3

Algae: the major microbial biomass in freshwater systems

In the freshwater environment, light energy conversion and related synthesis of carbon compounds is carried out by three major groups of organisms (primary producers) – higher plants (macrophytes), algae, and photosynthetic bacteria. Algae are the main microorganisms involved in this process and may be defined as simple plants (lacking roots, stems, and leaves) that have chlorophyll-a as their primary photosynthetic pigment and lack a sterile covering of cells around the reproductive cells. Although algae include prokaryote organisms (blue-green algae), the closely-related photosynthetic bacteria differ in terms of cell size, pigmentation, and physiology (strict anaerobes, not evolving oxygen) and are generally placed in a distinct category. Photosynthetic bacteria will be considered separately in Chapters 4 and 6.

In this chapter, and elsewhere in the book, the term 'algae' includes many different types of organism, and is really a term of convenience. Although commonly used, the term becomes problematic in the light of modern knowledge on the diverse origins of the group, with blue-greens fundamentally different from eukaryotic algae – and within these, heterokont algae quite distinct from other eukaryote organisms (Section 3.1.2, Table 3.3).

Algae as autotrophs and heterotrophs. In addition to requiring light (phototrophs) the majority of algae are also autotrophs – using carbon dioxide as their sole source of carbon. Algae also typically use inorganic sources of nitrogen, phosphorus, and sulphur as sole sources of these elements for biosynthesis. Although most algae are autotrophic, some do have the ability to assimilate organic compounds in certain environmental situations. Furthermore, although most algae are photoautotrophs in relation to bulk supplies of energy and inorganic nutrients, they frequently require small quantities of organic growth factors, including one or more of cobalamin, biotin, and thiamine (Swift, 1980). The development of heterotrophy is considered further in Section 3.11.

Algae as freshwater microorganisms. Freshwater algae range in size from microscopic organisms (unicellular and colonial) to macroscopic forms which are visible to the naked eye and appear plant-like. Planktonic algae are typically microscopic (micro-algae), and are part of the microbial community which is the main theme of this book. In contrast, various benthic or attached algae are macroscopic, and do not fit into the broad area of aquatic microorganisms. The green algae in

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Table 3.1 Major divisions of freshwater algae: microscopical appearance, motility, and typical habitat (data from Lee, 1997, Van den Hoek *et al*., 1995, and Wehr, and Sheath, 2003)

Algal division (class)	Index of biodiversity*	Typical colour	Typical morphology of freshwater species	Motility (vegetative cells/colonies)	Aquatic habitats	Typical examples
1. Blue-green algae ***Cyanophyta***	124	Blue-green	Microscopic or visible – usually colonial	Buoyancy regulation Some can glide	Lakes and streams Planktonic or attached	*Synechocystis Microcystis*
2. Green algae ***Chlorophyta***	302	Grass-green	Microscopic or visible – unicellular or filamentous colonial	Some unicells and colonies with flagella	Lakes, rivers, estuaries Planktonic or attached	*Chlamydomonas Cladophora*
3. Euglenoids ***Euglenophyta***	10	Various colours	Microscopic – unicellular	Mostly with flagella	Lakes and ponds Planktonic	*Euglena Colacium*
4. Yellow-green algae: **Eustigmatophyta Raphidiophyta Tribophyta**	90	Yellow-green	Microscopic – unicellular or filamentous	Flagellate and non-flagellate forms	Planktonic, benthic and epiphytic Wide habitat range	*Chlorobotrys Vischeria*
5. Dinoflagellates **Dinophyta**	37	Red-brown	Microscopic – unicellular	All with flagella	Lakes and estuaries Planktonic	*Ceratium Peridinium*
6. Cryptomonads ***Cryptophyta***	12	Various colours	Microscopic – unicellular	Mostly with flagella	Lakes Planktonic	*Rhodomonas Cryptomonas*
7. Chrysophytes† ***Chrysophyta***	72	Golden brown	Microscopic – unicellular or colonial	Some with flagella	Lakes and streams Planktonic	*Mallomonas Dinobryon*
8. Diatoms ***Bacillariophyta***	118	Golden brown	Microscopic – unicellular or filamentous colonies	Gliding movement on substrate	Lakes, rivers, estuaries Planktonic or attached	*Stephanodiscus Aulacoseira*
9. Red algae ***Rhodophyta***	25	Red	Microscopic or visible – unicellular or colonial	Non-motile	Mainly streams, some lakes Attached	*Batrachospermum Bangia*
10. Brown algae ***Phaeophyta***	4	Brown	Visible – multicellular cushions and crustose thalli	Non-motile	Lakes and streams Attached	*Pleurocladia Heribaudiella*

*Biodiversity: number of genera within the group in the USA (Wehr and Sheath, 2003)
†Including haptophyte and synurophyte algae

particular include large filamentous forms such as *Cladophora* and *Chara*, and description of these is limited to their ecological role as attached algae or periphyton.

Freshwater algae constitute a diverse group of biota and occupy a wide range of aquatic habitats. In this chapter we will consider this group of organisms in terms their taxonomic grouping, molecular characterization, variation in size and shape, activities within the freshwater environment, strategies for survival, and general biodiversity. Other aspects such as photosynthesis (Chapter 4) and competition for nutrients (Chapter 5) are considered elsewhere.

A. TAXONOMIC AND MOLECULAR CHARACTERIZATION

3.1 Major taxonomic divisions of freshwater algae

Freshwater algae do not occur as a formal taxonomic group of organisms, but represent a loose and diverse collection of divisions with members that are united by possession of the general features outlined previously. The heterogeneity of freshwater algae is emphasized by the fact that they are split between two of the major domains of living organisms (Table 1.2) – the Bacteria (a prokaryote group that includes blue-green algae) and the Eukarya (including all eukaryote algae). Although there is little consensus among phycologists in terms of exact groupings, they are separated here into 10 principle divisions or classes. Information on the major characteristics of these divisions is summarized in Table 3.1 (microscopical appearance, motility, and habitat) and Table 3.3 (biochemical and cytological characteristics), with a description of each group in Section 3.1.3. Further information on algal classification can be obtained from a range of standard texts including Lee (1997), Van den Hoek *et al*. (1995), Wehr and Sheath (2003), and John *et al.* (2002). Molecular characterization of freshwater algae is of increasing taxonomic and diagnostic importance, and is discussed in Section 3.3.

3.1.1 Microscopical appearance, motility and ecological features (Table 3.1)

Examination of environmental samples under the light microscope reveals a wide range in algal morphology and size (Figure 3.1 and Table 3.2), with variation from unicellular to colonial forms and (in fresh samples) differences in colour, presence or absence of an outer layer of mucilage, and motility.

Motility occurs both on solid surfaces (benthic algae) and within the water column (planktonic forms) and involves both active (mucilage extrusion, cilia and flagella) and inactive (buoyancy mechanisms) processes. Planktonic diatoms are non-motile, depending on water movement to maintain their position within the water column. Within the eukaryote algae, three groups – the euglenoids, dinoflagellates, and cryptomonads are entirely unicellular and are actively motile by flagella.

Algae are present in all freshwater environments including lotic and lentic systems, plus snowfields, aerosols, and a range of extreme aquatic situations (Section 2.15). Within lotic and lentic systems, certain algal groups (euglenoids, dinoflagellates, cryptomonads, and chrysophytes) show a preference for planktonic conditions, while others (blue-green algae, green algae, and diatoms) are equally planktonic or benthic. Although the great majority of freshwater algal species have a widespread geographic distribution (cosmopolitan), there are some species of chrysophytes, green algae, red algae, and diatoms (Figure 2.8) which are restricted (endemic) to certain geographic regions or particular water bodies. Some freshwater species are also able to survive in brackish (partly saline) water, which highlights the freshwater or marine affinities of the different major groups.

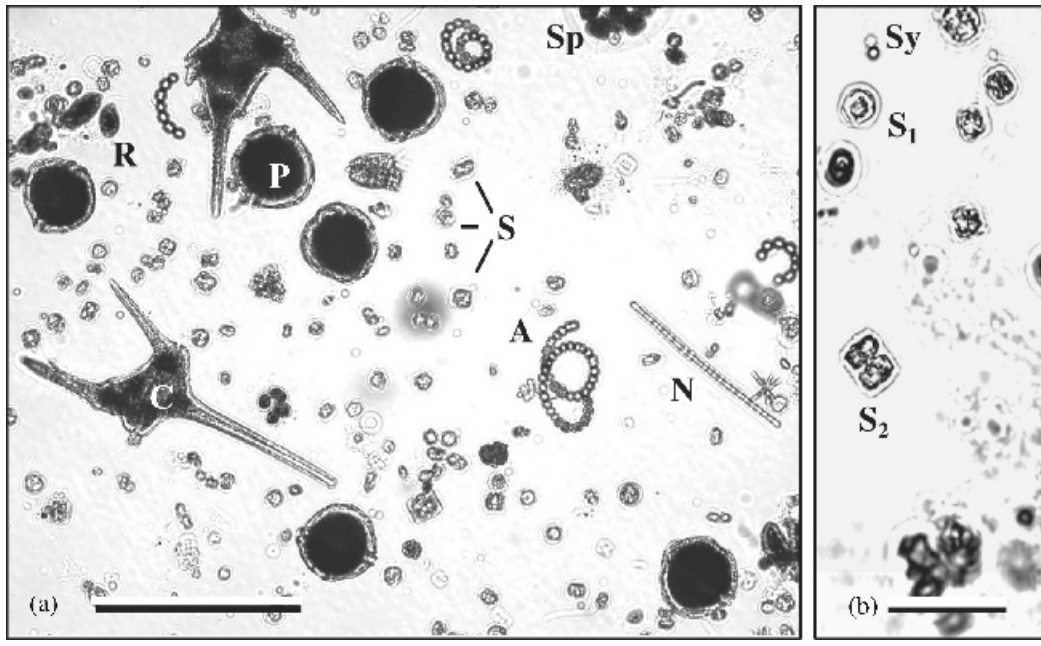

Figure 3.1 Taxonomic diversity in a mixed phytoplankton sample (a) low power view, showing blue-green algae (*Anabaena*, A; *Aphanizomenon*, N), green algae (*Sphaerocystis*, Sp), dinoflagellates (*Ceratium*, C; *Peridinium*, P), cryptomonads (*Cryptomonas*, R) and diatoms (*Stephanodiscus*, S) (scale–100 μm); (b) high power view, showing details of some of the smaller phytoplankton including *Stephanodiscus* in face (S1) and side view (S2-pair of cells), and the unicellular blue-green alga *Synechococcus* (Sy, pair of cells) (scale–25 μm). The cells have been fixed in iodine in preparation for cell/colony counts (see Table 3.2). In these fields of view, cell size (greatest axial dimension) ranges from ~180 μm (*Ceratium*) to ~2 μm (*Synechococcus*). Cell/colony shape ranges from spherical (*Peridinium*) to oval (*Cryptomonas*) to the extended forms of *Ceratium* (unicell) and *Aphanizomenon* (colony)

Table 3.2 Estimating major algal species populations in a mixed phytoplankton sample as organism counts and biovolumes (unpublished data from Andrew Dean, with permission)

Species	Count (cells or colonies ml^{-1})	% total phytoplankton count	Unit species biovolume (μm^3)	Total species biovolume × 10^5 (μm^3 ml^{-1})	% total phytoplankton biovolume
Stephanodiscus minutula	12000	78	380	46	13
Cryptomonas ovata	775	5	1050	8.1	2
Rhodomonas minuta	680	4	145	1.0	<1
Anabaena flos-aquae[*]	120	1	2165	2.6	<1
Synechococcus aeruginosa	30	<1	20	0.005	<1
Aphanizomenon flos-aquae[*]	480	3	1520	7.3	2
Ceratium hirundinella	30	<1	40,000	12	4
Peridinium cinctum	578	4	48,000	280	77

*Colonial species.

Phytoplankton population determinations are from the sample illustrated in Figure 3.1, and are presented as direct count and biovolumes of particular species:

- ***unit biovolume***: volume of a single cell or colony, estimated from linear measurements;
- ***total biovolume***: volume of the entire population of a particular species, determined as the product of until biovolume and cell count;
- ***percentage contributions*** of particular algae (e.g., *Stephanodiscus, Peridinium*) to the total phytoplankton count and total phytoplankton biovolume (shaded columns) differ markedly in relation to unit (cell or colony) size.

Table 3.3 Major divisions of freshwater algae: biochemical and cytological characteristics (data from Lee, 1997, Van den Hoek *et al.*, 1995, and Wehr and Sheath 2003)

Algal division (class)	Pigmentation*			Starch-like reserve	External covering	Chloroplast Fine-structure		Flagella (vegetative cells and gametes)
	Chlorophylls	Carotenes	Phycobilins			Outer membranes	Thylakoid groups	
1. Blue-green algae ***Cyanophyta***	**a**	β	+	Cyano-phycean starch$^{\alpha}$	Peptidoglycan matrices or walls	0	0	0
2. Green algae **Chlorophyta**	**a,b**	α,β,γ		True starch$^{\alpha}$	Cellulose walls, scales	2	2–6	0–many
3. Euglenoids ***Euglenophyta***	**a,b**	β,γ		Paramylon$^{\beta}$	Protein pellicle	3	3	1–2 emergent
4. Yellow-green algae: **Eustigmatophyta Raphidiophyta Tribophyta**	**a,c**	α,β		Chryso-laminarin$^{\beta}$	Cellulose in some	4	3	2 unequal (heterokont)
5. Dinoflagellates ***Dinophyta***	**a,c_2**	β		True starch$^{\alpha}$	Cellulose theca (or naked)	3	3	2 unequal (heterokont)
6. Cryptomonads ***Cryptophyta***	**a,c_2**	α,β	+	True starch$^{\alpha}$	Cellulose periplast	4	2	2 equal
7. Chrysophytes† ***Chrysophyta***	**a,c_1, c_2,c_3,**	α,β,ε		Chryso-laminarin$^{\beta}$	Pectin, plus minerals and silica	4	3	2 unequal (heterokont)
8. Diatoms† ***Bacillariophyta***	**a,c_1,c_2,c_3**	β,ε		Chryso-laminarin$^{\beta}$	Opaline silica frustule	4	3	1, reproductive cells only
9. Red algae ***Rhodophyta***	**a,d**	α,β	+	Floridean starch$^{\alpha}$	Walls with galactose polymer matrix	2	0	0
10. Brown algae† ***Phaeophyta***	**a,c_1,c_2,c_3**	β,ε		Laminarin$^{\beta}$	Walls with alginate matrix	4	3	2 unequal (heterokont) reproductive cells only

* Major pigments are shown in bold type Starch-like reserves $^{\alpha}$:$\alpha-1,4$ glucan $^{\beta}$:$\beta-1,3$ glucan

† Chrysophytes, diatoms and brown algae are sometimes grouped as classes within the phylum Heterokontophyta.

Major algal groups: biodiversity in freshwater and marine environments

Comparison of marine and freshwater systems The different divisions of freshwater algae differ in their relation to freshwater and marine environments. The degree of adaptation and evolution within these separate aquatic systems is indicated by the relative abundance and extent of species diversity that occurs for particular groups. None of the major algal divisions listed in Table 3.1 is exclusively freshwater, but certain groups exhibit greater abundance and diversity in freshwater systems – including blue-green and green algae. Other groups such as diatoms and chrysophytes are well-represented in both situations, while dinoflagellates, red algae, and brown algae show greater biodiversity in marine systems. Red algae and brown algae, in particular, have relatively few freshwater representatives.

Biodiversity in freshwater systems Biodiversity within the major algal divisions in freshwater systems is indicated by the range of habitats that are colonized and by the diversity of genetic, physiological, biochemical, and structural characteristics that occur within the group. The number of genera within divisions provides an index of phenotypic biodiversity and in the freshwater algae of North America (a well-characterized group occurring over a wide geographic area) ranges from >300 (Chlorophyta) to <5 (Phaeophyta) (Table 3.1).

3.1.2 Biochemical and cytological characteristics (Table 3.3)

Although biochemical and cytological features are important markers in distinguishing different algal groups (e.g., Descy *et al.*, 2000), they have limited use as aids to the identification of field samples since they typically require detailed laboratory (chemical and microscopical) analysis.

Biochemical features

Major biochemical features include pigmentation, storage products, external covering (cell wall) composition, and identity of osmotically-active low MW organic solutes (Table 4.5).

Pigmentation The pigmentation of algae is derived from three main groups of molecules – chlorophylls, carotenoids, and phycobilins (Van den Hoek *et al.*, 1995). The predominantly green colour of these organisms (from chlorophyll) is frequently modified by the presence of the other pigments. The golden-brown colour of diatoms, for example, arises due to the masking of chlorophyll by the accessory pigment fucoxanthin and blue-green algae have a bluish tinge due to phycocyanin.

Algal pigments are localized within the algal cell in association with the photosynthetic or thylakoid membranes. Chlorophylls are composed of a porphyrin ring system with a central magnesium atom, and occur as four main types – chlorophyll-a, -b, -c (c_1, c_2, and c_3) and -d. Chlorophyll-a (Figure 4.10) occurs in all photosynthetic algae as the primary photosynthetic pigment (the light receptor of photosystem I of the light reaction) and varies from 0.3–3 percent of algal dry weight. The other chlorophylls function as accessory pigments and have a limited but distinctive distribution within the different algal groups (Table 3.3).

Carotenoids are long-chain molecules that can be divided into two main groups: carotenes – oxygen-free hydrocarbons, and xanthophylls, their oxygenated derivatives. Of the four carotenes present in algae, β-carotene, occurs in all the algal groups while α-, γ- and ε-carotenes have a more restricted occurrence (Table 3.3). Xanthophylls occur as a wide range of molecules, with approximately 30 different types being recognized and forming a distinctive pattern of distribution within the different algal groups.

Phycobilins are water-soluble red or blue pigments located on (blue-green algae, red algae) or inside (cryptophytes) the photosynthetic membranes. The pigment molecule or chromophore is a tetrapyrrole and occurs in a combination with non-pigmented protein (the apoprotein) to form the phycobiliprotein. The blue chromophore is phycocyanobilin and the red chromophore phycoerythrobilin.

Storage products Algae contain a range of high and low molecular weight carbohydrate storage

products. The high MW starch-like compounds are either α-1,4 linked or β-1,3 linked glucans, and are diagnostic for particular algal groups (Table 3.3). Low MW storage carbohydrates include the sugars sucrose (important reserve in green algae and euglenoids) and trehalose (blue-green algae), with various glycosides and polyols (e.g., mannitol) being important in red and brown algae respectively.

Protein reserves include cyanophycin, present in blue-green algae as an important storage product of fixed nitrogen (Figure 5.15). Lipid reserves are also widely present in algae, and appear to be particularly prominent in dinoflagellates and diatoms. Polyphosphates are also a major storage product throughout the algal groups and are important in the luxury consumption of phosphate (Section 5.8).

Cell wall composition The outer covering of algae typically forms a continuous discrete structure (cell wall), which is variously referred to as a pellicle (euglenoids), theca (dinoflagellates), periplast (cryptomonads), and frustule (diatoms). The pellicle and periplast occur within the plasmalemma, the rest are external to it.

In general, algal cell walls are composed of two constituents – a skeletal or fibrillar component plus an amorphous matrix. The most common skeletal component is cellulose (a polymer of 1,4 linked β-D-glucose), but other macromolecules – including pectin, peptidoglycan (mucopeptide), and protein – may also be involved (Table 3.3). Amorphous mucilaginous components are an important part of cell wall structure in red and brown algae, and form a separate (mucilage) layer in many algal groups. Diatoms are unique in having a cell wall made of amorphous, hydrated silica that is associated with proteins, polysaccharides, and lipids (Fischer *et al*., 1999). Details of cell wall formation in diatoms are given in Section 5.10.

Cytological characteristics

Cytological features are of fundamental importance in distinguishing the different algal groups.

The most fundamental division within the algal assemblage, into prokaryotes (blue-green algae) and eukaryotes (other groups), is based on cell structure – with blue-green algae lacking a cell nucleus and the distinctive cytoplasmic organelles typical of eukaryote cells (Figure 3.2). The various fine structural and metabolic features that separate prokaryotes from eukaryotes, and distinguish blue-greens from other (eukaryotic) algae are shown in Table 1.2. The relatively 'primitive' state of blue-green algae in no way detracts from their ecological and evolutionary success within the freshwater environment.

Within the eukaryotic algae, other important cytological features include the fine-structure of the chloroplast, the number and appearance of

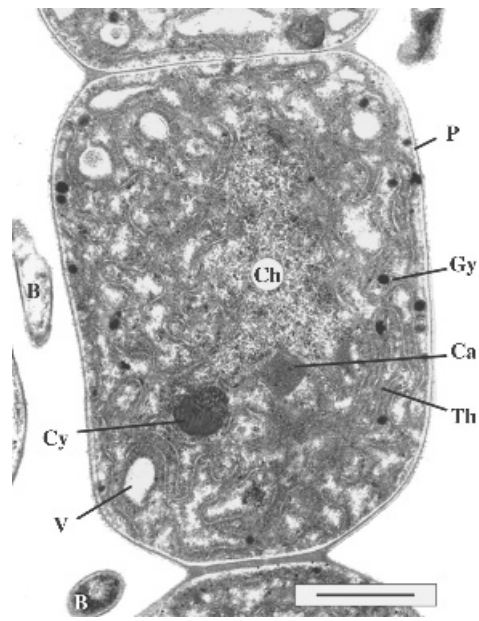

Figure 3.2 Prokaryotic identity of blue-green algae: Transmission electron micrograph of a section of *Anabaena*, showing:
Ch – central region of diffuse chromatin (no limiting membrane),
Cy – cyanophycin granule (nitrogen storage),
Ca – carboxysome (polyhedral body),
Gy – glycogen granule (cyanophycean starch),
Th – peripheral thylakoid membranes,
V – vacuole,
P – thin peptidoglycan cell wall
Epiphytic bacteria (**B**) are embedded in a layer of surface mucilage surrounding the *Anabaena* cell (not visible in this preparation) (scale – 1 μm)

flagella, and the fine structure of nuclear chromatin. Chloroplasts differ in terms of the number of outer membranes (two envelope membranes plus additional endoplasmic reticular membranes) and the grouping of thylakoids – ranging from single membranes (not grouped) to aggregates of up to six (Table 3.3). Considerable diversity occurs in relation to flagellar structure. Several groups of algae (Table 3.3) are united by the presence of unequal (heterokont) flagella, with a long forward-directed tinsel flagellum and a backward-directed smooth flagellum. The fine structure of nuclear chromatin is diagnostic for one particular group of algae, the dinoflagellates, which have distinctive permanently-condensed chromosomes (Figure 3.23). The reader is referred to standard texts on phycology (e.g., Van den Hoek *et al*., 1995) for a comprehensive review of algal cytology.

3.1.3 General summary of the different groups

General features of the different algal groups, with links to their reference in subsequent chapters in the book, are given below.

Blue-green algae (Cyanophyta)

Also referred to as cyanobacteria, this is the only prokaryote group of algae. *Cyanophyta* are important constituents of periphyton and phytoplankton communities, where they are present both as unicellular (picoplankton) and colonial forms. Blue-green algae are thought to have evolved during the early Precambrian era, when they were exposed to a reducing atmosphere and high levels of irradiation (particularly UV). During their long existence, they have colonized nearly all freshwater, marine, and terrestrial habitats, including such extreme environments as hotsprings (up to 70°C), hypersaline lakes, high arctic and alpine lakes, and hot and cold deserts. The general success of blue-green algae in aquatic environments has been attributed to the following

- Efficient light harvesting mechanisms, with ability to adjust to spectral differences by variations in accessory pigments.
- Continued photosynthesis at low concentrations of CO_2 and high pH. These conditions are particularly apparent during bloom formation in eutrophic waters, which eukaryotic algae are not able to tolerate.
- Resistance to damaging radiation, by producing a range of compounds that act as photoprotectants by absorbing short-wavelengths (Section 4.9.3).
- Temperature adaptations – different species can grow in extreme hot or cold environments. In temperate and tropical lakes, blue-green algae are able to maintain growth rates at high summer temperatures where other algae show temperature inhibition.
- Buoyancy mechanism for positioning in the water column. This energetically-efficient process allows blue-green algae to carry out diurnal migration within the water column between surface waters (high light and predation) and lower waters (high phosphate and nitrate concentrations, avoidance of photoinhibition).
- Efficiency in nutrient uptake. Blue-green algae show a whole range of features related to nutrient uptake:
  - they are able to dominate both oligotrophic (as picopoplankton) and eutrophic (large colonial greens) environments; in eutrophic conditions, dense algal blooms out-compete eutrophic algae;
  - some blue-greens can fix gaseous nitrogen, allowing them to grow at low N/P ratios;
  - blue-greens produce siderophores under conditions of iron stress, allowing them to scavenge Fe(III) at limiting environmental levels;
  - blue-greens do not require exogenous sources for vitamin requirements.
- They have chemical (toxins) and physical (large colonies) mechanisms to resist removal by filter-feeding zooplankton.

- They have evolved specialized symbiotic bacterial associations, which are particularly important in relation to heterocyst functions.

Some of these characteristics have specific importance in the ability of blue-green algae to form dominant blooms in eutrophic waters, and are discussed further in Section 10.6.

Green algae (Chlorophyta)

These comprise the most diverse class of algae. They are important as both planktonic and attached organisms, with morphologies ranging from simple unicells to complex colonial forms. Some macroscopic members of the green algae (*Chara*, *Cladophora*) have a higher-plant like appearance and are important members of the periphyton. Certain groups within the green algae have specific ecological requirements, including flagellated chlorophytes (nutrient-rich standing waters) and coccoid unicells and colonies (high light, nutrient, and temperature, standing waters). Desmids are more common in ponds and ditches that have low conductance and low to moderate nutrient levels.

Euglenoids (Euglenophyta)

In terms of general abundance and species diversity, this is a relatively minor group of freshwater algae. These organisms may become particularly abundant, however, in the phytoplankton of standing waters rich in nutrients where they may be readily identified under the microscope by their unicellular spindle-shaped morphology and active motility.

Yellow-green algae (Eustigmatophyta, Raphidiophyta and Tribophyta)

Yellow-green algae are a diverse group of freshwater organisms, occurring in a wide range of habitats, and with a large number of reported genera (at least 90 in North America). Although widespread, these algae are not very prominent members of the freshwater flora, since many are small coccoid forms that occur only in small numbers.

Dinoflagellates (Dinophyta)

Dinoflagellates are often relatively minor components of of lake phytoplankton in relation to species counts. The large size of these organisms, however, means that their biovolume contribution to phytoplankton is much more significant – and they often make a major contribution to overall algal biomass (Figure 3.1).

Along with colonial blue-greens, these algae are prime examples of *K*-selected organisms (Section 3.10.2), and tend to form blooms in temperate lakes towards the end of summer when stratification is stable and epilimnion nutrients are in decline. Under such conditions, these highly motile organisms are able to migrate into the nutrient-rich hypolimnion to obtain their supplies of nitrate and phosphate (Figure 3.22).

Cryptomonads (Cryptophyta)

These unicellular organisms are particularly diverse in temperate regions, where they typically occur as phytoplankton in lakes and ponds. The short cell cycle and ability for active growth of cryptomonads means that they are particularly common during the clear-water phase of the annual cycle in temperate lakes, along with other *r*-selected organisms.

Chrysophytes (Chrysophyta)

The majority of chrysophytes are unicellular or simple colonial forms, and are typically planktonic although attached forms do exist. They are typically associated with standing waters which have low to moderate nutrients, alkalinity, and conductance (pH slightly acid to neutral).

Diatoms (Bacillariophyta)

Diatoms initially appeared in the fossil record about 185 million years ago, and have been abundant in surface waters for the past 115–110 million years. The major biomass of diatoms occurs in marine systems, where they are the most important microbial primary producers and the major contributors to global carbon fixation.

These micro-algae are also of major importance in freshwaters, where they occur as both planktonic and attached (biofilm) organisms in lakes, streams, and estuaries. Diatoms may be unicellular or colonial, and constitute one of the largest classes of freshwater micro-algae (Fischer *et al*., 1999). The cell wall (frustule) is unique among living organisms in being almost entirely composed of silica (Section 5.10), and these organisms are well known for the intriguing species-specific design and ornamentation of this rigid and very dense structure.

Diatoms are divided into two main groups – centric diatoms (radial symmetry, typically planktonic) and pennate (bilateral symmetry, many benthic species), with further taxonomic subdivisions in relation to frustule morphology (Lee, 1997). The taxonomic composition of diatom communities provides a useful indicator of environmental characteristics, and has been widely used to monitor changes in the salinity (Section 2.15.3), nutrient status (Section 10.3), acidity (Section 3.7.3), and general hydrological disturbance of lakes.

Red algae (Rhodophyta)

Red algae are predominantly marine in distribution, with only 3 per cent of over 5000 species worldwide occurring in true freshwater habitats (Wehr and Sheath, 2003). Although freshwater red algae (such as the large filamentous alga *Batrachospermum*) are largely found in streams and rivers, these organisms may also occur as marine invaders of lakes and brackish environments.

Certain freshwater red algae in the littoral zones of the Great lakes Basin (USA), 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.

Brown algae (Phaeophyta)

As with red algae, brown algae are almost entirely marine – with less than 1 per cent 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.

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, and they lack the complex macro-morphology typical of the brown seaweeds.

3.2 Algal species: taxonomy and intraspecific variation

Although algal species are typically well-defined units, based principally on morphological criteria, there is often a wide variation within these taxa in terms of morphology, size and shape, molecular genetics, biochemistry, and microscopical analysis. This intra-speciic variation is important for two main reasons.

- *Algal taxonomy.* Intraspecific variation has relevance to the definition of individual species, and is also used in some cases to define subspecific taxonomic units (subspecies or strains).

- *Biodiversity.* Although biodiversity is normally considered in relation to the variety of species (see Section 3.13), variation within species is also important as a major source of diversity within the environment.

3.2.1 Taxonomy of algal species

Definition of algal species on morphological criteria may present problems where the criteria are not well-defined or where there is intra-specific variation. Morphological polymorphism and geographical variation have been noted for species of the blue-green algae *Microcystis* (Komarek, 1991) and *Anabaena* (Baker, 1991, 1992). Individual species

often appear to be a continuous transitional sequence or 'cline' of strains, with a gradation of taxonomic characteristics (Baker, 1991). Variation in environmental factors may also create a wide variation in cell and filament morphology (Gorham *et al*., 1964; Doers and Parker, 1988; Zirbel *et al*., 2000).

3.2.2 Chemical diversity within species – enzyme analysis, molecular groups, and elemental composition

In addition to key biochemical differences between the major algal groups (Table 3.3), variations in chemical composition also occur at genus to subspecies level. Chemical and biochemical analysis of intra-specific variation can be carried out in a variety of ways, providing information on such diverse aspects as enzyme composition, lipid composition (Hayakawa *et al*., 2002), isotope ratios, molecular groups, and elemental composition. All of these approaches combine to emphasize chemical diversity at the species and intra-specific level.

Enzyme analysis

In the blue-green algae, electrophoretic analysis of polymorphic enzymes (allozymes) has revealed distinct patterns of occurrence within *Anabaena* (Stulp and Stam, 1984) and *Microcystis* (Kato *et al*., 1991). Allozyme analysis of blue-green algae is relatively simple since individual organisms typically have a single allozyme for each polymorphic enzyme, compared to a pair of allozymes (cytosol and plastid) in higher (eukaryote) algae. The studies carried out on *Microcystis* (Kato *et al*., 1991) involved analysis of four polymorphic enzymes and demonstrated clear and consistent allozyme combinations for two morphologically-defined species, but marked heterogeneity in a third. The latter showed well-defined differences between strains, indicating clear genotypic variation at subspecies level.

Stable carbon and nitrogen isotope ratios

Isotope ratios within cells provide a very sensitive index of biochemical function, depending on both extrinsic (environmental) and intrinsic (metabolic) characteristics (Yoshii *et al*., 1999).

Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) ratios of phytoplankton vary spatially and temporally according to environmental conditions. The (δ^{13}C) value of microalgae is determined largely by the organism's metabolism and growth rate, with enhancement of the phytoplankton (δ^{13}C) level in temperate and tropical climates when growth is high. The δ^{15}N of phytoplankton is closely correlated with the form of nitrogen used and with algal growth rate. Within the ecosystem as a whole, δ^{13}C and δ^{15}N values differ markedly between biota, and show a close correlation with trophic status.

Molecular groups and elemental composition

Information on intraspecific biodiversity in relation to the chemistry of individual cells can only be obtained by microscopical techniques. These include the use of chemiluminescence to determine variation in nitrate concentrations (Villareal and Lipschultz, 1995), analysis of molecular species using Fourier-transform infra-red (FTIR) analysis (Sigee *et al*., 2002) and the determination of elemental composition using proton-probe analysis (Brook *et al*., 1988) and X-ray microanalysis (Sigee *et al*., 1998). All of these approaches indicate wide variability in the chemical composition of cells within particular species in environmental samples.

Infrared analysis FTIR analysis of individual algal cells gives information on the vibrational states of molecular groups present within major macromolecules (Sigee *et al*., 2002). Analysis of single colonies of the green algal *Pediastrum*, for example, gave very clear spectra with 12 distinct bands corresponding to a range of molecular groups (Figure 3.3). Significant differences between spectra occurred within a single environmental

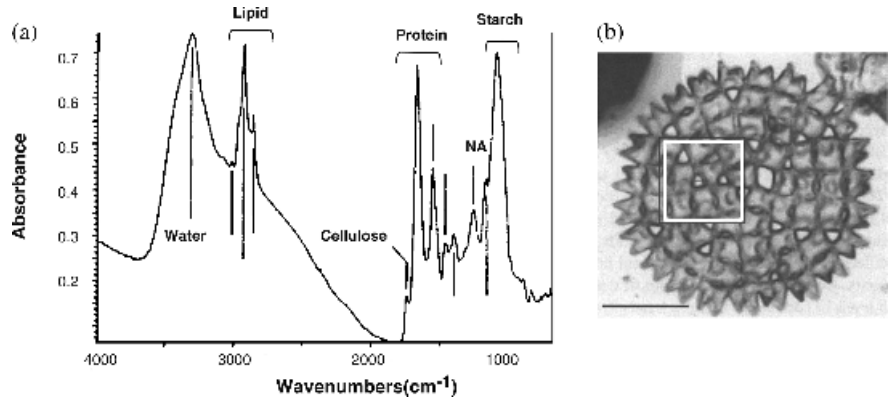

Figure 3.3 Molecular composition of algal cells – FTIR spectrum of the colonial green alga *Pediastrum* (figure taken from Sigee *et al*., 2002 and reproduced with permission from the European Journal of Phycology). (a) Fourier Transform Infra-red (FTIR) spectrum from colony (b). Some of the major bands, with vibrational modes, are: **Water**: ν(O–H) stretching **Lipid**: ($\nu_{as}(CH_2)$ stretching of methylene **Protein**: amide I & II **Nucleic acid** (**NA**) ν_{as}(>P=O) stretching of phosphodiesters **Starch**: ν(C–O) stretching (b) Single colony of *Pediastrum* – the spectrum was taken from the area marked by a white square (scale = 25 μm)

micro-population of this organism, demonstrating intra-specific heterogeneity at the cellular level.

X-ray microanalysis XRMA provides information on the elemental composition of biological micro-samples (Sigee *et al*., 1993), such as single cells and colonies. A detailed account of the application of this technique to cells of the dinoflagellate *Ceratium* (Sigee *et al*., 1999a) is given in Section 5.1.4 (see Figure 5.2). Significant differences in the elemental composition of *Ceratium* cells were noted both within and between micro-populations (in mixed phytoplankton samples) isolated from the lake environment.

Similar results have been obtained with other freshwater algae, including the demonstration of distinct cell sub-populations of *Anabaena* and *Microcystis* (Sigee and Levado, 2000) in relation to silicon content.

3.3 Molecular analysis

Extraction and sequencing of DNA has been carried out on laboratory and environmental samples of a wide range of freshwater algae. In the case of prokaryote (blue-green algae) organisms this has involved analysis of nucleoid DNA (Kaneko *et al*., 1996), while in eukaryote algae gene sequences present in nuclear, mitochondrial (Chesnick *et al*., 1996; Laflamme and Lee, 2003), and chloroplast (Reith, 1999) DNA have been examined.

DNA studies on freshwater (and marine) algae have provided novel approaches to:

- understanding phylogenetic relationships,
- molecular characterization and identification of major taxonomic groups, species and subspecies in environmental samples,
- identification and characterization of gene activity relevant to environmental and biotic interactions.

This volume is concerned particularly with the last two of these aspects, dealing respectively with the occurrence and activities of algae within the aquatic environment.

3.3.1 Molecular characterization and identification of algae

Reliable characterization of taxonomic groups is clearly important in environmental studies, since

the definition of particular units needs to be clear and to be established on a firm biological basis.

The use of molecular techniques has been particularly useful for blue-green algae, giving clearer resolution of species and sub-species (strains). The delineation of species within this group is currently based largely on morphological features such as the arrangement (e.g., filamentous, colonial, or single cells), size, and shape of cells. In many cases, however, closely related species have overlapping morphology and individual species show geographic or environmental variation. As a result, characterization and identifying species of blue-green algae in environmental samples can be confusing and difficult.

Molecular characterization of species or subspecies makes use of a particular DNA sequence to define the taxonomic unit. Use of this approach with a specific algal sample involves amplification of the particular sequence to produce enough DNA for analysis, followed by the analysis itself – using restriction fragment length polymorphism (RFLP) or direct sequencing. The choice of the DNA sequence to be amplified, and hence the appropriate PCR primer, is important for two reasons – prevention of amplification of contaminant DNA, and level of taxonomic resolution.

Avoidance of contamination

One of the potential problems with the PCR process is that non-target DNA may also be amplified in addition to target DNA. Amplification of bacterial DNA in blue-green algal cultures may be avoided by the use of bacteria-free (axenic) samples, or by designing blue-green algal-specific PCR primers that can discriminate between the two types of DNA. The latter may be achieved either by the use of well-characterized genes common to both organisms (e.g., 16s ribosomal RNA genes (rDNA)) where the gene sequence is different in target and contaminant situations, or by using gene sequences in the target organism that are not present in contaminants (e.g., genes that code for phycobilin pigments in blue-green algae).

Taxonomic resolution

Blue-green algae contain a number of well-characterized genes which are not present in bacteria and have potential for taxonomic differentiation. These include the genes encoding the main light-harvesting accessory proteins phycocyanin (*cpc*), allophycocyanin (*apc*) and phycoerythrin (*cpe*). The problem with using functional gene sequences for taxonomic purposes is that they are typically highly conserved and have sufficient sequence divergence for comparisons only at genus level and above. More variable gene sequences for use at species and sub-species level can be obtained by amplifying the non-coding DNA region between genes, referred to as the intergenic spacer (IGS) or internal transcribed spacer (ITS) sequences.

Detection of particular gene sequences in environmental samples can be carried out in two main ways – direct sequence analysis of the sample, or use of molecular probes (Table 3.4).

Direct sequence analysis Sequence analysis has been used to provide general information on

Table 3.4 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 and flanking regions	Bolch *et al*. (1996)
Flagellate nanoplankton	Small subunit 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 internal transcribed spacer-specific PCR assays for *Pfiesteria* (dinoflagellate)	Litaker *et al*. (2003)

species composition of poorly-characterized environmental samples and also to reveal variations on genotype composition within species.

Analysis of poorly-characterized samples. Molecular analysis has been particularly useful in the case of picoplankton, where traditional methods of identification are difficult due to the minute dimensions of these organisms. The use of culture techniques as part of the identification process also present problems since many of these organisms have stringent nutritional requirements.

This approach was used to study the species composition of picoplankton in Lake Baikal (Russia), where a mixture of autotrophic and heterotrophic organisms become prominent in summer (Semenova and Kuznedelov, 1998). These authors carried out isolation, PCR amplification, sequencing, and phylogenetic analysis of the picoplankton DNA. Comparative analysis of rRNA sequences coding for the 5′-terminal region of 16s rRNA genes led to the construction of a phylogenetic tree from the combined data set. This indicated the presence of seven autotrophic (algal) species, 15 heterotrophic species of picoplankton plus some unidentied organisms. Named autotrophic species included five blue-green algae and two green algae, while the heterotrophs comprised Actinomycetes and a range of bacteria.

Genotypic variations within species. Molecular techniques are increasingly being used to study genotypic variations within species. Studies by Bolch *et al*. (1996), for example, used the intergenic spacer between genes encoding the β- and α-phycocyanin subunits to study genetic variation within 19 isolates of blue-green algae belonging to three morphologically-defined species (morphospecies). Restriction enzyme digestion of the amplification products revealed clear fragment patterns that placed the strains within three genetically-defined species – *Anabaena circinalis*, *Microcystis aeruginosa*, and *Nodularia spumigena*. Within each species, differences in the fragment pattern (restriction fragment length polymorphism, RFLP) revealed further subdivision (polymorphism) into genetic strains. *M. aeruginosa* was particularly polymorphic, while the other two species were less so. Denaturing gradient gel electrophoresis of sections of the internal transcribed spacer between rRNA genes (Janse *et al*., 2003) has also proved useful in detecting intrageneric and intraspecific variation in *Microcystis*.

Recent studies by Kurmayer and Kutzenberger (2003) have used real-time PCR to investigate the frequency of microcystin genes in natural populations of *Microcystis* sp. sampled from Lake Wannsee (Germany). Two DNA sequences were monitored – the *mcyB* gene (as an index of microcystin production) and the intergenic region within the phycocyanin (PC) operon (to quantify total population). Phytoplankton microsamples from the lake showed that *mcy* genotypes made up only a small proportion (1–38 per cent) of the total *Microcystis* population. The mean proportion of *mcy* genotypes was relatively stable over time, indicating that the straight *Microcystis* cell count could be directly used to infer the number of microcystin-producing cells.

Identification of algae – 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 (Table 3.4).

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 (Pomati *et al.*, 2000). 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 USA (Section 10.1.4). 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).

3.3.2 Investigation of gene function in freshwater algae

Molecular techniques have been used to sequence and characterize a wide selection of genes in freshwater algae. The genome of *Synechocystis* (blue-green alga), for example, has been completely sequenced (Kaneko *et al*., 1996) and analysis of open reading frames (ORFs) has lead to the identification of over 3000 putative genes. These include genes for biosynthesis (amino acids, cofactors), cell processes (e.g., chemotaxis, cell division), metabolism (energy processes, fatty acids), nucleic acid activities (synthesis, transcription, translation), and energy transduction (photosynthesis and respiration). In the eukaryote algae, *chlamydomonas* has proved a model organism for the molecular genetic dissection of photosynthesis (Dent *et al*., 2001).

Molecular responses to environmental change

Many examples of gene expression in algae involve a response to environmental factors (Table 3.5), and molecular biology has particular potential in exploring the ways in which environmental change mediates these effects. These studies have been particularly intensive in blue-green algae, where the comparatively small size of the prokaryote genome makes the genetic system of these organisms more amenable to study compared to eukaryotes. Molecular responses to environmental change are considered in relation to light modulation of algal activities, algal responses to limiting nutrients, environmental control of differentiation, and responses to stress.

Light modulation of genetic activity Light activation of algal genes involves the response of specific photoreceptors and a signal transduction process leading to either transcriptional control of gene activity or post-transcriptional control of the gene products (Reith, 1999; Johnson and Golden, 1999).

Light responsive genes are particularly important for optimizing photosynthesis at low light levels, and encode proteins involved in pigment synthesis, chlorophyll-a binding proteins, and phycobiliproteins. Induction of these genes by different wavelengths of light suggests a complex modulation of gene activity within the constantly changing light conditions of the aquatic environment. This is considered further in Section 4.6.2 in relation to photoadaptation.

Light activation of gene activity is also important in other biological activities (Table 3.5) such as induction of DNA repair processes (Section 4.9.1), general modulation of the diurnal clock (Section 4.10.3) and aspects of nitrogen metabolism, including nitrogen fixation (Section 5.6.4) and assimilation (Section 5.5.3). Other light-mediated activities include the synthesis of microcystin-related proteins and the synthesis of gas vesicle proteins, and are discussed below.

Light modulated synthesis of microcystin and microcystin-related protein. Microcystin is an important secondary metabolite that is released by the blue-green alga *Microcystis*, and is a major toxin associated with bloom formation (Section 10.7.3). Synthesis of microcystin is carried out by a group of proteins, the microcystin synthetase complex, which is encoded by a cluster of genes including *mcy*A, *mcy*B, and *mcy*D (see Figure 3.4 and Dittmann *et al*., 2001). The microcystin gene cluster

Table 3.5 Some examples of environmentally-controlled genetic activity in algae, summarized in relation to four interrelated aspects – the response to light, uptake of nutrients, differentiation, and stress responses

Gene activity	Algae	Induction
Light-responsive genes	Blue-green algae	Light
Synthesis of photosynthetic proteins and pigments[a]		
DNA repair – photoreactivation of dimers[b]	*Chlamydomonas* (green alga)	UV-A and blue light
Synthesis of microcystin-related proteins[c]	*Microcystis* (blue-green)	Blue light
Gas vesicle formation[d]	*Pseudanabaena* (blue-green)	Low light intensities
Modulation of diurnal clock[e]	Blue-green algae	Light
Nutrient-related gene activity		
Nitrogen fixation: synthesis of nitrogenase and cyanophycin synthetase[f]	Blue-green algae	Low nitrogen levels
Induction of nitrate reductase[g]	General	Light, nitrate and carbohydrate concentration
Silicic acid transporter (SIT) proteins[h]	Diatoms	Silicic acid concentrations Factors controlling the cell cycle
Cell wall protein formation (frustulins)[i]	Diatoms	Factors controlling the cell cycle
Synthesis of flavodoxin[j]	*Synechococcus* and other blue-green algae	Iron depletion
Gene control of differentiation		
Heterocyst differentiation[k]	*Calothrix* (blue-green)	Red and green light
Differentiation of hormogonia[l]	*Nostoc* (blue-green alga)	Light intensity, culture medium
Conversion of vegetative cells to gametes[m,n]	*Chlamydomonas, Closterium* (green algae)	Two-stage process promoted by nitrogen starvation, followed by blue light signal
Response to environmental stress		
Switch to alternative transcription (σ) factors[o]	*Synechocystis* (blue-green alga)	Salt stress, heat shock, acute nutrient starvation

References: [a]Golden (1995); [b]Petersen and Small (2001); [c]Dittmann *et al*. (2001); [d]Damerval *et al*. (1991); [e]Johnson and Golden (1999); [f]Schneegurt *et al*. (2000); [g]Heldt (1997); [h]Martin-Jézéquel *et al*. (2000); [i]Fischer *et al*. (1999); [j]Porta *et al*. (2003); [k]Campbell *et al*. (1993); [l]Waaland *et al*. (1971); [m]Musgrave (1993); [n]Fukumoto *et al*. (2003); [o]Huckauf *et al*., 2000

also contains a gene that encodes an ABC transporter protein, suggesting that the microcystin is actively secreted by the algal cell. Cells that are active in microcystin synthesis also produce two microcystin-related proteins, MrpA and MrpB, encoded by genes *mrp*A and *mrp*B. Synthesis of these proteins:

- is closely coupled to microcystin synthesis – Mcy mutants fail to synthesize both microcystin and the microcystin-related proteins;
- is modulated by light – synthesis of MrpA is strongly promoted by blue light, and is inhibited by high levels of white light;
- is probably promoted by close proximity of other *Microcystis* cells (quorum sensing) – evidence for this comes from the close homology of MrpA with RhiA protein of *Rhizobium leguminosarum*, which is subject to quorum-sensing regulation, and has been shown to control the expression of a large number of genes – including peptide

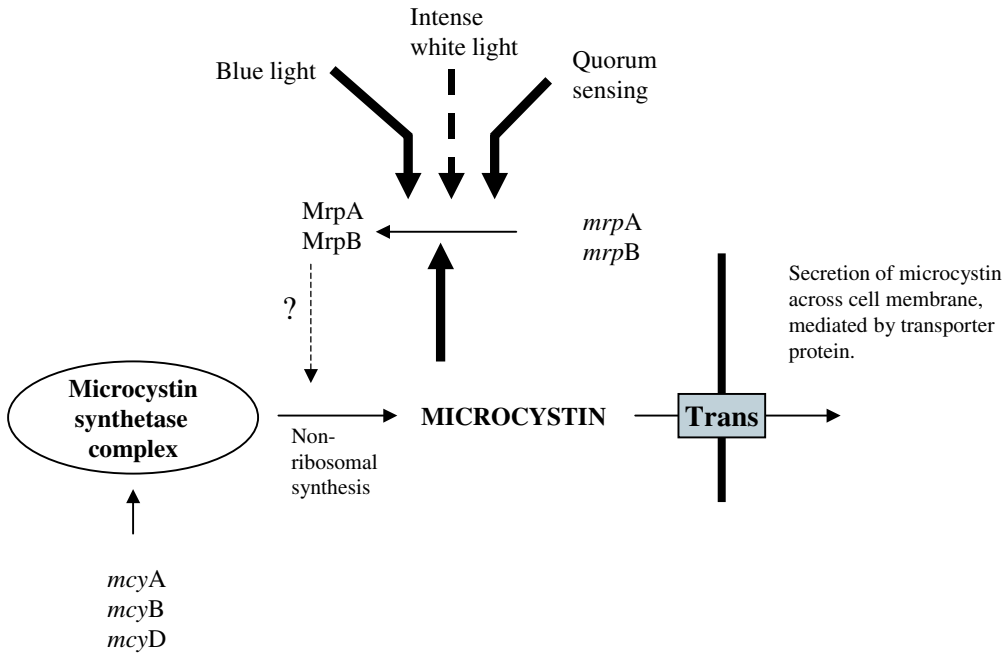

Figure 3.4. Hypothetical scheme to show possible feedback interactions between the synthesis of microcystin and microcystin-related proteins (Mrps): expression of Mrps is promoted (⟶) or inhibited (- - →) by external and internal factors (including microcystin synthesis) and may in turn modulate the synthesis of microcystin (based on Dittmann *et al*., 2001)

synthetase genes, exopolysaccharide genes and virulence factors.

The different responses of *mrp*AB transcript accumulation by mutant (no response) and wildtype (response) cells show that *mrp*AB transcription is microcystin-dependent, and that the response of the *mrp* genes to blue light is also microcystin dependent. Although the role of Mrp protein has yet to be clarified, the studies of Dittmann *et al*. (2001) suggest that it is part of a quorum sensing, light dependent system which controls the production and possibly the export of microcystin.

Induction of gas vesicle formation in blue-green algae. Formation of gas vesicles in these organisms is important in their migration and depth regulation within the water column (Section 3.8), and may be light-regulated. The induction of gas vesicle protein (GvpA) in the blue-green algae *Pseudanabaena* (Damerval *et al*., 1991) is particularly interesting since it is promoted by low but not high light intensity. Algae cultured at low light (5 μmol photons m^{-2} s^{-1}) produced 0.4kb transcripts of the gvpA gene, which were lost within 12 hours after transfer of the cells to high light (50 μmol m^{-2} s^{-1}) conditions. Transcription was resumed on transfer back to a low-light regime. The potential role of light-induced GvpA production in the regulation of buoyancy is summarized in Figure 3.20.

Algal responses to limiting nutrients The induction of genes which promote inorganic nutrient uptake is a key aspect in the ability of algae to respond to fluctuations in nutrient availability. This is seen, for example, in the induction of nitrate reductase by threshold levels of nitrate (Section 5.5.2) and by the Si-induction of silicic acid transporter (*SIT*) genes (Martin-Jézéquel *et al*., 2000;

Section 5.10.2). Inhibition of nitrogenase synthesis by threshold nitrate levels (Section 5.6) represents the reverse situation of nutrient suppression of gene activity.

Exposure to conditions in which nutrients are limiting may also trigger a molecular response. Limitation in Fe availability, for example, leads to the replacement (in both eukaryote and blue-green algae) of the electron transfer catalyst ferredoxin by the iron-free but functionally-equivalent protein flavodoxin (Porta *et al*., 2003). Environmental populations of planktonic micro-algae exhibit flavodoxin accumulation as a biochemical marker for conditions of iron depletion. In blue-green algae, flavodoxin is encoded by the gene *isiB*, which combines with *isiA* to form an operon under the control of the Fe-dependent repressor *Fur*. Decrease in external Fe concentration to sub-critical levels inactivates *Fur* and leads to expression of the *isiAB* operon. This operon is widely spread throughout blue-green algae, and in most cases is only responsive to the environmental parameter of Fe deficiency. The occurrence of flavodoxin accumulation and *isiAB* transcription as diagnostic markers for iron deficiency has led to the construction of a blue-green algal (*Synechococcus* sp. PCC 7942) reporter strain for freshwater environments (Porta *et al*., 2003). This has *luxAB* reporter genes fused to the *isiAB* promoter, and provides a means of assessing low Fe availability, as perceived by the test organism.

Environmental control of differentiation Environmental factors such as the concentration of inorganic and organic nutrients may act on their own or in combination with light to control differentiation processes. These include the differentiation of heterocysts and hormogonia in blue-green algae, and the conversion of vegetative to gamete cells in *Chlamydomonas* (Musgrave, 1993) The transition to gamete cells in this organism can be mediated by transference of a culture to a low-nitrate medium, and is an interesting example of differentiation within a haploid organism. Sexual differentiation in the green alga *Closterium*, is also controlled by the environmental nutrient and light regime (Fukumoto *et al*., 2003). In this organism, transcription of a sexual cell division-inducing pheromone occurs in mating type minus (mt-) cells only, and is suppressed by continuous dark and supplementation of a nitrogen source.

Control of transcription in prokaryotes is typically modulated by transcription proteins or sigma (σ) factors (Sections 3.4, 1.4.1, 4.10, 6.6.4). Sigma factors have also been identified in some eukaryote algae. In the red alga *Cyanidium*, for example, nuclear-encoded σ-factors have been shown to transcribe subsets of plastid genes for photosynthesis during chloroplast development (Liu *et al*., 1995).

Responses to stress Stress factors include all those environmental changes which have an adverse effect on biological function, and have been considered previously in relation to ecosystems (Section 1.6). They also operate at the cellular and molecular level, and freshwater algae are able to adapt to sudden but moderate changes such as salt stress, heat shock, acute nutrient starvation, and high light levels (Section 4.9) by a range of adaptive molecular processes.

Processes of microorganism acclimation to environmental stress are mainly regulated at the level of transcriptional activation or repression, where the change is acting on single genes. An example of this is the light induction of the DNA repair enzyme photolyase as a response to damaging light levels (Figure 4.23).

In addition to stress responses involving single genes, groups of genes may also be regulated in stressed cells by the activity of alternative sigma factors which replace the primary sigma factors that normally operate under favourable conditions (Huckauf *et al*., 2000). In blue-green algae, these alternative sigma factors have been identified mainly in group 3 of the σ^{70} family. The role of alternative sigma factors in stress responses has been studied in the single-celled blue-green alga *Synechocystis* sp. by examining the effect of mutagenesis on specific sigma genes (Huckauf *et al*., 2000). These studies identified three gene products (group 3 σ factors) as important in stress responses – regulatory protein RsbU (important in regenerating growth after nitrogen- and sulphur-starvation), SigF protein (required for the induction of salt stress proteins), and SigH (induction of heat-shock proteins).

B. SIZE, SHAPE, AND SURFACE MUCILAGE

3.4 Phytoplankton size and shape

Freshwater phytoplankton, composed of photosynthetic bacteria and algae, shows considerable variation in the size and shape of individual organisms (cells or colonies), as illustrated in Figure 3.1. Phytoplankton dimensions are important in relation to enumeration and assessment of biovolume, designation of size category (picoplankton to macroplankton), and biological activity.

3.4.1 Cell counts and biovolume

Enumeration of species within mixed phytoplankton samples is normally carried out after fixing the sample in iodine, allowing the cells and colonies to settle in a tall container, then transferring a concentrated aliquot of suspension to a counting chamber (e.g., Sedgwick rafter slide). Counts of individual species are usually made using a light microscope with inverted objectives, ensuring that adequate numbers of organisms are counted to achieve statistical validity.

Although counts of individual species provide useful information of the total numbers of organisms present, and can be used to study aspects such as population change, fungal infection rate, and biodiversity – they give little indication of contribution to the total phytoplankton biomass. In the phytoplankton sample shown in Figure 3.1, for example, the small diatom *Stephanodiscus minutula* was the most common alga present. On a direct count basis (Table 3.2), this organism accounted for 78 per cent of the total phytoplankton population, compared with only 4 per cent for the dinoflagellate *Peridinium*. If the volume (biovolume) of individual cells/colonies is taken into account, however, *Stephanodiscus* now records only 13 per cent of the total phytoplankton population, while *Peridinium* is 77 per cent. These biovolume determinations give a much better estimate of relative biomass and provide a baseline in terms of contribution to overall productivity.

3.4.2 From picoplankton to macroplankton

Freshwater phytoplankton can be divided into four main groups (picoplankton to macroplankton, Table 3.6) on the basis of size. The range of linear size seen in phototrophic planktonic organisms is from photosynthetic bacteria (0.2μm cell diameter) to large colonial algae such as *Microcystis* (2000μm colony diameter), and represents variation over four orders of magnitude. The smallest planktonic blue-green alga, *Prochlorococcus marinus*, is found in marine rather than freshwater systems and has a diameter ranging from 0.5 to 0.7μm, reaching concentrations of up to 4×10^5 cells ml^{-1} (Palinska *et al*., 2000).

Table 3.6 Size range of phytoplankton

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

*Biovolume values are based on a sphere (volume = $^4/_3 \prod r^3$)

Although freshwater planktonic algae might be regarded simply as a relatively uniform group of microscopic organisms, the linear size range of algae encountered in the lake environment is equivalent to the range of plant sizes seen in terrestrial environments such as a tropical rain forest. If biovolumes are considered, the size range from picoplankton to macroplankton extends over nine orders of magnitude (Table 3.6), and *Microcystis* is greater than the bacterial cell by a factor of 10^{12}.

The smallest organisms (picoplankton and nanoplankton – see Figure 3.5) have often been difficult in the past to detect and enumerate by conventional microscopy, and their role in the freshwater environment has been underestimated. The more recent use of fluorescent microscopy and the development of species-specific oligonucleotide probes (Caron *et al*., 1999) has improved this dramatically. A variety of micro-algae are now known to be widespread in the freshwater environment and in some cases to make a major contribution to the overall phytoplankton biomass. Studies by Happey-Wood (1988), for example, have shown that micro-chlorophytes account for more than 90 per cent of the algal cell count in some oligotrophic lakes in North Wales (UK), where they comprise more than 75 per cent of the algal biomass expressed as cell volumes. In other lakes such as Lake Baikal (Russia), the nanoplankton community is dominated by unicellular blue-green algae (Nagata *et al*., 1994) such as *Synechocystis limnetica*. In this oligotrophic lake, these organisms generate a major bloom in late summer (Figure 2.9) – accounting for about 60 per cent of the total annual primary production (Semovski *et al*., 2000).

3.4.3 Biological significance of size and shape

The major distinction between prokaryotic phytoplankton (blue-green algae, photosynthetic bacteria) and eukaryotic phytoplankton noted earlier has important implications for cell size, with most prokaryote cells having diameters $<5\,\mu m$. Some eukaryote planktonic organisms, such as heterotrophic nanoflagellates also approach this size range – with diameters typically $<10\,\mu m$ – but are ultimately limited by the need to accommodate a nucleus and various eukaryote organelles within the cell. In addition to size, the range of cell and colony shape is also remarkable, with many organisms having an elongate shape, further extended by spines or cellular processes (Figure 3.1).

The size and shape of phytoplankton has important implications for a range of biological functions, including physiological processes (surface exchange of materials, light absorption, ability for rapid growth), distribution in the water column (passive movement, sedimentation, motility) and resistance to ingestion by zooplankton.

Exchange of materials at the cell surface

The passive diffusion of solutes and dissolved gases across the surface of phytoplankton cells is a two-way process and is governed by Fick's Laws of Diffusion. For each solute, the rate of diffusion for the whole cell depends primarily on surface area, concentration gradient, and coefficient of molecular diffusion (see Section 3.7.1).

Cell size is an important aspect of this exchange process since the volume of the organism determines total metabolic demand (solute uptake) or excess (solute loss) – which drive the concentration gradient. With a particular concentration gradient and diffusion coefficient, the size-related surface area determines the total passive exchange that can take place. These two aspects of demand and exchange capacity are brought together in the surface area to volume (S/V) ratio. Organisms with a low S/V ratio have potential problems since the area over which exchange can take place is low in relation to requirements. Spherical organisms have the smallest possible ratio, since:

$$S/V = \frac{4\pi r^2}{4/3\pi r^3} = \frac{3}{r} \qquad (3.1)$$

where r = radius of sphere. For single-celled spherical algae, an increase in size will reduce the S/V ratio. This has important evolutionary implications, where the maintenance of a high S/V ratio during the evolution of large-sized organisms is achieved by the development of elongate shapes and by

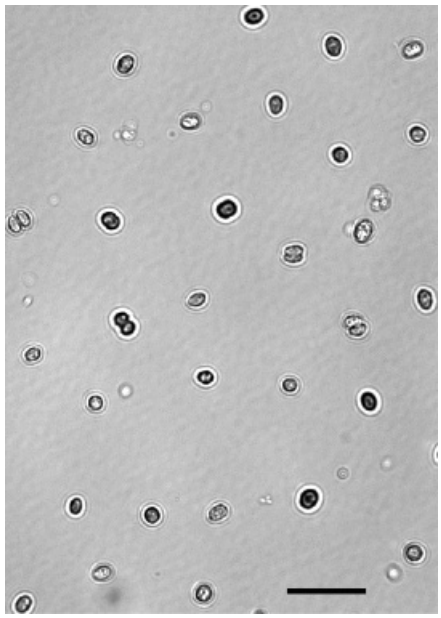

Figure 3.5 *Synechococcus aeruginosa* (Cyanophyceae): this single-celled blue-green alga is a common member of the nanoplankton, with a cell diameter of approximately 2–5 μm. This sample was obtained from a mid-summer *Synechococcus* bloom which developed in a stratified eutrophic lake at a time when epilimnion nutrient concentrations were still high (soluble nitrogen: 1 mg l^{-1}, soluble phosphate: 50 μg l^{-1}, scale −20 μm)

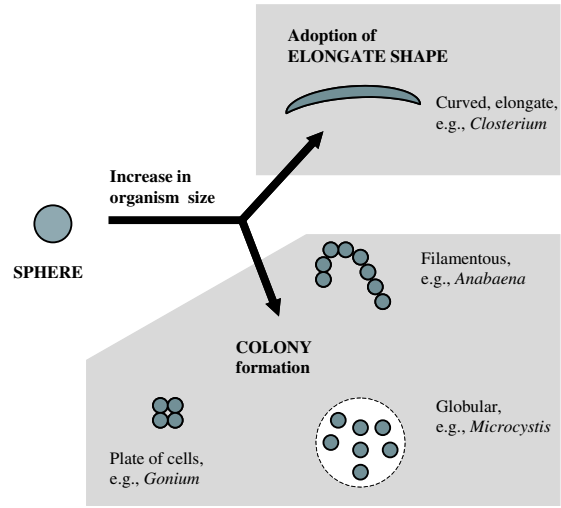

Figure 3.6 Hypothetical scheme to show maintenance of a high S/V ratio during the evolution of larger-sized phytoplankton. Algae avoid an evolutionary decrease in surface area to volume (S/V) ratio that would occur by simple increase in spherical size by adopting elongate shapes or forming colonies. This is particularly the case for *K*-selected organisms, which include the dinoflagellate *Ceratium* (with elongate spines) and the colonial blue-green algae *Anabaena* (filamentous) and *Microcystis* (globular). In colonial algae, the key S/V ratio is that of individual cells rather than the whole colony since diffusion of metabolites occurs directly to and from the separate cells

colony formation (Figure 3.6). Many large-sized *K*-selected planktonic algae that inhabit stable, crowded environments (Section 3.10.2) have a colonial form (*Anabaena, Microcystis*) or have elongate extensions (*Ceratium*).

The ecological importance of organism size in relation to nutrient transfer is also indicated by a comparison of algal species which dominate low-nutrient and high-nutrient environments. Within major algal groups such as Chlorophyceae and Cyanophyceae, the smaller single-cell organisms tend to dominate oligotrophic sites, while larger colonial forms tend to predominate in eutrophic systems. The higher surface/volume ratio of microplanktonic algae leads to a more efficient uptake of nitrate and phosphate, giving these organisms a competitive advantage in nutrient-limiting conditions. The smallest free living lake biota, bacteria, have an even greater competitive advantage over algal cells in low-nutrient conditions (Section 6.12). Although unicellular picoplankton and nanoplankton are particularly characteristic of oligotrophic systems, they also occur in eutrophic lakes where they may form dominant populations. This is seen in Figure 3.5, with a mid-summer bloom of *Synechococcus*, and is also recognized in Reynolds' (1990) classification of algal succession in relation to lake nutrient levels – where hypereutrophic lakes are characterized by a sequence of unicellular algae (Table 10.4).

Light absorption and cell size

Absorption of light by pigments contained within a suspension of cells is different from the situation

where the same amount of pigment is dispersed throughout the whole liquid medium. This packaging effect has implications for the absorption of light by phytoplankton populations and the attenuation of irradiance within water columns.

Light absorption by individual particles (e.g., algal cells) in suspension is governed by the size of the particles (d) and the absorption coefficient (a), and may be considered either in terms of absorption by the whole cell or absorption per unit pigment molecule (pigment-specific absorption).

In the case of an entire spherical cell, the 'efficiency factor for absorption (Q_a)' may be defined:

$$Q_a = \frac{\text{energy absorbed within the sphere}}{\text{energy incident on its geometrical cross section}} \quad (3.2)$$

Q_a directly varies with particle size (d) and absorption (a) coefficient and can be used to determine the light absorption of an entire population of cells in suspension (Morel and Bricaud, 1981).

In contrast to whole cells, pigment-specific light absorption increases with decreasing cell size and decreasing internal molecular shading. Under conditions of equal pigment concentration, small cells would have higher light absorption per unit pigment with implications for the efficiency of photosynthesis. Cell size is also important in the absorption of UV radiation and the damage to pigment and other macromolecules during the process of photoinhibition. In general, smaller phytoplankton cells have higher rates of UV absorption and are thus more prone to photoinhibition, as discussed in Section 4.9.4.

Small size as an ecological response

Small-celled unicellular algae have a low biomass, short cell cycle and rapid growth rate. These organisms are adapted to the exploitation of transient new environments, (where conditions for growth are time-limited) and situations of environmental stress. Such conditions favour organisms that follow an opportunistic or *r*-selection strategy (see Section 1.2.6).

Conditions of time-limited growth Small-celled (pico- and nanoplanktonic) algae tend to dominate the phytoplankton of sub-polar lakes, where the period of summer growth is restricted. In Lake Baikal (Russia), for example, there is only a short period (July–September) of warm (10–15°C) surface water, during which autotrophic nanoplankton predominate (Figure 2.9). These organisms reach counts of up to 2×10^6 cells ml^{-1} within the euphotic zone (about 15m), with the blue-green alga *Synechocystis* as the major organism.

Conditions of environmental stress The inverse relationship between the size of freshwater biota and the effects of both internal and external environmental stress factors has been noted previously in Section 1.6 (Figure 1.13).

Zooplankton grazing pressure operates as a major internal stress factor during the seasonal cycle of many temperate lakes, with a pronounced shift to small (r-selected) algae during the clear-water phase (Figure 3.14). External stress, such as heavy-metal pollution, may also have a dramatic effect. Studies by Cattaneo *et al*. (1998), for example, demonstrated a marked decline in the size of algae (diatoms), protozoa (thecamoebians), and zooplankton (cladocerans) in Lago d'Orta (Italy) over a 50-year period. This change in the mean size of individual organisms began with the onset of copper (and other metal) pollution, and was primarily due to a shift in taxonomic composition within the different groups. Reduction in body size also occurred within a single taxon, the diatom *Achnanthes minutissima*.

Passive movement of cells and colonies

Phytoplankton units (single cells or colonies) are exposed to continuous random water movement within the water column. This ranges from oscillations at the molecular level, causing Brownian motion of the smallest phytoplankton cells (along with bacteria and viruses), to larger-scale water movement which occurs as turbulence.

In conditions of turbulence, the fixed flow to which small phytoplankters are exposed is a laminar shear, causing the organism to move (translate) and

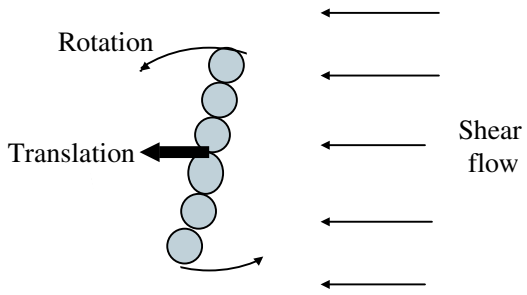

Figure 3.7 Displacement of phytoplankton in flowing water; linear colonies and single cells undergo forward movement (translation) and rotation under conditions of linear flow

rotate (Figure 3.7). Such passive rotation is important in increasing the nutrient flux, and needs to be taken into account in considering the uptake of nutrients in turbulent conditions. It is also important in other planktonic processes such as aggregate formation and predator–prey interactions, since it determines the volume swept by the phytoplankter and hence the likelihood of encounter with other particles or with the appendages of herbivores.

Laboratory studies on the passive movement of chain-forming diatoms such as *Skeletonema* (Karp-Boss and Jumars, 1998) under conditions of steady shear flow demonstrated periodic rotation. In diatoms where the chain forms a rigid structure, the period of rotation depended on the magnitude of the shear rate and the axis ratio (major axis/minor axis) of the colony.

Sedimentation within the water column

Sedimentation is the downward movement of non-motile plankton (typically with a rigid cell wall, lacking cilia or flagella) relative to the adjacent water medium, in response to gravitational forces ($g = 9.8081$ m s^{-2}).

Sedimentation occurs where the density of a particle exceeds that of the surrounding liquid medium, which is 1000 kg m^{-3} for pure water. In the case of phytoplankton cells, the protoplasm is rich in macromolecules which are denser than water, including proteins ($\sim$1300 kg m^{-3}), carbohydrates ($\sim$1500 kg m^{-3}), and nucleic acids ($\sim$1700 kg m^{-3}). These molecules are contained within an aqueous cytosol, and the overall density of phytoplankton cells or colonies is typically $>$1050 kg m^{-3}. This may be considerably increased by the presence of dense inorganic inclusions such as polyphosphate bodies ($\sim$2500 kg m^{-3}) and opaline silica cell wall material ($\sim$2600 kg m^{-3}). Conversely, cell density can be reduced by the internal presence of low-density lipids (860 kg m^{-3}), gas vacuoles, and the occurrence of external mucilage.

The passive rate of sedimentation of a spherical body under conditions of laminar flow, is given by the Stokes equation:

$$\nu_s = 2gr^2(\rho - \sigma)(9\eta)^{-1} \qquad (3.3)$$

where ν_s is the terminal velocity of the particle (m s^{-1}), g is gravitational acceleration (m s^{-2}), η is the coefficient of viscosity of the liquid medium (kg m s^{-1}), ρ and σ are the density of the particle and liquid medium respectively (kg m^{-3}), and r is the particle radius (m).

According to this equation, for spherical cells, size and density are key factors affecting the rate of sinking. Major factors that can cause deviation from this equation are non-laminar flow and non-spherical shape. Most algae are not exactly spherical, and their deviation from a sphere (ϕ_r) will result in a larger surface area and a greater frictional resistance, thus reducing the sinking rate. This can be incorporated into a modified Stokes equation:

$$\nu_t = 2gr^2(\rho' - \rho)/(9\eta \cdot \phi_r) \qquad (3.4)$$

where ν_t is the terminal velocity of the particle (m s^{-1}) and ϕ_r is the coefficient of form resistance (deviation from sphericity).

The variety of shapes seen in phytoplankton cells and colonies will thus have a major effect on sinking rates, and the development of attenuated shapes such as elongate colonies and the presence of spines may be interpreted in part as an evolutionary development to reduce passive sinking and maintain

cells within the zone of light availability. Although many phytoplankton cells/colonies have developed attenuate morphology, the extent to which this occurs (as measured by surface area/volume ratio) remains within strict limits – see Section 4.3.1.

For buoyant particles such as gas-vacuolate blue-green algal cells (where the density is less than water) the value for ν_t is negative, representing a rate of upward flotation.

Size and motility

The size range of flagellate algae is limited by the propulsive force of the flagella (Fenchel, 1991). These organisms typically have a regular flagellar beat frequency of about 50 Hz, with a flagellar length not exceeding 50 μm. The swimming velocity of cells that propel themselves by one or a few flagella is typically about 0.1–0.2 mm s^{-1}.

In order to swim, the swimming velocity (forward propulsion) must exceed the sinking velocity. With spherical cells of density 1.1, Stoke's law predicts sinking velocities of 0.1–0.2 mm s^{-1} for cells with a diameter in the range 25–50 μm. This sets the upper size limit for flagellates. The size range of typical flagellates that use flagella for locomotion is about 4–20 μm, with many (particularly the heterotrophic nanoflagellates) having diameters <10 μm. A decrease in size to this level allows for greater motility due to a decrease in the gravitational vector. An alternative strategy to decreasing size in relation to flagellar motility is to adopt other forms of movement. Many of the larger flagellates (e.g., large euglenoids) do not swim, but are capable of gliding over surfaces. Another possibility for circumventing the problem of cell size in flagellates is to regulate buoyancy, and reduce sinking velocity. This is seen in the dinoflagellate *Noctiluca*.

Resistance to ingestion by zooplankton

Grazing of phytoplankton cells by zooplankton is the major factor that limits algal growth in the freshwater environment. Ingestion of algae by zooplankton can be limited by a range of algal characteristics including size and shape, surface chemistry, presence of mucilage, and algal toxins (Section 9.8.3).

Many algae have evolved a strategy to reduce zooplankton grazing by maximizing size (greatest axial linear dimension), including single cells (e.g., dinoflagellates *Ceratium*, *Peridinium*) and colonies (e.g., blue-green *Anabaena*, *Microcystis*).

3.4.4 Variation in size and shape within phytoplankton populations

The size and shape of planktonic algae clearly have considerable adaptive value since they have direct influence on both growth (compare *r*- and *K*-selected cells) and loss (sedimentation, zooplankton ingestion) rates. These parameters are thus important in competition between algae, determining the abundance of particular species within the aquatic environment and their individual contribution to community biomass (Duarte *et al*., 1990). Differences in size and shape will also lead to differences in surface area/volume (S/V) ratios of cells and colonies – with resulting differences in physiological activities (see previous section).

Most studies on size/shape variation in phytoplankton populations have concentrated on differences between species (mixed populations), but size plasticity within species may also be important.

Variations in size and shape within mixed populations

Phytoplankton populations sampled from a particular site typically contain a mixture of different species with an apparently unrestricted range of morphologies, varying from spherical unicellular and colonial forms to single cells and colonies with highly extended and irregular shapes (Figure 3.1).

In such a situation, it might be expected that derived parameters, such as S/V ratios would also extend over a wide and unrestricted range. This was

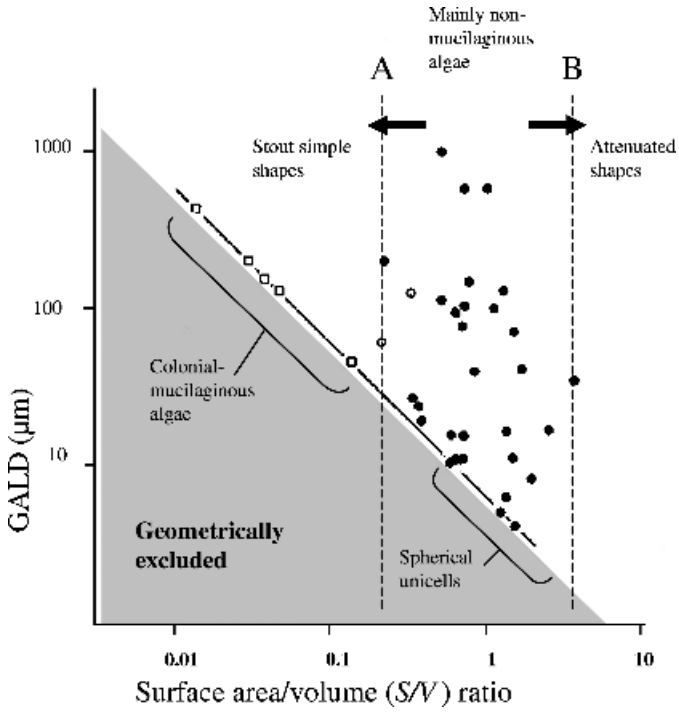

Figure 3.8 Phytoplankton size and shape: log/log plot of GALD and surface area/volume ratio. Individual points show values for a range of unicellular and colonial algae. Lower (A) and upper (B) limits of the S/V ratio for most algae are indicated by the broken lines (based on a figure from Reynolds, 1990)

initially investigated by Lewis (1976) who examined the relationship between unit size (expressed as the greatest axial linear dimension – GALD) and the surface/volume ratio of the 27 most abundant phytoplankton species in a tropical lake. Values were plotted for various theoretical shapes and for phytoplankton data (similar to Figure 3.8). The major conclusion was that phytoplankton S/V ratios were conserved within a range much narrower than expected by random choice of shapes. Unlike the theoretical shapes, phytoplankton organisms occupied quite a narrow band, with particular sizes ranging from spherical (low S/V ratio) to attenuate (high S/V ratio) shapes. Extending the Lewis plot to a wider range of planktonic algae (Reynolds, 1990; Figure 3.8) indicates that conservation of S/V ratios holds particularly for non-mucilaginous algae, but not for mucilaginous colonial forms.

In non-mucilaginous phytoplankton, a lower limit to the S/V ratio is to be expected in terms of algal physiology, since a point is reached at which the surface is no longer able to provide metabolite exchange levels appropriate to volume requirements. The presence of an upper S/V boundary may seem surprising, however, since it is not immediately clear in terms of cell physiology why there should be a limitation to the development of attenuate shape and high S/V ratios. The upper S/V limit may relate to the need for a critical level of sedimentation to ensure continuous replacement of the microenvironment adjacent to the cell surface. This replacement is required for efficient nutrient uptake and for the loss of unwanted metabolites.

The occurrence of mucilaginous colonial algae such as *Microcystis* below the lower S/V limit emphasizes the separateness of cells within the colony. These aggregations are not limited by size since metabolites are able to diffuse to and from individual cells through the mucilage matrix. The physiologically relevant S/V ratio in these organisms is that of individual cells rather than the whole colony (Figure 3.6).

Size variation within species

Considerable size plasticity occurs within species, with both single cell and colonial algae showing great variation in overall dimensions. Although this plasticity partly reflects the variation expected within a normal distribution, evidence suggests that algae are also able to modulate size in relation to functional parameters such as absorption of light and general competitive ability. These functional parameters may in turn relate to environmental features such as phytoplankton community biomass, water turbulence, and nutrient availability.

Light absorption Control of colony size in blue-green algae, for example, may be important in controlling sedimentation and optimizing light absorption. In studies on a nutrient-rich lake, Robarts and Zohary (1984) noted that light absorption by the dominant alga *Microcystis aeruginosa* increased with reduction in colony size (at comparable lake water chlorophyll-a concentrations), and that the euphotic zone accordingly increased with colony size.

General competitive ability Algal size may also be important in relation to general competitive ability. Comparative studies (Duarte *et al*., 1990) of different lakes have suggested that the average size of phyoplankton tends to increase with increasing community biomass. In the case of Florida lakes, for example, these studies have shown that the average size of individuals within genera tended to increase as the ½ power of their biomass within the community. These observations have been interpreted as evidence for size-dependent differences in competitive ability, with small algae being better competitors in sparse communities and large algae competing more effectively in dense communities. The adaptive value of algal size is emphasized by the fact that genera capable of large size variations tend to thrive in both sparse and dense communities, and are more ubiquitous than genera with a limited size plasticity.

Water turbulence (Steinberg and Geller, 1990) may have a direct physical effect on cell and colony size. Conditions of high turbulence, occurring in systems such as dammed rivers, lead to algal populations of small mean size. This was particularly apparent in algae such as *Microcystis aeruginosa* and *Coelosphaerium kuetzingianum*, where the reduction in size under turbulent conditions resulted in these species occurring as small single cells within the picoplankton.

3.5 Mucilaginous and non-mucilaginous algae

In addition to differences in size and shape, freshwater algae also vary considerably in the presence of extracellular mucilage. This is seen particularly clearly in the lake environment, where some species of planktonic algae have cells embedded in a large volume of mucilage – while others have no apparently detectable mucilage at all. The presence of mucilage is not readily observed by bright-field light microscopy, but can be seen under phase contrast (Figure 3.9) and by special preparation techniques such as negative staining and fluorescence microscopy of lectin-stained preparations. Atomic force microscopy (AFM) can also be used to provide high-resolution information on the topography and material properties of the mucilage layer of living algae. Recent AFM studies on three diatoms, for example, have revealed differences in mucilage surface nanostructure and in the dynamic properties (adhesion and stretching) of surface polymer chains (Higgins *et al*., 2003).

An outer layer of mucilage increases overall size and confers a distinctive surface chemistry to the algal cells. Mucilage has an important role in the biology of these organisms and has ecological implications in terms of biogeochemical cycles. Even algae that are regarded as 'non-mucilaginous' typically have a surface layer of polysaccharide material. Combined carbohydrate analysis and atomic force microscopy demonstrated a thin sugar-rich layer of surface mucilage in the diatom *Pinnularia* (Chiovitti *et al*., 2003), for example, which would be difficult to see under the light microscope. This thin mucilage layer in diatoms has particular importance in separating the cell wall from the external aquatic medium and reducing silica solubilization in living organisms (Figure 5.24).

3.5.1 Chemical composition of mucilage

Mucilage is composed of a complex macromolecular network enclosing a water matrix. Although water is the major component (>95 per cent) by weight, the chemistry of mucilage is dominated by the macromolecular component and the exposed sugar and charged groups which are associated with this. Relatively little is known about the detailed chemistry of algal mucilage, although chemical analyses have been carried out on the surface slime of a number of blue-green algae. In *Microcystis flos-aquae*, for example, where individual cells are embedded in a globular mass of surface slime, the macromolecular component is almost entirely polysaccharide, with no detectable protein (Plude *et al*., 1991). Gas chromatographic analysis of the polysaccharide indicates a composition closely similar to higher plant pectin, with galacturonic acid as the main sugar plus minor quantities of neutral sugars (galactose, glucose, xylose, mannose, and rhamnose). The surface mucilage of other blue-green algae is also polysaccharide-based, but differences occur in sugar composition and detectable levels of protein are present in some cases. Variations in chemical composition may account for differences in mucilage appearance and consistency in different algae.

Surface sugars

Surface sugars are an important biochemical feature of both mucilaginous algae (where they are exposed throughout the mucilage layer) and non-mucilaginous algae, and have been studied using lectin probes. These multivalent carbohydrate-binding proteins are highly specific to particular sugar groups and include molecules such as concanavalin A (ConA – binds to mannose, glucose, and N-acetylglucosamine) and peanut agglutinin (PNA – binds to galactose and N-acetylgalactosamine).

Using a panel of seven fluorescent-labelled lectins, Sengbusch and Muller (1983) demonstrated great variety in the occurrence of surface sugars in selected species from the major algal groups. The results showed clear patterns of lectin-binding which appeared to be species specific, but not specific to the major algal group. Within the aquatic environment, surface biochemistry is clearly an important aspect of biodiversity – with each individual species expressing its own particular combination of sugar molecules at the environmental interface. The functional role of these surface sugars has not been elucidated, but they have clear potential as receptor molecules for communication (within and between algal species), species-pathogen recognition, and the attachment of a wide range of organisms (including protozoa, fungi, bacteria, and viruses).

3.5.2 Role of mucilage in phytoplankton activities

The presence of a layer of surface mucilage can be seen as an ecological strategy that has evolved in all the major algal groups and affects a number of characteristics – including an increase in unit (single cell or colony) size, approximation to a spherical shape, decrease in overall density, and the acquisition of a characteristic surface chemistry. This mucilage is important for both planktonic and benthic micro-algae.

Phytoplankton

For planktonic algae, surface mucilage influences a number of biological activities.

Sinking rate The effect of surface mucilage on the sinking rate within the water column is defined by the Stokes Equation (3.3). The speed of sedimentation will depend on the balance between increase in unit size (increasing the sedimentation rate) and the decrease in density (decreasing the rate).

Grazing and digestion by zooplankton Surface mucilage is an important factor in the selection of food particles by zooplankton (Section 9.8.3) – influencing the size and shape of the algal cell or colony and also its surface chemistry. Once the alga has been ingested, the presence of a surface layer of mucilage may also prevent digestion within the

zooplankton alimentary canal, allowing the algal cells to be voided in a viable state.

Association of epiphytic biota Mucilage provides an increased area for attachment and a solid substratum for a range of epiphytic organisms – including bacteria, protozoa, fungi, and other algae. In addition to providing a site of attachment for these organisms mucilage may also provide a medium within which motile epiphytic organisms can move, and in some cases provides an major source of nutrients.

Surface mucilage is particularly important in large colonial algae, where an extensive phycosphere community can develop forming a self- contained micro-ecosystem (Section 6.13, Figure 6.23).

Adsorption of anions and cations Although physical binding (adsorption) of anions and cations occurs at the surface of both mucilaginous and non-mucilaginous algae, recent studies have shown that algae with a large amount of mucilage tend to have a higher capacity for ion adsorption. This has potential significance for the uptake of nutrients from the aquatic environment and the ability of algal cells to resist heavy-metal toxicity.

Laboratory studies (Figure 3.9) on Cu adsorption by cultured algal cells demonstrate a linear relationship between adsorption (expressed as log Cu per unit dry mass) and Cu concentration in the medium (log values). This indicates a close fit to the Freundlich adsorption isotherm, with the mucilage behaving kinetically as a monolayer containing heterogeneous binding sites. These adsorption characteristics are not restricted to algal cells, but are also typical of other microorganisms such as fungi (Sag *et al*., 1998).

The heterogeneous binding sites in algal mucilage include positively and negatively charged groups, which promote the adsorption of both anions and cations. The degree of adsorption and the balance between anion and cation binding depends on the composition of the mucilage. In the case of *Microcystis flos-aquae*, for example, the mucilage is composed largely of galacturonic acid, and charge attraction of cations to carboxyl groups is the main factor in ion adsorption (Plude *et al*., 1991). In addition to carboxyl groups, sugars with particular configurations of hydroxyl groups can also complex some metals (Angyal, 1972). The ability of mucilage to chelate Fe may be mediated by the presence of specific iron-binding compounds (siderophores) such as hydroxamic acid, catechols and phenols.

Benthic algae

Surface mucilage is important for benthic microalgae such as diatoms, where it is involved in attachment and locomotion.

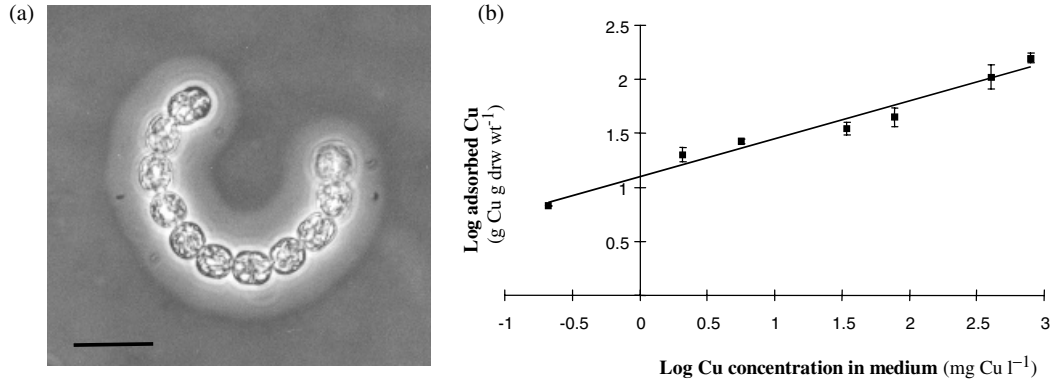

Figure 3.9 Adsorption of cations by surface mucilage of *Anabaena*. (a) Phase contrast image of a single filament of *Anabaena spiroides*, showing a 5 µm-thick mucilage sheath (scale = 10 µm). (b) Freundlich isotherm plot for Cu adsorption by cultured *Anabaena* cells, showing a close fit ($p < 0.01$) to the model. The Freundlich adsorption capacity constant (K_f) was 12.6, indicating a much greater capacity for adsorption compared with non- mucilaginous algal species (K_f values 0.4 –3.6) (Unpublished data from Christina Tien, with permission)

Some benthic diatoms are able to glide over solid surfaces by the extrusion of mucopolysaccharide filaments through special 'mucilage pores,' leaving a mucilaginous trail over the substratum. Motility in these organisms is restricted to two major groups – the pennate diatoms (where the mucilage pores occur along the central ridge, or raphe), and centric diatoms with special surface projections (labiate processes). Studies on the pennate diatom *Navicula* (Edgar and Zavortink, 1983) have shown that the mucus filaments are produced by secretory vesicles along the length of the raphe slit, and that control of secretion is mediated via actin filaments. Mucilage secretion is a periodic event and, in response to an appropriate stimulus, occurs via mucilage pores which typically occur at the ends of the raphe. The mucilage is released into the raphe, streaming in one direction until it hits the fixed substratum – when it forces the diatom to move in the opposite dirdection. In pennate diatoms, the direction of movement follows a curved or straight trajectory (depending on the shape of the raphe), and observed speeds vary from 2–14 μms^{-1} at room temperature (Lee, 1997). Speed of movement can vary with the type of substrate, ranging in *Nitzschia* from 2.7 μms^{-1} on glass to only 0.8 μms^{-1} on soft agar.

3.5.3 Environmental impact and biogeochemical cycles

Many mucilaginous algae, including blue-green algae such as *Microcystis* and *Anabaena*, have worldwide occurrence throughout a range of freshwater systems. These organisms often form large populations in natural waters, with the high levels of surface mucilage influencing the chemical and ecological properties of the ecosystem. Studies on a temperate eutrophic lake (Tien *et al*., 2002) showed that cell-associated mucilage occupied 0.1–7×10^{-3} per cent of lake water volume within the epilimnion over the annual cycle, reaching the highest value during the autumn bloom of *Microcystis*. Seasonal changes in the total volume of associated mucilage reflected the succession of mucilage-forming species, but did not correlate with the concentration of soluble extracellular polysaccharide (EPS) in the lake water. Soluble EPS ranged in concentration from 2.5–60 mg l^{-1}, with peaks in the spring diatom bloom ('non-mucilaginous') and the late clear-water phase – but not the *Microcystis* (mucilaginous) bloom. This suggests that surface mucilage (cell-associated polysaccharide) does not simply diffuse into the surrounding water, and that the soluble lake water EPS concentration relates more to other factors such as algal secretion (Section 4.7.2), and bacterial and zooplankton activity.

Whatever the derivation of soluble EPS, it has a combined role with surface mucilage in the adsorption of cations from lake water – influencing the availability of trace nutrients, sequestering toxic metals, affecting pH, and playing a role in several biogeochemical cycles. EPS has been implicated, for example, in the manganese cycle where it is involved in the oxidative precipitation of manganese nodules in certain lakes (Richardson *et al*., 1988).

C. ACTIVITIES WITHIN THE FRESHWATER ENVIRONMENT

3.6 Benthic algae: interactions with planktonic algae and ecological significance

3.6.1 Planktonic and benthic algae

Benthic algae are present in the bottom sediments of almost all aquatic systems, where they require adequate light to carry out photosynthesis and growth. In terms of primary productivity, they form a distinct community (periphyton) from pelagic algae (phytoplankton), which photosynthesize, grow, and spend their vegetative phase in the water column. The two communities are ecologically related, however, since individual species contribute to both populations and dynamic interactions occur between the two groups.

Differences between benthic and planktonic algae

Some general distinctions separate these major groups of algae (Sterenson *et al*., 1996). Phytoplankton is typically composed of micro-algae (<200 μm diameter), while periphyton is a mixture of micro- and macro-algal (>200 μm) species. Particular taxonomic groups, such as filamentous green algae and pennate diatoms are typical of benthic conditions, while volvocales and centric diatoms are characteristic of the pelagic environment. Various morphological and physiological features are also diagnostic. The attachment structures of filamentous algae and the raphe, mucilage pads, and stalks of pennate diatoms are clear adaptations to the benthic environment. Conversely, the development of motile mechanisms (flagella, gas vacuoles) is typical of planktonic forms. Other differences that have been suggested include a higher specific gravity (higher settling speed) in benthic species, and adaptation to greater nutrient diversity in the benthic environment.

Differences between planktonic and benthic algae also occur in relation to the structure and location of the community. In contrast to the dispersed state of phytoplankton, benthic algae are typically closely associated within a densely packed periphyton mat. This influences the growth of cells and their rates of primary production and nutrient uptake. Internal shading results in a wide variation in photosynthetic activities within the dense periphyton biomass, leading to major differences in the photosynthesis-irradiance (*P–I*) response curves between phytoplankton and periphyton communities (Section 4.3). The location and close packing of benthic algae also influences the way in which these biota are grazed. In contrast to the dispersion feeding of phytoplankton by (largely crustacean) zooplankton, benthic algae are largely removed by the scraping and gathering activities of benthic molluscs and insect larvae (Section 9.13).

Benthic and planktonic phases

Freshwater algae share one important characteristic with other lake microorganisms (such as bacteria, fungi, and protozoa) – individual species typically have both pelagic (present in the main water body) and benthic (associated with sediments) phases. In species which are mainly planktonic, the benthic phase may simply be a metabolically inactive and resistant spore which over-winters in the sediments. In species that are primarily benthic, the planktonic phase may originate by detachment of single cells or colony fragments, or by the formation of gametes and zoospores as part of the life cycle. Pelagic phases are particularly important in benthic organisms for dispersal and colonization of new environments.

The occurrence and interactions of planktonic and benthic algae depend primarily on light availability to the sediments (depth of water column) and the displacement of unattached algae by the water current, both of which relate to the hydrology of the water body. Some of the interactions between planktonic and attached phases are illustrated in Figure 3.10, which shows two contrasting environmental situations. In deep lakes, where lack of light penetration prevents growth of benthic algae, sediment populations are mainly resistant spores – which are in seasonal equilibrium with planktonic populations. Although there are no attached benthic algae, attached algae do occur as epiphytes which are in balance with free planktonic populations. These epiphytic algae are associated with larger colonial algae as part of the algal microcosm (Figure 6.23) and also with other lake organisms. The unicellular blue-green algae *Cyanothece* and *Synechococcus* are occasionally observed as epiphytes within colonies of *Gomphosphaeria*, and the euglenoid alga *Colacium* occurs as a green epibiont on the surface cuticle of microcrustaceans and rotifers (Wehr and Sheath, 2003). In contrast to deep lakes, shallow river systems typically have extensive populations of actively growing algae on sediments and as epiphytic growths on macrophytes, both of which relate to planktonic phases in terms of detachment and recruitment.

In lakes, periphyton communities are associated particularly with the littoral zone, and are spatially separated from the main water body which is dominated by phytoplankton. Although lake periphyton are subject to some physical disturbance

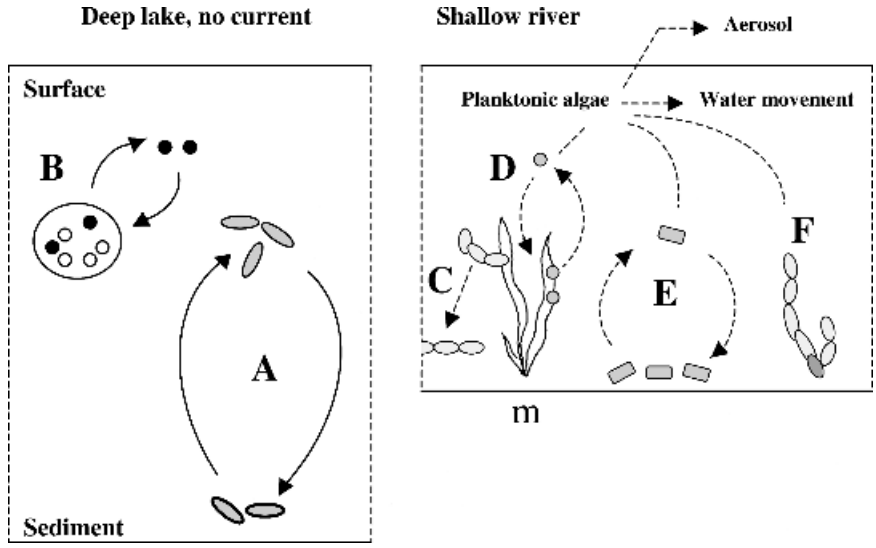

Figure 3.10 Diagram showing dynamic interactions between freely pelagic and non-pelagic (benthic or attached) phases of algae in contrasting lake and river situations (not to scale)
Deep lake: sediments are not within the photic zone, so there are no photosynthetic benthic algae. **A**. Benthic resistant spores give rise to planktonic populations, and vice versa. **B**. Some free planktonic algae occur in equilibrium with attached (epiphytic) populations associated with large colonial algae and other organisms.
Shallow river. High light levels support vigorous growths of attached epiphytic and benthic algal communities, which are detached by the current to form phytoplankton:

- *Epiphytic algae* present on macrophytes (m) include filamentous and unicellular forms: **C.** filamentous algae may detach to form filament masses (metaphyton); **D.** unicellular algae are in equilibrium with planktonic populations.
- *Benthic algae* (periphyton): **E**. attached micro-algae (e.g., diatoms) interchange with planktonic cells by detachment and settlement; **F**. macroscopic filamentous algae can also detach (giving rise to planktonic fragments) and settle

(wave action) they do not experience the flow characteristics of river systems, with the continuous displacement and detachment of microorganisms which is important in lotic dispersal.

Competition between planktonic and benthic algae

Competition between planktonic and benthic algae for light and nutrients shows close similarities to the interactions that occur between phytoplankton and higher plants (macrophytes) – Section 4.8. In both cases, benthic and pelagic primary producers exhibit various competitive interactions, which in the case of phytoplankton and periphyton may involve (Vadebonceur *et al*., 2003):

- more rapid uptake of nutrients from the water column by phytoplankton due to the smaller surface/volume ratio of pelagic algae, and the fact that nutrient uptake by periphyton is constrained by boundary layer kinetics;
- closer access of periphyton to sediment nutrients, allowing them to regulate the availability of these growth resources to phytoplankton;
- attenuation of light by phytoplankton, limiting periphyton productivity.

These interactions may lead to different responses by pelagic and benthic algae under conditions of changing light and nutrients, resulting in inverse relationships between population development in the two groups of organism. Eutrophication resulting from fertilization experiments, for example, may lead to increased phytoplankton biomass – with a compensatory decline in the level of periphyton. This transition was observed in shallow, oligotrophic Greenland lakes (Vadebonceur *et al*., 2003), with a eutrophication switch from benthic to pelagic dominance of primary productivity. The change in productivity was accompanied by a corresponding shift from periphyton to phytoplankton in the diets of zoobenthos, as demonstrated by carbon stable isotope analysis. Although benthic and pelagic habitats in these lakes were energetically linked through food web connections, eutrophication depleted and finally eliminated the benthic primary production pathway.

3.6.2 Lake Periphyton

Although the algal population of lakes is normally considered in terms of phytoplankton, benthic algae also make a major contribution to lake ecology and to primary productivity. This is particularly the case for shallow lakes, where a large part of the bottom receives enough light to support photosynthesis and periphyton (Section 1.2.4) can dominate carbon fixation. Even in deep lakes, where the area of periphyton is restricted to the shoreline (littoral zone), the role of periphyton in the whole-lake carbon budget can be substantial (Lowe,1996). Lakes with steep sides, such as Lake Tahoe (USA) have relatively narrow littoral zones with a correspondingly lower proportion of periphyton carbon fixation.

The development of periphyton communities in lakes is strongly influenced by environmental variables such as light, turbulence, water chemistry, macrophyte dominance, and grazing pressure. Climate is also important, and the seasonal changes which determine periphyton development in a dimictic lake of temperate zones are clearly quite different from those operating in a permanently frozen (amictic) lake in Antarctica. In temperate lakes, the periphyton community receives light every day, but experiences great seasonal fluctuations. Periphyton in polar lakes experiences alternating 6 month periods of light and dark, and is permanently under ice.

Lentic periphyton communities are often complex and highly structured, and are composed of both autotrophic (algae) and heterotrophic (fungi and bacteria) components. The algae occur as two major growth forms:

- Large filamentous algae (mainly green algae and blue-green), which are firmly attached to the sediment or to macrophytes, and extend into the water medium. Filamentous algae with coarse cellulose cells walls, such as *Cladophora*, can themselves support extensive populations of epiphytes. *Cladophora* creates an important microhabitat for epiphytic microbes and communities of invertebrates, increasing the surface area of the eulittoral zone by an estimated factor of >2000.

- Single-celled and small colonial algae, present on rocks (epilithon) and large plants or algae (epiphyton). These algae include a wide range of diatoms, plus blue-green and some red algae.

Although these different growth forms and taxonomic groups are intermixed within the benthic community, periphyton often shows a clear shoreline zonation – with an upper eulittoral zone dominated by green algae and diatoms and a lower sublittoral zone dominated by blue-green algae and diatoms. These two zones are determined by differences in light availability, water turbulence, seasonal variation, and macrophyte dominance (Figure 3.11).

The eulittoral zone roughly corresponds in depth to the epilimnion of the water column, and shows similar seasonal variations in light and temperature. Algae within this zone show clear seasonal variation, and are characterized by species which have firm attachment to the substratum. Many of these

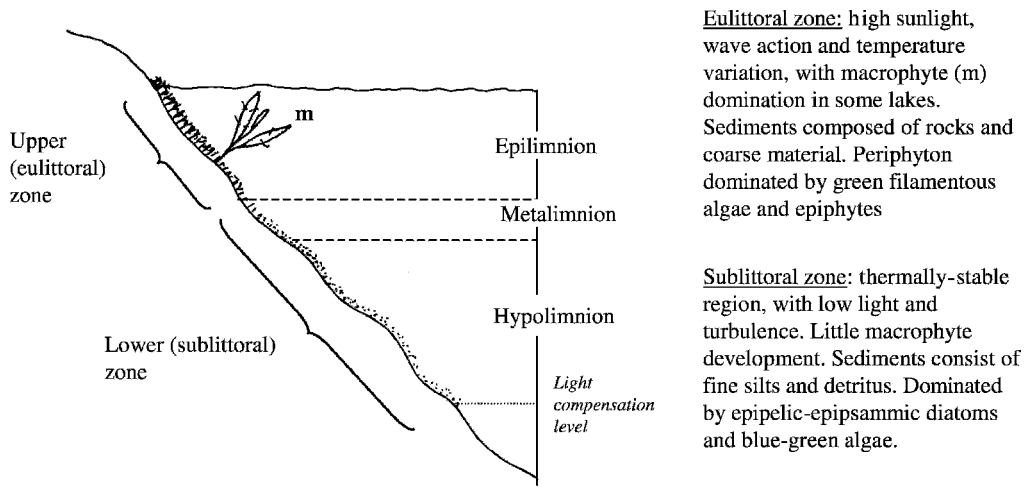

Figure 3.11 Periphyton distribution and habitat variation in a thermally-stratified lake (based on a figure from Lowe, 1996)

Although the littoral periphyton community can be grouped into two broad zones, changes in periphyton and environmental parameters also form an interrelated continuum with depth. In addition to the above factors, variation in grazing also has an important impact on community diversity.

algae grow best under conditions of water movement (rheophilous), and the eulittoral community is closely similar to the periphyton of well-lit streams. The species composition of the eulittoral zone varies with water quality (Table 3.7). In high nutrient (eutrophic) hard-water lakes, dominant filamentous genera include green algae (*Cladophora*, *Ulothrix*, *Oedogonium*) and occasionally the red alga, *Bangia*. The epilithon and epiphyton of these lakes are dominated particularly by diatoms such as *Navicula* and *Cymbella*. Oligotrophic softwater lakes tend to have a shift from filamentous to unicellular algae, with a preponderance of diatoms and desmids (*Cosmarium*, *Staurastrum*). Diatoms are particularly important in these environments, and in oligotrophic Lake Tahoe (USA), for example, the eulittoral zone is dominated by stalked diatoms such as *Gomphoneis*. Filamentous algae which do occur in these conditions are represented by members of the Zygnemataceae (green algae) such as *Mougeotia*.

Below the eulittoral zone, where wave motion, light energy and temperature variation are reduced, a marked change occurs in the periphyton community. The distribution and abundance of periphyton populations in the sublittoral zone is also strongly influenced by the fine silts and detritus that occur below the zone of wave activity. These deep, thermally stable, lentic habitats support a rich assemblage of algal species. Although relatively few studies have been carried out on these environments, various workers have reported communities of diatoms and blue-green algae. Caljon and Cocquyt (1992), for example, reported 227 diatom taxa from the surface sediments of Lake Tanganyika, and Roberts and Boylen (1988) discovered extensive communities of epipelic filamentous and colonial blue-green algae in the deep littoral zone of an Adirondack lake (USA).

This deep-water algal periphyton can only occur where the euphotic zone extends down to the lake sediment, and is typically not present in eutrophic

Table 3.7 Comparison of algal periphyton in eutrophic and oligotrophic lakes (adapted from Lowe, 1996)

	Oligotrophic, soft-water lake	Eutrophic, hard-water lake
Eulittoral Zone	**Unicellular and colonial epilithic algae**	**Filamentous and epiphytic algae**
Filamentous forms	**Green algae**: (Zygnemataceae) *Mougeotia*	**Green algae**: *Cladophora, Ulothrix, Oedogonium* **Red algae**: *Bangia*
Unicellular and simple colonial	**Green algae:** (Desmids) *Cosmarium, Stuarastrum* **Diatoms:** *Eunotia, Tabellaria*	**Blue-greens:** *Chamaesiphon Lyngbya* **Diatoms**: *Cocconeis, Cymbella*
Sublittoral Zone	**Extensive growth of diatoms and blue-green algae**	**Few taxa, below the photic zone**
Filamentous and colonial	**Blue-greens:** *Hapalosiphon, Calothrix*	
Unicellular	**Diatoms:** *Eunotia Navicula*	

lakes. Changes in the light regime of lake sediments and the periphyton community may arise secondarily due to changes in nutrient levels and other chemical effects. The deep-water periphyton habitat has been eliminated in many lakes, for example, where increased phytoplankton levels resulting from eutrophication have lead to a reduction in the depth of light penetration. Conversely, acidification of poorly-buffered lakes tends to have a direct impact on phytoplankton (more than periphyton), reducing phytoplankton populations, increasing light penetration, and secondarily increasing periphyton biomass (Lowe, 1996).

3.6.3 Benthic algae in flowing waters

In rivers, benthic algae become the main algal component in the ecosystem due to the displacement of phytoplankton by the water current, and its subsequent loss from the system. These algae are frequently attached to the substratum and include both macroscopic filamentous forms (*Cladophora*, *Spirogyra*, *Chara*, *Vaucheria*) and microscopic forms (particularly diatoms) which are part of the periphyton assemblage. Attached algae may be grouped in terms of the substratum, with some present on higher plants and macro-algal surfaces (epiphytes), while others are associated with inorganic substrates such as rocks (epilithic algae), sand (epipsammic algae), and fine sediments (epipelic forms). Dispersal of algae within freshwater systems occurs primarily by water movement of detached single cells and fragments of filaments, which contribute to the limited plankton occurring in this environment (Fig. 2.14). It seems likely that some algae may also enter the atmosphere in aerosols and undergo aerial dispersal. A wide selection of Chlorophyta (including *Chlorella*, *Chlorococcum*, and *Chlamydomonas*) have been recovered from aerial samples, with a much smaller range of chrysophytes and blue-green algae also identified (Brown *et al*., 1964).

3.6.4 Ecological role of benthic algae

Benthic algae play a major ecological role in streams, wetlands, estuaries, and shallow lakes, where they are important in primary production, chemical transformation, retention of nutrients by sediments, physical stabilization of sediments and the provision of habitats for other organisms (Stevenson, 1996).

Primary production by benthic algae

Although the main source of energy in streams was once thought to be detritus from terrestrial origin, it

is now realized that primary production by benthic algae is equally important in many mid-sized streams (Minshall, 1978). Benthic algae are also significant primary producers in shallow lakes, ponds, and wetlands. In wetlands, photosynthetic activity and growth of epiphytic algae (with phytoplankton) occurs at those times of year when higher plants can be out-competed due to low macrophyte growth and high algal turnover rates (Section 4.8).

Chemical transformation

In many aquatic ecosystems, benthic algae have an important role as chemical modulators, transforming inorganic chemicals into organic forms. In low-nitrogen habitats, for example, benthic blue-green algae play a key part in the nitrogen cycle – converting N_2 to NH_3 and amino acids. In medium to high nitrogen habitats benthic algae are also involved in the daytime uptake of nitrate, leading to a diurnal variation in the concentrations of this nutrient in some streams. Benthic algae are also primary harvesters of inorganic phosphate in stream nutrient spiralling (Case Study 5.1) and in the removal of phosphate from inflow steams at the edge (littoral zone) of lakes and wetlands.

Nutrient retention by sediments

Benthic algae on surface sediments intercept the passage of inorganic nutrients into the water body, acting as important sinks prior to release into the water column. Epiphytic algae also have a particularly important role to play on wetlands, where macrophytes pump nutrients out of the sediments into the main water body. Epiphytic algae present on the macrophyte surfaces trap nutrients before they reach the water column, returning them to the sediments when the algae settle to the bottom.

Physical stabilization of sediments

Substrata in a wide variety of aquatic habitats are stabilized by benthic algae, which prevent displacement of the sediment at times of aquatic disturbance. This stabilization ranges from the presence of epipelic diatoms and other unicellular algae on estuarine mudflats to the extensive overgrowth of filamentous forms (blue-greens, *Vaucheria*) on sands and other coarse sediments. In addition to this stabilization effect, benthic filamentous algae can also trap inorganic materials, leading to sediment build-up in regions of algal colonization.

Habitats for other organisms

Communities of benthic algae are important habitats for many other aquatic organisms. These communities provide refuges from high current flow, protection from predators, and access to other organisms (potential food supply) within the localized area. Hummocks of *Chara*, for example, support a considerable density and diversity of aquatic invertebrates in streams where sand particles provide conditions which are otherwise too unstable and low in nutrient to support invertebrate populations. Cladophora and other filamentous algae support considerable numbers of epiphytes, along with populations of small invertebrates such as chironomids, amphipods, and small meiofauna. Even algal biofilms, dominated by diatoms, can support substantial numbers of chironomids and meiofauna.

3.7 Temporal activities of freshwater algae

Freshwater environments are relatively unstable and subject to continuous change, varying from transient alterations in environmental factors (e.g., light intensity) to longer-term changes in hydrology and climate. The ability of algae to respond to changes in their microenvironment, and in the ecosystem as a whole, is clearly important to their biological success.

Temporal activities of freshwater algae (Figure 3.12) vary with the time interval that is being considered (fractions of a second to hundreds of years) and include short-term cellular events,

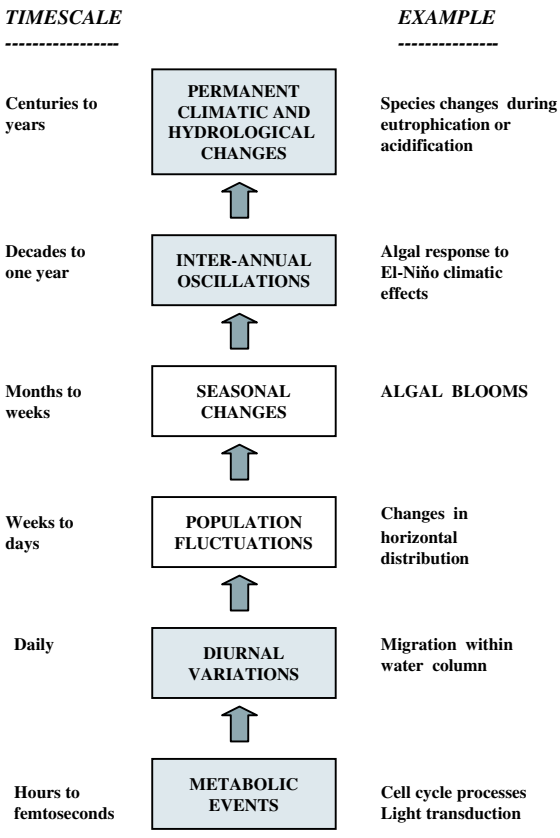

Figure 3.12 Temporal activities of freshwater phytoplankton. The time scale may be arbitrarily divided into short-term (bottom shaded) boxes, medium-term, and long-term (top boxes) periods

medium-term changes in algal succession, and long-term alterations to the ecosystem. Some of these aspects are considered below, while others are discussed elsewhere – including diurnal activities (Section 4.10) and algal bloom formation (Section 10.6).

3.7.1 Short-term changes: molecular and cellular processes

Physiological processes within algal cells occur over periods ranging from femtoseconds (10^{-15}s, e.g., light harvesting and electron transfer processes) to minutes and hours (cell cycle events).

Rapid molecular events: light harvesting and electron transfer in photosystem II

In eukaryote algae, the initial harvesting of light and subsequent generation of high-energy electrons provides a good example of rapid molecular processes at the short end of the timescale shown in Figure 3.12. These processes occur in the 400kD photosystem II light-harvesting complex (LHC II) present in plastid thylakoid membranes and involve the activities of two separate molecular structures – the antenna complex and photochemical reaction centre (Figure 3.13).

Light photons are collected by chlorophyll molecules within the antenna complex. The energy of excitation from a single photon is then transferred to chlorophyll P680 within the photochemical reaction centre, raising the energy of an electron in the reduced molecule from its ground state to a highly excited state. This high-energy electron is then captured by a pheophytin (Pheo) acceptor molecule, converting chlorophyll P680 to $P680^{+}$ in one of the most powerful biological oxidation reactions. The reduced state of $Pheo^{-}$ then stimulates electron capture by the quinone acceptor Q_A, with subsequent serial electron transfer to Q_B, plastoquinones, cytochrome b6/f, and on to Photosystem I. At times of inactivity, the reaction centre is in an 'open' condition, with P680 in a reduced state and the electron acceptors Pheo, Q_A, and Q_B in an oxidized form. Photon excitation of P680 initiates the flow of electrons within the PSII reaction centre and on to the plastoquinone pool.

The time course of these molecular events is extremely rapid (Kolber and Falkowski, 1993). Following the initial impact of a photon of light, excitation of P680 occurs within 100fs, reoxidation of Q_A- occurs within 0.6 ms, and the onward passage of electrons from the plastoquinone pool occurs within 2–15 ms, depending upon water temperature. A single linked Photosystem II pathway can process up to 66 reactions per second at near freezing temperatures and up to 500 at about 50°C, provided that the PSII reaction centre continues to receive the next electron the instant that it reopens. Under such conditions, the pathway would be operating at full capacity and the rate of photosynthesis

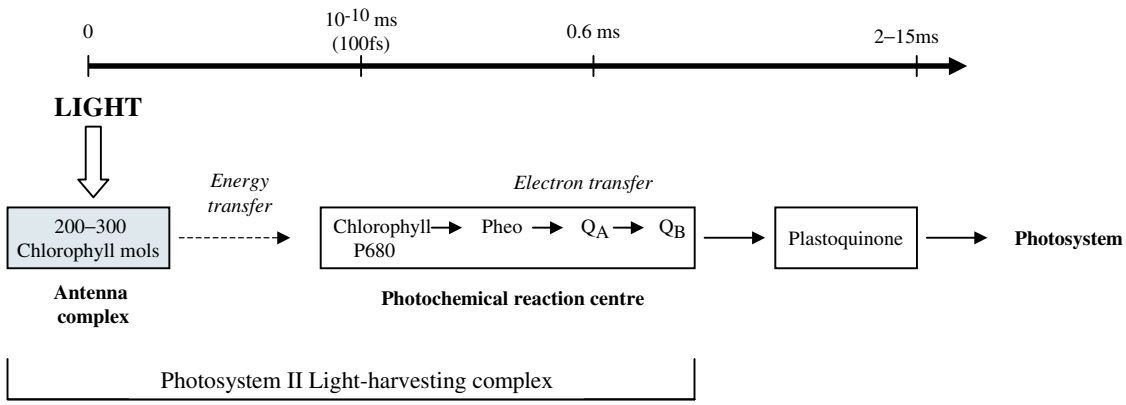

Figure 3.13 Rapid molecular events (femtoseconds to milliseconds) within the Photosystem II Light-Harvesting Complex (LHCII) of eukaryote algae (see also Figures 4.6 and 4.24)

would be at the point of light saturation by the incident photon flux density.

Light harvesting and electron transfer in the PSII light-harvesting complex initiate the process of photosynthesis in eukaryote algae (Section 4.2) and are a key part of the light-dependent reactions occurring in the thylakoid membranes (Figure 4.4). Further information on the PSII photochemical reaction centre is given in relation to photoinhibition and the effects of UV-B radiation on algal cells (Section 4.9, Figure 4.24).

Cell cycle processes

Cell cycle processes provide an example of longer-term physiological processes, operating over periods of minutes to hours. These processes include aspects such as new cell-wall formation, which is initiated once cytokinesis has occurred. In the case of diatoms, this involves the formation of a silicon deposition vesicle and the activities of special cell-wall forming proteins (Figures 5.28 and 5.29).

The duration of the cell cycle, from daughter cell, through growth and division, to the next daughter cells, varies considerably between algal species. Under conditions of optimum temperature, nutrient supply, and light, duration depends primarily on cell size – with the shortest cell cycles being recorded for picoplankton such as *Synechococcus* (where duration may be as low as 2 hours). Minimum cycle length for nanoplanktonic algae such as *Chlamydomonas* and *Chlorella* are about 6 hours, while larger single-celled organisms (e.g., dinoflagellates) and colonial forms typically have much longer cell cycles.

The overall duration of the cell cycle depends on the time required for doubling of the biomass, which in turn depends on the uptake of sufficient quantities of nutrients such as carbon, nitrogen, and phosphorus. Where this uptake occurs as a simple diffusion process (e.g., CO_2), the minimum time taken for completion of the activity can be calculated in terms of physical parameters and cell size.

Time required for diffusion of CO_2 in *Chlorella* The number of moles of a solute (n) which can diffuse across an area of cell membrane (a) per unit time (t) depends on the gradient in solute concentration (C_o) at the cell surface (dC_o/dx) and the coefficient of molecular diffusion (m) of the substance. The value for 'n' can be determined (Reynolds, 1997) from the equation:

$$n = a\,m(dC_o/dx)t \qquad (3.5)$$

Using the calculations of Reynolds (1997), a single spherical cell of *Chlorella* with a diameter of about 4×10^{-6} m would have values for surface area (a) and volume (v) of 50.3×10^{-12} m^2 and 33.5×10^{-18} m^3 respectively, and a carbon content of 0.63×10^{-12} mol. Aquatic concentrations of

CO_2 in air-equilibrated conditions range from 11–23 μmol CO_2 l^{-1}. With an external CO_2 concentration of 11 μmol CO_2 l^{-1} and a molecular diffusion coefficient (m) of about 10^{-9} m^{-2} s^{-1}, the rate of solute diffusion (n) would be 275×10^{-18} mol s^{-1}. This assumes a constant diffusion gradient, with depletion of CO_2 from a water thickness equal to the cell radius. At this rate, the time taken to deliver the entire doubling requirement of carbon to the cell by simple diffusion would be about 2300 s or just over 38 minutes. Once carbon has entered the cell, the time requirements (per cell cycle) of natural daylight for photosynthesis and the duration of carbon fixation have been estimated at about 25 minutes and 4.4 hours respectively.

With other nutrients, temporal values for the doubling requirement uptake by *Chlorella* of nitrogen and phosphorus (by simple diffusion) have been calculated at 540 s (9 minutes) and 2360 s (just over 39 minutes) respectively (Reynolds, 1997).

Responses to environmental change may involve a gradation of temporal processes. The up-regulation of nitrate reductase (NR) during light-mediated nitrate assimilation, for example. involves both short-term (within minutes) regulation by an activation/inhibition system and longer-term (within hours) light-induced gene activation and NR induction (Figure 5.13).

3.7.2 Medium-term changes: algal succession

Algal succession involves temporal changes in the biomass and species composition of natural populations. These changes are an important aspect of aquatic ecosystems, since they typically define the major microbial biomass (micro-algae) within the environment and have a major impact on other fresh water biota.

Algal succession will be considered in relation to two particular examples – (a) seasonal changes in lake phytoplankton (1 year period), and (b) algal succession in the development of stream biofilm and periphyton communities (a period of weeks to months). Although these transitions provide good examples of medium-term changes in the time scale sequence (Figure 3.12), long-term changes in phytoplankton succession are also an important aspect of lake development (see next section). Seasonal changes in lake phytoplankton and biofilm succession are similar in some respects. In both cases there is a clear algal transition, with early successional species being replaced by late successional species in a defined and predictable sequence. Algal succession in phytoplankton differs from that of biofilms in being relatively long-term, and being determined to a large extent by abiotic factors (e.g., light and temperature). Unlike biofilms, seasonal changes in phytoplankton populations do not involve permanent change either in the biota or in the physical environment.

The changes in species composition which occur during pelagic and benthic algal succession often involve interactions between algae. These interactions may be direct or indirect and are of four main types – induced environmental change, species competition, species antagonism, and non-competitive adaptation (Tuji, 2000; McCormick and Stevenson, 1991).

- *Induced environmental change*. Early successional species bring about changes in the local environment which either facilitate or inhibit the establishment and growth of subsequent species.

- *Species competition*. Competition arises when organisms depend on the same consumable resource and when that resource is present in limited supply. Consumption of this resource by one species limits access by other species and reduces their growth and development. Competition occurs in relation to three major resources – light, space, and nutrients.

- *Species antagonism*. Where direct inhibition of other species is mediated via anti-microbial compounds such as antibiotics.

- *Non-competitive adaptation*. Species change is determined largely by adaptations to particular environmental conditions which occur at different times in the sequence rather than by direct interactions between species.

Species succession in phytoplankton populations are driven mainly by non-competitive adaptation

(e.g., early spring growth of diatoms under conditions which other algae cannot tolerate) and species competition. In contrast to this, community changes in biofilms are more equally determined by all of these factors.

Seasonal changes in phytoplankton populations

In temperate climates, well-marked changes in the total biomass and species composition of lake phytoplankton occur over the annual cycle. Individual species show bursts of population growth, which typically last for 30–100 generations, and can react rapidly to shifts in environmental conditions (Heinonen *et al*., 2000). Seasonal changes in a typical temperate eutrophic lake are shown in Figure 3.14 and can be separated into four major phases: spring diatom bloom, clear-water phase, mixed summer/autumn bloom, and overwintering phase (Steinberg and Geller, 1993).

Spring diatom bloom The massive increase in population of diatoms seen in spring is triggered by

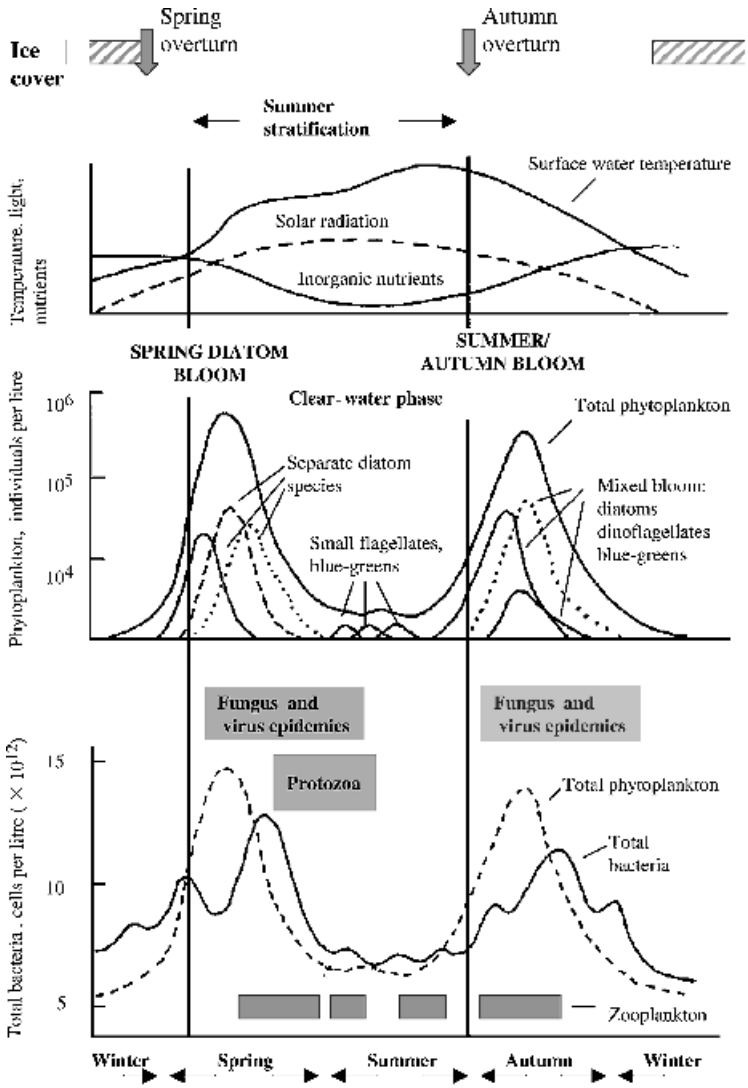

Figure 3.14 Effects of seasonal changes in the physical environment on microbial populations in the pelagic zone of a temperate lake (based on a figure from Horne and Goldman, 1994) Increases in solar radiation lead to the development of a spring diatom bloom, which reaches a peak after stratification. The bloom becomes limited due to depletion of inorganic nutrients in the epilimnion, and is followed by a clear-water phase (low algal biomass) and then a late summer/autumn bloom. Epidemics of fungi and viruses that infect the phytoplankton contribute to the termination of the blooms. Zooplankton grazing is particularly intense in the clear-water phase and involves a sequence of crustacean and rotifer populations throughout the summer and autumn period. Heterotrophic bacteria feed on the excretory products (dissolved organic carbon) of the phytoplankton and have major peaks which follow the algal blooms (Straskrabova and Komarkova, 1979). Populations of ciliate and flagellate protozoa, which graze the bacteria, follow on from the bacterial peaks

a rise in water temperature and an increase in light intensity and daylength. These changes initially occur in the unstratified lake, where diatoms are well suited to the turbulent state of the water and are able to grow under conditions of relatively low light intensity.

Early in the spring diatom bloom the lake becomes stratified, leading to the isolation of the surface layer (epilimnion). Nutrients within this layer – particularly Si and P – become depleted, ultimately falling to levels which may limit diatom growth. Limitation may also occur due to fungal infection and zooplankton grazing, leading to a crash in the diatom population.

Clear-water phase The end of the diatom bloom is characterized by a period of low algal biomass and high zooplankton population. Under conditions of high zooplankton grazing pressure, algae are restricted to species with rapid growth and short cell cycles (*r*-selected species, Section 3.10.2). Zooplankton populations occur as irregular and patchy groups within the water column, and development of algal populations is transient within the rapidly changing biotic environment.

During this phase, phytoplankton is dominated by small unicellular algae, particularly cryptomonads and green algae. Towards the end of the clear-water phase the development of significant numbers of inedible algal species leads to a decline of zooplankton and the start of the summer bloom.

Mixed summer/autumn bloom The mixed summer/autumn algal bloom develops under conditions of increased temperature, day length and light intensity, and decreasing epilimnion nutrients (particularly soluble N and P). This phase is characterized by a succession of blue-green algal populations and dinoflagellates (all of which are inedible to zooplankton) and diatoms. The occurrence of high populations of inedible algae means that the zooplankton population is maintained at a low level at a time when the algal biomass reaches maximum seasonal levels. The final part of the summer bloom occurs with the autumn overturn. Destratification of the lake results in a large-scale increase in inorganic nutrients (N, P, Si) which promotes the growth of a wide range of algae – including diatoms – at the end of the growing season.

Over-wintering phase Progressive reduction in temperature and light leads to reduction in algal growth. Circulation of algae within the water column also leads to phytoplankton populations, with many algae sinking to the bottom of the lake. Throughout winter, phytoplankton biomass is limited and is dominated by diatoms and blue-green algae.

The four major phases seen in the above sequence may vary in magnitude within an individual lake from one year to the next. This can be seen in Figure 9.12, where the annual cycle for Rostherne Mere (UK) in the year 2000 was unusual in having the spring diatom bloom repressed by intense calanoid copepod grazing (Section 9.8). This led to a 'clear-water' phase which was dominated by the expected *r*-selected algae (particularly cryptomonads), but these developed to bloom proportions in this normally low-biomass phase.

The seasonal sequence also varies considerably with lake trophic status and water circulation characteristics. Oligotrophic and continuously mixed (polymictic) lakes tend to have diatom dominance throughout the entire summer period. In stratified lakes, increasing nutrient level causes a progressive decrease in the extent of the early seasonal diatom bloom (Table 10.4).

Algal succession in the development of stream biofilm and periphyton communities

Benthic colonization of stream environments frequently leads to the development of a periphyton community. This is dominated by attached algae, but also contains a wide range of associated microorganisms and invertebrates. The development and continuation of the periphyton community involves four major stages (Figure 3.15):

- initial colonization of the exposed surface,
- development of a diatom biofilm,
- colonization of the biofilm by filamentous algae to form a mature periphyton community,

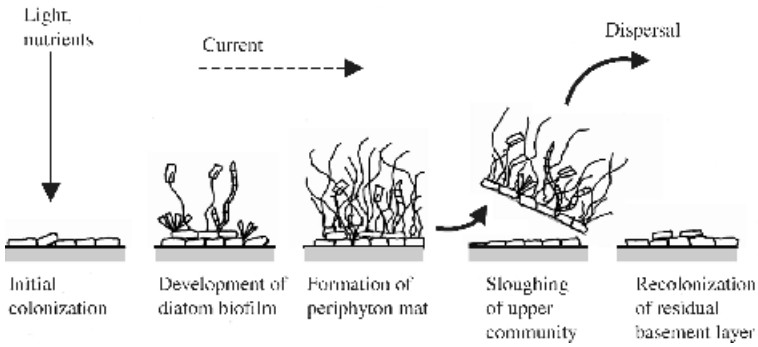

Figure 3.15 Accumulation of benthic algal cells on river substrata and the process of periphyton mat development (based on a figure from Tuchman, 1996)

- loss of fragments of the community due to the stream current, leading to exposure of earlier surfaces and regeneration of a new community.

Colonization and biofilm development Diatoms are particularly important in the initial colonization of exposed surfaces and the formation of an algal biofilm. Various authors (Tuji, 2000; McCormick and Stevenson, 1991) have demonstrated a clear diatom succession, with transition from early colonizing (phase I) to mid-successional (phase II) and late-successional (phase III) species. The position of a particular species within this time sequence depends on its growth form, growth dynamics, and adaptation to a high- or low-light environment (Table 3.8).

Table 3.8 Diatom succession in algal biofilms (information from McCormick and Stevenson, 1991, and Tuji, 2000)

Species	Growth form	Growth Dynamics	Light Adaptation
Early colonizers (phase I)	*Attached diatoms*		High irradiance species High G_{max} and I_s values
Gomphonema angustatum	Short chains of cells	Settlement from high population level in water column	
Meridion circulare	Attached rosettes	Fast immigration due to rapid reproduction after attachment	
Surirella ovata	Single cells or pairs	Fast immigration due to rapid attachment	
Mid-successional species (phase II)	*Attached diatoms*		Low irradiance species Low G_{max} and I_s values
Cymbella sp., *Gomphonema olivaceum*	Vertical growth via long mucilaginous stalks	High growth rate maintained in dense algal conditions	
Late-successional species (phase III)	*Unattached, tangled diatoms*		Low irradiance species Moderate G_{max} and very low I_s values
Fragilaria vaucheriae	Unicellular	High growth rate maintained in dense algal conditions	
Aulacoseira varians	Chain forming		
Cocconeis placentula	Prostrate		

Light adaptation parameters: G_{max} – estimated maximum growth rate, I_s – estimated light intensity at half G_{max}

The rate of colonization of the newly exposed surface (r), which occurs by settlement from planktonic populations, depends on the summation of several processes (McCormick and Stevenson, 1991):

$$r = (R - D) + (I - E) \qquad (3.6)$$

where r = rate of accumulation of benthic diatoms, R = reproductive rate, D = death rate, I = immigration rate, and E = emigration rate.

In the above study, five diatom species dominated the colonization sequence – with three phase I and two phase II species. Early successional species were adapted in different ways for rapid colonization of the substrate by having high population counts in the water column (high immigration rate), high probabilites of attachment (low emigration rate), or high levels of division (high reproductive rate) on the exposed surface. Early colonizers had a limited growth form, which restricted their development to the immediate surface of the substratum. Mid-succession species were slow to establish, but then had high growth rates. The ability of these phase II diatoms to develop mucilaginous stalks gave them a vertically-extended growth which allowed them to displace earlier species by out-competing them for light, nutrients, and space. The final phase of diatom development is characterized by the entry of diatom species which cannot attach to the substratum, but become entangled within the vertical community of stalked diatoms. These phase III diatoms include both unicellular organisms and chain-forming species.

With development of the biofilm, there is a transition from high irradiance (early colonizers) to low irradiance (subsequent colonizers) species (Tuji, 2000) in line with the development of a vertical structure to the community. Early colonizers have high values for maximum growth rate (G_{max}) and half-saturation coefficient (I_s) – (Table 3.8). Phase II diatoms produced stalks at low-light intensities, increasing their elevation within the biofilm, and showing clear low-light adaptations. Phase III species were highly adapted to low irradiance, with very low I_s values. These diatoms are able to survive the very low-light levels within the middle of the dense diatom community, where they show substantial growth (moderate G_{max} values). Comparison of the irradiation responses of these three groups of biofilm diatoms shows that the efficiency of light is closely linked to successional strategy.

In disturbance-prone ecosystems such as rapid-flowing head-water streams, algal succession may not progress beyond the formation of a diatom biofilm. In less stressful environments an extensive periphyton community may develop.

Maturation of the periphyton community

Further development of the biofilm involves the formation of a mixed algal community of stalked diatoms, filamentous green and blue-green algae, forming a loosely associated upper canopy. Resources become limiting at the base of the mat as cell densities increase, forming a vertical resource gradient. Cells in the upper canopy experience conditions of high light and access to inorganic nutrients in the water column, while cells in the lower part of the periphyton mat experience low light and low nutrient levels (Tuchman, 1996). Photoadaptation to low light involves an increase in photopigment concentrations to maximize photon collection. Heterotrophy may also be important as a survival mechanism for basement cells.

As the periphyton mat grows and extends into the water medium, periodic sloughing due to current shearing forces results in exposure of the original substratum or regions of the lower canopy. Recolonization of this exposed surface results in a continuation in the cycle of biofilm development.

In biofilm succession, where community change is occurring over a short (2–3 week) period, seasonal changes in the environment are not important. Algal succession is determined initially by non-competitive adaptation (establishment mechanisms) followed by induced environmental change (substrate formation), species competition, and possibly species antagonism.

3.7.3 Long-term changes: variations over a number of years

Inter-annual oscillations

Although lakes and reservoirs of temperate regions typically show a repeated annual pattern of seasonal

change, these freshwater systems also show variations from one year to the next (Hoyos and Comin, 1999). Such inter-annual variability is an intrinsic feature of many water bodies and is caused by climatic fluctuations and the effects of human activities. Regional and local climatic variations are responsible for differences in a wide range of parameters which have a direct influence on phytoplankton populations, including physical (timing of ice break-up, mixing depth, water residence time), chemical (concentrations of inorganic nutrients), and biological (over-wintering survival of fish and zooplankton populations) characteristics. As a result of these variations, phytoplankton populations show marked annual differences in overall productivity and the precise sequence of species succession. Lake Sanabria (Spain) provides a good example of this, where climatic variations in rainfall have a marked effect on water residence time, affecting the accumulation of nutrients and the development of blue-green algal blooms (Hoyos and Comin, 1999).

In many cases, local conditions relate to broader climatic trends, such as the El-Niño Southern Oscillation. This low frequency (within a decade) variation has been shown (Anderson *et al*., 1996) to correlate well with observed inter-annual variability in temperate lakes such as those of the Wisconsin area (USA). Distances over which these climatic influences extend may be considerable, as shown by the sensitivity of lakes in southern Europe to climatic variability caused by ocean–atmosphere interactions in remote areas of the South Pacific (Rodo *et al*., 1997).

Long-term permanent changes in lakes

Long-term changes to lakes also involve more permanent alterations than the inter-annual oscillations seen previously, extending beyond a 10 year period and in some cases to hundreds of years. These chronic changes relate to internal factors (such as the build-up of organic material in the lake, leading to long-term eutrophication) or to external changes such as heavy-metal pollution, prolonged inflow of nutrients (eutrophication), and the effects of acid rain (acidification).

Confirmation of long-term changes in general ecology and resident phytoplankton requires an extended data series, which can be obtained by analysis of lake sediments. In many lakes, uninterrupted deposition of both phytoplankton and benthic algae occurs over a prolonged time period, leading to the formation of well-preserved layers in chronological sequence. Analysis of diatoms within these sediment cores is particularly useful, since these organisms can be readily identified from their preserved frustules and are useful indicators of particular pH and nutrient conditions.

Long-term changes in algal populations have been demonstrated from sediment analyses in relation to the following.

- *Heavy-metal pollution*. Studies by Cattaneo *et al*. (1998) on the sediments of Lago d'Orta (Italy) showed taxonomic shifts in the populations of algae (diatoms) and other lake biota (thecamoebians, cladocerans) over a 50 year period, as a response to industrial effluent contamination (primarily copper pollution). These studies have also demonstrated the long-term inverse relationship between size and environmental stress (Sections 1.6 and 3.4.3).

- *Eutrophication*. Various indices of algal species composition can be employed in the assessment of trophic status (Section 10.3.1), including the proportion of araphid pennate/centric diatoms (A/C ratio). This has been used by Byron and Eloranta (1984) to study long-term changes in Lake Tahoe (USA), where sediment analyses demonstrated a dramatic shift in the A/C ratio during the 1950s (Figure 10.3). These diatom changes indicate a sudden transition from ultra-oligotrophic to oligotrophic status at a time of increased lakeshore human habitation.

- *Acidification*. Studies on the sediments of Loch Dee (UK) have demonstrated a long-term acidification of the lake (pH 6.3 to pH 5.6) over a 130 year period (Flower *et al*., 1987). During this time, diatoms typical of near-neutral conditions (*Cyclotella comensis*, *Asterionella formosa*) have been replaced by diatoms more adapted

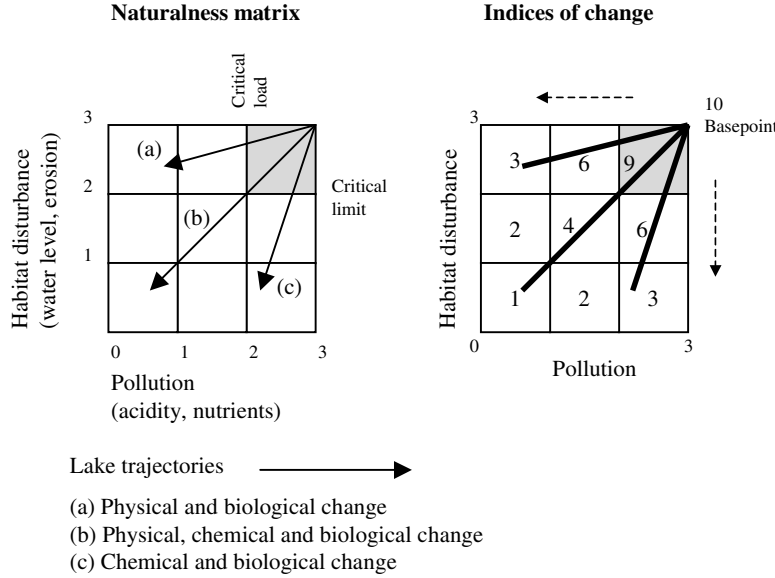

Figure 3.16 Diagrammatic representation of long-term biological changes in lakes in relation to pollution and habitat disturbance. *Left*: hypothetical changes over time for three lakes (a), (b) and (c). *Right*: quantitative expression of these changes in relation to the original (or natural) state, with 1 = most changed, 9 = least changed, and 10 = pristine. Restoration of the lakes, with changes in phytoplankton and other biota back to the original state, would involve a return to the 'target box' (shaded square) (based on a figure from Battarbee, 1999)

to a lower pH (*Eunotia veneris*, *Frustulia rhomboides*).

With many lakes, long-term effects not only involve changes in water quality but also physical disturbance, including changes in water level and erosion. These different effects are largely independent and can be visualized in terms of a 'naturalness index', representing the degree to which a lake has changed in relation to its natural baseline. This concept has been developed in relation to lake rehabilitation, where the direction of restoration would be towards a target ecosystem of minimal habitat disturbance and pollution, represented by the 'target box' in Figure 3.16 (Battarbee, 1999).

3.8 Phytoplankton distribution within the water column

The vertical distribution of phytoplankton in a stratified lake is highly dynamic and is closely related to environmental parameters and their seasonal/diurnal changes within the water column. It has major importance for the growth and survival of the algae concerned, and also has implications for the biology of other organisms such as bacteria, protozoa, and zooplankton. The vertical distribution of phytoplankton is considered initially in relation to a particular lake (case study), with subsequent discussion of diurnal migration and vertical positioning.

Case study 3.1 Vertical zonation of Phytoplankton in a stratified lake

Distribution of algae in the water column of a mixed (unstratified) lake may be relatively uniform, but the development of stratification imposes major vertical differences in the physical–chemical environment and in the occurrence of algae. A specific example of algal zonation is illustrated in Figure 3.17, which shows

the mid-day vertical pattern of different phytoplankton groups during bloom formation in a stratified eutrophic lake (Rostherne Mere, UK). At this time of year, the lake was dominated by dinoflagellates (mainly *Ceratium hirundinella*) and blue-green algae (mainly *Anabaena minima*), populations of which showed high levels in the epilimnion (0–4 m), low levels in the metalimnion (4–8m) and moderate levels in the hypolimnion (10–22m). Other algal groups–diatoms, green algae, chrysophytes and cryptophytes occurred at much lower biomass levels, but had a broadly similar distribution.

These patterns of algal distribution relate to zonation in the physico-chemical environment (Figure 3.18), with highest algal populations occurring under conditions of high light intensity, temperature, and oxygen concentration (epilimnion). Algal populations were at a minimum under conditions of severe oxygen depletion (metalimnion), rising to moderate levels in the lower part of the water column – where oxygen levels were restored and concentrations of major nutrients (P, N, Si) were at a maximum.

Although the distributions of particular groups were broadly comparable, differences in detail did occur. Dinoflagellates and blue-green algae had their highest surface concentrations within the top 2 m of the surface, for example, while diatoms peaked at a depth of 2–4 m, and green algae at 4–6 m. Individual species also showed major differences in their vertical distribution, suggesting that different algae have different strategies determining their position in the water column.

In general, the distribution of algae at any one point in time within the water column of a lake will depend on three major factors – active migration, passive movement, and pre-existing population levels (Figure 3.19).

3.8.1 Active Migration of Algae

Active migration involves the ability of algae to move themselves independently within the water body and is mediated by the intracellular production of gas vacuoles (buoyancy mechanism) or by some type of active motility (flagella or streaming movements). Algae (such as diatoms and non-motile chlorophytes) which do not possess either of these mechanisms are not capable of independent movement within the water column. During the period of major growth within the water column, migration of phytoplankton involves two distinct but interrelated activities – diurnal migration (large-scale movement) and depth regulation (small-scale vertical positioning) in relation to external stimuli. The entry of algal cells into the water column from the benthic sediments at the beginning of the growth phase is a further major migratory activity of these organisms (Section 3.10.1).

Individual algae and algal groups differ in the extent to which they exhibit diurnal migration or vertical positioning (Table 3.9). For three of the groups (chrysophytes, cryptophytes, and green algae) diurnal migration is not well documented, but these algae do show strong vertical positioning within lake strata – in some cases over long periods of time (Sandgren, 1988b). Other groups, such as dinoflagellates and blue-green algae, are more defined in their patterns of diurnal migration, but also show vertical positioning within their diurnal cycle.

Diurnal migration of algae

Many freshwater algae have the ability for diurnal migration between the lake surface (epilimnion) and deeper layers (metalimnion or hypolimnion), moving up the water column during the morning and down later in the day. This migration allows these organisms to combine day-time photosynthesis in the euphotic zone with night-time uptake of nitrates and phosphates in the nutrient-rich hypolimnion, and has been investigated particularly in relation to dinoflagellates and blue-green algae.

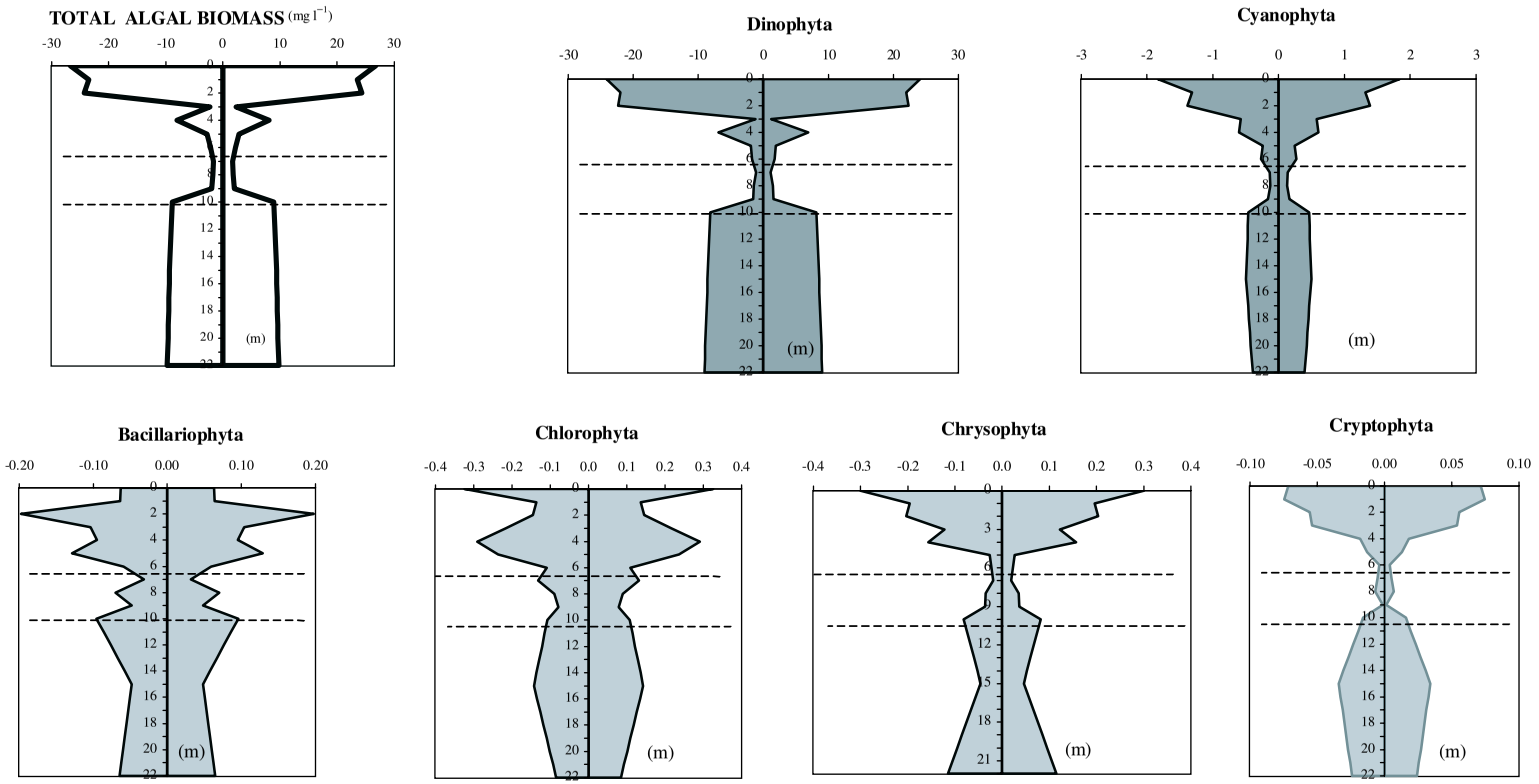

Figure 3.17 Mid-day phytoplankton distribution in the water column of a stratified eutrophic lake (Rostherne Mere, UK) during the late summer bloom. The biomass of total hytoplankton, and algae within different taxonomic groups, is expressed as mg l^{-1} (total width of each graph) and shows considerable variation with depth. The main thermocline is indicated by broken lines. The vertical distribution in total algal biomass (top left) may show major differences from the profile of chlorophyll concentration (Section 4.6.3) (taken, with permission, from Levado, 2001)

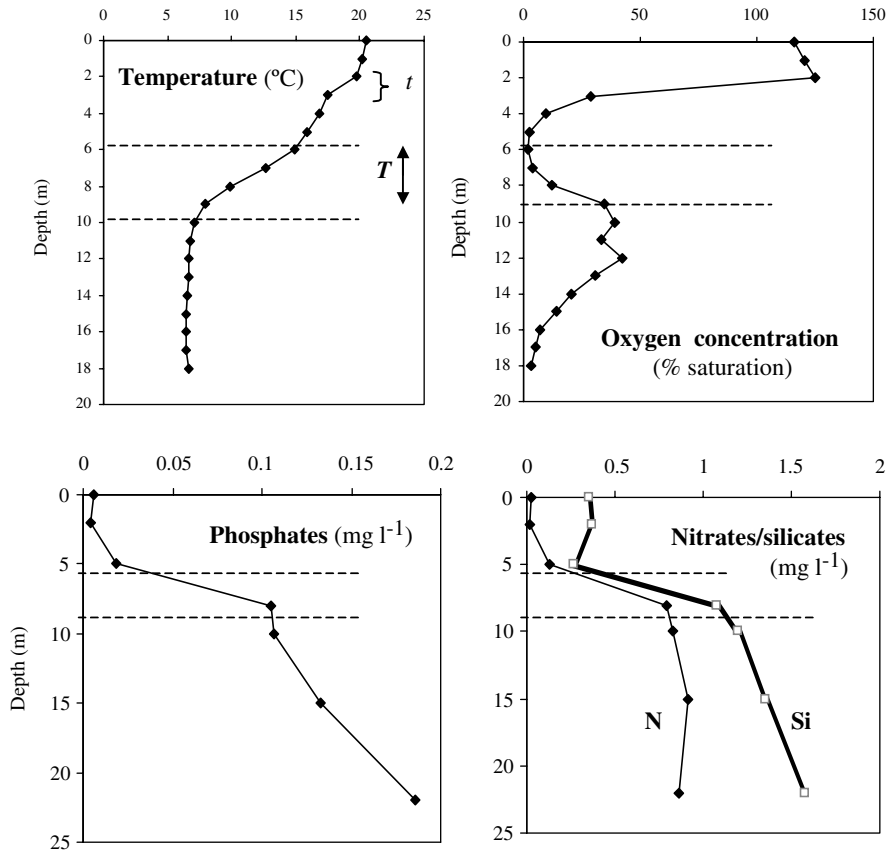

Figure 3.18 Physico-chemical characteristics of the water column during the late summer (August) bloom of a eutrophic lake (Rostherne Mere, UK). The water column shows clear thermal stratification, with a well marked thermocline (*T*) at 6–10 m, and a hypolimnion below 10 m which is stable at 6°C. The epilimnion is biphasic, with a temporary thermocline (*t*) at 2–3 m. Depth changes in oxygen concentration are highly complex, with supersaturation (reaching 130 per cent) at the top of the epilimnion – typical of an intense surface bloom, dropping to minimal levels in the lower epilimnion and thermocline. The upper hypolimnion has a broad peak of oxygen concentration (reaching 50 per cent saturation) corresponding to a region of high algal biomass (see Figure 3.17). Inorganic nutrients (phosphates, nitrates, silicates) are almost completely depleted in the epilimnion, but reach high levels in the hypolimnion (taken, with permission, from Levado, 2001)

Diurnal migration of dinoflagellates As with blue-green algae, the diurnal movement of dinoflagellates relates to their strong migratory mechanism and their ability to dominate stratified lakes in late summer. At this time of year, these algae are only able to maintain their growth by nutrient uptake from the lower parts of the water column. Some evidence for hypolimnetic uptake has been provided by studies of Mitchell and Galland (1981), where sampling of *Gymnodinium* at Lake Mahinerangi (New Zealand) in the early morning showed that cells located in the hypolimnion (12 m depth) had higher concentrations of phosphorus compared with cells present at the lake surface.

Dinoflagellates, such as *Peridinium* and *Ceratium*, are strongly motile and positively phototactic, with clear patterns of diurnal migration – rising to the lake surface during the light period and descending

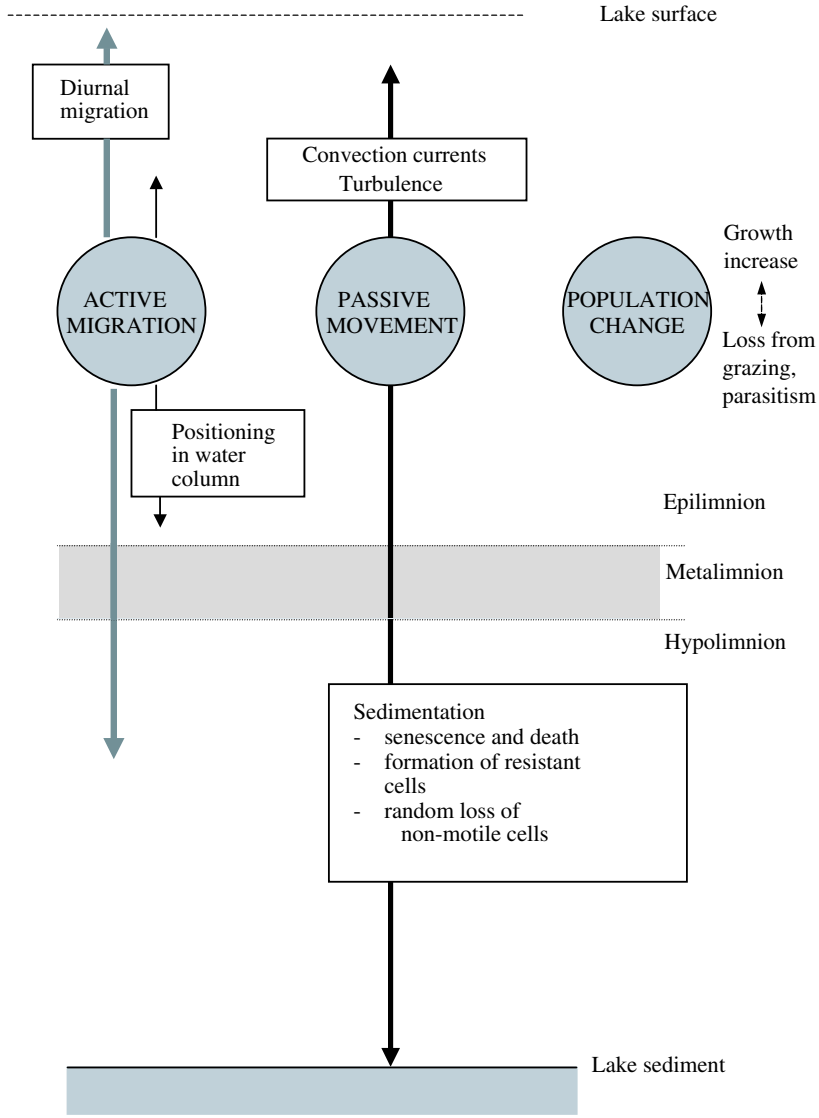

Figure 3.19 Factors affecting the occurrence and distribution of phytoplankton in the water column of a stratified lake

or dispersing at night. The diurnal migration of these organisms is closely tied to a circadian rhythm in their phototactic response, which is high in the first half of the day and subsequently declines. Patterns of dinoflagellate diurnal migration vary with light gradients, and are also influenced by temperature variations, nutrient limitation, and population age (Pollingher, 1988).

Buoyancy migration and gas vacuole formation in blue-green algae Buoyancy migration in blue-green algae is promoted by the formation of gas vesicles (Waaland and Branton, 1969), which are present within larger membrane-bound gas vacuoles. The only other group to possess these gas vesicles are bacteria, and they are almost exclusively restricted to microorganisms living in

Table 3.9 Migratory characteristics of motile algae within the water column of standing waters

Algal group	Diurnal migration	Positioning in water column
Blue-green algae[a]	Effective buoyancy mechanism with well-defined diurnal migration	Unicellular algae often form stratified populations in oligotrophic lakes Colonial forms show rapid positioning
Green algae[b]	Demonstrated in *Pandorina*, but not well documented for other motile greens	Various motile greens reported to occur in restricted depth zones
Dinoflagellates[c]	Typically strongly motile and phototactic with well-defined diurnal migration	Vertical positioning in relation to light and oxygen (avoidance of anoxia)
Cryptomonads[d]	Demonstrated for some species	Many occur as stably stratified populations
Chrysophytes[e]	Demonstrated for some species in special (very stable, shallow epilimnion) habitats, but not a general characteristic	Many have ability for phototactic positioning in defined strata, e.g., metalimnion, stable population under ice

References: [a]Paerl (1988); [b]Happey-Wood (1988); [c]Pollingher (1988); [d]Klaveness (1988); [e]Sandgren (1988a)

aquatic habitats. They have been found in over 150 species of prokaryotes present in 11 phyla within the Kingdom Eubacteria (including blue-green algae) and two phyla in the Kingdom Archaebacteria (Table 1.2).

Gas vesicle structure and composition. In all the organisms studied, gas vesicles have a similar cylindrical morphology and are constructed from homologous proteins. These features, together with fundamental gene sequence homologies, suggest that the ability to migrate within the water column via gas vesicle/buoyancy regulation has evolved once in an ancestral prokaryote, and is now an important feature of several major groups of aquatic prokaryotes.

Gas vesicles are cylindrical structures, occurring as stacks within gas vacuoles. These reduce the specific gravity of cells below that of the surrounding aquatic medium, allowing the organism to rise and become positioned at a particular depth within the water column. A full account of the structure, synthesis, and role of gas vesicles in regulating buoyancy has been given by Walsby (1994). These inert, gas-filled structures are produced by the synthesis of two key proteins – GvpA and GvpC. GvpA forms the main framework of the vesicle, and is a small MW (7.4 kDa) hydrophobic protein that is arranged in a linear crystalline array. GvpC is a larger (22 kDa) protein which stabilizes the structure by adhering to the inside of the GvpA framework. GvpC contains a highly conserved motif of 33 amino acid residues, and differs markedly from GvpA in having s preponderance of hydrophilic residues. The molecular genetics of vesicle synthesis has now been well characterized, with two genes (*gvpA* and *gvpC*) encoding the two major proteins.

Gas vesicle formation and collapse. The buoyancy of blue-green algal cells is lowered by loss of gas vesicles (due to collapse at high turgor pressure) and is raised by vesicle increase. Formation of new gas vesicles occurs by a process of self-assembly, following the expression of GvpA and GvpC by activation of *gvpA* and *gvpC* genes. In some blue-green algae, activation of *gvp* genes is constitutive (occurring after existing vesicles have been destroyed by pressure) while in others (e.g., *Pseudanabaena*) it is induced at low light intensity. Dissolved gases subsequently enter the gas vacuole by diffusion, and the buoyant cell rises in the water column.

Role of vesicles in diurnal migration. Diurnal migration results from alternating phases of decreasing (daytime) and increasing (nighttime)

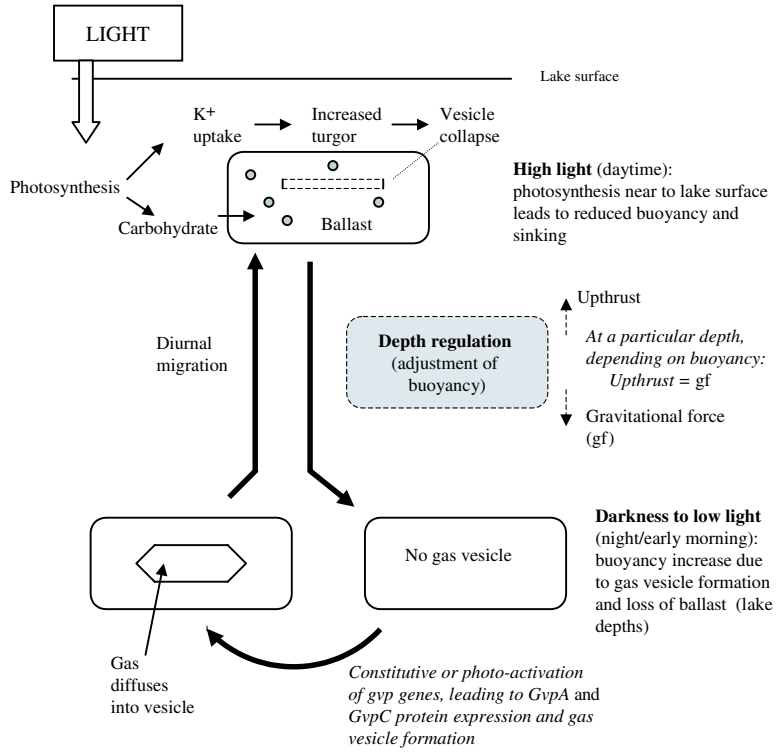

Figure 3.20 General scheme for buoyancy control of diurnal migration and regulation of depth in blue-green algae, based on data from a variety of species (Walsby, 1994). Periodic diurnal migration (⟶) results from an alternating decrease in buoyancy during daytime, and an increase at night/early morning. For simplicity, a single gas vesicle is shown (not drawn to scale, and with surrounding gas vacuole omitted). Carbohydrate ballast – ○

buoyancy (Figure 3.20). During daytime, high rates of photosynthesis at the lake surface result in decreased buoyancy, causing the organism to sink in the water column at the end of the light period. The decreased buoyancy can arise in three ways:

- formation of photosynthetic product, resulting in the formation of carbohydrate storage material (mainly glycogen), which acts as ballast – increasing the specific gravity of cells;
- photosynthetically-mediated uptake of K^+ leads to an increase in turgor pressure, which may lead to collapse of the gas vesicles and a drop in buoyancy.
- inhibition of *gvpA* and *gvpC* gene expression may occur at high light intensity, preventing the formation of new gas vesicles.

Different blue-green algae appear to differ in the relative extent to which these three processes occur.

Reverse processes occur at night, with removal of ballast, loss of turgor (K^+) and formation of new vesicles all contributing to an increased buoyancy and a rise in algae within the water column at the beginning of the next light period.

In conditions of bright light, the algae may position themselves at a particular depth (or depths) within the water column (see next section). At this point, the light intensity promotes sufficient ballast formation to counteract the upward thrust from the gas vesicles, which are continuously formed during the division process.

In some situations, environmental conditions may prevent ballast formation, leading to a loss of buoyancy control. This has been reported in conditions of iron depletion, where lack of uptake of Fe (III) results in a block of photosynthesis. Under such

conditions, the blue-green algae are trapped at the surface of the lake, unable to carry out either diurnal migration and or depth regulation. Inability to escape from the damaging radiation causes death to a large part of the population within a relatively short (~2 hour) period.

Diurnal migration and dissolved organic carbon accumulation Diurnal migration of phytoplankton occurs as a direct response to changes in the abiotic lake environment (light regime), and can in turn lead to further abiotic changes, particularly in relation to water chemistry. Various studies have demonstrated diurnal changes in the concentration of dissolved organic carbon (DOC) in lakes (Burney, 1994), which in some cases can be linked to migration of algae within the water column and the daytime release of DOC in the epilimnion.

Analysis of surface water in Lake Havgardsjoen (Sweden), for example, demonstrated a huge mid-day pulse of DOC (>129 mg l^{-1}), which appeared to relate to the presence of migratory populations of blue-green algae and dinoflagellates at this time (Sødergren, 1984). In subsurface water, DOC increased during the day to reach a maximum of 15 mg l^{-1}, subsequently decreasing to less than 10 mg l^{-1} at night. Elevated daytime concentrations of DOC have also been demonstrated in other lakes, including Lake Eyrie, USA (diurnal range 2.3–4.2 mg l^{-1}, Nalewajko *et al*., 1980) and Lake Oern, Denmark (diurnal range 2.7–3.5 mg l^{-1}, Søndergaard, 1984). In other cases, where diurnal fluctuations of DOC have not been demonstrated, the lack of correlation to algal migration has been variously attributed to high levels of externally-derived DOC (obscuring the internal fluctuations), close coupling of DOC utilization to its release, and lakes where *in situ* biological activity is minimal.

More recent studies (Burney, 1994) have concentrated on specific groups of compounds rather than total DOC, demonstrating diurnal fluctuations in the lake water concentrations of a range of algal exudates – including dissolved free amino acids (DFAA), dissolved mono- and disaccharides, and the metabolic regulator cyclic adenosine 3′:5′-monophosphate (cAMP).

Vertical positioning in the water column

Many motile algae are able to position themselves by active movement (taxis) at particular depths in the water column in response to external stimuli. These include gravitational field (gravitaxis), light direction and intensity (phototaxis), temperature and chemical (chemotaxis) gradients – including variations in oxygen concentration. Recent studies on phototaxis in phytoplanktonic flagellates (Clegg *et al*., 2003) have shown that the preferred light level matches the optimal irradiance for growth in some organisms, but is sub-optimal in others, suggesting that light may also be used as an ecological indicator of other environmental conditions.

Blue-green algae carry out depth regulation by adjustment of buoyancy (Figure 3.20). This involves control of gas vesicle gene expression, collapse of vesicles by alteration of turgor pressure, and formation of carbohydrate ballast by photosynthesis. Stabilization at a particular depth occurs when the opposing forces of upthrust and gravitation are equal.

Depth regulation may involve the long-term establishment of stable populations at fixed depths in the lake (no diurnal migration) or temporary positioning in relation to ambient conditions (combined with diurnal migration).

Long-term stratified algal populations In thermally stratified lakes, chrysophytes, cryptophytes, flagellate green algae, and unicellular blue-green algae (picoplankton) often occur as long-term stable populations at fixed depths within the water column. In some situations, these algae are restricted to the metalimnion layer where they persist throughout summer at reduced temperature and very low light. These adverse environmental conditions are compensated for by reduced zooplankton grazing, close proximity to nutrient rich hypolimnion and the ability of many algae to supplement reduced carbon fixation by heterotrophic nutrition. Such metalimnetic algae are actively growing cells, forming a substantial biomass (chlorophyll maximum) and are not simply sinking out of the epilimnion layer. Lakes that develop metalimnetic maxima are often of low

nutrient status, with low productivity, clear water, and a euphotic zone extending into the metalimnion (Klaveness, 1988). The importance of motility in establishing algal zonation is emphasized by depth analysis of green algae, with motile greens such as *Pandorina* and *Volvox* typically forming narrower depth zones than non-motile greens such as *Sphaerocystis* and *Oocystis* (Happey-Wood, 1988).

Temporary positioning of algae Many motile algae (including flagellate and buoyant species) are able to make rapid and short-term depth adjustments within the water column in relation to changing growth-related environmental factors. In some cases, the responses of individual algal species to particular stimuli have been studied under controlled laboratory conditions. These laboratory studies often employ continuous monitoring of single cells and whole populations of algae exposed to different stimuli, and are particularly useful since they reveal:

- the type and precision of the response,
- changes in motility,
- changes in short-term responses within the diurnal cycle,
- adverse effects of high light intensity (photoinhibition) on algal orientation and motility (see Section 4.9).

Results obtained from laboratory studies suggest that the upward movement of flagellate algae within the water column at low light intensities is typically mediated by positive phototaxis, supported by negative gravitaxis (Hader, 1995). At higher levels of illumination the upward movement of cells is normally reversed with a change to negative phototaxis. In contrast to this, some organisms show a change from positive phototaxis to diaphototaxis (movement perpendicular to the incident light beam) at higher light intensity. This has been demonstrated particularly in dinoflagellates where it provides an effective mechanism for cells and populations to stay at a selected level within the water column.

The wide variation in vertical positioning of micropopulations or individual cells within the water column at any point in time suggests considerable heterogeneity within the overall population in terms of environmental responses. Although algae such as *Ceratium* and *Peridinium,* for example, may show large-scale lake-surface accumulation in lakes, individual cells are distributed throughout the water column. These cells are in stable equilibrium with their microenvironment and have higher chlorophyll contents compared with cells higher present at a higher light intensity (Sandgren, 1988a). The vertical spread of these cells is limited, however, to aerated regions of the water column. Dinoflagellates are very sensitive to oxygen concentration, and their vertical migrations do not extend into anoxic hypolimnia.

Algae also differ markedly in the speed of their positioning. Blue-green algae in particular are capable of very rapid vertical movements, with upward speeds of about 3m hour^{-1} being recorded for *Microcystis* and *Aphanizomenon* (Paerl, 1988). Such migration rates greatly exceed the flagellar swimming speeds of motile eukaryote algae such as dinoflagellates and green algae. The vertical distribution of these organisms may be quite complex, with persistent buoyancy leading to the accumulation of surface scums overlying populations of migrating cells.

3.8.2 Passive movement of algae within the water column

Passive movement of algae within the water column is brought about by water movement (convection currents, wind turbulence) and sedimentation.

Wind disturbance of the epilimnion can have a major effect on the distribution of algae in lakes, breaking stratification and disrupting the migration and positioning patterns noted earlier. Surface accumulation and bloom formation of blue-green algae, for example, requires a well-stratified and static water column for full development. In certain situations, convection currents may also be important for water movement, and have been implicated in algal

mixing within the epilimnion (Happey-Wood, 1988).

Passive sinking of algae (sedimentation) within the water column potentially affects all algae, since all objects are subject to gravitational force. The tendency for sedimentation is counteracted by algal motility, specific buoyancy mechanisms, and water turbulence, and is influenced by intrinsic features of cell size, shape, and density as defined by the Stokes equation (see previously). Sedimentation of algae within the water column is a continuous process resulting from senescence and cell death (loss of motility mechanism), formation of resistant cells (increased density), and random loss of non-motile cells from the euphotic zone. Sedimentation of diatoms, which are maintained in suspension by water turbulence, is increased by the development of a static water column at the onset and progression of stratification.

Sedimentation is of particular significance to diatoms because of their high density (silica cell wall) and lack of planktonic motility. These organisms have evolved either in the direction of reducing the rate of sedimentation or by adapting their life cycle to periodic sinking. The nanoplanktonic diatom *Stephanodiscus hantzschii* provides an example of the former, where the evolution of small cell size reduces sinking rate to <0.05 m day^{-1}, and promotes planktonic existence. At the other end of the scale, the filamentous diatom *Aulacoseira granulata* has a high density (1.13 g ml^{-1}) and sedimentation rate (>1 m.day^{-1}) and has evolved a life cycle where planktonic existence is restricted to a turbulent part of the annual cycle (Sommer, 1988).

3.9 Freshwater algae and nutrient status of the environment

The development of phytoplankton populations in lakes and rivers is related to a range of environmental factors, including temperature, light, soluble gases, and levels of dissolved inorganic nutrients in the water body. Concentrations of silicate, nitrate, and phosphate, in particular, influence the growth of algae and the species present within the ecosystem. The quantitative effects of inorganic nutrients in promoting algal growth are considered in Chapter 5, and the role of algae in the biological effects of environmental nutrient enrichment (eutrophication) is considered in Chapter 10.

3.9.1 Phytoplankton species composition and lake nutrient status

The trophic state of freshwater lakes has a major impact on the type of algae that dominate the ecosystem and in the seasonal phytoplankton succession (Table 10.4).

Ecological preferences in lakes

General ecological preferences of different algal groups within the lake environment are summarized in Table 3.10. It should be emphasized, however, that individual species and groups can often exist in a wide range of environments, so this summary must be regarded as a very broad overview. The presence or absence of particular algal groups and species has considerable diagnostic value in the assessment of lake trophic status, as discussed in Section 10.3. Although nutrient status (particularly the concentrations of phosphates and nitrates) is clearly important, other environmental aspects such as temperature, pH, salinity, and water turbulence are also relevant. Some of these aspects, such as oligotrophic status and acidity, tend to be correlated, with ologotrophic algae such as chrysophytes being tolerant of both conditions.

In green algae, blue-green algae, and to a lesser extend the chrysophytes, the broad spectrum of morphology is paralleled by a related range of ecological preferences. In each of these phylla, the small unicellular subgroups tend to be particularly characteristic of oligotrophic waters, while the large colonial forms dominate eutrophic lakes. The ability of small, single-celled algae to exist and out-compete larger colonial forms at low nutrient level can be related to their higher surface/volume ratio and resulting greater efficiency in nutrient uptake (see previously).

The major environmental requirements of planktonic diatoms are water turbulence and high silicon

Table 3.10 General ecological preferences (standing waters) for algae within the major taxonomic groups (information taken mainly from Sandgren, 1988b, and Wehr and Sheath, 2003)

Group	Typical member of group	Preferred nutrient status	Physico-chemical preferences	General occurrence
Blue-green algae				
• Small coccoid forms	*Synechococcus, Aphanothece*	Range from oligotrophic to eutrophic waters	Some adapted to low light regimes, altered spectral conditions, high salinity	Particularly important in large oligotrophic and mesotrophic lakes
• Large colonial algae	*Anabaena, Microcystis*	Mesotrophic–eutrophic Many can grow at low N/P ratios	Establish massive blooms under conditions of low turbulence and high irradiation Can tolerate low CO_2, high O_2 and high pH	Common in productive lakes throughout temperate and tropical regions Frequently occur as late summer dominants
Green algae				
• Small flagellates	*Chlamydomonas*	Common in oligotrophic lakes	Some species of *Chlamydomonas* typical of acid conditions	Occur in a wide range of standing waters,
• Colonial flagellates	*Eudorina, Volvox*	Mesotrophic to eutrophic waters	Often found in temporary conditions – ponds and rain pools	Do not usually form dominant populations.
• Desmids	*Staurastrum, Cosmarium*	Oligotrophic to mesotrophic lakes (low conductance and low Ca content)	Greatest diversity seen in waters with pH 4–7	Greatest diversity occurs on the sediment surface in shallow areas. Relatively few species are truly planktonic

• Non-motile coccoid and colonial	*Chlorella, Sphaerocystis*	Oligotrophic to eutrophic lakes	Suited to early stratification when light is not limiting and turbidity/turbulence are limited	Ubiquitous and widely-distributed
Dinoflagellates	*Ceratium, Peridinium*	Typically dominant in mesotrophic to eutrophic lakes	Prefer abundant light. Avoid salinity (halophobic), anoxic conditions. Growth and migration disturbed by turbulent conditions	Frequently occur as late summer dominants in temperate mesotrophic and eutrophic lakes
Cryptomonads	*Rhodomonas, Cryptomonas*	Found over a wide range of nutrient status	Can tolerate variable salinity and deviant chemical composition	Wide range of spatial and temporal niche occupation in temporal lakes
Chrysophytes	*Ochromonas* (oligotrophic) *Dinobryon* (meso/eutrophic)	Typically dominant in oligotrophic lakes	Low summer temperatures Low conductivity. Prefer neutral–acid lakes	Common in north temperate oligotrophic lakes. Also seen in alpine, arctic, and some tropical lakes
Diatoms	*Cyclotella*[1], *Asterionella*[2] *Stephanodiscus*[3]	Preferences range from oligotrophic[1] to mesotrophic[2] and eutrophic[3] conditions	Non-motile plankton, so need water turbulence to stay in suspension. Many adapted to low light, low temperature and survival on sediments	Occur throughout all standing water systems as planktonic and benthic populations Dominate most temperate lakes in winter and spring

level. Although this group does not show any overall preference in terms of nutrient status, individual members have strict trophic preferences over the whole spectrum of nutrient levels.

Eutrophication and phytoplankton change

Increased inorganic nutrient status (particularly in relation to nitrate and phosphate availability) leads to changes in phytoplankton composition and biomass. Although such nutrient changes are normally considered in relation to human activities (anthropogenic eutrophication – Chapter 10), they may also occur as natural processes.

The term 'eutrophication' was originally used in reference to the natural ageing process of lakes, and for a long time it was assumed that oligotrophic lakes were 'primitive', 'evolving' to eutrophic water bodies as part of a natural progression. Although this has certainly occurred in some cases, the long-term continuation of oligotrophic lakes such as Lake Baikal (Russia) indicates it is not universal.

In lakes where 'natural eutrophication' occurs, long-term inflow of water carries soluble nutrients into the lake where they are taken up by phytoplankton, sequestered within biomass, and deposited onto sediments in a process of continued accumulation. This leads to increased 'internal loading' and an overall increase in nutrient status. The degree to which such accumulated uptake can occur depends partly on the mean annual concentration of nutrient which occurs in the system. In the case of phosphorus, for example, this may be expressed (Vollenweider and Kerekes, 1980):

$$[P_\lambda] = [P]_q\,(\tau_p/\tau_w) \qquad (3.7)$$

where $[P_\lambda]$ is the mean annual phosphorus concentration in the water body, τ_p and τ_w are the respective mean retention times of phosphorus and water in the system, and $[P]_q$ is the average phosphorus concentration in the inflow. The values τ_p and τ_w depend on the internal hydrology (area loading, water circulation, mean depth) of the lake, and $[P]_q$ depends on the external hydrology and catchment area. In sub-polar lakes such as Lake Baikal, the limited phytoplankton growth season will also impose a restriction on nutrient uptake and biomass accumulation.

3.9.2 Nutrient status of river environments – effect on benthic algal biofilms

Benthic algal biofilms dominate the river environment and contain a complex mixture of algae including green algae, blue-greens, and diatoms. The algal composition of biofilms is influenced by a range of interrelated environmental factors including water flow, water depth, light regime, chemistry, nutrient status (eutrophication), pollution, and grazing. The effects of these parameters have been investigated particularly in relation to diatoms (Lock *et al.*, 1984; Round, 1993) since these organisms:

- occur throughout all rivers – diatoms comprise the largest and most prevalent group of algae in river systems,
- can be rapidly and easily sampled,
- have species that are very sensitive to water quality (chemistry), eutrophication, and pollution – the ecological requirements of species are well documented,
- have a rapid growth cycle and respond rapidly to perturbation of the environment.

Rivers form a chemical and biological continuum, with increases in nutrient content from the upper reaches to the estuary. Longitudinal changes in physical and chemical parameters are paralleled by qualitative and quantitative changes in the algal biofilm. These have been summarized in some detail for diatoms by Round (1993), who has suggested longitudinal subdivision of increasingly eutrophic rivers into five major zones (Table 3.11).

The increasing state of eutrophication seen in Table 3.11 would be typical of a river that commences with clean water at the source (Zone 1), followed by increasing input of nutrients (agriculture,

Table 3.11 Diatom transition along a river course with increasing nutrient pollution – division into five major zones (information taken from Round, 1993)

Zone	Physical Parameters and Water Quality	Dominant Diatom Species	General Aspects of Biofilm
ZONE 1 Clean water in uppermost reaches	High water flow pH 3.6–4.1 Clear, well-aerated water	*Eunotia exigua* *Achnanthes microcephala* Small-celled species Directly attached to the stone surface	Little development of an associated mucilage community
ZONE 2 Nutrient richer, higher pH	pH 5.6 – 7.1 Clear, well-aerated water	*Hannaea arcus* *Fragilaria capucina* *Achnanthes minutissima*	Development of a mucilaginous biofilm with complex community
ZONE 3 Nutrient rich	pH 6.5 – 7.3	*Achnanthes minutissima* *Cymbella minuta* *Amphora pedunculus* *Cocconeis placentula*	Wide range of diatom species Complex, well-structured community
ZONE 4 Eutrophic with restricted diatom flora	Moderate organic pollution Decline in water quality – high nitrogen levels	*Gomphonema parvulum*	Decline in water quality is indicated by loss of the *Amphora*, *Cymbella*, *Cocconeis* complex
ZONE 5 Diatom flora grossly restricted by organic pollutant influx	Highly eutrophic, organically polluted water Extremely poor water quality	*Navicula atomus* *Nitzschia palea* Small-celled diatoms	Limited diatom community

sewage) lower down (Zone 5). Individual rivers vary in their development of these zones. Most eutrophic rivers that occur in the UK, for example, are typical of Zone 3 over most of their length.

As with the variation in lake nutrient status seen earlier, changes in river trophic status are also accompanied by other parameters such as pH and oxygenation. Different trophic states (Zones 1–5) are characterized by particular dominant species and also by groups or assemblages of diatoms. The *Cymbella*, *Amphora*, *Cocconeis* complex seen in Zone 3, for example, is typical of moderately rich waters, but is lost with the pollution input seen in Zones 4 and 5.

D. STRATEGIES FOR SURVIVAL

3.10 Strategies for survival: the planktonic environment

Complex interactions and constraints within the freshwater environment have resulted in the evolution of a wide range of strategies for survival. This is particularly the case for planktonic algae, which have to contend with a number of interrelated growth-limiting factors, including:

- competition with other algae for light, space, and nutrients,

- limiting light conditions – this includes elimination from the euphotic zone, caused by gravitation and water movement,
- adverse winter conditions of light and temperature (seasonal variation in temperate lakes),
- fungal and viral attack,
- grazing activities of zooplankton, protozoa, and fish.

Planktonic algae have evolved different ways of dealing with these constraints. These include various strategies for maintaining their position in the water column (Section 3.8), avoidance of grazing (Section 9.8), and adaptations to low light (Section 4.6) and nutrient (Section 5.8) levels – all of which lead to major differences between algae in structure, physiology, and life style. Two further aspects will be considered here, the ability of algae to exploit environmental conditions in a particular part of the seasonal cycle by adopting a meroplanktonic life style, and adaptations to unstable and stable environments by *r*- and *K*-selection. The development of heterotrophic nutrition in relation to light limitation (and high nutrients) is considered in Section 3.11, and the adoption of strategies to survive the limitations of snow environments in Section 3.12.

3.10.1 Meroplanktonic algae

The majority of lake algae are holoplanktonic, being present within the water column over much of the annual cycle, and competing with other algae for light and nutrients during this period. In contrast to this, meroplanktonic algae have evolved a relatively limited planktonic existence, with a large part of the annual cycle being spent as a dormant phase on the lake sediments. Meroplanktonic algae include the diatom *Melosira* and the blue-green alga *Microcystis*, which have their planktonic phase at different times in the annual cycle of temperate lakes.

The evolution of the meroplanktonic state allows particular phytoplankton species to grow over a limited phase of the seasonal cycle to which they are adapted. This may either be a time of low competition from other algae (*Melosira*) or when competition is intense (*Microcystis*).

Melosira

Many diatoms in freshwater environments are able to escape the effects of intense competition in the top part of the water column by mechanisms such as subthermocline growth or prolonged vegetative survival on sediments (Wehr and Sheath, 2003). *Melosira* presents a good example of the latter, being present on the sediments for most of the year, but moving into the water column at a time when the populations of other algae are generally limited.

Melosira is a filamentous diatom with thick cell walls (high cell silicon content) and a high rate of sedimentation, requiring water turbulence to keep in suspension. Studies by Lund (1954) have shown that populations of this alga rise from the lake sediment in autumn, persisting over winter as planktonic cells (Figure 3.21). Although water inorganic nutrients are present at elevated concentrations over winter, algal growth does not commence until spring, when increases in light and temperature trigger nutrient uptake and a brief population increase. This is brought to a close by stratification, which results in a reduction in turbulence and sedimentation of

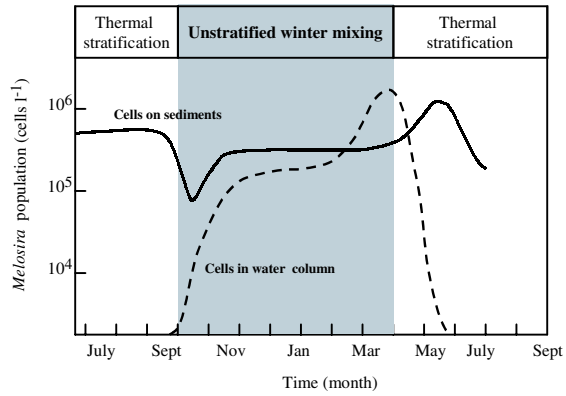

Figure 3.21 Seasonal cycle of the meroplanktonic alga, *Melosira italica* in Blelham Tarn (English Lake District) (figure adapted and redrawn from Horne and Goldman, 1994, based on the studies of Lund, 1954)

cells to the lake bottom. Cells with stored nutrients are able to survive on the lake sediment until the next planktonic phase. The early population increase in spring makes use of the brief improvement in growth conditions that occurs prior to stratification, and precedes the growth of other phytoplankton species – thus avoiding competition.

Melosira is closely related to another filamentous diatom, *Aulacoseira*, which has been mentioned previously in relation to the endemic flora of Lake Baikal, Russia (Figure 2.8) and the formation of early-season blooms below ice (Figure 2.3).

Microcystis

This blue-green alga (Figure 6.24) overwinters as vegetative colonies on the lake sediment, where it persists until early summer, when some of the colonies rise into the upper water column in response to increased light intensity (Figure 3.22). Successful recruitment of benthic cells to the water column requires a preceding phase of high water clarity (Reynolds, 1997) and light penetration, possibly involving the photo-activation of gas vesicle formation (Section 3.8.1). Other seasonal changes that trigger activation include increasing temperature and the onset of anoxic conditions. As the colonies enter the water column, passive dispersion may also be promoted by wind-induced turbulence.

Recent studies (Brunberg and Blomqvist, 2003) have shown that recruitment from shallow areas of the lake is particularly important. Initial (early summer) abundance of *Microcystis* on surface sediments in shallow and deep areas of Lake Limmaren (central Sweden) ranged from 25–28 $\times$ 10^6 colonies m^{-2}. Subsequent recruitment to the water column (over a 17 week period) amounted to 12.9 $\times$ 10^6 colonies m^{-2} for shallow water (i.e., 50 per cent of original abundance) and 2.3 colonies m^{-2} for deep sites (8 per cent of original abundance). These data indicate a clear link between planktonic populations of this alga throughout the lake and benthic populations in shallow regions, which in the case of deep lakes means the peripheral littoral zone.

Initial inoculum levels in the water column are quite low, but continued growth leads to planktonic populations which dominate the lake towards the end of the stratification period – at a time of low nutrient concentrations. Populations of *Microcystis* are able to survive nutrient-limiting conditions in the top part of the water column by the initial use of stored materials from their dormant phase and by diurnal migration into the nutrient-rich hypolimnion. The ability to migrate into nutrient-rich regions of the water column give *Microcystis* a

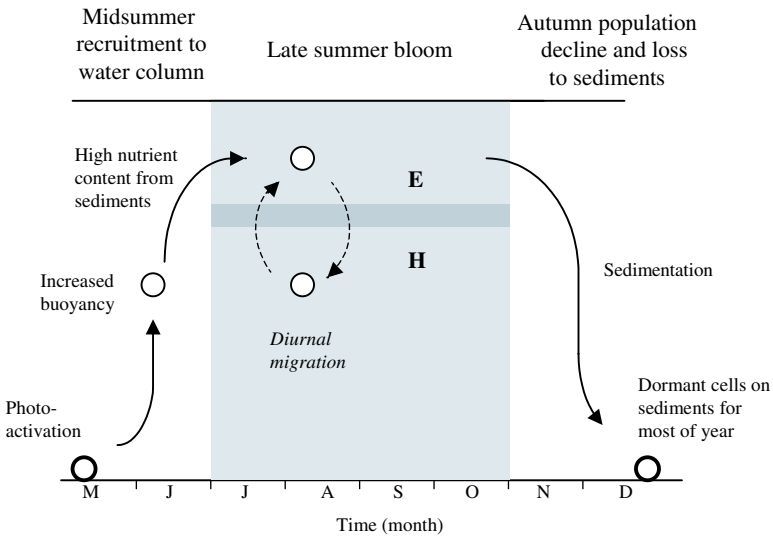

Figure 3.22 Annual cycle of the meroplanktonic colonial blue-green alga *Microcystis*. Colonies of *Microcystis* (O) are present in the water column over a limited period, spending most of the year in a dormant state on lake sediments. During the summer bloom, a period of intense phytoplankton competition, diurnal migration into the nutrient-rich hypolimnion (H) makes the organism independent of declining epilimnion (E) nutrient concentrations

competitive advantage over less motile algae at a time when algal populations in the epilimnion are dense and competition is high. *Microcystis* does, however, have to compete with other algae such as dinoflagellates, which are also able to migrate into the nutrient-rich hypolimnion, and the seasonal progression of many eutrophic lakes involves summer dominance by either *Microcystis* or *Ceratium*. Whether a particular season favours *Microcystis* or *Ceratium* dominance appears to depend on which population develops first. 'Microcystis years' have been linked to early recruitment of colonies into the water column, correlating with early conditions of light penetration and water clarity (Reynolds, 1997).

Populations of *Microcystis* come to the end of their bloom phase in autumn, falling to the bottom of the lake as resistant cells where they may remain viable for a number of years.

3.10.2 Strategies for unstable and stable environments: r-selected and K-selected algae

R-selection and *K*-selection represent adaptive strategies to exploit two contrasting environmental situations (Section 1.2.6). *R*-selected species are characterized by small size, high growth rate and short cell cycle – and are able to increase in population rapidly under conditions of low population density and low species competition. These organisms are particularly prominent in temperate lakes during the clear-water phase. During this time, grazing pressure by herbivores is patchy and intense, and algal growth is limited to short periods in those parts of the lake with lower densities of zooplankton. *K*-selected species, conversely, typically have large size, low growth rate, and long cell cycles and are adapted to conditions of high population density and high competition.

Examples of *r*-selected and *K*-selected phytoplankton are given in Table 3.12, with data for growth rate and cell/colony size. Growth rate can be expressed as the generation time (the time taken for the population to double) or the exponential growth constant (k') – which is derived from the exponential growth equation:

$$N_t = N_0 e^{k'} \tag{3.8}$$

where N_0 = biomass at zero time, N_t = biomass at time t, k' = the exponential growth constant.

The value k' can be used as a measure of growth rate, and in the case of *r*-selected algae is typically

Table 3.12 Growth rate and size differences between various *r*-selected and *K*-selected algae. Maximum exponential growth rates, k', are shown for laboratory cultures grown under continuous saturating illumination, at temperatures in the range 20–25°C (taken partly from Reynolds, 1990)

Species	Exponential growth constant (k') (ln units day^{-1})	Approximate size of single cells or colonies*
***r*-selected species**		
Chlamydomonas spp	2.29–2.91	upto 40 μm
Chlorella pyrenoidosa	2.15	3–5 μm
Synechococcus sp.	2.01	4–15 μm (depending on species)
Scenedesmus obliquus	1.52	
Cryptomonas erosa	0.83	32 μm
***K*-selected species**		
*Aphanizomenon flos-aquae**	0.98	upto 2 mm
*Anabaena flos-aquae**	0.78	upto 2 mm
*Microcystis aeruginosa**	0.48	upto 2 mm
Ceratium hirundinella	0.21	upto 400 μm

>1, while *K*-selected algae typically have values <1. Values for k' tend to decrease with increase in organism (single cell or colony) size.

Dinoflagellates as K-selected organisms

Of all the algal groups, dinoflagellates provide some of the best examples of *K*-selected species – as indicated by the very low k' value for *Ceratium hirundinella* (Table 3.12). The evolution of these organisms has been dominated by the development of huge amounts of nuclear DNA, reaching levels of 200 pg cell^{-1} in *Gonyaulax* – compared to levels of 0.05–3 pg cell^{-1} for other eukaryote cells. The evolution of these very high levels of nuclear DNA (most of which is genetically redundant) has paralleled unique methods of packaging (Sigee, 1984), with dinoflagellate DNA being permanently condensed as chromosomes (Figure 3.23) which are largely stabilized by divalent cations and not conventional eukaryote histones. The high levels of dinoflagellate DNA directly correlate with large cell size and result in the long cell cycle – due to the length of time required for DNA replication (interphase) and chromosome separation (karyokinesis).

The dinoflagellates *Ceratium* and *Peridinium* are common examples of *K*-selected organisms in moderate to high nutrient waters, often dominating the rather static lake environment in late summer when the water column is stably stratified and grazing is limited. Alternative domination of late summer standing waters by *Ceratium* or *Microcystis* has been discussed in the previous section.

3.11 Heterotrophic nutrition in freshwater algae

Although the majority of freshwater algae are photoautotrophic, carrying out photosynthesis and forming complex organic compounds from inorganic precursors, some also have the facility for heterotrophic nutrition. These organisms are able to use external organic compounds for energy, metabolism, and growth (Sanders, 1991; Tuchman, 1996).

The ability of some algae to supplement their autotrophic life style by the uptake of complex organic carbon from the environment occurs in two separate ways:

- *organotrophy*: direct uptake of soluble organic molecules by absorption over the cell surface, and
- *phagotrophy*: ingestion of particulate organic matter.

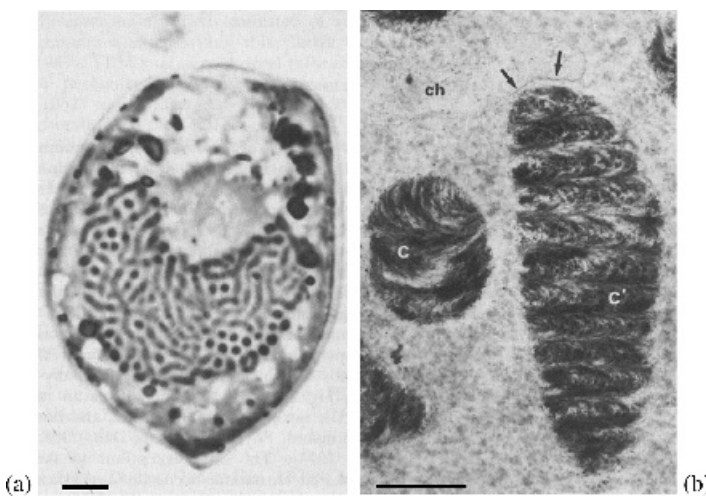

Figure 3.23 Distinctive nuclear fine-structure in dinoflagellates:
(a) Light micrograph of thin section of fixed interphase cell of *Prorocentrum*, showing the large central nucleus with numerous condensed chromosomes (scale = 2 μm)
(b) Electron micrograph-detail of transverse (c) and longitudinally (c′) sectioned chromosomes, showing the distinct fibrillar appearance. One of the chromosomes is attached (arrows) to an intranuclear cytoplasmic channel (scale = 0.5 μm). Figure taken from Sigee (1984) and reproduced with permission of the Linnean Society of London.)

Table 3.13 Transition from autotrophy to heterotrophy in flagellate algae (vertical arrows emphasize the transition from facultative to obligate heterotrophy)

Mode of Nutrition	Characteristics
Autotrophy	Synthesis of all required organic compounds from inorganic sources. Algae use light energy (phototrophy) for this
Heterotrophy	Requirement for external organic compounds – either as specific metabolites or as a general carbon source
Organotrophy	Uptake of soluble organic molecules at cell surface
↓ Auxotrophy	Metabolic requirement for specific external metabolites (vitamins, growth factors)
↓ Photo-Organotrophy	Facultative organotrophs, able to obtain carbon from CO_2 or soluble organic compounds Energy from light
↓ Chemo-Organotrophy	Facultative or obligate organotrophs obtain carbon from organic compounds Energy from organic compounds
Phagotrophy	Uptake of particulate matter by phagocytosis
↓ Mixotrophy	Facultative phagotrophs, able to carry out photosynthesis and phagotrophy
↓ Phagotrophy	Obligate phagotrophs Feeding via food vacuoles for the uptake of particulate matter

In each case, the transition from autotrophy to heterotrophy occurs along a gradient of nutritive states (Table 3.13), ranging from facultative organotrophy and phagotrophy (where requirement for external organic compounds may be minimal) to obligate states (where dependence is total). These facultative states include auxotrophy, photo-organotrophy and mixotrophy (Table 3.13). The development of heterotrophy has arisen in response to two quite different sets of environmental situations as follows.

- *High external nutrients*. Nutrient enrichment of the environment, with high external concentrations of soluble organic compounds may occur in various ways. These include large-scale enrichment of aquatic systems due to agricultural pollution, as well as micro-enrichment of epiphytic and benthic algae from vascular plants or via exudates/faeces of molluscs, insects, crustaceans, and other organisms crawling over the benthic flora. The presence of soluble organics presents the opportunity for nutrient absorption at the cell surface and favours growth of organotrophic algae.
- *Limitations to photosynthesis*. Conditions of low light intensity and/or limiting inorganic nutrients, may cause severe impairment of photosynthesis and thus the facility for autotrophic nutrition. This situation has lead to the development of mixotrophy and phagotrophy (Wilcox and Wedemeyer, 1991; Raven, 1997; Li *et al*., 2000).

There are many situations of low light intensity where facultative heterotrophy would provide advantages over obligate autotrophy. On a large spatial and temporal scale, conditions of light limitation result from seasonal, geographic, and hydrological factors. Seasonal effects include snow-covered ice on lakes and rivers, turbid spring floodwaters, and autogenic (self) shading within late-summer algal blooms. The geographic influence of latitude is particularly severe in polar and subarctic regions, and hydrological influences include the development of algal communities in deep lentic and lotic ecosystems. On a more local scale, seen for example in forest streams, shading from surrounding terrestrial vegetation may impose severe light limitation, and at the microlevel – shading

Table 3.14 Auxotrophic requirements for vitamin B_{12} (cyanocobalamin)

Algal group	General requirement	Specific requirement
Euglenoids	All require B_{12} (photoauxotrophic)	Absolute requirement for *Euglena gracilis* – 4900 to 22 000 molecules of B_{12} needed for cell division[a]
Chrysophytes	Many phagotrophs, also require B_{12}	*Oochromonas danica* can synthesize all vitamins except B_{12} and several fat-soluble vitamins[b]
Prymnesiophytes	Generally require either thiamine or vitamiun B_{12}	*Coccolithus huxleyi* needs only thiamine[c]
Diatoms	Most diatoms need B_{12}, except some estuarine species	Estimated at 5–13.8 molecules μm^{-3} for cultures of *Skeletonema costatum*[d]

References: [a]Carrell (1969); [b]Stoltze *et al*. (1969); [c]Lee (1997); [d]Guillard and Cassie (1963).

within benthic algal communities (periphyton) reduces light penetration to the basal layer.

3.11.1 Organotrophy

Organotrophy (also referred to as saprotrophy or osmotrophy) may either involve the specific uptake of particular metabolites or the bulk non-specific uptake of organic materials as a simple carbon source. This uptake takes place over the whole cell surface and occurs either via active transport at the plasmalemma or by pinocytosis.

Auxotrophy: specific uptake of particular metabolites

With some algae, organotrophy involves a specific requirement for small amounts of particular metabolites. This is referred to as auxotrophy, and differs markedly from the more general situation where there is bulk uptake of organic carbon at the surface, with no molecular specificity. Many photosynthetic algae, when cultured in the laboratory, show a metabolic requirement for small quantities of particular organic molecules in their surrounding medium. These organisms may lack the ability to synthesize the complete suite of macromolecules required for growth, and require small levels of organic supplements such as vitamins (particularly vitamin B_{12}; thiamine, and biotin) and organic growth factors.

Requirement for vitamin B_{12} has been demonstrated in various groups of algae (Table 3.14), where it occurs irrespective of both photosynthetic (euglenoids) and phagocytic (chrysophytes) activity. The use of defined media for laboratory cultures permits precise estimation of the required level of B_{12} supplementation, which in the case of *Skeletonema* may be as low as 5 molecules μm^{-3} (Table 3.14). The need for specific vitamins may have important ecological implications. This has been demonstrated for the marine prymnesiophyte *Coccolithus huxleyi*, which requires the vitamin thiamine but not B_{12}, it is thus able to grow regularly and out-compete other algae in the vitamin-deficient offshore waters of the Sargasso sea (Lee, 1997).

Photo-organotrophy and chemo-organotrophy: non-specific uptake of organic materials as a simple carbon source

Many laboratory studies have shown the ability of planktonic and benthic algal species to be cultured heterotrophically in complete darkness, with the addition of organic supplements (Tuchman, 1996). The carbon supplements typically involve the addition of one to three simple organic substrates to the normal growth medium, including sugars (e.g., glucose, fructose), organic acids (acetate, lactate), amino acids (leucine, glycine), glycerol, and urea. The algae include blue-greens, chrysophytes, dinoflagellates, diatoms, and green algae.

Organotrophy may be particularly important to planktonic diatoms, where short-term periods of low

turbulence can cause the organisms to drop out of the photic zone. The ability of diatoms such as *Cyclotella cryptica* (Hellebust, 1971) to grow heterotrophically in the dark allows the alga to survive on sediments prior to resuspension in the water column when turbulence returns. This organism is able to use glucose as the sole carbon source, requiring a 24 hour lag period for metabolic adjustment to the heterotrophic mode of nutrition.

The ability of many freshwater algae to grow under controlled laboratory conditions in the dark in the presence of a carbon source provides strong experimental evidence for the wide occurrence of organotrophy. In some cases, direct uptake has been demonstrated using radiolabelled compounds such as [^{14}C]-glucose or [^{14}C]-leucine. Although organic carbon uptake under conditions of prolonged darkness provides critical evidence for organotrophy, many algae carry out this process in the light. The energy sources required for carbon uptake in these two situations are respectively organic compounds (chemo-organotrophy) and solar radiation (photo-organotrophy).

Photo-organotrophy In autotrophic algae, the dark-fixation of CO_2 is coupled to the generation of ATP and NADPH by light-dependent processes as part of the normal process of photosynthesis. Recent studies (reported in Tuchman, 1996) have demonstrated that this coupling may become disrupted in the presence of external organic compounds in the light. Illumination of photosynthetic reaction centres leads to automatic activation of light-dependent reactions, with no switch-off until the quality/quantity of irradiance decreases below the threshold for pigment stimulation. Associated light-independent reactions can be deactivated in the light if adequate concentrations of organic carbon substrates are available in the surrounding environment. If this occurs, the Calvin cycle becomes blocked and carbon fixation ceases. Light dependent reactions continue to function, providing energy which can be used to actively transport exogenous carbon substrates into the cell, assemble energy-rich storage compounds or simply be channelled into other cellular metabolic pathways.

Photo-organotrophy can be regarded as a facultative activity in which the balance between autotrophy and heterotroophy depends on substrate availability, and possibly other environmental factors as well.

Chemo-organotrophy Chemo-organotrophs obtain energy from organic compounds and include both facultative and obligate species.

Many facultative organotrophs normally exist by phagotrophy, but are able to take up soluble organics in appropriate circumstances. In these organisms, soluble organic nutrient may be taken up with particulate material and be absorbed across the phagocytic vacuole membrane. Several of these algae have been cultured heterotrophically in the laboratory on high nutrient liquid media, including various chrysophytes (species of *Ochromonas* and *Poterioochromonas*) and dinoflagellates (species of *Crypthecodinium*, *Oxyrrhis*, and *Gyrodinium*). In all cases the required concentration of organic solutes in the culture medium was much higher than normally encountered in environments where the algae are found, suggesting that organotrophy is not the normal mode of nutrition of these organisms under environmental conditions.

Algae with an absolute dependency on organotrophic nutrition typically live in organically polluted environments such as low pH sites enriched with animal waste or sewage works. Such obligate heterotrophs include several colourless euglenoid algae (*Astasia*, *Cyclidiopsis*) and other organisms normally regarded as protozoa (*Chilomonas*, *Polytomella*). Flagellates living in such environments tend to be out-competed by bacteria for nutrients due to their lower surface to volume ratio. For this reason, nutrient levels need to be very high for the flagellates to survive, and in most environments saprotrophy alone is insufficient to support the growth of a flagellate population.

3.11.2 Phagotrophy

The evolutionary development of phagotrophy is largely a feature of flagellate algae, and has been

observed in three major groups of organisms – chrysophytes, dinoflagellates, and prymnesiophytes. The trend towards phagotrophy in algae may have given rise to a wide range of flagellates that are now completely heterotrophic and are of indeterminate taxonomic status. Many of these are very small (<10 μm diameter) and are part of a mixed assemblage referred to as heterotrophic nanoflagellates (HNFs). The ecological importance of HNFs in planktonic food webs has been emphasized in Chapter 2, and is considered further in Chapter 9.

The capture and ingestion of particulate food by flagellate algae involves a wide range of prey – including bacteria, blue-green algae, eukaryote algae, protozoa, and metazoan gametes. Algal phagotrophs are raptorial feeders, obtaining prey by direct interception. This is in contrast to filter and diffusion feeding, which has been adopted by some non-algal phagotrophs such as protozoa and some indeterminate flagellates. Once the prey has been caught, various mechanisms have evolved for the ingestion process. This range of activities is seen particularly well in the dinoflagellates, where a large number of species are known to feed phagotrophically (Wilcox and Wedemayer, 1991). Some of these dinoflagellates (e.g., *Amphidinium cryophilum*) are able to ingest food through a cytoplasmic extension (peduncle), drawing whole cells or parts of cells into a nascent food vacuole prior to digestion. Other dinoflagellates envelop their prey in a membranous structure, carry out external digestion, then take up the nutrients. *Noctiluca* uses a flexible adhesive tentacle to bring food material to a point of ingestion – the cytostome.

Other algae simply ingest particulate food material at the cell surface, with the formation of a phagocytic vesicle. Where this occurs, the food source appears to relate mainly to the size of the phagotroph, with (large) dinoflagellates ingesting small eukaryotes such as cryptophytes. Smaller chrysophytes and prymnesiophytes ingest mainly bacteria (Table 3.15). Environmental and laboratory studies have demonstrated a range of factors which may promote phagotrophic activity – including prey density, light intensity, and inorganic nutrient (particularly N and P) concentrations. Recent studies (Skovgaard and Hansen, 2003) have suggested that toxin production by Prymnesiophytes may be important in motile prey immobilization and ingestion.

Mixotrophy: the ability to carry out phagotrophy and photosynthesis

Various algae are able to carry out both photosynthesis and phagotrophy. With increasing development of the phagotrophic habit, the algal cell moves from dependence on photosynthesis (obligate phototroph) to only limited dependence (facultative phototroph). The balance between phototrophic and heterotrophic nutrition has shifted even further with some algae, where the photosynthetic capability of the algal cell has been lost (non-phototrophic) but the cell is dependent on ingested algae for intermittent photosynthesis (facultative symbiosis) and heterotrophic nutrition via phagocytosis. These three states are illustrated within the dinoflagellates (Table 3.15), where there is a wide range of heterotrophic nutrition.

Obligate phototrophs In obligate phototrophs, such as the estuarine dinoflagellate *Gyrodinium galatheanum* (Li *et al*., 2000), photosynthesis is an essential source of carbon at all times. The rate of phagotrophy increases with light intensity up to a particular saturation level and this organism is not able to grow in the dark – even in the presence of food. In *Gyrodinium galatheanum,* where there is a light-dependent feeding pattern, phagotrophy is clearly not a response to low light conditions. The major role of phagotrophy in obligate phototrophs is probably to supply additional nutrients (N and P) which are needed for photosynthetic carbon assimilation. Phagotrophy may also be important in supplying trace organic growth factors, since feeding (at a reduced rate) also occurs in nutrient-rich conditions.

Facultative phototrophs Photosynthesis is not essential for the growth of these algae. The dinoflagellates *Fragilidium subglobosum* (Skovgaard, 1996), and *Amphidinium cryophilum* (Wilcox and Wedemeyer, 1991) are facultative phototrophs and

Table 3.15 Mixotrophy in dinoflagellates and chrysophytes: the balance between phototrophy and heterotrophy in selected species (obligate phototrophs are indicated by shading)

		Heterotrophic Nutrition		
Organisms	Phototrophy	Mode of nutrition	Stimulated by	Food Source
Dinoflagellates				
Gyrodinium galatheanum[a]	Obligate	Facultative phagotrophy	High light Low inorganic nutrients	Cryptophytes
Fragilidium subglobosum[a]	Facultative	Facultative phagotrophy (obligate at low light levels)	Low light	
Amphidinium cryophilum[b]	Facultative	Facultative phagotrophy (obligate at low light levels)		Other dino-flagellates
Gymnodinium acidotum	Not phototrophic (rely on ingested organism)	Facultative Symbiosis		Cryptophytes
Gymnodinium aeruginosum[c]		Obligate periodic phagotrophy		
Chrysophytes				
Dinobryon cylindricum[d]	Obligate phototroph	Facultative phagotrophy		Bacteria
Poterioochromonas malhamensis[d]	Facultative phototroph	Facultative phagotrophy and saprotrophy		Bacteria Glucose uptake

References: [a]Li *et al*. (2000); [b]Wilcox and Wedemayer (1991); [c]Fields and Rhodes (1991); [d]Raven (1997).

phagotrophs – able to grow equally well phototrophically or completely phagotrophically in the dark. The cold-water dinoflagellate *Amphidinium cryophilum* has been described as a photosynthetic winter species, and grows in freshwater ponds which are frequently covered in ice and heavy snow. The phagotrophic habit is a clear environmental adaptation, supplementing carbon and other nutrient input overwinter and allowing the organism to grow at a time when irradiance (and photosynthesis) is reduced by short days, low sun angle, and ice/snow cover. At low light levels, cells feed phagotrophically and are almost colourless, while at high light levels they feed much less frequently (if at all) and are brightly pigmented (Wilcox and Wedemayer, 1991).

Non-phototrophic algae Two species of algae, *Gymnodinium acidotum* and *Gymnodinium aeruginosum* can be regarded as non-phototrophic, in that they appear to have completely lost their own chloroplasts and their ability to carry out autonomous photosynthesis. Instead, they rely on ingested cryptophyte cells for short periods of imported photosynthetic activity (symbiosis) followed by periodic phagocytosis (Figure 3.24). Resistant cysts of these algae germinate in the laboratory to release colourless motile cells, which remain colourless and die within a few days unless they are mixed with cryptophytes (Fields and Rhodes, 1991). Ingestion of cryptophytes leads to digestion of the cryptophyte nucleus, but chloroplasts (and other cytoplasmic organelles) are retained intact and functional over a period of about 12 days, supplying the host cell with photosynthetic products. Within this time period, the dinoflagellate has to ingest more cryptophytes to maintain the symbiotic association and stay alive. The colourless cell released

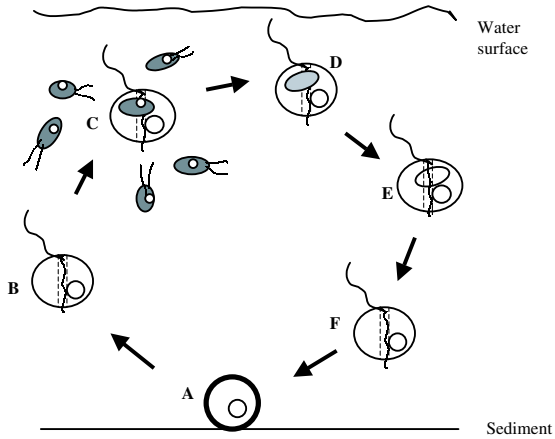

Figure 3.24 Environmental cycle of *Gymnodinium acidotum*, (based on the laboratory studies of Fields and Rhodes, 1991).
(A) Resistant cyst, resting on lake sediment, (B) Released colourless, motile cell, migrates up to the surface water (positively phototactic) and populations of cryptophytes. (C) Ingestion of cryptophyte cells. (D) Symbiotic phase – within a few days, the cryptophyte nucleus has been digested but chloroplasts remain functional. (E) Completion of phagotrophy – chloroplasts and other cryptophyte organelles undergo digestion. (F) Colourless dinoflagellate, needs to ingest more cryptophyte cells, or will encyst from the cyst is positively phototactic, and is presumably carried into the surface waters of its lake environment to make contact with populations of cryptophyte cells. Growth and division of the dinoflagellate will continue during the period of the cryptophyte bloom, but will cease when this terminates resulting in cyst formation and dormancy until the next opportunity for symbiosis occurs. This association between dinoflagellate and cryptophyte is of interest in relation to the ecology of the lake, but also has evolutionary relevance as a possible early stage in the permanent incorporation of actively functional foreign plastids in recipient host cells.

Obligate and facultative phototrophs have also been observed in phagotrophic chrysophytes (Raven, 1997). *Dinobryon cylindricum* (obligate phototroph) cannot survive in continuous darkness, but ingestion of bacteria (bacterivory) is needed for rapid sustained growth in light. Bacterivory supplies up to 25 per cent of the carbon in *Dinobryon* cells, but probably has a more important role in supplying major inorganic nutrients and growth factors. Studies by Auclair (1995), for example, have suggested that ingestion of blue-green algae is a major source of Fe for phagotrophic chrysophytes in Canadian Lakes.

3.12 Survival in snow and ice: adaptations of cryophilic algae

A wide range of algae are adapted to grow and survive in low temperature (snow and ice) environments. As noted earlier (Section 2.17), such environments occur over a wide area of the Earth's surface and support very distinctive ecosystems with organisms exposed to a range of adverse environmental conditions. The most notable feature in such environments is the absence of free water for most of the year, so algae spend most of their time in a frozen and inert physiological state.

The biological features of snow algae have been looked at particularly in snow packs, where a burst of physiological activity occurs during the annual melt process (Hoham and Duval, 2001). The colonization of melting snow by these organisms and their subsequent growth to high population levels within the snow matrix represents one of the more remarkable sets of adaptations to a particular freshwater system.

3.12.1 Major groups of cryophilic algae

Cryophilic (snow and ice) algae include representatives from all of the major groups noted earlier in this chapter (Tables 3.1 and 3.3). Some of the typical species routinely encountered in low temperature situations are summarized in Table 3.16, together with their typical locations and other ecological characteristics. As with other freshwater systems, true red algae (class Rhodophyta) are poorly represented. Reports of blooms of 'red algae' in snow environments typically refer to high populations of green algae with carotenoid pigmentation or – less commonly – to high levels of other algae such as dinoflagellates.

Table 3.16 Taxonomic diversity and habitats of snow and ice algae

Algal Group	Representative species	Typical snow and ice habitat
Blue-green algae	*Phormidium frigidum*	Aerobic or anaerobic mats under ice
	Lyngbya martensiana	
	Anabaena cylindrica	Ponds and ice surface
	Nostoc commune	
Green algae	*Chlamydomonas nivalis*	Melting snow-packs and ponds
	Chloromonas brevispina	Can form red blooms, with populations of 10^5–10^6 cells ml^{-1} of snow water
	Chloromonas nivalis	
Euglenoids	*Notosolenus sp.*	Melting snow-packs and ponds
	Euglena sp.	
Dinoflagellates	*Gyrodinium sp.*	Snow surfaces, occasionally forming red blooms
	Gymnodinium pascheri	
Cryptomonads	*Cryptomonas frigoris*	Melting snow-packs and ponds
Chrysophytes	*Chromulina chionophila*	Melting snow-packs and ponds
	Ochromonas sp.	
Diatoms	*Navicula sp.*	Widely present in aerobic conditions of polar lakes, particularly in algal mats
	Nitzschia sp.	
	Pinnularia sp.	

Different algae tend to occupy their own specific environments within snow and ice systems. Some areas of melting snow are very rich in algae, particularly flagellates that belong to the order Volvocales of the green algae, including *Chlamydomonas* and *Chloromonas*. Other algal flagellates in snow include chrysophytes, euglenoids, cryptomonads, and dinoflagellates, some of which are illustrated in Figure 3.25. Individual organisms are found both as actively motile cells and non-motile resistant spores, depending on snow conditions.

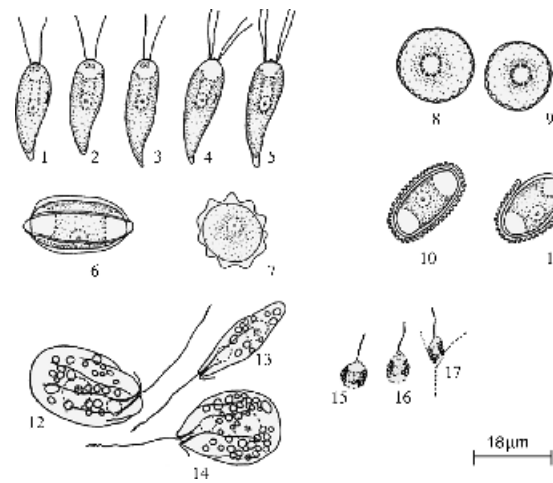

Figure 3.25 Typical examples of snow algae found in southwestern United States.

Chloromonas nivalis, a green alga. 1–3: vegetative cells with two flagella, papilla, two apical contractile vacuoles, centrally located nucleus and parietal cup-shaped chloroplast. 4–5: motile zygotes (planozygotes) with four flagella, otherwise similar to vegetative cells. 6–7: zygospores with central nucleus surrounded by parietal chloroplast. (Note two large lipid bodies, one at each pole of the cell, 6.)
Chlamydomonas nivalis, a green alga. 8–9: resting spores with smooth cell wall, red protoplast, and central pyrenoid surrounded by starch plates. *Chloromonas brevispina*, a green alga. 10–11: zygospores with central nucleus surrounded by parietal chloroplast. (Note two large lipid bodies, one at each pole of the cell and shedding of primary wall with spines and exposure of inner smooth secondary wall, 11.)
Notosolenus sp., a colourless euglenoid. 12–14: cells with ridges on pellicle, central to posterior nucleus, numerous paramylon bodies, one contractile vacuole at anterior end of cell, and two flagella, the longer projected anteriorly, the shorter posteriorly. Cells in narrow diameter view (13) and wide diameter view (12–14).
Chromulina chionophila, a golden alga. 15–17: cells with a parietal, band chloroplast, one contractile vacuole, a single flagellum, and 8–25 chrysolaminarin bodies. (Note three rhizopodia extending from the plasmalemma, 17.)
Figure taken from Hoham and Duval (2001) and reproduced with permission of Cambridge University Press

Algae without an actively motile stage, such as blue-green algae and diatoms, are not normally found in snow packs, but do occur in other low-temperature locations. In Antarctic dry valley lakes, for example, algal mats present under the ice are dominated by blue-green algae in the genera *Lyngbya* and *Phormidium* (Wharton *et al*., 1983). Benthic (pennate) diatoms in the genera *Navicula*, *Nitzschia* and *Pinnularia* are common in such mats under aerobic conditions, where they can be seen gliding between filaments of the algae. Green algae are rare in below-ice samples, but are present in surface ice and snow, along with blue-green algae such as *Anabaena* and *Nostoc*. Many of the cryophilic algae noted in Table 3.16 are restricted to ice and snow environments and are not found in temperate lakes or other water bodies.

3.12.2 Life cycles of snow algae

Studies on the life cycles of snow algae have been carried out mainly in relation to green algal flagellates – the dominant algae in the snow environment.

The active phase of the life cycle occurs in spring or summer when water is released as the snow melts, nutrients and gases become available and light penetrates into the snowpack. The life cycle of *Chloromonas* (Figure 3.26) is typical of snow algae and involves four main phases – zygospore germination, colonization of the snowpack, population increase, and formation of zygotes. These phases correlate with physical and chemical factors at the time of snowmelt.

- The active phase begins with germination of resting zygospores at the bottom of the snowpack. These spores are present either on the snow/soil interface, or on the snow/snow interface in persistent snowfields. Germination of these spores is triggered by the persistence of air temperature above freezing over several days and by increased light levels, and involves division by meiosis to produce biflagellate zoospores.

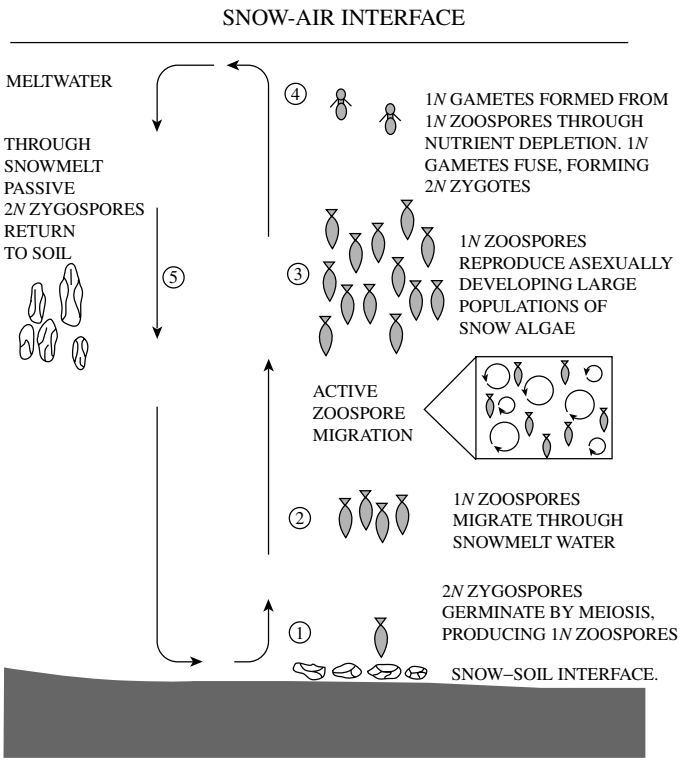

Figure 3.26 Life cycle of the snow algal flagellate *Chloromonas* in a melting snowpack. The cycle commences with zygospore germination (1), followed by active zoospore migration and positioning in the snowpack (2), population increase (3), and formation of zygospores (4), (5). Algal cells are present in the haploid (1N) or diploid (2N) state. Figure taken from Hoham and Duval, 2001, and reproduced with permission of Cambridge University Press.

- The zoospores swim in the liquid meltwater around the snow particles towards the upper part of the snowpack. This is the dispersive or colonization phase of the life cycle and leads to vertical positioning of the zoospores within the snowpack in relation to irradiance levels and spectral composition.

- Zoospores reproduce asexually, generating large populations of snow algae. Visible blooms of snow algae often occur a few days after germination.

- Nutrient depletion results in the differentiation of zoospores to form gametes. These fuse to form resting zygotes, which eventually deposit on the soil surface when the snowpack has melted – or remain on old snow in persistent snowfields. Populations of snow algae stay in the same locality from year to year. In conditions where the snow completely melts, the spores remain dormant through summer and may undergo meiosis during the first freezes in autumn. They are subsequently covered in new snow and germinatewhen conditions ameliorate the following year.

3.12.3 Physiological adaptations of snow algae

Survival and active growth in the snow environment requires the ability to adapt to a relatively brief growth period and to withstand high levels of solar irradiation, low nutrients and temperature, high acidity, and extended periods of desiccation.

Brief growth period

Many snow algae are motile and single-celled. These characteristics reflect the need for free water (for movement and growth) and the relatively brief time period (snow melt) when this may be available. During this brief growth phase, motility, unicellularity, and reproductive strategy are all important in making optimal use of the environment.

Rapid colonization and motility Motility is important for colonization of the snowpack when free water (melt water) becomes available. Having flagella allows the cells to move within the water film that surrounds snow crystals at the time of snow melt. Other snow microbes that are not flagellates (non-motile algae, fungi, and bacteria) are passively moved within the snow, melt water.

Rapid population growth: *r*-selected species Snow algae are typically good examples of *r*-selected species, single-celled with small size and short cell cycle. Such organisms show maximum increase in population under appropriate (i.e., free water) conditions, and are able to dominate the new environment within a short period of time.

Reproductive strategy The production of resting spores is an essential part of the life cycle of snow algae, ensuring survival between the brief periods of growth. The fastest route to producing these resting spores would be by asexual reproduction, since more time is needed to complete the sexual phase of the snow algal life cycle. Field observations suggest that in the most severe environments, where the snowpack is inconsistent from year to year, asexual reproduction may be favoured by natural selection (Hoham and Duval, 2001). This ecological strategy, however, would lead to a decrease in genetic diversity and algae living in such environments may be headed for evolutionary extinction.

Protection from harmful irradiation

The exposed, often high-altitude habitats of snow algae means that these organisms frequently have to tolerate excessive levels of irradiation. Shortwave radiation values as high as 86 000 lux, for example, have been measured in exposed alpine snow containing *Chlamydomonas nivalis* (Mosser *et al*., 1977). Ultraviolet radiation is particularly high in snow environments due to reduced atmospheric shielding (alpine regions) or where the stratospheric ozone layer is relatively thin (polar regions). The

damaging effects of solar radiation, particularly UV light, are discussed in Section 4.9. Specific effects on snow algae have been recorded for *Chlamydomonas nivalis*, where 90 minutes of UV-B irradiation impaired photomovement, motility, and velocity (Hader and Hader, 1989). Exposure to UV radiation has also been shown to reduce photosynthesis by as much as 25 per cent in 'red snow' and 85 per cent in 'green snow'.

As with other algae in exposed environments (Section 4.9.6) snow algae have demonstrated a wide range of high-light and UV-protection responses, including the intracellular production of the following.

- *Photoprotectants*. These include secondary carotenoid pigments such as astaxanthin (see below) and shikimate pathway metabolites, acting as a passive radiation shield.

- *Antioxidants*. In *Chlamydomonas nivalis*, these are known to arrest photoinhibitory damage by the reduction of free radical production in the thylakoid membrane.

- *Repair systems*. A photoreactivation enzyme in *Chromulina chionophilia* is known to repair UV damage to chlorophyll and other photosynthetic pigments.

Astaxanthin: the major pigment of 'red' snow algae One of the most conspicuous features of snow algae is the development of red pigmentation. In various green algae, including the widespread snow alga *Chlamydomonas nivalis*, the major red pigment has been identified as the xanthophyll astaxanthin. Large accumulations of this pigment occur within the cell in extra-chloroplastic lipid droplets, where they are considered to reduce photoinhibition and cell damage.

The development of red pigmentation occurs at high levels of irradiation, but also depends on nutrient levels, particularly nitrogen availability (Figure 3.27, Bidigare *et al*., 1993). Cells of *Chlamydomonas* collected from exposed snow slopes remained green when nutrient levels supported a high growth rate. Such cells maintain a high turnover of the Q_b protein that reduces the cells, susceptibility to photoinhibition from damaging wavelengths of light. The effects of nutrient limitation depends on the level of incident radiation. Under conditions of nitrogen depletion but continued high light level, growth rate is reduced and chlorophyll breakdown is paralleled by the synthesis of astaxanthin. At low light levels, chlorophyll is not degraded and the cells remain green.

In addition to red astaxanthin pigment, orange and yellow-orange carotenoids also accumulate in vegetative cells and zygotes of some algae. Orange carotenoid accumulation, for example, occurs in the polar lipid bodies of zygotes of *Chloromonas* (Figure 3.25). The orange zygotes are located in the upper snow layers where irradiation levels are highest, while zygotes collected from the lower snow layers lack carotenoid accumulation and are usually green (Hoham and Duval, 2001).

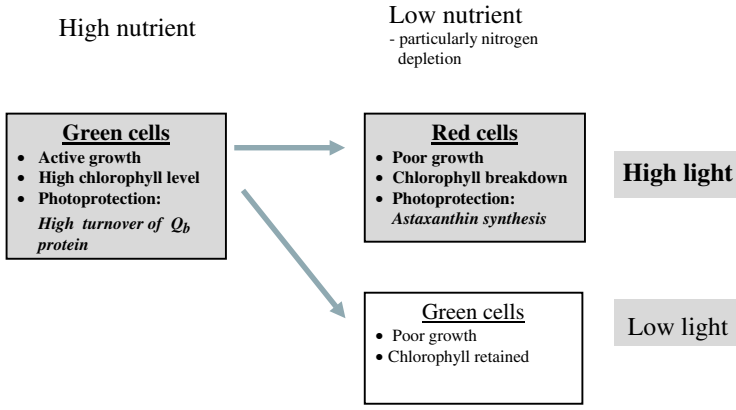

Figure 3.27 Influence of light and nutrient on pigmentation of snow algae. High light conditions occur in exposed environments, low light in areas covered by forest canopy (based on information in Hoham and Duval, 2001)

Nutrient limitation

Nutrient levels are frequently very limited in snow environments (see Chapter 2). Adaptations of snow algae to low-nutrient conditions include the requirement for mineral but not organic molecules, their ability to remain inert over long periods as resistant zygotes, and the ability of motile stages to migrate towards nutrient sources (chemotaxis). Snow algae rapidly colonize habitats where nutrients are high, including snow surrounding sea bird colonies in Antarctica.

The small size of (unicellular) snow algae, with high surface area/volume ratios, also optimizes nutrient absorption under limiting conditions.

Temperature

Various workers have queried whether snow algae are adapted specifically to low temperatures or are simply surviving at the edge of their temperature range. In order to answer this question, algae have been isolated and their growth characteristics studied under controlled laboratory conditions. Hoham (1975) has suggested that true snow algae are obligate cryophiles (or psychrophiles), growing optimally below 5°C, abnormally at 10°C, and not surviving over this limit. Obligate cryophiles include species of *Chloromonas* and *Chlainomonas* and strains of *Chlamydomonas nivalis*. Other snow algae, such as *Raphidonema nivale,* are able to grow at temperatures up to 15°C in the laboratory, and qualify as facultative cryophiles.

Specific adaptations to life in the cold include the ability to photosynthesize at low temperatures and the occurrence of a variable fatty acid ratio.

Fatty acid ratio Various studies have indicated higher ratios of monounsaturated/saturated fatty acids in snow algae compared with their temperate relatives. Such ratios are considered to have a cryoprotective function since high levels of unsaturated fatty acids cause increased membrane fluidity, which is important in maintaining membrane transport at low temperatures. It has been suggested that the growth temperature range of an organism depends primarily on the ability to regulate its membrane fluidity, and the ability to maintain this fluidity at low temperature. In cold-adapted organisms, the lower temperature limit is thus not determined by membrane constraints, but by the freezing properties of aqueous solutions inside and outside the cell.

Photosynthesis

Photosynthetic optima for snow algae show some variation between species. Many snow algae show maximum photosynthetic activity at −3 to 4°C (Hoham, 1975; Mosser *et al*., 1977) while others, such as *Chlamydomonas nivalis*, photosynthesize optimally at 10–20°C, but retain substantial activity at −3 to 4°C. The ability of snow algae to carry out photosynthesis at freezing temperatures is a key factor in their survival.

Acidity

The pH of snow which contains algae has been widely sampled in different continents and has indicated universally acidic conditions, with pH values typically in the range 4–6.2. Laboratory studies on *Chloromonas pichinchae*, which normally grows at pH 4.9–5.2 in nature, have demonstrated a growth optimum at pH 6. Other isolates of *Chloromonas* indicate adaptation to more acid conditions. Little is known about the biochemical and physiological mechanisms which underlie this low pH tolerance.

Desiccation

Desiccation presents the final challenge for many snow algae, which have to survive long periods of potential water loss. This is particularly the case for resistant spores. In many of these cells, the presence of thick complex walls, with primary and secondary layers, may reduce desiccation.

E. BIODIVERSITY IN THE ALGAL COMMUNITY

Preceding sections of this chapter have emphasized the huge diversity of living organisms (biodiversity) that occurs within this major group of freshwater biota. This has been noted in relation to taxonomic features, molecular characteristics, size and shape, environmental preferences, interactions with other biota, and strategies for survival. This section considers diversity in relation to species composition.

3.13 Variety of freshwater algae: indices of species diversity

The assessment of diversity in freshwater biota is complex, both in terms of the criteria used (see above) and the target organisms being assessed. These may be particular taxonomic groups (e.g., blue-green algae) or broad taxonomic assemblages (all algal species), and may be assessed in relation to a single point in time, an extended time period (e.g., a phases of the seasonal cycle), or a particular micro-habitat.

The biodiversity of freshwater algae within a specific environment is normally considered in reference to the range of species present. Assessment of biodiversity in aquatic habitats is important for a number of reasons, including:

- comparison of different natural habitats (e.g., different lake systems) in relation to geographic, hydrological, and specific environmental parameters;
- assessment of the effects of human activities (or their effects) such as chemical pollution, eutrophication, and global warming;
- understanding fundamental aspects of community structure dynamics.

In this section, algal species diversity will be considered specifically in relation to one particular situation – the lake planktonic environment.

3.13.1 The Paradox of Phytoplankton Diversity

One of the remarkable aspects of the lake environment is the large number of phytoplankton species that is present at any one time. Between 30 and 40 algal species may be routinely identified in mid-summer samples from the water column of a temperate eutrophic lake, the final total depending on the skills of the identifier and the number of samples examined. Such species diversity appears as a paradox (Hutchinson, 1961), since it might be expected that a single group of organisms (algae) competing for resources within a uniform and relatively unstructured environment would have low diversity due to competitive elimination. Explanations for the high diversity of freshwater phytoplankton are based on the fact that interactions in the planktonic environment are highly complex, and fall into three main areas as follows

- *Environmental complexity.* Although the freshwater environment might appear to be relatively uniform, particularly within the epilimnion, there are some important variations. This is particularly the case for the biotic environment, with very patchy distributions of algae and zooplankton resulting in micro-environmental variation in nutrient availability, algal competition, and grazing pressure.
- *Diversity in adaptive strategies*. As noted earlier, algae have responded to a range of environmental constraints in a number of ways with different strategies for survival. Competition between algae will not, therefore, involve a single characteristic relating to a single environmental feature (e.g., relative ability to take up phosphate ions from a phosphorus-limited epilimnion), but a whole range of adaptations relating to multiple environmental characteristics.
- *Non-adaptive interactions*. This approach to phytoplankton diversity postulates that there is

insufficient time for competitive interactions and adaptations to completely and fully operate, since interactions in the freshwater environment are essentially short-term. Community composition at any one time is considered to be determined more by the pre-existing situation than by current species adaptations. This situation is dominated by historical factors and the pre-existing community (referred to as 'founder-effects').

3.13.2 Biodiversity indices

Biodiversity within a group of organisms may be determined numerically in relation to a variety of parameters (referred to as importance values) including biomass, productivity, and organism count (Odum, 1997). Most estimates of species diversity are determined from species counts, which can be used to generate bio-indices of three main types – species richness, species evenness/dominance, and a combined index of biodiversity.

Species richness

This index relates to the total number of species present in the population sample – the greater the number of species the greater the measure of biodiversity. Species richness may be assessed in two main ways as follows.

Total number of species (*S*) Species richness is often determined simply in relation to the total number of species. The major problem with this index is that the value (S) may depend on sample size – the bigger the sample the more species there are likely to be.

Margalef index (*d*) This index avoids the complication of sample size, by incorporating the number of individuals (N) in the sample. The index thus represents a measure of the number of species relative to the total number of individuals present in the sample, where

$$d = (S - 1)/\log N \qquad (3.9)$$

Species evenness/dominance

The evenness of species occurrence is also an important measure of biodiversity. Considering two hypothetical samples (I and II), each composed of four species (A, B, C and D):

Sample I. Total 100 individuals; species A (25), B(25), C (25), and D (25).
Sample II. Total 100 individuals; species A (94), B (2), C(2), and D(2).

Sample I would be regarded as more diverse than sample II, though species richness is the same. Sample I has greater evenness of species occurrence, but a lower dominance of any species. Sample II is the converse.

Species evenness may be determined as *Pielou's evenness index* (J'), where:

$$J' = H'(\text{observed})/H'\,\text{max} \qquad (3.10)$$

and $H'_{\max}$ is the maximum possible diversity that would occur if all species were equally abundant.

Dominance is the converse of evenness, and can be determined using the Simpson index (D), which relates the number of individuals in each species (n_1) to the total number of individuals in the sample (N), where:

$$D = \sum (n_1/N)^2 \qquad (3.11)$$

Comparison of indices for species evenness and dominance over a number of population samples shows a clear inverse relationship.

Combined index of biodiversity

Clearly, species richness and evenness (or dominance) both contribute towards mixed population diversity, and a most useful approach would be to bring these together as a single value. The Shannon–Wiener Diversity Index is the most common combined measure of diversity, taking into account richness, evenness, and abundance of the community structure and assumes that individuals are randomly sampled from an infinitely large population

(Mason, 2002). The equation below is used to calculate the Shannon–Wiener Index:

$$H' = \sum P_i(\ln \mathrm{P_i}) \qquad (3.12)$$

where H' is diversity and P_i the proportion of individuals found in the ith species (estimated as n_i/N (total number)).

3.13.3 Numerical comparison of phytoplankton populations

Lake nutrient status

In general, lakes with a very high nutrient status have relatively low species diversity (Margalef, 1958). Studies by Reynolds (1990), for example, compared UK lake sites of decreasing nutrient status – Blelham enclosures (fertiliser-enriched, never *P*-limiting), Crose Mere (rarely *P*-limiting), and Windermere (chronically *P*-limiting). Both mean and maximum seasonal diversity (Margalef index) increased along this sequence, with maximum levels in Lake Windermere. Further reduction in lake nutrient status towards oligotrophy results in a decrease in phytoplankton diversity. This was demonstrated by Debacon and McIntyre (1991), who obtained seasonal Shannon index values in the range 0.5–2.5 for the ultra-oligotrophic Crater lake in Oregon (USA).

Seasonal changes in phytoplankton biodiversity

The major seasonal changes that occur in phytoplankton biomass, with the succession of spring diatom bloom, clear-water phase, and mixed summer algal bloom (Figure 3.14), are also accompanied by changes in biodiversity. An example of this is presented in Figure 3.28, which shows changes in algal biomass, with related indices of species richness, species dominance, and general biodiversity in a typical temperate eutrophic lake (Levado and Sigee, unpublished observations).

During this annual sequence, the spring diatom bloom was a period of moderate species richness, with low dominance – since the diatom population

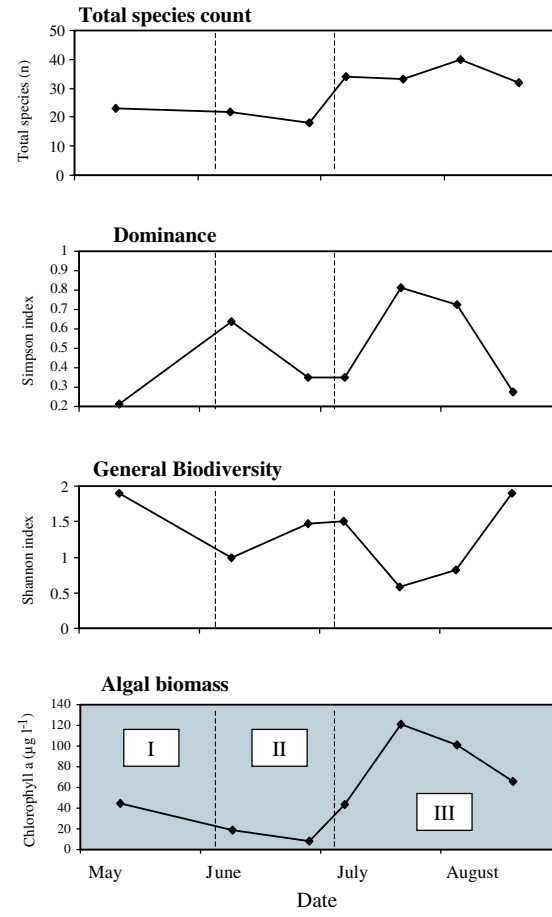

Figure 3.28 Seasonal changes in indices of phytoplankton biodiversity in a temperate eutrophic lake. Vertical broken lines demarcate the end of the diatom bloom (I), the clear-water phase (II), and the late summer mixed algal bloom (III). (Taken with permission, from Levado, 2001.)

was composed of a balanced mixture of species. Increased dominance and low biodiversity (Shannon index) occurred in the early clear-water phase. This was characterised by a relatively small number of *r*-selected species, with dominance by green algae such as *Ankyra, Chlamydomonas* and *Sphaerocystis*.

Species richness reached highest levels during the development of the mixed summer algal bloom, emphasising the wide range of species that grew towards the end of lake stratification (see

Figure 3.17). This build up in species richness towards the end of the growth period is in line with the laboratory studies of Odum (1962), who studied the transition of a fresh culture of algal species to a mature steady-state situation. Initially the culture was dominated by a few rapidly-growing species, but eventually a steady state mixed population of high biomass and biodiversity was reached. Park (1980) has suggested, in relation to terrestrial ecosystems, that the increase in species richness occurring during ecological succession is due to an increased availability of habitat niches. In the case of the aquatic environment, we can consider 'habitat niches' as the ability of different phytoplankton species to compete and exploit the environment in different ways, with the diversity of competitive strategies noted previously.

The maximum values for species diversity that occurred during the summer bloom were matched by minimum indices for the combined diversity index. This was because of developing high population levels of colonial blue-green algae and dinoflagellates, resulting in a high index of dominance. Increased summer dominance in this lake (Figure 3.28) was associated with the conditions of intense competition that relate to very high phytoplankton biomass ($>100\,\mu g$ chlorophyll l^{-1}) and signals the tendency of highly-adapted *K*-selected species to out-compete other algae.

3.13.4 Biodiversity and ecosystem function

Various aspects of ecosystem function relate to biodiversity within the biological community, particularly the diversity of primary producers (see Chapter 1). Seasonal changes in phytoplankton biodiversity, for example, relate to aspects of lake community dynamics such as algal productivity and ecosystem stability.

Biodiversity and algal productivity

Biodiversity is at a minimum during the period of minimum productivity (clear-water phase), with increased biodiversity typically being associated with periods of higher algal growth (spring diatom bloom and mixed summer bloom). The relationship between biodiversity and productivity breaks down, however, under conditions of intense summer bloom formation – when dinoflagellates or blue-green algae may completely dominate the lake and cause a marked reduction in species richness.

Biodiversity and ecosystem stability

Ecosystem stability may be defined in various ways (see Chapter 1), including the degree of community change and species diversity.

On both of these criteria, the clear-water phase can be seen as a time of high instability within the annual cycle. During this time there are major changes in the biomass and species content of phytoplankton populations, with zooplankton populations also undergoing rapid changes and maintaining a high grazing pressure (high stress level) on phytoplankton. Biodiversity (measured as species richness or Shannon index) typically reaches low levels during this phase.