulcus (s) on *Melosira*; 4 spines on margin of *Stephanodiscus*; 5 valve shapes: (a) isopolar, (b) heteropolar, (c) asymmetrical, (d) symmetrical. Reproduced, with permission, from Bellinger, 1992.

filament breakage easily occurs (Fig. 4.9). The length of filaments within a population of *Aulacoseira* depends on the proportion of separation valves to linking valves.

- Gelatinous stalks. Biofilm diatoms such as *Rhoicosphenia* (Fig. 4.67) and *Gomphonema* (Fig. 2.29) have gelatinous stalks, which attach the diatom to the substratum and join small groups of cells together as a single colony. In these diatoms, the stalk material is secreted via apical pores. Some species of *Cymbella* also produce a gelatinous tube-like filament.

1.10.3 Ecology

Diatoms are ubiquitous in both standing and running freshwaters – occurring as planktonic, benthic, epiphytic (on higher plants and other algae) and epizoic (on animals, such as zooplankton) organisms. They are equally successful as free floating and attached

Table 1.13 Centric and Pennate Diatoms

Characteristic	Centric Diatoms	Pennate Diatoms
Symmetry	Radial	Bilateral
Examples*	*Stephanodiscus* (4.58) *Cyclotella* (4.62)	*Pinnularia* (1.13, 4.71) *Navicula* (4.73)
Gliding motility	Non-motile	Some (raphid) diatoms are actively motile
Plastids	Many discoid plastids	Two large plate-like plastids
Sexual reproduction[†]		
Initiation	Independent formation of gametes from parent cells	Pairing of parental cells prior to gamete production
Egg cells	Oogamous – production of one or two eggs per parent cell	Isogamous
Sperm cells	4–128 sperm per parent cell. Each with a single flagellum bearing two rows of mastigonemes	Few amoeboid, non-flagellate sperm cells
Ecology	Mainly planktonic, typical of open water	Planktonic, epiphytic and benthic forms

*Figure numbers in brackets.

[†] Hasle and Syvertsen, 1997.

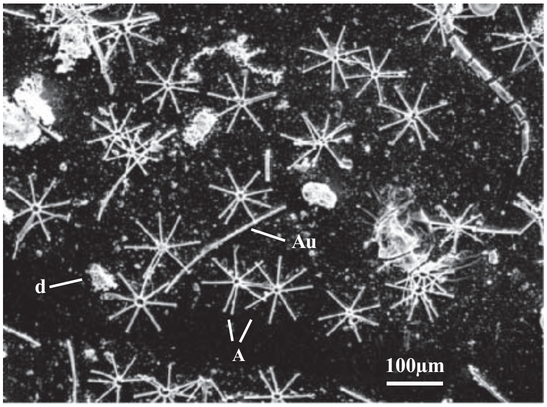

Figure 1.16 Spring diatom bloom in a eutrophic lake. SEM view of lake surface phytoplankton sample (March), showing numerous stellate colonies of *Asterionella* (A) with some filamentous *Aulacoseira* (Au). The large amount of debris (d) occurs due to the complete mixing of the water column at this time of year. See also Figs 2.8 (seasonal cycle), 4.8 (live *Aulacoseira*) and 4.42 (live *Asterionella*). Photo: Sigee and Dean.

forms, where they may be respectively important in plankton bloom formation and biofilm development.

Planktonic diatoms: bloom formation

In many temperate lakes, diatoms such as *Asterionella* and *Tabellaria* dominate the phytoplankton population in Spring and early Summer (Fig. 1.16), at a time when inorganic nutrients (N, P, Si) are at high concentration, light and temperature levels are rising and lake turbulence is maintained by moderate wind action. At this point in the seasonal cycle, diatoms are able to out-compete other micro-algae due to their tolerance of low temperature and low light conditions, coupled with their ability to grow in turbulent water. Autumn blooms of *Fragilaria* and *Asterionella* are also a common feature of many lakes, with the diatoms part of a mixed phytoplankton population.

Diatoms such as *Aulacoseira italica* are regarded as meroplanktonic, with major growth in very early Spring, but spending the rest of the year as resistant cells on lake sediments. Other diatoms, such as *Asterionella* and *Tabellaria* also form major blooms in Spring, but are additionally present as minor constituents within the mixed phytoplankton population over much of the annual cycle – and are regarded as holoplanktonic.

Attached diatoms: biofilm formation

Diatoms are major components of biofilms, where they are present early in the colonization sequence and also within the mature periphyton community. See later for examples of diatoms in river biofilm (Fig. 2.23), reed biofilm (Fig. 2.29) and as attached epiphytes (Fig. 2.28).

1.10.4 Diatoms as bioindicators

The major use of diatoms as bioindicators of water quality (both lotic and lentic systems) is detailed in Chapter 3. These algae can be isolated from existing live populations (contemporary analysis) or from sediments (fossil diatoms) and quantitative information on water quality from species counts can be obtained from taxonomic indices, multivariate analysis, transfer functions and species assemblage analysis.

Contemporary analysis

Many diatom species have distinct ecological preferences and tolerances, making them useful indicators of contemporary ecological conditions. These preferences relate to degree of water turbulence, inorganic nutrient concentrations, organic pollution, salinity, pH and tolerance of heavy metals. Round (1993) also lists a number of other advantages in using diatoms as indicators – including easy field sampling, high sensitivity to water quality but relative insensitivity to physical parameters in the environment and easy cell counts. Diatoms are also the most diverse group of algae present in freshwaters, making them the most ideal assemblage for calculation of bioindices (Section 3.4.5).

Diatoms, once cleaned and mounted for identification, make excellent permanent slides which can form an important historical record for a location. Although there are hundreds of diatom species that may be present only dominant species need to be used in an assessment. Benthic diatoms have been particularly useful in assessing water quality of rivers, and the wide range of indices that have been devised is discussed in Chapter 3.

Lake sediments

Diatoms, more than any other group of algae, are used to monitor historical ecological conditions from sediment analysis – as discussed in Section 3.2.2. The major role of diatoms in this lies in the resistance of the diatom frustule to biodegradation, coupled with ease of identification from frustule morphology and the wide range of species with clear ecological preferences see in freshwater environments.

The importance of diatoms in environmental analyses is indicated by the European Diatom Database Initiative (EDDI). This key web-based site includes electronic images and data handling software, and is particularly useful for lake sediment analysis (Section 3.2.2). Other databases, such as the stream benthic database of Gosselain *et al*. (2005), are more applicable to contemporary environmental analysis.

1.11 Red algae

Red algae (Rhodophyta) are predominantly marine in distribution, with only 3% of over 5000 species worldwide occurring in true freshwater habitats (Wehr and Sheath, 2003). Although termed red-algae, the level of accessory pigments (phycoerythrin and phycocyanin) may not be sufficient to mask the chlorophyll – resulting in an olive-green to blue (rather than red) coloration. The relatively common river alga *Batrachospermum*, for example, typically appears bluish-green in colour (Fig. 4.4) – giving no clue to its rhodophyte affinity.

Major cytological features of red algae (Table 1.3) include the absence of flagellae, presence of floridean starch as the major food reserve, characteristic photosynthetic pigments (chlorophyll-*a* only, presence of phycobilins) and distinctive plastid structure (unstacked thylakoids, no external endoplasmic reticulum).

Many of the freshwater species (which occur mainly in streams and rivers) are quite large, being visible to the naked eye when occurring in reasonable numbers (Sheath and Hambrook, 1990). The range of morphologies include gelatinous filaments (e.g. *Batrachospermum*), pseudoparenchymatous forms (e.g. *Lemanea*) and a flat thallus of tiered cells (*Hildenbandia*). Many of these shapes can be an advantage in resisting the forces exerted in swiftly flowing waters.

Although freshwater red algae (such as the large filamentous alga *Batrachospermum*) are largely found in streams and rivers, Rhodophyta may also occur as marine invaders of lakes and brackish environments. Certain freshwater red algae in the littoral zones of the Great Lakes Basin (United States), for example, appear to be originally marine and to have lost the capacity for sexual reproduction. These include the filamentous red alga *Bangia atropurpurea* (Lin and Blum, 1977), which reproduces only by asexual monospores – in contrast to marine species which undergo alternation of generations and carry out sexual reproduction. Attached red algae (e.g. *Chroodactylon ramosum*) also contribute to the epiphytic flora of lake periphyton.

1.12 Brown algae

As with red algae, brown algae (Phaeophyta) are almost entirely marine – with less than 1% of species present in freshwater habitats (Wehr and Sheath, 2003). These species are entirely benthic, either in lakes or rivers, and have a very scattered distribution.

Cytological features of this group (Table 1.3) include heterokont flagella (reproductive cells only), presence of laminarin as the major food reserve, characteristic photosynthetic pigments (chlorophyll-*a* and -*c*, β and ε carotenes) and distinctive plastid structure (triple thylakoids, enclosing endoplasmic reticulum). Freshwater brown algae include genera such *Pleurocladia* and *Heribaudiella*, and are the least diverse of all freshwater algae. Their morphologies are based on a relatively simple filamentous structure (forming mats or crusts), and they lack the complex macro-morphology typical of the brown seaweeds. The freshwater brown-algae have not been fully studied and their ecological characteristics are not well known. All are benthic and may be recognized by their possession of large sporangia (Wehr, 2003).