Microalgal ecology

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Introduction

Over the past few decades, microalgal ecology has been dominated by research on free-living planktonic forms (phytoplankton). Thus, while the importance and significance of benthic microalgae (microphytobenthos) (e.g. Macintyre *et al.* 1986, Wainwright *et al.* 2000), and epiphytic microalgae (Moncrieff *et al.* 1992) are acknowledged, they are not the focus of this review. The review will also exclude parasitic microalgae (e.g. Chapman & Henk 1985, Bouarab *et al.* 2001) and symbiotic microalgae (e.g. Lewin 1995, Goodson *et al.* 2001), as well as terrestrial microalgae (see Evans & Johansen 1999, Metting 1981). Although they are not strictly "algae", prokaryotic species are included in the review because they are inseparable from algal components of the phytoplankton.

Microalgal ecology (particularly of marine species) has tended to become more distinct from other ecological disciplines over the past 25 years. Ecological principles developed in terrestrial ecosystems have been extensively applied to microalgae (see Harris 1986, Reynolds 1984), but the principles governing their success are different, being a consequence of their small size, physiological flexibility, and the dominance of physical and chemical processes in the aquatic environments they inhabit. Microalgal ecology has a very strong dependence on physics, chemistry and geochemistry and studies have increasingly focussed on smaller scales of spatial and temporal variability. The problems in dealing with such scales have resulted in a greater reliance on technology than is found in many of the sub-disciplines of terrestrial ecology (e.g. Falkowski & La Roche 1991). In some ways, this has helped microalgal ecology avoid becoming trapped in traditional questions and traditional methods (something Peters (1991) has termed the "crisis" in ecology), but can be criticised for favouring technology over theory (Peters 1991).

In general, concepts have transferred well between freshwater and marine systems, though the overall tendency (with important exceptions) has been for those working in fresh water to focus on organisms and populations, while marine phytoplankton ecologists have tended to look more at community and ecosystem processes.

Recent developments

Physical influences on algal ecology

Although algal biophysics will be dealt with in a separate review in this volume (Koehl & Jumars), it is important to acknowledge that the involvement (and even dominance) of physics in

microalgal ecology has been an important research focus in the past 25 years (Imberger 1998, Daly & Smith 1993). Critical processes affected by physics include light acquisition (e.g. Lewis *et al.* 1984), sinking (e.g. Brzezinski & Nelson 1988) and nutrient acquisition (e.g. Karp-boss *et al.* 1996). Moreover, the entire structure of of microalgal ecosystems is shaped by turbulence (e.g. Spigel & Imbeger 1987, Franks 1995).

Estimating primary production

Estimating primary production has been something of an obsession for microalgal ecology in both fresh and marine waters. In part, this has been driven by the development of simple methods (e.g. Steeman Nielsen 1952), but recognition of climate change has also placed a premium on accurate estimates of global primary production, particularly oceanic contributions to the global carbon budget (see Falkowski 1994, Geider *et al.* 2001).

Accurate estimation of primary production (i.e. biomass accumulated over some period of time) depends on determining biomass and estimating productivity (i.e. instantaneous rates of increase of biomass). In terms of measuring biomass, although microscopy has largely been replaced by technological advances, it is still a useful methodology. Inverted microscope methods (Lund *et al.* 1958) coupled with cell volume-specific equations (Strathman 1967) are one of the few ways to determine biomass of individual species within a natural assemblage (e.g. Montagnes *et al.* 1994, Verity *et al.* 1998), but the method remains time-consuming and automated identification of species have not developed as rapidly as once thought possible (Gorsky *et al.* 1989; see Medlin, this volume). Flow cytometry is increasingly applied to small algal species (Li 1994,), though chain-forming and colonial species are still problematic.

^{14}C methods

In terms of measuring productivity, the ^{14}C method (celebrating its 50th anniversary with the British Phycological Society this year, see Williams *et al.* 2002) remains the most widely used, and misunderstood, method. Introduced by Steemann Nielsen (1952), ^{14}C quickly became the method of choice for the determination of marine micro-algal productivity. It is a deceptively simple procedure; add a known amount of ^{14}C bicarbonate to a water sample and incubate for a period of time. The phytoplankton fix CO_2, including the added tracer, they are recovered by filtration and the total radioactivity in the phytoplankton can be accurately measured. If the total carbon dioxide concentration (dissolved CO_2, HCO_3^- and CO_3^{2-}) is known, then the proportion of added tracer which is incorporated into phytoplankton is the same as the proportion of total CO_2 in that water sample which is fixed. It has the illusion of being as simple a technique to determine a chemical parameter such as salt or nitrate concentration. Over thirty years ago, Ian Morris, with Charles and Clarice Yentsch, wrote in relation to the use of the ^{14}C technique *"Finally, it seems pertinent to point out that although we appear to have inherited a very simple technique for estimating carbon fixation, the method is only as good as our knowledge of how carbon is fixed. Until this is certain, our knowledge of the productivity of the open ocean will still be in question"* (Morris *et al.* 1971). With the passage of 30 years, are we any closer to understanding exactly what is measured by the ^{14}C technique?

It is critical to recognise that the ^{14}C does not measure primary production — that is, the increase in biomass of the micro-algae within a natural assemblage (c.f. Dring & Jewson 1982). During whatever period of time the incubation takes place, the ^{14}C method measures the assimilation of carbon, some of which goes to the biosynthesis of cellular material but some of which is lost by respiration, or released as dissolved organic carbon; and some of the label will end up in grazing organisms which feed on the phytoplankton. In addition, any phytoplankton assemblage is made up of a collection of different phytoplankton species, with varying intrinsic generation times, different responses to light and nutrient limitation or requirements for trace elements or other cofactors. So the quantity of ^{14}C fixed is a mean rate of activity of the diverse mixed assemblages.

There are three basic approaches to the use of ^{14}C to estimate primary production: a) incubation of samples *in situ* at the depths from which water samples were obtained, b) incubation in natural light with screens to simulate light attenuation with depth, and c) incubation with artificial light to quantify the relationship between light and carbon fixation (P vs. E curves),

followed by use of these photosynthetic parameters to model primary production in relation to a depth profile of chlorophyll concentration (Sakshaug *et al.* 1997). No method is ideal and considerable attention has been paid to the complications inherent (see Leftley *et al.* 1983, Marra 2002). *In situ* incubations, particularly coupled with size fractionation experiments to determine the activity of different cell sizes, could be argued to be the most realistic approach since the phytoplankton assemblage experiences the most natural light quantity and quality. However, there are complications: phytoplankton cells are returned to the depths at which they were sampled, but they are held at constant depths, unlike natural assemblages which move vertically within the light gradient of the surface mixed layer (Uncles & Joint 1983). The same uncertainties apply to incubations on-deck with natural light (generally used because of the obvious logistical advantage that primary production can be assessed whilst a ship is underway), but other artefacts may also be introduced due to changes in temperature of the incubated samples from in situ conditions, and changes in light spectrum with depth that are difficult to simulate with screens. The third approach of quantifying photosynthetic parameters has been used extensively to estimate primary production, but strictly the approach gives information about photosynthesis, not production. The method suffers from problems relating to light quality since most artificial light sources of intensity sufficient to saturate photosynthesis have an unrealistic spectral composition with too much red light. However, photosynthetic characteristics have been widely applied and have the advantage of allowing the modelling of phytoplankton dynamics.

All of these methods share the disadvantage that the phytoplankton assemblages are contained within bottles for periods of time, varying from a few hours (P vs. E experiments) to a day (*in situ* incubations). In the case of short term incubations, it is necessary to assume that the rate measured can be extrapolated to a longer time scale and will relate to the generation time of the phytoplankton cells within the assemblage. Longer incubations of 24 h or so are more compatible with the actual generation times of the phytoplankton but it is usually assumed that the longer an assemblage is enclosed, the more likely that artefacts may be induced by the bottles. Although considerable attention has been directed at showing why incubation artefacts are a problem (e.g. Leftley *et al.* 1983), in reality, these effects may sometimes be overstated. For example, Massana *et al.* (2001) have shown that significant changes in bacterial composition occur after enclosure in bottles only after more than 2 days. Given that the generation time of bacteria is generally much shorter than of phytoplankton, enclosure in a bottle may not radically affect the phytoplankton assemblage. Moreover, evidence suggesting that incubation artefacts may be less serious than sometimes thought were provided in a recent Lagrangian experiment. Rees *et al.* (2001) measured primary production and nutrient uptake within a mesoscale eddy in the north Atlantic. A patch of water was labelled with the tracer sulphur hexafluoride (SF6) which enabled precise calculation of dispersion of the patch, and allowed repeated sampling over 5 days. At the same time, daily estimates of nutrient uptake were made using bottle incubations. The uptake of nitrate and phosphate based on bottle incubations explained 76% of the observed change in nitrate and 77% of the observed change in phosphate concentration in the labelled patch. That is, there was very good agreement between bottle estimates of nutrient uptake and the changes occurring in an unenclosed natural assemblage.

It is not surprising that there are doubts about what the ^{14}C technique measures because new and significant primary producers continue to be discovered in the oceans. For nearly 40 years after the introduction of the ^{14}C technique, we were completely unaware of the presence of *Prochlorococcus* (Chisholm *et al.* 1988) — an organism considered by some to be the most abundant on the planet. It was only slightly earlier that the importance of picoplankton in general was recognised (Waterbury *et al.* 1979). Recently anoxygenic photosynthetic bacteria, previously thought to be restricted to sediments, have been shown to be widespread in the sea (Kolber *et al.* 2001). Unlike other well-characterised photosynthetic bacteria which synthesise their photosynthetic apparatus and perform photoassimilation of organic compounds only under anoxic conditions, these marine photosynthetic bacteria are active in the well-oxygenated conditions of the surface ocean. Estimates of their abundance vary from ~5 – 10% (Kolber *et al.* 2001) to <0.5% (Goericke 2002) of the phytoplankton within an assemblage. Whatever the true

abundance, the photosynthetic bacteria will almost certainly utilise light for photoassimilation of organic compounds and will not be truly autotrophic. They appear to be a very diverse group and recently, Béjà *et al.* (2002) have shown a very wide molecular diversity of these hitherto unknown aerobic anoxygenic phototrophs.

More serious and less well understood problems with ^{14}C methods involve the consequences of isotope distribution within the phytoplankton cells. If ^{14}C is added to an assemblage, the label is assimilated in proportion to the ^{12}C / ^{14}C ratio of the bicarbonate in sea water. There is relatively little discrimination against ^{14}C (on the order of a few percent), however, at the point at which ^{14}C is added, the phytoplankton cells are composed entirely of ^{12}C. As photosynthesis proceeds, the cells will become increasingly labelled with ^{14}C, eventually reaching equilibrium with the inorganic carbon sources. However, incubations are rarely long enough for the cell composition to reach equilibrium and some of the ^{14}C fixed is respired and lost as $^{14}CO_2$ rather than incorporated into biomass. This process has been modelled by Williams & Lefèvre (1996) who provide a very clear description of the problems of using ^{14}C to estimate production.

Additional uncertainties relate to the process of grazing by zooplankton on phytoplankton cells as they are fixing ^{14}C label. Since most primary production measurements are done in bottles of <100 ml capacity, mesozooplankton with typical abundance of a few animals per m^3 are unlikely to be enclosed in every bottle (though this will increase variability between replicates), and so microzooplankton grazing would be expected to represent a more significant problem. Microzooplankton grazing is often of the same order as phytoplankton production, so a significant proportion of the ^{14}C collected in the microbes filtered at the end of the experimental incubation may in fact be present in microzooplankton which have ingested labelled phytoplankton. Few direct experiments have attempted to quantify any potential redistribution of label, but Joint & Pomroy (1983) did time-course experiments which measured ^{14}C label in three size fractions (>5µm, between 5 and 2µm and <0.2µm). Although this study did not make concomitant measurements of microzooplankton grazing, there was no evidence of increased labelling of larger size fractions after 12 h incubation than after 3h. If microzooplankton grazing was a significant process, such an increase would be expected.

Finally, a more basic concerns underlined by Morris *et al.* (1971) was the issue of carbon fixation in the dark. Measurement in both light and dark bottles was a standard methodology before the introduction of ^{14}C, when production was measured by changes in O_2 using Winkler titrations. In this case, the dark bottle was essential since changes in O_2 concentration in the dark are a measure of respiration of the total assemblage (net community respiration) and variations in O_2 concentrations in the light were due to both respiration and photosynthesis. Subtraction of the rate of change in O_2 in the dark (D) from that in the light (L), gives a measure of photosynthesis. However, the same arguments do not apply to the ^{14}C technique, and it is unclear exactly what the dark bottle results represent. For example, beta-carboxylation reactions in heterotrophic organisms may or may not occur at the same rate in the light and dark, and chemolithotrophic bacteria, which also fix CO_2, may or may not be inhibited by light. In many oceanic provinces, the rate of ^{14}C fixation in the dark is a small proportion of that in the light and, if the dark bottle is subtracted, it probably will make little difference to the estimate of primary production. However, in large provinces, particularly in oligotrophic regions, dark fixation is a very high proportion of that measured in the light (Prakash *et al.*1991). Markager (1998) found that a significant part of the dark ^{14}C uptake was abiotic in waters with low phytoplankton biomass. Different researchers use dark incubation results in different ways, and there can be very large variations in the calculation methods even if common data are used (see Richardson 1991).

Alternative methods for estimating production

The limitations of the ^{14}C method have been well-appreciated and an enormous number of attempts have been made to find other means of estimating productivity. These have included measurement of parameters related to the cell-cycle (e.g. Falkowski & Owens 1982, Vaulot 1992), correlations of growth and ATP content (e.g. Sheldon & Sutcliffe 1978) or nucleic acid content (e.g. Berdalet & Estrada 1993), measurement of protein synthesis (Lean *et al.* 1989) ,

enzyme measurements (e.g. Collos *et al.* 1993), and immunochemical methods (Orellana & Perry 1992).

Another set of methods has been developed by exploiting measurements of active fluorescence using techniques including addition of photosynthetic inhibitors (Cullen & Renger 1979), pulse-amplitude-modulated instruments (Schreiber *et al.* 1995), and *in situ* profiling instruments based on pump-and-probe or fast-repetition-rate methods (Kolber & Falkowski 1993, Kolber *et al.* 1998). There have been relatively few inter-comparisons of methods (e.g. Boyd *et al.* 1997), but the fluorescence methods have proven their worth in enrichment experiments (see below), and instruments to make these measurements are increasingly available.

Remote sensing of primary production

Satellite remote sensing is now making rapid advances in mapping the global distribution of surface chlorophyll concentrations and providing data at spatial and temporal scales which were undreamed of only a few years ago (Joint & Groom 2000). The challenge is to estimate production as well as phytoplankton biomass on these scales. Much progress has been made in utilising surface pigment concentrations to estimate primary production and several approaches have been taken, utilising empirical and semi-analytical models. Kyewalyanga *et al.* (1992) described a series of spectral models which were subsequently used by Platt *et al.* (1995) to estimate global primary production, based on photosynthesis characteristics of phytoplankton. Alternatively, Behrenfeld & Falkowski (1997) have implemented a light-dependent, depth-independent model which requires a smaller number of parameters. Their simple model explained 86% of the variance between measured and modelled production estimates for a data set of nearly 1700 estimates of primary production. Semi-analytic approaches, such as that proposed by Morel (1991), calculate primary production to the base of the euphotic zone, using the vertical chlorophyll profile, photosynthetically usable radiation (*i.e.* spectral PAR weighted by the spectral phytoplankton absorption), a function relating carbon production to usable light. This approach has been successfully applied to a number of situations, including the upwelling region off NW Spain (Joint *et al.* 2002) where satellite remote sensing was used to quantify the annual increase in primary production due to wind-driven upwelling processes.

Thanks to remote sensing, it has been possible to estimate the contribution of marine phytoplankton to total global carbon fixation; this is now thought to be approximately equal to terrestrial productivity (Field *et al.* 1998) at *ca.* 50 Pg C yr^{-1}. However, to date there only been a few attempts to estimate the potentially large errors involved in these estimates (Joint & Groom 2000).

Limiting factors

A dominant theme of microalgal ecology in the past few decades has been the identification of limiting factors. In examining limiting factors, an important distinction is between factors that limit total biomass (often termed "standing crop") from those that limit rate of increase in biomass (i.e. productivity). Thus, for example, while the concentration of a major macronutrient such as nitrogen may limit the ultimate biomass that microalgae can achieve, the availability of light may limit the growth rate, *i.e.* the time it will take to achieve that biomass. Unfortunately, because of the variability in recycling rates, the declining efficiency of acquisition systems at very low concentrations and the emerging concept of co-limitation, even the definition of limitation is difficult (Cullen *et al.* 1992, Falkowski *et al.* 1992).

The classical view has been that freshwater systems tended towards phosphorus limitation, while marine systems tended towards nitrogen-limitation (see Kilham & Heckey 1988, Howarth 1988), but recent research has clearly demonstrated that this is a gross oversimplification. For example, there is considerable evidence that phosphorus limitation may play an important role in marine microalgal ecology. Local examples of P-limited marine systems have often been identified in coastal regions (e.g. Harrison *et al.* 1990), but geochemists have long argued that phosphorus should ultimately limiting production in longer time scales (see Smith 1984). Interestingly, this hinges on how important nitrogen fixation proves to be (see below), and this may, in turn depend on the availability of iron (see Falkowski *et al.* 1998, Tyrell 1999). As well, Karl (1999) hypothesised that so-called "domain shifts" in the

North Pacific lead to variability between periods of N and P limitation, which have important biogeochemical consequences.

Improved analytical methods and clean sampling techniques have led to a reconsideration of metal ion limitation of microalgae. This idea has a considerable history (see deBarr 1994), and the first important studies were in freshwater where analytical issues were much simpler (see Howarth & Cole 1985). Iron and molybdenum limitation were especially important in freshwaters owing to their importance in nitrogen-fixing organisms that often dominate in freshwater. While metal limitation of freshwaters seems less likely due to their proximity to the geological sources and their lower pH, freshwaters also have high concentrations of organic matter, so the issue of complexation of metals become important. In the last two decades, however, the concept of iron limitation in marine systems has been a major research focus. Older studies of metals limitation were hampered by the problems of analysis and contamination, and it was really only with the work of Martin and colleagues (e.g. Martin *et al.* 1990) that trustworthy evidence began to emerge. This culminated in open-ocean iron enrichment experiments in the equatorial Pacific (Kolber *et al.* 1994, Behrenfeld *et al.* 1996) and Antarctic (Boyd *et al.* 2000). The physiological and ecological data from these experiments leave little doubt that growth rate limitation and biomass limitation are both occurring. The literature now abounds with work on other trace metals ranging from zinc and cobalt to nickel and selenium (e.g. Price & Morel 1990, Hutchins & Bruland 1995).

Carbon limitation has also received attention. There is evidence that carbon dioxide in freshwaters can be decreased to the point where it may limit phytoplankton (e.g. Talling 1976, Jaworski *et al.* 1981), but this was rarely considered in marine water prior to the work of Riebesell *et al.* (1993). Whether CO_2 can limit phytoplankton productivity depends on physical constraints and also the nature of carbon-concentrating mechanisms that the microalgae possess, and though there are examples of CO_2 draw-down in nature (e.g. Cooper *et al.* 1996), the phenomenon is not always simple (Watson *et al.* 1994).

With changes in atmospheric ozone becoming apparent, attention has also been focussed on potential effects of increased UV radiation on phytoplankton (e.g. Smith *et al.* 1992). A range of effects has been found in short-term experiments in marine (e.g. Behrenfeld *et al.* 1993, Cullen & Neale 1994) and freshwaters (e.g. Furgal & Smith 1997). However, detecting such effects in longer term has proven more difficult (e.g. McMinn *et al.* 1994).

With such attention focussed on identifying limiting factors, new techniques have been developed (see Beardall *et al.* 2000). Classic enrichment experiments using incubations (e.g. Ryther & Guillard 1959, Lean & Pick 1981) continue to be used, but new methods for identifying limitation based on photosynthetic characteristics (Geider *et al.* 1993) or molecular methods based on specific proteins have also been used (Scanlan *et al.* 1997, La Roche *et al.* 1995). Ecosystem-level experiments (*sensu* Carpenter *et al.* 1995) have also been used to determine whether control is "bottom-up" (i.e. by nutrients) or "top-down" (i.e. by grazers) (see Elser & Hassett 1994, Pace *et al.* 1999). Generalisations about the factors governing trophic cascades have emerged from freshwater systems, but not yet from marine environments.

Loss factors

For many years, the emphasis in microalgal ecology was placed on growth and ecological loss terms, usually identified as grazing and sedimentation, were less often considered. A considerable body of work on freshwater losses has accumulated for tractable small-lake ecosystems (see sections of Reynolds 1984, Wetzel 1995), but there was recognition that closure of budgets in most marine ecosystems was more problematic (Walsh 1983).

Presently, a much larger number of potential loss factors are acknowledged. Viruses are now recognised as important agents of mortality in microalgae (Proctor & Fuhrman 1990, Maranger & Bird 1995), though their quantitative significance continues to be uncertain (Fuhrman 1999). It is now also appreciated that phytoplankton mortality in aquatic ecosystems can result directly from bacteria (Cole 1982, Imai *et al.* 1993), and that other causes of mortality can be modified by bacteria (Brussaard & Reigman 1998). Parasites of freshwater and marine phytoplankton are known to including flagellates (Kuhn 1998, Erard le Denn *et al.* 2000), chytrids (e.g. Bruning 1991) and fungi (Holfeld 1998). These agents can be widespread (Tillman *et al.*

1999), and responsive to environmental variables (Bruning 1991) but their quantitative significance has rarely been assessed.

Recent work has begun to examine the possibility that there is a component of "natural mortality" in phytoplankton communities. This has been well considered in freshwater systems (see Reynolds 1984), but has rarely received attention in marine waters. Unlike the case in lakes, there is a considerable body of evidence to show that classical loss terms cannot account for observed losses in many marine environments (e.g. Brussaard *et al.* 1995). Moreover, indirect evidence of mass lysis in ecosystems (Agusti *et al.* 1998, Kirchman 1999), and evidence from cell vital staining that a considerable portion of cells in nature may not be viable (Veldhuis *et al.* 2001) suggest that there are undefined processes at work. Culture work has shown that physiological stresses can lead to mortality events that are abrupt and have similarities to apoptotic pathways in multicellular organisms (Berges & Falkowski 1998, Segovia *et al.* in press).

Allelopathy and chemical interactions

The ecological interactions between algae other groups has been an active area of research in the past few decades. There are many examples of allelopathic effects between different algal species (e.g. Maestrini & Bonin 1981), though the ecological significance of these findings often remains unclear. Nor are effects necessarily one-way: very recent work has demonstrated reciprocal allelopathic interactions between cyanobacteria and dinoflagellates, which may be a key factor in determining community structure in some lake ecosystems (Sukenik *et al.* 2002, Vardi *et al.* 2002). Effects of microalgae on other groups such as bacteria have also been noted (e.g. Kellam & Walker 1989). Intriguingly, recent research has demonstrated that algae may also affect their grazers, though feeding deterrents or toxins (Wolfe *et al.* 1997), or more indirectly; for example, aldehyde compounds produced by some diatom species can actually inhibit copepod reproduction (Ban *et al.* 1997, Miralto *et al.* 1999).

A special case of microalgal chemical interactions concerns toxic algal blooms. These comprise mainly cyanobacteria in freshwaters (e.g. Codd *et al.* 1999), and a wide range of groups including dinoflagellates, prymnesiophytes and diatoms in marine systems (Smayda & Reynolds 2001). There are also indications that interactions with bacteria are an important element in toxicity (e.g. Doucette 1995), and concerns have been raised that recent environmental change and mobility are leading to trends towards increases in blooms of such species (Hallegraeff 1993). It is important to recognise that though the effects are perceived largely in human terms, blooms also affect fish (e.g. Burkholder *et al.* 1992) and marine mammals (Hernandez *et al.* 1998).

Phytoplankton composition

The concept that phytoplankton composition is not simply linked to availability of nutrients in the environment, but actually determines environmental concentrations has been important in algal ecology for well over 50 years (see Redfield 1958). Formalised as the Redfield atomic ratio of 106C: 16N: 1P, the concept remains useful, despite recognition that it can vary widely and that there were biases in the data used to establish it (Takahashi *et al.* 1985). Moreover, the stoichiometry is largely maintained at higher trophic levels (Elser & Hassett 1994). The concept of the Redfield ratio has driven considerable ecological research and it has been used a basis for interpreting interspecific resource competition (see Rhee &Gotham 1980). It has been proposed that phytoplankton organisms must be growing optimally in order to display the ratio (and thus to suggest that open-ocean phytoplankton growth is not nutrient-limited), but this may not hold for light-limited growth (Goldman *et al.* 1979, Goldman 1986). As well, biogeochemical information has been derived from comparing deep-water ratios with those in surface waters (Pahlow & Riebesell 2000). Fascinatingly, after such considerable research, the biological basis for this ratio is still not understood and the question itself is rarely posed (Falkowski 2000).

Algal diversity: paradoxes and new discoveries

In the past few decades, the ecological question "why are there so many species?" (which peters 1991 has described as "intractable"), has found a special expression in microalgal ecol-

ogy in Hutchinson's (1961) "paradox of the plankton"; how can so many species with apparently similar resource requirements share such apparently homogeneous environments? The significance of the question has largely faded, with a variety of solutions posed, including physical environmental variability, small inequalities in resource competition and higher level trophic control (Petersen 1975, Sommer 1996). It has also become clear that niches can be very sharply defined and partitioned along gradients that are not at first clear; Moore *et al.* (1998) have demonstrated such partitioning for open ocean *Prochlorococcus* species.

In both freshwater and marine systems, recognition of the diversity and ecological importance of very small phytoplankton, the picoplankton (defined as those species less than 2 µm in diameter), has been an important trend (e.g. Stockner 1988), driven by the rise of flow cytometry as a method. As noted above, prokaryotes such as *Synechococcus* and *Prochlorococcus* have been a major focus of research (Campbell *et al.* 1994, Carrick & Schelske 1997), but picoeukaryotes have also been recognised (Simon *et al.* 1994), including the a new algal class, the Pelagiophyceae (Anderson *et al.* 1993).

Ecological modelling

As in other areas of ecology, modelling has, for five decades or more, played an important part in developing understanding of microalgal ecology. Early on in this period, the models were essentially conceptual, sometimes expressed in words or, more usually, were quantitatively descriptive, comprising equations fitted by long-hand derivations to experimental or observational data. With the advent of computers, biologists were encouraged to explore larger data sets with multiple regressions or to seek statistical components or to distinguish criteria for separation an classification. From the mid 1980s, the personal computer and a powerful armoury of appropriate software packages have brought modelling increasingly within the grasp of all numerate biologists.

In the first category of models, one of the oldest and most useful was Smith's (1936) solution to the light dependence and saturation of subsurface aquatic photosynthesis. There have been detailed variations to the Smith equation and there are now alternatives to estimating photosynthetic rates (such as by fluorescence) without resort to light and dark bottles but which nevertheless rely on the shape and quantitative components of the photosynthesis/light curve (Behrenfeld *et al.* 2002). A recent verification of the *in-situ* rates of biomass increase reconstructed for a deep-stratified, continuously light-limited population of *Planktothrix rubescens* in the Zürichsee, Switzerland (Bright & Walsby, 2000) serves as a powerful vindication of the original modeller's skill. Similarly, the measurement of nutrient uptake at the surfaces of nutrient-starved cells was soon recognised to conform to a saturable function of concentration, describable by a Michaelis-Menten formulation (Dugdale, 1967). The modification proposed by Droop (1974) allowed for the internal accumulation of nutrient and, incidentally, explained the ability of cells to maintain growth on the internal quota, even after the "limiting" nutrient had been exhausted. The recognition of interspecifically differing affinities for nutrients present in low concentrations in the medium was at the base of the resource competition models introduced by Tilman (1977).

Independently of the physiology of uptake, the limnologists' long-standing appreciation of microalgal biomass being frequently a direct function of the nutrient (typically phosphate) was given expression by the fitted regressions of Vollenweider, eventually in the form noted by Vollenweider & Kerekes (1980). This powerful function represents an average of carrying capacities over a range of lakes and gives no more than a guidance about the biomass fluctuations in any one of them - it is a mistake to predict, much less manage, the algal populations represented by a given nutrient load. Nevertheless, it remains in frequent use by managers and biologists alike: its robust simplicity works well for deep, temperate lakes; its alleged failures have helped us to understand the role played by hydraulic retention, tropicality, shallowness and nutrient recycling in the microalgal behaviour of many other types of lake and to diagnose conspicuously wide responses of systems subject to deliberate load reductions (SAS, 1989).

Various statistically complex analytical models have been made increasingly accessible for sorting and allying data from large sets (see, for example, Ter'braak & Smilauer, 1998), used by hopeful ecologists in pursuit of the all-important patterns upon which ecological theory must be based. Indeed, many may use it just for this purpose but it is better when a testable hypothesis already exists or can be formulated on the basis of the analytical results. To show a discontinuous

distribution is rarely an end in itself.

One of the true values of modelling is to be able to verify explanations for phenomena, to test the sensitivity of models in the light of other influences. The ability to predict or hindcast is often viewed as a measure of model quality, especially if they are based upon processes and not just derived from statistical distributions. The best models generally set out to give the best fit simulation on the basis of the least information; their complexity should be increased no more than is needed to improve the simulation, whether this seeks simplicity, precision or generality (Levins, 1966). Such useful models of microalgal carrying capacities, based on the interplay of optimum resource conversion with supply, may be compounded into more complex process-based simulations as PROTECH (Reynolds *et al.*, 2001), in which the simultaneous growth-rate responses of individual species are built into a community response and larger-scale, compositional changes, generally considered still to be too difficult to model, begin to be amenable to simulation and the diagnosis of the critical precursors to changed composition.

Coming closer to the end of the half-century, one of the most exciting of the recent modelling approaches has been the "artificial neural network" (ANN), which tool is effective in modelling community composition. Information is extracted from complex compositional data by comparison with an array of environmental signals and, like a nervous system, critical sensitivities are diagnosed. Thus "trained" against existing data, the network is used to simulate outcomes against test conditions to determine the likelihood and nature of the modelled responses. Many more are likely to emulate the application of Recknagel *et al.* (1997) to microalgal behaviours.

As computers become more capacious and faster and approaches become more sophisticated, so the power of the modellers increases. The danger that understanding might not keep up with analytical ability has always threatened but there is little doubt that model development has been a powerful component to the advances in microalgal ecology over the last half century.

Microalgae and biogeochemical cycles

The role of microalgae in global biogeochemical cycles extends well beyond their role as primary producers (see Falkowski 1994). While a discussion of biogeochemistry is well beyond the scope of this review, the important microalgal contributions to these cycles deserve mention.

The concept of "new production" was introduced in the late 1960's (Dugdale & Goering 1967). New production is that primary production which uses nitrate or atmospheric forms of nitrogen (i.e. those generated outside of the ecosystem, in the atmosphere or deep water), rather than forms of nitrogen such as ammonium and urea that can be regenerated in the water column. The importance of this difference is that in a balanced ecosystem, carbon fixed in association with regenerated form of nitrogen is likely to result in short-term carbon sequestration (lost as CO_2 again as the nitrogen is regenerated), while carbon fixed in association with newly available nitrogen from the atmosphere or deep water can be transferred to longer-term reservoirs in the sediments or in higher trophic levels. This concept was developed into an "F-ratio" from which carbon export could be predicted (Eppley & Peterson 1979). The concept of new production has proved extremely useful in conceptualising material cycles in different ocean regions, (Eppley 1989, Dugdale & Wilkerson 1992).

Another biogeochemical cycle with an important algal component is sulphur. Lovelock & Margulis (1974) proposed that global climate could be controlled through emissions of sulphur compounds by microalgae in a homeostatic way. Conditions that lead to climate warming are likely to promote microalgal growth; some phytoplankton use the sulphur compound dimethylsulphopropionate as osmoregulant and, as a result of cell lysis or grazing, there will be increase emissions from these cells of dimethylsulphide (DMS). DMS released to the atmosphere could then act as a cloud nucleating agent and act to reduce irradiance reaching the ocean surface (see Schwartz 1988, Malin & Liss 1992). Elements of this idea are well accepted and many issues surrounding microalgal DMS production have been examined (see Watson & Liss 1998), however, the interpretation of these processes as indicating that the earth functions as 'super-organism' (the so-called Gaia hypothesis) remain controversial (Lovelock 1997).

Microalgae also appear to be critical in understanding the production and use of dissolved organic matter (DOM) in aquatic ecosystems. There is a considerable pool of DOM in natural

waters (McCarthy *et al.* 1997, Perakis & Hedin 2002), and it can be labile (Kirchman *et al.* 1991, Ammon & Benner 1994). To what extent phytoplankton are capable of using it, and to what extent they are responsible for its production remains largely unquantified (Collos 1992, Bronk *et al.* 1994).

Ecological processes: novel and newly important

The past few decades have led to re-evaluation of the ecological roles of microalgae in a number of ways. The traditional assumption that N_2-fixation by cyanobacteria is a process that is important in freshwaters, but not in marine systems, has been strongly challenged (see Howarth *et al.*1988). Significant nitrogen fixation in many marine systems has been demonstrated (e.g. Carpenter & Romans 1991, Karl *et al.* 1997), and it is now clear that even unicellular cyanobacteria are capable of N_2 fixation (Zehr *et al.* 2001). Such findings have significantly changed paradigms about new production as well (Zehr & Ward 2002).

It has also become apparent that the ecological roles of microalgae encompass heterotrophy and mixotrophy (see Jones 2000). The flexibility of algal metabolism means that traditional divisions between autotrophs and heterotrophs are becoming more difficult to make (e.g. Lewitus & Kana 1995), and thus our understanding of ecosystem dynamics based on simple categories is being challenged. To make matters even more confusing, the phenomenon of kleptoplasty (whereby a heterotroph is able to assimilate a functioning chloroplast from its prey for some period of time) is present in algal groups (e.g. Lewitus *et al.* 1999).

The future

Paradoxically, future advances depend on resolving basic issues that are as old as microalgal ecology, e.g. how can very low concentrations of nutrients that are rapidly turned over be measured?, how can we separate microalgae from other organisms in aquatic environments, how can we distinguish living and dead biomass in bulk measurements?, how can we determine the balance between heterotrophic and autotrophic metabolism in microalgae and assign meaningful ecological roles? The answers to these questions are unlikely to come quickly or simple, so in the following sections, we have focussed on more general issues and likely advances.

Measuring on appropriate spatial and temporal scales

Spatially, it has always been clear that even the largest coordinated programmes leave oceans and freshwaters chronically under-sampled. Satellite remote sensing is now making rapid advances in mapping the global distribution of surface chlorophyll concentrations. For the foreseeable future, ocean colour satellites will be functional and are providing data at spatial and temporal scales which were undreamed of only a few years ago (Joint & Groom, 2000). The challenge is to estimate production as well as phytoplankton biomass on these scales. However, to date there only been a few attempts to estimate the errors involved in these estimates from remote sensing and they can be quite large (Joint & Groom, 2000). Further work is required to refine remote sensing algorithms and to obtain more realistic estimates of the uncertainties involved in the methodology. Nevertheless, satellite remote sensing of marine primary production will be a major tool for use now and in the future in the study of the contribution of marine microalgae to global productivity.

A great deal of what we know about marine and freshwater ecology has been learned from long-term measurements. There are now a number of time series (e.g. the Windermere lakes, George 2002, and oceanic locations like the Hawaiian Ocean Time Series (HOTS), Karl *et al.* 2002). These programmes have been difficult to fund and maintain, but have provided virtually the only means to distinguish long-term changes (such as those resulting from anthropogenic influences) from normal inter-annual variability (events such as the El Niño Southern Oscillation and the North Atlantic Oscillation). Even such detailed time series can be too intermittent. It is clear that the only way to get sufficient resolution in time is to have some form of continuous monitoring such as that offered by moored arrays of instruments. Large data sets based on

multi-instrument arrays and autonomous instruments are now becoming available from ambitious projects such as the "Longterm Ecosystem Observation at 15 m depth" (LEO-15), based in the coastal waters off New Jersey (Glen *et al.* 2000, http://marine.rutgers.edu/mrs/leo/leo15.htm).

Automatic identification of microalgal species

It is becoming clear that microalgal assemblages are diverse in terms of species and that species have diverse ecological functions that are not necessarily represented by their biomass, represented as particulate carbon or chlorophyll (c.f. Reynolds 1984). There are few studies that attempt to determine the activity of individual phytoplankton species within natural assemblages. Simple methods such as size fractionation of ^{14}C fixation (Joint & Pomroy, 1983) have been useful in giving information on production of picoplankton, but there are few methods that can estimate the growth rate of single species within a population. These data are essential if we are to understand how individual species can come to dominate the wide diversity of phytoplankton within an assemblage to form blooms, many of which are almost unialgal in nature.

The basic problem is that the only commonly available method to enumerate and identify a microalgal species is microscopy (and sometimes even electron microscopy) and it is very time consuming to make a microscope-based analysis of a phytoplankton assemblage. Methods such as HPLC measurement of group-specific photosynthetic pigments (Mackey *et al.* 1996) or flow cytometry of natural samples (Li 1994) do not have the required level of discrimination. Similarly, molecular approaches such as analysis of 16S and 18S ribosomal RNA genes can provide an indication of total diversity, but they are not quantitative at present. It is becoming clear that to make progress in studying individual species and cells within a natural population, we need breakthroughs in computer-based image analysis and automatic identification of phytoplankton. Coupling Automated Neural Networks with flow cytometry has been attempted (e.g. Balfoort *et al.* 1992), but there is great potential for combining such methods with RNA or DNA probes, and potentially developing simple microcassette formats for identifying presence and absence of particular species (Jonker *et al.* 2000). Once that is reliably available, it will be possible to apply allometric theory (Joint & Pomroy, 1988) to estimate growth rate from biomass of all of the species within a population.

Ecological interactions

Once we have succeeded in identifying and enumerating species, we must begin the complex process of understanding of the interactions between them. Moving from the individual organism to an ecosystem is conceptually challenging because there is an element of "self-assembly" inherent in ecosystems which creates more complex "emergent properties". Reynolds (2001) has proposed a detailed framework in which to examine such systems. Interestingly, this approach also help interpret which scales of variability in the environment are meaningful with respect to specific organisms, e.g. short-term fluctuations in irradiance due to variations in cloud cover and changes in vertical mixing due to variations in local wind speeds prove to be critical in primary productivity.

A particular area of weakness in our understanding of ecological interactions concerns loss processes. It is clear that much (and possibly most) primary production in specific ecosystems is not directly grazed by conventional consumers (see Reynolds 1984, 1998). Determining the ecological importance or viruses, and pathogens such as bacteria, as well as fungal and algal parasites will be an important area of work. Moreover, assessing the significance of "intrinsic" mortality is a wide-open area.

Understanding of cell-to-cell interactions, be they involved in communicating information or allelopathic in nature, must move to a deeper level that the phenomenological. One process that is increasingly perceived as important in bacterial dynamics is cell-to-cell communication by chemical signal molecules. Bacteria are capable of complex assemblage behaviour, a phenomenon which has become known as "quorum sensing" and which relies on the accumulation of a signal molecule to a threshold concentration at which target structural genes are activated (Dunny & Winans 1999). Quorum sensing signal molecules enable individual bacterial cells to sense when the minimal population unit or "quorum" of bacteria has been

achieved and so initiate a concerted population response (Swift *et al.* 2001). Quorum sensing modulates a variety of physiological processes including secondary metabolism, virulence and biofilm development in a variety of plant, animal, soil-borne and marine bacteria. Could it be that microalgae can also influence other cells through chemical pheromones? Given that bacteria evolved long before algae and that quorum sensing has probably been important throughout, it would be surprising if similar mechanisms were not present in microalgae. We speculate that a form of quorum sensing may well prove to be an important process explaining the success of marine and freshwater phytoplankton.

Part of the key to resolving such issues will be the ability to dissect the large pools of dissolved organic matter within marine and freshwater systems in order to characterise the constituents, their variability in nature and their physiological effects. The chemical expertise and the methodologies are likely to be advanced and will require substantial collaborative efforts with other disciplines.

Applying ecological information effectively

Speaking of ecology in general, Peters (1991) commented that: "the problems that ecology should solve are not being solved...they are worsening, growing more imminent, more monstrous". Certainly, the continuing degradation of marine and freshwaters must be sobering for microalgal ecologists. There is a temptation to believe that solutions to these problems are political and that politicians take little notice of ecologists, however, recent events suggest otherwise.

For example, the realisation that microalgal growth is limited by iron in large areas of the ocean has led to the idea that iron fertilisation may provide a means to reduce atmospheric CO_2. In turn this has led to a growing market for carbon removal technologies and trading in carbon credits. Concerted efforts on the part of microalgal ecologists (e.g. Chisholm *et al.* 2001) have been crucial to raising awareness and effectively communicating the short-sightedness of such approaches. In terms of water policies, the European Union's Water framework directive and has effectively brought ecological assessments to the forefront (Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a Framework for Community Action in the Field of Water Policy, OJ L327, 22 December 2000; see also Moss, this volume). The objective of "general protection of the aquatic ecology, specific protection of unique and valuable habitats" and the concept that "ecological protection should apply to all waters" is coupled in this document to requirements for maintaining "good ecological status" and "good chemical status". In turn, "good ecological status" is defined in terms of "the quality of the biological community, the hydrological characteristics and the chemical characteristics". Unfortunately, such criteria are difficult to objectively legislate and at present are phrased as "allowing only a slight departure from the biological community which would be expected in conditions of minimal anthropogenic impact". The challenge for microalgal ecologists in providing the data to determine baselines and establish monitoring criteria is clear.

Finally, an important element in future progress may well prove to be social rather than conceptual or technological. Interdisciplinary work with chemists, physicists and engineers is strong within some areas of algal ecology, but, paradoxically, often weaker between the sub-disciplines of ecology. Freshwater microalgal ecology has tended to pay far greater attention to individual organisms and has embraced ecological theory to a very high degree. In many cases, methodologies are traditional and the technology rather low. In contrast, marine microalgal ecology has tended to be more process-oriented and have stronger links to biogeochemistry than ecology. Organism-based measurements are rarer and the range of techniques applied and the level of technologies used are considerably higher. Despite efforts to integrate the disciplines (e.g. initiatives such as joint meetings organised by the American Society of Limnologists and Oceanographers), many national funding agencies still divide the fields and there are significant barriers in communication to overcome. Similarly, within the marine discipline, there tends to be a divide between those studying micro- and macroalgae. There is much to be gained crossovers in methods and theories and it is probably most likely that individuals (e.g. students and postdoctoral fellows) are the key to bridging such gaps.

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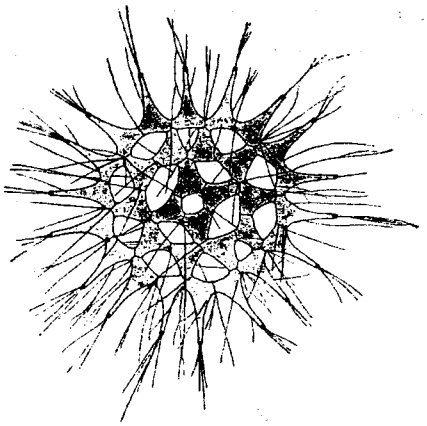

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COLLECTED REVIEWS TO CELEBRATE THE JUBILEE OF THE BRITISH PHYCOLOGICAL SOCIETY

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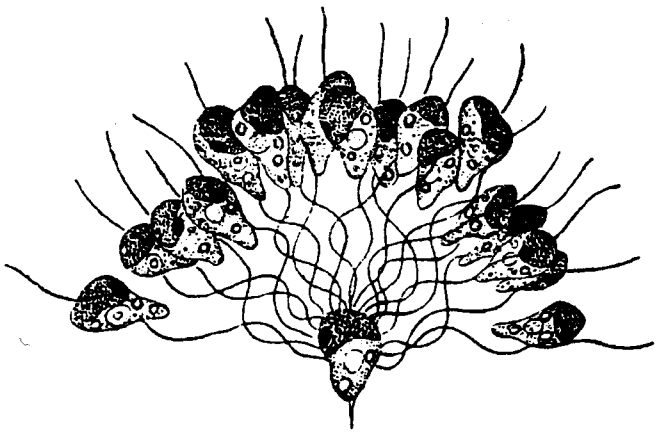

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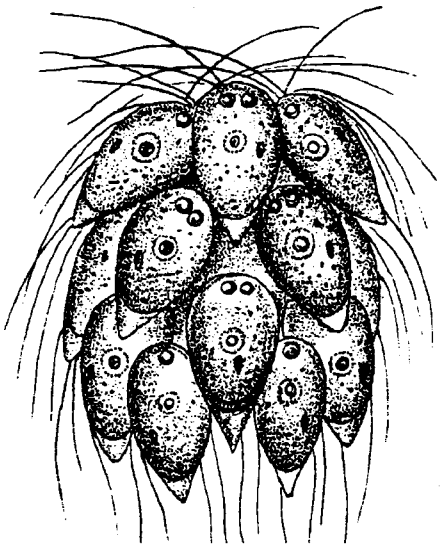
The vignettes that grace the foot of several pages come from classic books and papers on phycology – and from a time when scientific drawings could be beautiful as well as informative.



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