Use of Flow Cytometry to Measure Biogeochemical Rates and Processes in the Ocean

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Abstract

An important goal of marine biogeochemists is to quantify the rates at which elements cycle through the ocean's diverse microbial assemblage, as well as to determine how these rates vary in time and space. The traditional view that phytoplankton are producers and bacteria are consumers has been found to be overly simplistic, and environmental metagenomics is discovering new and important microbial metabolisms at an accelerating rate. Many nutritional strategies previously attributed to one microorganism or functional group are also or instead carried out by other groups. To tease apart which organism is doing what will require new analytical approaches. Flow cytometry, when combined with other techniques, has great potential for expanding our understanding of microbial interactions because groups can be distinguished optically, sorted, and then collected for subsequent analyses. Herein, we review the advances in our understanding of marine biogeochemistry that have arisen from the use of flow cytometry.

INTRODUCTION

The first use of flow cytometry to study marine microbes—titled simply "Analysis of phytoplankton by flow cytometry"—was by Trask et al. (1982) and was quickly followed by several cornerstone publications (Olson et al. 1983, Yentsch et al. 1983). Over the past three decades the field has blossomed. Flow cytometers work on the principle of laser excitation of a single particle with a specific wavelength of light, and subsequent quantification of light scattered by and fluoresced from that particle. These basic light signals are then used to investigate particle characteristics and define sample sorting routines. For a more detailed description of the strengths and limitations of flow cytometry, which are beyond the scope of this review, the reader should examine the comprehensive text by Shapiro (2003).

Initial studies in the marine field used flow cytometry as a tool to quantify the abundance of small autotrophic and heterotrophic cells that previously were quantified by laborious manual microscopy methods (e.g., del Giorgio et al. 1996, Olson et al. 1993). Improvements in both speed and function (e.g., sorting) rapidly diversified the use of flow cytometry as a tool in marine microbial ecology. A key advantage of analyzing samples by flow cytometry is the ability to sort target cells for subsequent analysis, such as determination of radiotracer incorporation (Lebaron et al. 2002), cellular activity (Zubkov et al. 2001), nucleic acid content (Lebaron et al. 2001), and total protein content (Zubkov et al. 1999). As a result, flow cytometers, both analyzers and sorters, have become a common fixture in marine institutions and university departments. It has been a decade since several extensive reviews on the application of flow cytometry to marine sciences were published (Collier 2000, Legendre et al. 2001, Veldhuis & Kraay 2000). These reviews did an excellent job of discussing immunolabeling applications, but our knowledge has grown significantly over the past decade, particularly in the areas of physiological probes and taxon-specific biogeochemistry. Rather than focus on the literature that cites flow cytometry as a means to enumerate marine microbes or the work covered by previous reviews, we focus specifically on those studies that have used flow cytometry to investigate biogeochemical rates and processes in the ocean. These studies have become increasingly common in the literature (**Figure 1**) and have altered our view of the

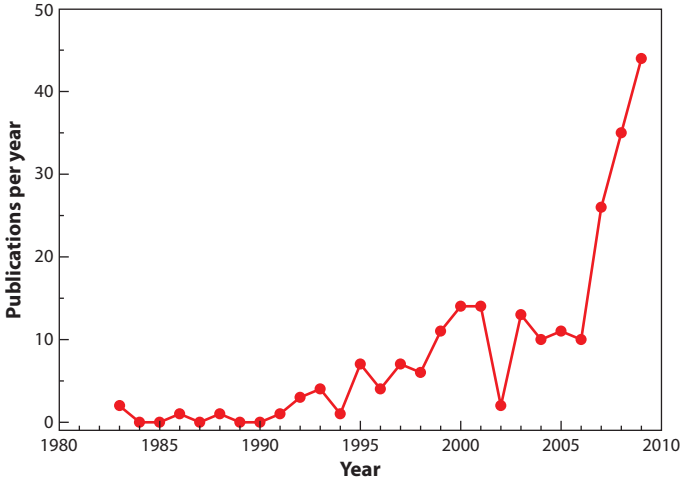

Figure 1

Noncumulative plot of publications per year presenting data on marine microbial physiological rates and biogeochemical processes that were generated by the use of flow cytometry. Citation numbers are current through the end of 2009 as referenced by an ISI Web of Science abstract search.

microbial ocean. This review focuses on advances in our understanding of which organism does what and at what rates. We review recent literature on the application of flow cytometry to the study of (*a*) cellular characteristics and physiological processes, (*b*) marine biogeochemical cycles and rate processes, (*c*) ecotoxicology, marine pathogens, and harmful algae, (*d*) continuous and remote observations, and (*e*) the burgeoning field of environmental metagenomics. We conclude with our view of future advances in flow cytometry technology.

CELLULAR CHARACTERISTICS AND PHYSIOLOGICAL PROCESSES

Cellular Biomass and Characteristics

Most current marine ecosystem models, whether static (where the microbial community structure is defined, e.g., Moore et al. 2004) or emergent (where the microbial community structure within the model can change, e.g., Follows et al. 2007), require fundamental information about cellular properties, such as cellular nutrient quotas and ratios, cellular uptake kinetic parameters (half saturation concentration and maximum uptake rate), and growth rates, for proper parameterization. However, for many microbial functional groups (in this review, we use microbial to refer to both heterotrophic bacterial and autotrophs collectively, and heterotrophic bacteria and autotrophs or phytoplankton when referring to groups separately), these have been determined only in the laboratory. Application of flow cytometry techniques to marine biogeochemistry has begun to provide a wealth of data on these parameters in situ.

Autotrophs. Biomass of a specific autotrophic population, when coupled with uptake rates by the same population in the same elemental units (see below), allows the calculation of particle turnover rates. Because every photosyntheic organism with photosystem II has chlorophyll, it is arguably the best known and most widely used proxy for autotrophic biomass, but it is for that very reason that it is difficult to determine chlorophyll in subpopulations of mixed assemblages using traditional chlorophyll fluorescence techniques. Chlorophyll fluorescence intensity quantified by flow cytometry has been shown to scale with cellular chlorophyll levels, but the relationship is not constant for all phytoplankton due to differences in intracellular pigment structure (i.e., the package effect) or even for the same phytoplankton due to differences in growth conditions (Sosik et al. 1989). So although chlorophyll fluorescence in discrete populations can be estimated and chlorophyll converted to carbon units using an appropriate carbon:chlorophyll ratio, the errors can be quite large. A more direct measure of autotrophic carbon biomass can be determined by calibrating cellular carbon content to the geometric mean forward scatter signal, which scales with cell diameter (e.g., DuRand et al. 2001). This approach is robust and has shown that whereas cyanobacteria are numerically dominant in the Sargasso Sea, it is the rare but much larger eukaryotic phytoplankton that often dominate the autotrophic biomass (DuRand et al. 2001).

With the increased access to flow cytometers, phytoplankton ecologists are also beginning to compile global autotrophic cell abundance maps that highlight some macroecological patterns (e.g., Li 2009); it would be a reasonably simple step forward to convert these population-specific abundance maps to population-specific carbon maps. Such an activity would further link satellite oceanography with biogeochemistry and help validate satellite backscatter/particulate carbon (e.g., Pabi & Arrigo 2006) and phytoplankton composition algorithms (e.g., Alvain et al. 2008). This would further enhance our understanding of autotrophic carbon cycling by exploiting the sampling resolution of satellites.

Heterotrophic bacteria. Due to a lack of pigments and small size, estimation of heterotrophic bacterial carbon is more complicated than for autotrophs. Methods are available, such as biovolume/carbon conversions (Bratbak 1985) and X-ray microanalysis (Gundersen et al. 2002, Heldal et al. 1985), but both are laborious, and for the latter it is very difficult to get absolute quantification. New flow cytometry methods have improved both accuracy and speed of fluorescence-based estimates, but the method is compromised when populations of unknown bacterial species or complex natural bacterioplankton communities are analyzed (Robertson et al. 1998). The use of specific stains has shown promise in quantifying biomass of mixed populations of cells. For example, the SYPRO family of stains, developed in the mid-nineties to stain protein gels, has been shown to stain proteins in intact cells. In culture and in natural populations, the average cellular fluorescent intensity of SYPRO-stained cells has been shown to correlate very well with the average cellular protein content (Zubkov et al. 1999). As a result, with proper calibration, cellular protein content can be rapidly quantified by flow cytometry and changes in population-specific carbon biomass calculated using appropriate carbon:protein conversions. This is a very sensitive and rapid technique that makes it suitable for larger oceanographic sampling programs, though we still lack a comparable technique for autotrophs.

Growth Rates and Metabolic Activity

The wide range of fluorogenic assays (**Table 1**) developed primarily for the biomedical field has been a boon to marine microbial ecologists, resulting in the ability to investigate rates of physiological processes and answer questions like, Are all microbes active? and Are they all equally active?

Autotrophs. Quantifying autotrophic metabolism (i.e., growth rate) in the ocean is a long-standing goal in oceanography that is intimately coupled to biomass as the product of the two yields rates of primary production. Following the changes in cell number of natural populations to estimate growth rate is an elegantly simple idea; however, it is compromised by difficulties in conducting a truly Lagrangian study (where a discrete parcel of water is sampled through time), as well as by small-scale patchiness and time-variable loss processes. A variety of physiological methods based on flow cytometry have been used sporadically (see Li 1993 for review), including cell cycle analysis (e.g., Mann & Chisholm 2000), turnover of intracellular lipids (e.g., Yentsch & Campbell 1991), cellular esterase activity (e.g., Dorsey et al. 1989), and photosynthetic electron turnover (e.g., Olson & Zettler 1995). Cell cycle analysis and lipid turnover techniques require sampling periods of at least one division cycle (minimum of ~24 h), but both methods directly measure cell division, which is, by definition, growth rate. The esterase activity and photosynthetic electron turnover assays require much shorter sampling durations but measure processes preceding cell division. Both have been shown to correlate well with measured growth rates determined by changes in cell number in culture. All of these techniques can provide growth rates of specific autotrophic taxa using inherent properties (e.g., size and chlorophyll content). However, there has been very little work in quantifying autotrophic growth rates using methods linked to flow cytometry since Li's review in 1993, thus these methods have not been readily incorporated into routine procedures. The sensitivity of flow-cytometric analysis is critical for the successful application of these methods to many of the ocean gyre domains that are dominated by small cells (<2 μm) and where use of microscopy techniques is too time-consuming or inappropriate.

Heterotrophic bacteria. In stark contrast to the autotrophs, a voluminous body of literature exists on population-specific heterotrophic metabolism. Microautoradiography has been used for

Table 1 Examples of fluorescent assays used to probe physiological functions and cellular characteristics in marine microbes

Process/Characteristic	Probe/Assay	Excitation/Emission	Interpretation	Reference(s)
Physiological process				
Electron transport system activity	5-cyano-2,3-ditolyltetrazolium chloride	450/630	Reduced by electron transport chain	Kaprelyants & Kell 1993
Cell membrane potential	bis-(1,3-dibutylbarbituric acid) trimethine oxonol [DiBaC4(3)]	490/516	—	Del Giorgio & Bouvier 2002
Cellular viability	5-chloromethyl fluorescein diacetate	492/517	Fluorescence quenched by exposure to heavy metals	Satoh et al. 1999
Metabolic activity: esterases[a]	fluorescein diacetate[b]	495/519	Hydrolyzed by intracellular esterases	Dorsey et al. 1989
Metabolic activity: esterases	calcein blue AM	494/514	Hydrolyzed by intracellular esterases	Jacobsen et al. 1997
Alkaline phosphatase activity	ELF-97	345/530	Hydrolyzed and immobilized as a crystal precipitate	Duhamel et al. 2008
β-galactosidase activity	fluorescein-di-β-D-galactopyranoside	495/519	—	Miao et al. 1993
Cellular characteristics				
Cellular lipid granules	Nile red	552/636	—	Elsey et al. 2007
Cellular protein content	SYPRO® red	550/630	—	Zubkov et al. 1999
Quantitative DNA content	PicoGreen and SYTOX® green (autotrophs)	502/524	dsDNA (with longer Stokes shift in the presence of ssDNA)	Veldhuis et al. 1997
Quantitative DNA content	DAPI (bacteria)	358/460	dsDNA (AT), some binding to cell membranes	Button & Robertson 2001
Cell membrane integrity	BacLight™[c]	470/535 (SYTO9™) and 530/620 (PI)	—	Jacobsen et al. 1997
Cell membrane integrity	calcofluor white	440/510	Stains cell wall chitin	Davey & Kell 1997
Extracellular-bound polysaccharides	concanavalin-A FITC	495/519	Binds specifically to mannose and glucose	Waite et al. 1995
Intracellular pH	carboxy-SNARF-1 AM	549/585	—	Leyval et al. 1997

[a]Because of the central role of esterases in cell metabolism, esterase activity is commonly used to assess relative cellular metabolism.
[b]Fluorescein diacetate derivatives 5-carboxyfluorescein diacetate and sulfofluorescein diacetate have also been used.
[c]Commercially available dye combination with SYTO9™/PI. Similar dye combinations also work.
Abbreviations: DAPI, 4,6-diamidino-2-phenylindole; ELF, enzyme-labeled fluorescence; FITC, fluoroscein isothiocyanate; PI, propidium iodide; SNARF-1-AM, seminaphtorhodafluor-1-acetoxymethylester.

decades to determine metabolic activity and the incorporation of specific substrates by individual cells using radiolabeled substrates (e.g., Brock 1967). While microautoradiography is very labor intensive, flow cytometry offers a number of time-saving alternative techniques (reviewed in Pernthaler & Amann 2005). For example, marine bacteria that are actively respiring are capable of reducing the tetrazolium salt CTC, producing a brightly fluorescing formazan that can be detected with a flow cytometer (del Giorgio et al. 1997). Subsequent work demonstrated that this approach lacked sensitivity and was most useful for determining which cells were most active among the growing cells (Sherr et al. 1999). An alternative to CTC is bromodeoxyuridine (BrdU), which is a nonradioactive analog of thymidine that bacteria and eukaryotes incorporate into DNA (Urbach et al. 1999). BrdU has also been combined with CARD-FISH (catalyzed reporter deposition–fluorescence in situ hybridization). FISH uses oligonucleotide or polynucleotide probes that target rRNA to allow one to identify and count the different bacterial taxa in environmental samples (Amann et al. 1990), and CARD-FISH is a much more sensitive technique that can be used to quantify water column bacteria, even if they have low ribosome content or are not growing rapidly (Sekar et al. 2004). The higher sensitivity of CARD-FISH also makes the approach more amendable to flow cytometry, and although this is common in the medical literature, there are no references that we can find that have combined BrdU with flow cytometry in the marine literature—clearly an open avenue of research.

Nucleic acid double staining (NADS) (**Figure 2**) is one approach to differentiating active, live cells from inactive, dead cells. The technique is based on the assumption that for a given cell to be active, it must have an intact membrane capable of maintaining an electrochemical gradient. Double staining protocols combine a probe that targets nucleic acids and is permeable to the membrane (e.g., SYTO family of stains) with a second probe that is impermeable to an intact membrane (e.g., propidium iodide). When both probes stain the nucleic acids, the second probe, if it can enter the cell, will quench the first. If no quenching is observed, the membrane is intact and the cell is defined as alive. If the first probe is partially or fully quenched, then the cell is defined as damaged or dead, respectively (Barbesti et al. 2000). This approach has been used successfully in both marine and freshwater environments (e.g., Gregori et al. 2001) and has been optimized by Falcioni et al. (2008). The ability to rapidly distinguish between active and compromised or dead cells is a valuable tool to study the effect of environmental variables, such as the effect of light, on bacterial viability (e.g., Alonso-Sáez et al. 2006).

Interestingly, the use of these nucleic acid stains has led to a controversial observation. In marine systems, two populations of heterotrophic bacteria are often found, as delineated by their nucleic acid content: high (HNA) and low (LNA). Several studies have used physiological probes and radioisotopic tracers to determine the activity and viability of these two different heterotrophic populations. Early studies attributed much of the substrate uptake to HNA cells (Lebaron et al. 2001, Servais et al. 2003), which is consistent with the observation that upon addition of nutrients it is the HNA cells that respond (reviewed by Bouvier et al. 2007). Recent evidence suggests, however, that the specific growth rate of LNA cells may in fact equal or even exceed that of the HNA cells due to reductions in protein per cell that allow for similar growth rates under suboptimal conditions (Longnecker et al. 2005, Mary et al. 2006, Scharek & Latasa 2007, Zubkov et al. 2001).

Viruses. With more sensitive photomultiplier tube detectors and better optics, flow cytometers are increasingly used to measure the abundance of viruses in natural systems (e.g., Marie et al. 1999). A detailed analysis of various protocols used to enumerate double-stranded DNA viruses with flow cytometry was undertaken by Brussaard (2004a), although Tomaru & Nagasaki (2007)

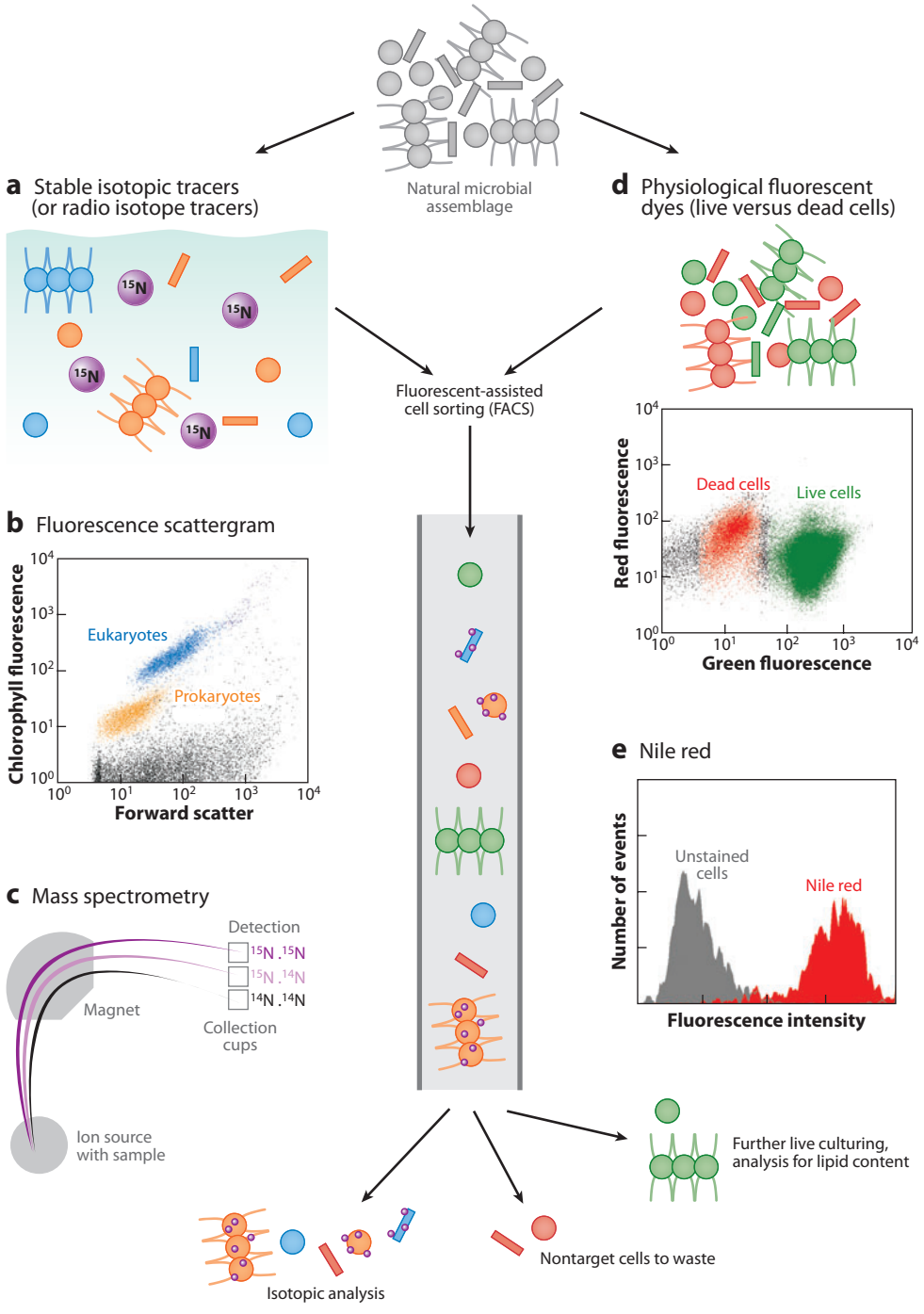

Figure 2

Schematic representation of possible strategies for combining flow cytometry with other methods to enhance biogeochemical studies in the ocean. (*a*) Incubation of natural assemblages with isotopic tracers (^{15}N in this example) that can be (*b*) sorted based on inherent cellular characteristics and then (*c*) analyzed via mass spectroscopy to determine stable isotope content. (*d*) Assessment of cell viability in natural populations (sample from Sargasso Sea euphotic zone) after nucleic acid double staining (in this instance with SYTO9™/ propidium iodide). Viable cells can then be sorted to start new cultures, or (*e*) other physiological stains can be used or a variety of other post-sort analyses can be conducted.

found that flow-cytometric counting approaches may underestimate the abundance of small DNA or RNA viruses.

Viruses are important agents influencing the composition of phytoplankton and bacterial communities, and viral infection is a key pathway that affects the transfer of energy and organic matter within the microbial loop (Suttle 2005, Wommack & Colwell 2000). Flow-cytometric analysis has been a boon for studies of viral infection in a range of habitats (see Brussaard 2004b for review). Viral infection, more accurately the loss of physiological activity upon viral infection, has been studied in *Phaeocystis pouchetii* and *Micromonas pusilla* using a membrane-impermeant nucleic acid dye (SYTOX green) to detect dead cells and a membrane-permeant dye (calcein AM, which, prior to fluorescent detection, must be hydrolyzed by intracellular esterases (and therefore active cells) into a green fluorescent form (Brussaard et al. 2001). This staining protocol is similar to the NADS staining protocol in that it is an assay done on live cells, but it differs from the other protocols in that the Calcein-AM must be hydrolyzed intracellularly to be fluorescent. So unlike the NADS procedures, succesful application of this procedure is dependent upon intracellular enzyme activity, not assumptions about membrane potential and integrity. Flow cytometry has also been used to study viral lysis in toxic dinoflagellates (Mizumoto et al. 2008) and heterotrophic flagellates (Massana et al. 2007) using similar protocols. One potential application of flow cytometry that has not been fully exploited is the study of viral-mediated particle aggregation/disaggregation and its role in carbon export (e.g., Jorio et al. 2006). Malits & Weinbauer (2009) observed that the formation of aggregates, which commonly happens in the marine environment due to physical turbulence, was reduced in a phytoplankton bloom that had been infected by viruses. Presumably this was due to some change (in either quantity or quality) in the production of saccharides—the glue that holds aggregates of autotrophs together—such that the aggregates did not form. Flow cytometry can be employed to follow viral dynamics, as part of studies focused on the biological sequestration and export of carbon to the ocean interior.

With the development of techniques for flow-cytometric viral enumeration, in addition to those used for autotrophs and heterotrophs, it is now possible to track changes in abundance of several members of the microbial food web through time (Larsen et al. 2001), along a salinity gradient (Schapira et al. 2009), and in a wide range of environments (e.g., Corzo et al. 2005). The possibilities for studies of community composition change seasonally, between systems, and in response to variation in environmental variables are enormous.

Nutrient Acquisition and Stress Responses

Although it is important to quantify rates of growth and total metabolic activity in marine microbes, it is equally important to quantify processes controlling the observed growth and metabolic rates. Ultimately, it is this control information that is fed into ocean ecosystem models to determine which microbial groups and how many are present in the model output. Here we review a number of approaches that have been used to study nutrient acquisition and stress with respect to nitrogen, phosphorus, and iron.

Nitrogen. Jochem et al. (2000) were the first to develop an immunolabeling protocol to detect nitrate reductase in the diatom *Skeletonema costatum*. Fluorescence intensity from the labeled anti-nitrate reductase antibody was found to be linearly related to nitrate reductase protein concentration (as quantified by Western blot) but displayed much better quantitative resolution at the low protein concentrations due to both the higher sensitivity of the fluorescence assay and the extreme sensitivity of flow cytometry. This immunolabeling assay, made successful by the combination of the speed and sensitivity of flow cytometry, led to some interesting findings. For

example, Jochem et al. (2000) demonstrated that when diatoms were grown in the presence of ammonia, nitrate reductase synthesis was repressed. This assay combined with flow cytometry was sensitive enough to show early stages of derepression of nitrate reductase synthesis following the addition of nitrate (in simulated upwelling experiments). Surprisingly, this technique, or derivatives thereof, has not been used to resolve outstanding questions of taxonomic variability in ammonium suppression of nitrate uptake (e.g., Dortch 1990).

Orcutt et al. (2008) used a similar protocol but with a novel fluorochrome. One of the primary problems with organic fluorochromes is the wide emission peak that limits the ability to label single cells with multiple fluorochromes. Orcutt et al. (2008) successfully conjugated the nitrate reductase antibody to quantum dots. Quantum dots are semiconducting nanocrystals, generally composed of a CdSe/ZnS (core/shell) crystal, that form stable aqueous suspensions and are biocompatible (Medintz et al. 2005). The most important characteristic of quantum dots is that they have very narrow emission spectra, allowing the possibility of labeling single cells with more than one fluorochrome simultaneously. However, there are some drawbacks to combining quantum dots with flow cytometry, in particular, the relatively slow radiative decay rate for quantum dots relative to organic fluorochromes (Alivisatos 2004), but this can be partially compensated for by reducing the flow stream rate and allowing particles to stay in the laser longer. The second drawback relates to labeling cell surface versus internal targets, a drawback for all methods of fluorescent detection, not just flow cytometry. Although theirs was only a method development study, Orcutt et al. (2008) showed the feasibility of using quantum dots as a fluorochrome in marine phytoplankton research.

Phosphorus. It is generally accepted that increases in alkaline phosphatase activity are indicative of inorganic phosphorus stress. There is a convenient fluorescent bioassay called enzyme linked fluorescence (ELF-97) for alkaline phosphatase activity that is based on the cleavage of a phosphate moiety from a soluble nonfluorescent substrate resulting in an insoluble fluorescent product (Telford et al. 2001). Because the product is insoluble, initial work with this assay in marine microbes focused on the determination of the presence or absence of alkaline phosphatase activity, as well as the intracellular location of the enzyme activity (e.g., Dyhrman & Palenik 1999, Gonzales-Gil et al. 1998) (**Figure 3**). Gonzales-Gil et al. (1998) were the first to combine flow cytometry with the ELF-97 assay in a study of phosphorus limitation in marine dinoflagellates, under the assumption that phosphorus limitation resulted in induction of alkaline phosphatase. They found that not all dinoflagellates induce ELF-detectable activity and that for those species that do, levels of induction are not the same despite similar stress conditions. It was suggested that if fluorescence intensity of the hydrolyzed product were normalized to some proxy for cell size (e.g., forward light scatter), which is also determined on each particle, then a semiquantitative index of alkaline phosphatase activity could be generated to compare across taxonomic groups. Nedoma and colleagues (Nedoma et al. 2003) have used this assay and flow cytometry in a time course to quantify the rate of hydrolysis for individual populations (**Figure 3**). Moreover, with the sensitivity of flow cytometry, these analyses have been expanded to marine bacteria (e.g., Duhamel et al. 2008). Although the general method may have limited ability to definitively assess phosphorus limitation, due to constitutive versus inducible expression, acid versus alkaline phosphatases, and other vagaries of cell physiology, it will have significant value in quantitatively comparing potential rates of organic phosphorus utilization between different marine microbial populations. In addition, if carefully conducted, investigators should be able to compare the same population of microbes between regions and begin to build a more detailed description of how microbial physiology responds to changes in phosphorus biogeochemistry.

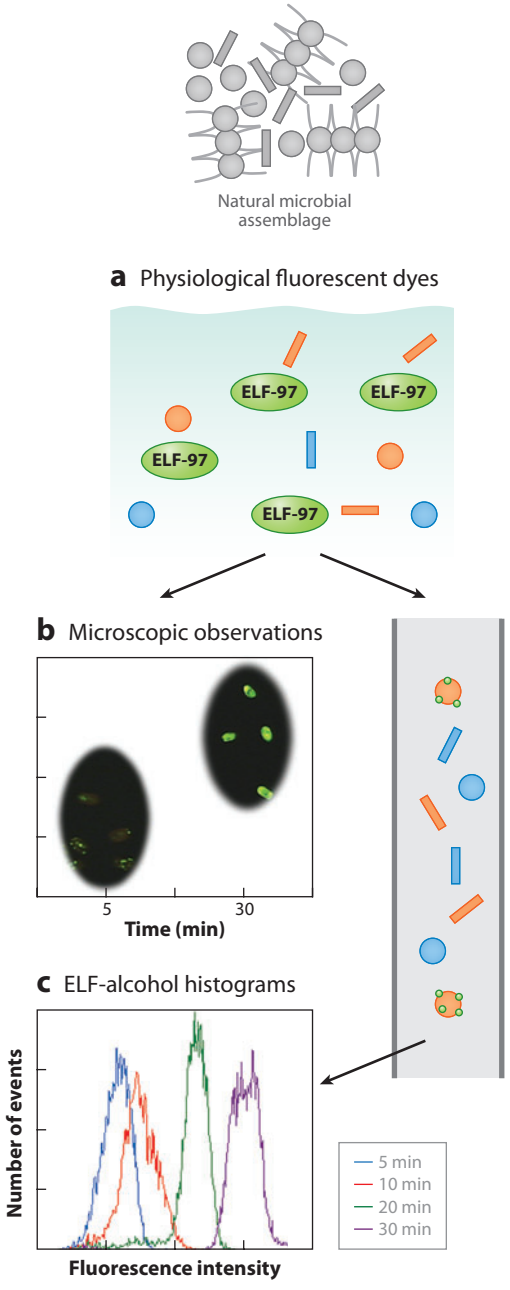

Figure 3

Schematic representation of combining flow cytometry with the physiological stain protocol ELF-97. (*a*) Natural populations are incubated with a nonfluorescent, soluble substrate that is (*b*) precipitated upon hydrolysis by the intracellular enzyme alkaline phosphatase. (*c*) Flow cytometry not only accelerates the process of sample analysis but can quantify fluorescence per cell over time, allowing the calculation of rates for quantitative comparison between different microbial populations. Abbreviation: ELF, enzyme-labeled fluorescence.

Iron. Similar to the flow cytometer–based nitrate reductase immunolabeling protocol above, Rivers and colleagues (Rivers et al. 2009) developed an antibody protocol to the iron-stress gene *IdiA* in the marine cyanobacterium *Synechococcus*. Culture and field testing showed that this antibody successfully detected differences in expression level with depth between two ecotypes despite similarities in ambient iron concentrations. This observation has had a profound affect on the field of microbial ecology. Nearly five decades ago, subtle differences in phytoplankton physiology were hypothesized as an explanation for the Paradox of the Plankton (briefly, how such a diversity of phytoplankton could be supported in a nearly constant physical and chemical environment; Hutchinson 1961). The advent of molecular oceanography only exacerbated the conundrum by pointing out that within species of phytoplankton (as would have been defined by Hutchinson) there can be many ecotypes. Here the speed of the flow-cytometric analysis allowed for quantitation of *Synechococcus* ecotypes that were a very low fraction of the total population. An initial limitation of this protocol was the use of the fluorochrome fluorescein, which requires that cells first be bleached to reduce autofluorescence and improve detection. However, with a selective choice of fluorochromes, or perhaps conjugation to quantum dots, this problem can be overcome.

Another means of assessing iron limitation is through the use of microbial bioreporter strains. Two iron bioreporter strains have been developed for the marine environment, one bacterial, *Pseudomonas putida* FeLux (Mioni et al. 2005), and one cyanobacterial (Boyanapalli et al. 2007). These strains increase cellular luminescence in response to iron limitation and to date have been quantified using a luminometer. However, recent advances in time-gated luminescence flow cytometry (Jin et al. 2007) suggest it may be possible to combine the speed and cell-specific capabilities of flow cytometry with these bioreporters to further enhance the applicability of these important tools.

MARINE BIOGEOCHEMICAL CYCLES AND RATE PROCESSES

As marine biogeochemical models become more complicated, by including multiple plankton groups, and migrate away from static elemental stoichiometry (i.e., the Redfield ratio; Flynn 2010), it is becoming increasingly important to generate data at the functional group level. This is, in our opinion, where flow cytometry and cell sorting shine and provide a clear path forward to continued advancements in the future.

Bacterivory

Bacterivory is a process that is often excluded from marine ecosystem models despite the suggestion that upwards of ~25% of bacterial standing stock can be grazed daily (Sherr et al. 2002) and therefore is a significant flux in the biogeochemical cycling of carbon. Early studies quantifying bacterivory used bacterial cells labeled with fluorescent markers [e.g., fluorescein isothiocyanate (FITC); Keller et al. 1994] to follow the incorporation of bacterial prey into autotrophic grazers, which were themselves discriminated by their own chlorophyll fluorescence. These early studies observed both preferential grazing based on prey morphology and increased grazing under light and nutrient limitation, suggesting an inducible mixotrophic nutritional mode (Keller et al. 1994). Advances in molecular biology and fluorescent proteins have allowed the genetic modification of bacterial prey lineages, which circumvents potential alteration of prey characteristics such as viability and behavior or cell surface characteristics upon staining (Fu et al. 2003) that in the past may have negatively impacted rates of bacterivory.

Zubkov and colleagues have pioneered a new flow cytometer–based technique using radioisotope labeling to quantify bacterivory (e.g., Zubkov & Tarran 2008). The approach uses

a pulse/chase format that follows the accumulation of a radioisotopic tracer in the predator. This protocol requires flow-cytometric sorting of both prey and predator to determine activity per cell, and if total cell numbers are also determined, then a mass balance of isotope can be generated and ultimately the metabolic assimilation efficiency calculated, another valuable parameter for ecosystem models. Using this method, Zubkov & Tarran (2008) showed that in the oligotrophic temperate and tropical North Atlantic small (<5 μm) nanoautotrophs are responsible for 40–95% of total bacterivory, in contrast to earlier work that suggesting >80% of total bacterivory was conducted by nanoheterotrophs (Sherr & Sherr 1994). These data support prior research of high bacterivory under low nutrient conditions (Keller et al. 1994) but questions the current view of who eats whom and how this process should be included in ecosystem models.

Following along the lines of organic nutritional modes, First & Hollibaugh (2009) showed that ciliates in natural populations can feed on dissolved organic matter (DOM). Using a FITC-labeled dextran compound, where cellular uptake was quantified by flow cytometry, they demonstrated a saturating feeding curve. They further found that degradation of the dextran consumed more energy than was gained, thereby reducing ciliate growth rates. This novel application of a flow cytometer–based technique holds promise for studying mixotrophy in autotrophic microbes and reconciling the growth advantages (or disadvantages) of DOM uptake that have been hypothesized to translate into competitive dominance (e.g., some harmful algal bloom species).

Elemental Cycles

The last decade has seen an increase in the application of the general methods first proposed by Li (1994) and Rivkin et al. (1986) to use radioisotopic tracers and flow cytometry to generate information on the rates at which discrete phytoplankton populations are assimilating nutrients. While the data set is still relatively small (**Table 2**), there is enough information to begin to make some generalizations.

Carbon. Given the importance of carbon as the "currency" in oceanography, there are surprisingly few studies that directly measure cell-specific or population-specific carbon uptake by open ocean phytoplankton, relative to other biogenic elements (**Table 2**). We are aware of only three data sets, the original one by Li (1994), a recent study by Jardillier et al. (2010), and another, unpublished data set of the primary author of this article that attempts to quantify population-specific primary production of the pico- and nanoplankton that numerically dominate in the open ocean (**Table 2**). Four autotrophic populations, *Prochlorococcus*, *Synechococcus*, small and large eukaryotes, from the low-nutrient central ocean gyres that were sorted using flow cytometry, accounted for the overwhelming majority of the whole community (nonsorted) primary production. As expected, primary productivity rates scale with size, but population contributions to whole community primary production vary by place and group depending on the relative abundance of cells. At two of the three stations sampled, large eukaryotes, despite being approximately two orders of magnitude less abundant, accounted for >50% of measured carbon fixation (Li 1994). The more extensive (20 total stations) study by Jardillier et al. (2010) in the same general ocean region suggests that *Prochlorococcus* and the small eukaryotes contribute equally to carbon fixation (**Table 2**). Information of this type is critical to understanding differences between numerical dominance and biomass dominance; whereas *Prochlorococcus* almost always numerically dominates the autotrophic microbial population throughout the subtropics, its contribution to biomass is much more modest.

In higher-productivity coastal and upwelling regions as well as the open ocean, larger, and numerically rare, phytoplankton are very important and may be missed due to the generally small volume of sample sorted (volume analysis rates are generally lower than 1 mL min^{-1}). To

overcome the problem, large incubation volumes should be concentrated to increase particle events to match the speed of modern electronics and therefore capture the contribution of these larger cells. Work by the authors' laboratories and others (e.g., Zubkov et al. 2007) suggests that this processing step does not introduce any bias in population structure or genetic ecotypes. This step is particularly critical when using stable isotopes that require significant sample mass, and therefore sample concentration is absolutely necessary to keep sort times reasonable. In addition, as not all research programs have a flow cytometer at sea, sample fixation and storage must be taken into account (Silver & Davoll 1978). The issue of fixation is an important one, and most investigators have a preferred method with no community-wide concensus. Both glutaraldehye and formaldehyde have proved effective, when samples are subsequently stored at −80°C or in liquid nitrogen until analysis. Although the later perhaps leads to lower cell loss, the former is preferred when conducting cell cycle analyses. New users should make decisions about protocol based upon which specific analyses they plan on conducting.

Recently, the basic ^{14}C-flow cytometry technique has been used to study carbon chemoautotrophy in the Black Sea (Jost et al. 2008). Below the oxycline, multiple bacterial populations were identified, each with a different side scatter signature, which is often used as a proxy for internal cellular complexity (i.e., organelles, vesicles, etc.). Not all bacteria took up CO_2 the same way, however. The high right angle scatter populations (i.e., the larger cells) had higher uptake rates and contributed 65–100% of the total uptake. The measured cell-specific uptake rates suggest high growth rates, although the identity of the organisms remains unknown.

Nitrogen. The first study to use the stable nitrogen isotopic tracer ^{15}N with flow-cytometric sorting was Lipschultz (1995). Whereas this study sorted size ranges of particles rather than populations per se, it provided the first convincing evidence to support the long-standing dogma that nanoplankton preferentially assimilate ammonium and that microplankton preferentially assimilate nitrate. Using this flow cytometer–stable isotope protocol (**Figure 2**), Casey et al. (2007) followed this study with the first cell- and population-specific estimates of nitrogen uptake by natural populations of *Prochlorococcus*. In Casey et al. (2007), reduced nitrogen uptake accounted for ∼85–90% of the total measured nitrogen uptake (nitrate, nitrite, ammonium, and urea), with urea uptake being the single largest contributor. The most interesting finding of this study, however, was that some fraction of the natural population assimilated nitrate. This conclusion was surprising because at that point no cultured strains had been shown to grow on nitrate. This observation has since been confirmed by genomic research identifying the genomic islands containing nitrate transporters and nitrate reductase in *Prochlorococcus* (Martiny et al. 2009). Given the significant contribution of *Prochlorococcus* to primary production and autotrophic biomass in the ocean (Partensky et al. 1999), even if only 10% of nitrogen nutrition is due to nitrate, this equates to a substantial increase in nitrate assimilation and the autotrophic groups that are responsible. Nitrate uptake by picoeukaryotes showed that they had a higher uptake rate, relative to carbon, than did the cyanobacteria, suggesting that in the open ocean small eukaryotes may prefer nitrate, a finding recently confirmed by natural abundance stable isotope analysis of flow cytometrically sorted autotrophic populations (Fawcett et al. 2010).

The ability to sort distinct microbial populations also allows us to study long-standing questions of organic and inorganic nutrient cycling. It has generally been assumed that autotrophic phytoplankton consume predominantly inorganic nutrients, whereas heterotrophic bacteria consume predominantly organic nutrients. Work in the eutrophic Chesapeake Bay (**Figure 4**) shows that this generalization is reasonably accurate, with phytoplankton accounting for >90% of measured ammonium and nitrate uptake by the whole microbial community (**Figure 4*a*,*b***). In contrast to long-held beliefs that phytoplankton cannot compete with bacteria for small organic molecules

Table 2 Summary of population-specific biogeochemical rate measurements for populations sorted or analyzed by flow cytometer–isotope protocols[a]

Location	Cruise date	Tracer	Depth (m)	Uptake rate (pmol L^{-1} h^{-1})				Reference(s)
				HBac	*Prochlorococcus*	*Synechococcus*	Eukaryotes	
Atlantic Ocean								
NADR	May–July	^{3}H-AA mix	~5 m	8.6 ± 4.4	—	0.6 ± 0.4	—	Casey et al. 2009
West–NAST	May	^{3}H-AA mix	1–120 m	60.1 ± 6.1	8.6 ± 4.3	0.4 ± 0.3	—	Michelou 2009
West–NAST	Sept	^{3}H-AA mix	1–120 m	20.7 ± 4.9	1.5 ± 0.9	0.4 ± 0.3	—	Michelou 2009
East–NAST	May–July	^{3}H-AA mix	~5 m	6.7 ± 3.2	0.1 ± 0.1	—	—	Casey et al. 2009
NADR	May–July	^{3}H-leucine	~5 m	16.1 ± 3.5	—	1.1 ± 0.7	—	Michelou et al. 2007
West–NAST	May–July	^{3}H-leucine	~5 m	5.1	0.3 ± 0.1	—	—	Michelou et al. 2007
East–NAST	May–July	^{3}H-leucine	~5 m	6.8 ± 2.3	0.5 ± 0.4	0.1 ± 0.1	—	Michelou et al. 2007
East–NAST	Sept–Oct	^{3}H-leucine	~5 m	7–24[b] (17–34)	0.8–13	—	—	Mary et al. 2008
West–NAST	May	^{3}H-glucose	1–120 m	19.7 ± 9.6	3.0 ± 1.3	0.3 ± 0.2	—	Michelou 2009
West–NAST	Sept	^{3}H-glucose	1–120 m	12.4 ± 5.4	0.3 ± 0.1	0.5 ± 0.3	—	Michelou 2009
West–NAST	Oct, Sept, Mar	^{33}P-PO_4	1–120 m	17.1 ± 8.9	6.2 ± 4.0	1.6 ± 1.4	—	Michelou 2009, Casey et al. 2009
West–NAST	Oct, Sept, Mar	α^{33}P-ATP	1–120 m	0.20 ± 0.17	0.01 ± 0.01	0.02 ± 0.02	—	Michelou 2009
East–NAST	Sept, May	^{33}P-PO_4	~5 m	9–200	20	0.9–20	0.2–0.4	Zubkov et al. 2007
West–NAST	Aug–Oct	^{13}C-$HC0_3$	~100 m	—	8.1 ± 4.6[c]	0.7 ± 0.6	6.3 ± 4.6	M. W. Lomas, unpublished data
East–NAST	Oct	^{14}C-$HC0_3$	1 or 60 m	—	0.4–4.5[c]	0.1–8.2	1.9–20.1	Li 1994
NATR	Jan	^{14}C-$HC0_3$	5 or 20 m	—	228 ± 87[m]	143 ± 131[m]	197 ± 99[m]	Jardillier et al. 2010
East–NAST	Sept–Oct	^{35}S-Met	~5 m	19 ± 20[d] (100 ± 131)	—	—	—	Mary et al. 2006
East–NAST	Oct–Nov	^{35}S-Met	~5 m	0.6–5[b] (3–8)	0.02–6[e]	—	—	Mary et al. 2008
NATR	Sept–Oct	^{35}S-Met	~5 m	13 (30)	—	—	—	Mary et al. 2006
WTRA	Sept–Oct	^{35}S-Met	~5 m	6 (21)	—	—	—	Mary et al. 2006
SATL	Sept–Oct	^{35}S-Met	~5 m	11 ± 5 (27 ± 15)	—	—	—	Mary et al. 2006
SATL	Sept–Oct	^{35}S-Met	~5 m	—	1.9 ± 1.0	1.6 ± 1.4	—	Zubkov & Tarran 2005
SATL	Sept-Oct	^{35}S-Met	3–10 m, 50–150 m	—	0–6.3[f] (2–7.5)	—	—	Zubkov et al. 2004
SATL	Sept–Oct	^{35}S-Met	~5 m	0.7–1.4[b] (3–5)	1.5–10	0.02–1.0	—	Mary et al. 2008
West–NAST	Aug–Oct	^{15}N-NO_3	~100 m	—	0.2 ± 0.1[g]	0.1 ± 0.1	0.7 ± 0.4	Casey et al. 2007

West–NAST	July	^{35}S-DMSPd	20 m	0.034 ± 0.012	—	0.004 ± 0.004	—	Vila-Costa et al. 2006
East–NAST	Mar, Oct	^{35}S-DMSPd	~1 m	25 ± 16	0.4 ± 0.07	9 ± 5	0.6 ± 0.3	Vila-Costa et al. 2006
SARC	June	^{35}S-DMSPd	1–40 m	0.8–1.7[h]	—	—	—	Zubkov et al. 2002
Indian Ocean								
MONS/ARAB	Sept	^{35}S-Met	0–60 m	8–24	4–19	0.5–9	—	Zubkov et al. 2003
MONS/ARAB	Sept	^{35}S-Met	0–100 m	0.2–11.6	—	—	—	Zubkov et al. 2006
Coastal seas								
Baltic Sea	Feb	^{14}C-HCO_3	116–150 m	0.0–62.5[c]	—	—	—	Jost et al. 2008
Boothbay Harbor	June	^{15}N-NH_4	~2 m	—	—	—	4–45[k]	Lipschultz 1995
Boothbay Harbor	June	^{15}N-NO_3	~2 m	—	—	—	5–10	Lipschultz 1995
Chesapeake Bay	Aug–Sept	^{15}N-NH_4	~2 m	0.36 ± 0.47[i]	—	—	0.387 ± 0.529[j]	Bradley et al. 2010b
Chesapeake Bay	Aug–Sept	^{15}N-NO_3	~2 m	—	—	—	0.001 ± 0.001[j]	Bradley et al. 2010b
Chesapeake Bay	Aug–Sept	^{15}N-urea	~2 m	0.04 ± 0.04[i]	—	—	0.036 ± 0.044[j]	Bradley et al. 2010b
Chesapeake Bay	Aug–Sept	^{15}N-AA mix	~2 m	0.06 ± 0.01[i]	—	—	0.036 ± 0.018[j]	Bradley et al. 2010b
LEO-15	July	^{15}N-NH_4	1–14 m	0.014 ± 0.01[i]	—	—	0.138 ± 0.201[l]	Bradley et al. 2010a,b
LEO-15	July	^{15}N-NO_3	1–14 m	0.003 ± 0.003[i]	—	—	0.031 ± 0.050[l]	Bradley et al. 2010a,b
LEO-15	July	^{15}N-urea	1–14 m	0.074 ± 0.085[i]	—	—	0.401 ± 0.457[l]	Bradley et al. 2010a,b
LEO-15	July	^{15}N-AA mix	1–14 m	0.013 ± 0.012[i]	—	—	0.020 ± 0.025[l]	Bradley et al. 2010a,b
Gulf of Mexico	May	^{35}S-DMSPd	~1 m	0.42 ± 0.14	—	0.11 ± 0.02	—	Vila-Costa et al. 2006
Pensacola Bay	June	^{35}S-DMSPd	~1 m	0.10 ± 0.02	—	0.01 ± 0.01	0.002 ± 0.001	Vila-Costa et al. 2006

[a]Locations follow nomenclature of Longhurst biogeographical provinces and include North Atlantic Drift province (NADR), east and west North Atlantic Subtropical Gyre provinces (NAST), North Atlantic Tropical province (NATR), West Tropical Atlantic province (WTRA), South Atlantic Gyre province (SATL), Atlantic Subarctic province (SARC), Indian Monsoon Gyre province (MONS), and the Northwest Arabian Sea Upwelling province (ARAB). Only studies where population-specific uptake rates could be reasonably estimated using data provided in the original publication are included. All uptake rates are in units of picomol L^{-1} h^{-1} except where noted, which is primarily in the higher-productivity coastal systems.

[b]LNA and HNA.

[c]Units: nmol C L^{-1} h^{-1}.

[d]Original units: pmol L^{-1} d^{-1} converted to h^{-1} by dividing by 24. LNA as is; total Bpl in parentheses.

[e]Range estimated from min/max in cellular activity and cell abundance from figures.

[f]Original units: pmol L^{-1} d^{-1} converted to h^{-1} by dividing by 24. HL Pro as is; LL Pro in parentheses.

[g]Original units: nmol N L^{-1} d^{-1} converted to h^{-1} by dividing by 24.

[h]Cells not flow sorted but bacterial subpopulation 1, *Roseobacter*, dominated uptake and was the only bacterial group related to DMSPd uptake.

[i]Units: μmol L^{-1} h^{-1}, estimated as 2*x* the difference between phyto and GF/F fractions in original manuscript, based on the assumption of 50% bacteria retention as detailed by authors.

[j]Units: μmol L^{-1} h^{-1}; phyto fraction in original manuscript includes flow-sorted, small eukaryotes plus >35-μm eukaryotes.

[k]3–53-μm sorted fraction.

[l]Units: μmol L^{-1} h^{-1}; phyto fraction in original manuscript includes flow-sorted eukaryotes.

[m]Units: nmol L^{-1} h^{-1}.

Abbreviations: AA mix, commercially available algal extract containing a mix of 16 amino acids; Bpl, bacterioplankton; DMSPd, dimethylsulfoniopropionate; HL Pro, high-light-adapted *Prochlorococcus*; HNA, high nucleic acid; LL Pro, low-light-adapted *Prochlorococcus*; LNA, low nucleic acid; Met, methionine.

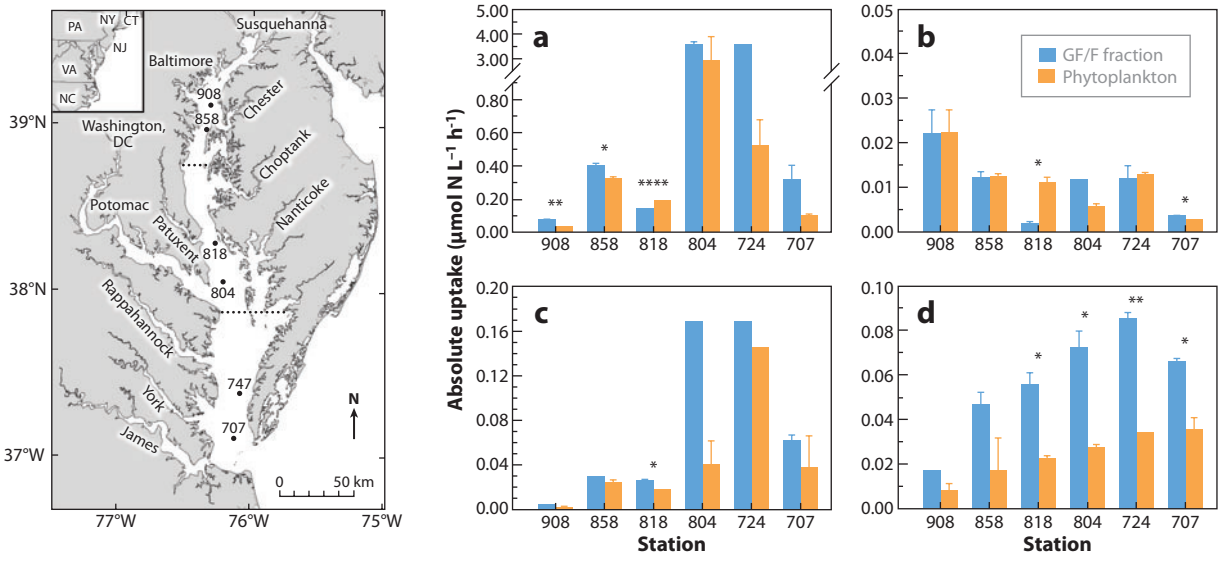

Figure 4

Map of Chesapeake Bay showing the location of six sites where absolute uptake rates of (*a*) ammonium, (*b*) nitrate, (*c*) urea, or (*d*) an amino acid mixture were measured into the GF/F fraction (nominal pore size 0.7 μm; *blue bars*) and flow-cytometrically sorted phytoplankton fraction (*orange bars*). Asterisks denote significant differences between fractions, as determined using student's t tests (*p < 0.1, **p < 0.05, ***p < 0.01, ****p < 0.001). Adapted with permission from Bradley et al. 2010b.

like amino acids, phytoplankton accounted for ~50% of amino acid uptake by the whole microbial community across a wide range of nutrient concentrations and ratios. If this is found to be the case in other systems, including the open ocean, then nutrient biogeochemical models will have to be substantially revamped.

Radioisotopic tracers are more sensitive than stable isotopic tracers; however, due to the lack of a practical nitrogen radioisotope tracer (half-life of ^{13}N is 10 minutes), a large amount of work has been done using tritiated (^{3}H) or sulfur (^{35}S)-labeled amino acids to study the contribution of small, dissolved organic nitrogen (DON)-containing compounds to nitrogen metabolism. Both marine cyanobacterial groups, *Prochlorococcus* and *Synechococcus*, from the Atlantic Ocean and Arabian Sea, have been shown to assimilate methionine (Zubkov et al. 2003, Zubkov & Tarran 2005), and data suggest that *Prochlorococcus* can effectively compete with the average heterotrophic bacteria for methionine in the ocean in its optimal growth range. Use of a tritiated amino acid mix suggests a different conclusion, with heterotrophic bacteria being the more important DON consumer in the same geographic range. Interestingly, maximal cell-specific uptake rates are found at the northern/southern extremes of *Prochlorococcus* extent (e.g., Zubkov & Tarran 2005). So although *Prochlorococcus* becomes a better competitor for amino acids as it nears the geographic limits of its extent, the lower cell abundances result in an overall lower contribution to DON cycling. These observations are confounded by different sampling locations, substrates, and isotopic tracers quantified to represent amino acid uptake but clearly show that microbial cycling of DON is more complicated than originally envisioned.

Another possible explanation for the observed differences is that light was shown to stimulate the uptake of amino acids and leucine to a greater degree in *Prochlorococcus* than in heterotrophic bacteria (Michelou et al. 2007), perhaps because only the LNA bacteria show light stimulation (Mary et al. 2008). Research suggesting different levels of activity between HNA and LNA bacteria

(discussed above), and the relatively higher abundance of LNA bacteria, raises significant uncertainties about multiplying an average cell-specific uptake rate by the total bacterial abundance.

Phosphorus. Despite recent reports that the subtropical North Atlantic and Pacific gyres are phosphorus limited, only a limited number of studies have combined ^{33}P and flow cytometry to investigate rates of phosphorus uptake. All of these have been in the North Atlantic and none included concurrent nitrogen uptake rates. At the population level, heterotrophic bacteria dominate inorganic phosphate uptake in both the western (Michelou 2009) and eastern North Atlantic (Zubkov et al. 2007). This is due entirely to higher cell abundances as cell-specific uptake rates were similar across the gyre for the same populations (Casey et al. 2009, Zubkov et al. 2007). In contrast to phosphate uptake, uptake of dissolved organic phosphorus (DOP; using ATP as a model molecule) was much higher in the western North Atlantic and often equivalent in magnitude to phosphate uptake; whereas, in the eastern North Atlantic, DOP uptake never exceeded 17% of phosphate uptake. This was primarily due to the strong physiological preference for DOP in *Synechococcus* and eukaryotic phytoplankton, relative to heterotrophic bacteria (Michelou 2009). Despite total phosphorus consumption being higher in the eastern North Atlantic, due to higher cell numbers, similar rates of primary production in the west suggest gradients in C:P uptake ratios that differ between autotrophic groups (Casey et al. 2009, Zubkov et al. 2007). This hypothesis cannot be evaluated at present due to the lack of taxon-specific carbon uptake data, but it has important implications for global biogeochemical models that allow for variable elemental stoichiometry in microbial groups.

Silica. Whereas, to date, no studies have been published that explicitly use flow cytometry to explore the marine silica cycle, it is possible to use this method given that both stable and radioisotopes of silica have been used in oceanography, though there are limitations in the sensitivity of the stable isotope and the supply of the radioisotope. In addition to these techniques, there is another fluorescent assay that has been shown to reflect silica deposition. Shimizu et al. (2001) and Leblanc & Hutchins (2005) show that the fluorescent dye [2-(4-pyridyl)-5{[4-dimethylaminoethyl-aminocarbamoyl)-methoxy]phenyl}oxazole] (PDMPO) may be a suitable, nontoxic tracer of internal silicic acid concentrations and rates of silica uptake and deposition. The dye has different emission peaks in the presence/absence of silica, and this ratio has been shown to scale with internal silica concentration (Shimizu et al. 2001). In live cells, newly deposited silica yields yellow-green fluorescence that is related to biogenic silica deposition. When small, non-chain-forming diatoms like *Minidiscus* spp. (common in the Sargasso Sea) are present, flow cytometry can be used and speed up sample analysis over manual microsopic methods. Moreover, use of the flow cytometer can provide estimates of cell carbon and thus generate more information on the biogeochemical coupling of carbon and silica in this important autotrophic group.

Sulfur. Organic sulfur cycling has received a great deal of attention due to its role as a climate-active gas. Hydrolysis of dimethylsulfoniopropionate (DMSP) to dimethyl sulfide (DMS) by the microbial food web and its oxidation to sulfate once in the atmosphere feed cloud condensation nuclei, which increases cloud albedo and ultimately reduces solar irradiance at the earth's surface, providing a negative climate feedback (Charlson et al. 1987). Heterotrophic bacteria are thought to be the primary consumers of DMSP (Kiene et al. 2000, Zubkov et al. 2002), although there is one record of assimilation of DMSP by the cyanobacterium *Synechococcus* (Malmstrom et al. 2005). Using the same basic flow cytometry–radioisotope technique described above (and in **Figure 2**), Vila-Costa et al. (2006) have shown that, in fact, many picoplankton from a diversity of marine

systems assimilate dissolved DMSP (DMSPd) (**Table 2**), contributing upwards of 10–34% of total DMSPd uptake. Interestingly, it was the heterotrophic bacteria, members of the *Roseobacter* lineage (Zubkov et al. 2002), and *Prochlorococcus* that showed a physiological preference for the uptake of DMSPd. These findings are particularly important as cyanobacteria are generally not considered to be producers of DMSPd (Matrai & Keller 1994) so would be a net sink for organic sulfur in the ocean, if these results are found to be representative of the broader ocean. Given the climate feedback linked to sulfur cycling in the ocean, this is a research question that needs to be resolved to improve predictive models of future climate.

Particulate and Dissolved Organic Matter Cycling

As discussed above, flow cytometry allows researchers to get away from bulk analyses of biotic particles. The other advantage of flow cytometry is the ability to separate live from dead particulate organic matter (POM). Eglinton et al. (1996) and Minor et al. (1998) combined flow-cytometric sorting with dual-temperature mass spectrometry to investigate sources and freshness of POM. Minor et al. (1998) sorted particles from the Mid-Atlantic Bight into phytoplankton and detritus classifications based on forward light scatter (proxy for size) and presence/absence of chlorophyll fluorescence and found the phytoplankton fraction to be enriched in proteins and sterols, whereas the detritus was enriched in polysaccharides, suggesting that stressed or dying phytoplankton are not a significant fraction of the detrital pool. In contrast, Eglinton et al. (1996), in a similar exercise but with samples collected from the Peru upwelling system, observed that detrital material was more similar to fresh phytoplankton, suggesting a tighter linkage to primary production.

Although the marine POM pool is clearly dynamic, the DOM pool is much larger and perhaps equally dynamic. DOM, operationally defined as organic matter that passes a specified filter pore size cutoff (generally <0.7 μm), includes microparticles such as colloids and microgels. Orellana et al. (2007) used flow cytometry to demonstrate the abundance and importance of marine microgels to dissolved organic carbon (DOC) cycling. Flow cytometry was absolutely critical to studying these microgels because normal handling procedures for DOC (i.e., filtration) would either disrupt or remove the microgels from the sample. Orellana et al. (2007) were able to identify enzymes that suggested one source of this material as the sloughing off of dead cell membranes. One potential source is the blue-fluorescent protein that these researchers found associated with the frustules of marine diatoms. This protein is unique in that it is UV-excitable and may therefore be useful as a means to trace the origin of these microgels.

CONTINUOUS AND REMOTE OBSERVATIONS

The acquisition of long-term data sets has become critically important in addressing questions of global change. The marine environment is notoriously patchy, with large variability observed in both time and space. Traditional sampling approaches, in which researchers occupy a site or sites for a period of time aboard a ship or along a transect, are ill equipped to define this variability. The need for approaches that can make continuous observations remotely is critically important to study processes that occur on small temporal scales at a range of spatial scales and to cost-effectively acquire long-term time-series data sets (Paul et al. 2007). Flow cytometry is uniquely suited to fill these needs. Flow cytometers can make observations at the level of a single cell in near real time, could be deployed as an array to collect data over broad geographic regions, and can be operated remotely to collect time-series observations. Although the field of remote, flow-cytometric observations is in its infancy, this is an important area for future study. Here,

we review a number of instruments that incorporate flow cytometry principles that are currently being used to monitor marine systems.

A pioneering approach in the area of remote observations was the CytoBuoy, which is a flow cytometer attached to a moored buoy (Dubelaar & Gerritzen 2000, Dubelaar et al. 1999). The development of a moored cytometer had to overcome many of the challenges inherent in other remote instrument packages. First and foremost is the requirement for adequate power, which remains a challenge. A second factor, biofouling, is less of an issue by the very nature of flow cytometry. The sample is imbedded in a particle-free sheath fluid, which makes flow cytometers relatively impervious to biofouling; however, a constant supply of particle-free sheath fluid remains a challenge (Dubelaar & Gerritzen 2000). In one approach, Swalwel et al. (2009) were developing a flow cytometer for phytoplankton that does not require a continuous supply of clean sheath water but rather images a core of water passing through the instrument. The inclusion of self-aligning optics allows the instrument to operate for prolonged times with minimal maintenance. This instrument has successfully completed several sea trials and is being prepared for longer deployments.

Another moored instrument is the FlowCytobot, which is an automated flow cytometer that can analyze cells at the pico- and nanoplankton levels. The FlowCytobot was first deployed at the LEO-15 cabled mooring site off the coast of New Jersey (Olson et al. 2003). One goal of the remote deployment was to estimate daily specific growth rates of *Synechococcus* (Sosik et al. 2003). The fact that phytoplankton increase in cell diameter during the day due to growth was the basis for this approach. The FlowCytobot measured diel changes in the cell-size distribution for the population. The data indicate that the abundance of *Synechococcus* declines in autumn as a function of a decline in specific growth rate, as opposed to grazing or physical advection that was previously assumed (Sosik et al. 2003).

The FlowCytobot's capabilities were expanded to allow the analysis of larger cells in the 10- to >100-μm range by the inclusion of a high-resolution camera (Olson & Sosik 2007). This size group, which includes many diatoms and dinoflagellates, is particularly important in coastal and more eutrophic systems. The new instrument, the Imaging FlowCytobot, uses the flow cytometer to measure chlorophyll fluorescence and video technology to capture images of organisms for identification. Previously this large size group of cells had been studied with the FlowCAM, which is also an imaging flow cytometer used in the laboratory or aboard a ship (Sieracki et al. 1998).

It is envisioned that one day instruments such as those described above could be placed on commercial vessels for continuously monitoring phytoplankton populations in the oceans on a scale rarely achieved, much like the Continuous Plankton Recorder program operated by the Sir Alister Hardy Foundation for Ocean Sciences that has been ongoing since 1931.

ECOTOXICOLOGY, MARINE PATHOGENS, AND HARMFUL ALGAE

Flow cytometry has also been used in a number of other, more applied aquatic fields. The ability of flow cytometers to assess vital cell functions has made them useful in toxicity testing—often referred to as ecotoxicity testing. A number of parameters have been used as the end point in toxicity tests, including population growth rates and enzyme activity, specifically esterase activity (e.g., Obst et al. 1988). Esterase activity is commonly monitored using fluorescein diacetate (FDA)—a low-molecular-weight, lipophilic, nonpolar compound capable of penetrating cell plasma membranes—or its derivatives. FDA is nonfluorescent, but when hydrolyzed by intracellular esterases, the hydrophilic fluorescent product fluorescein is formed, which is then measured using flow cytometry. Several studies used these methods with microalgal cells and demonstrated

that there is good agreement between esterase activity measured with flow cytometry and inhibition of growth (Faber et al. 1997, Hampel et al. 2001).

Flow cytometry has also been used with freshwater marine algae to assess the bioavailability of contaminants (e.g., Stauber et al. 2002). Flow-cytometric analysis of cultures is superior to traditional algal growth inhibition tests because environmentally realistic cell densities can be used; the differentiation between live and dead cells can be automated (as discussed previously); more than one algal species can be used simultaneously (e.g., Yu et al. 2007); and the approach can provide information on mechanisms of toxicity, all of which are necessary for the development of predictive models (e.g., Levy et al. 2008, Rioboo et al. 2009). In one example, copper toxicity was tested in three algal species simultaneously to determine the effect of algal–algal interactions (Yu et al. 2007) previously found to be more important than contaminant speciation alone (Stauber & Davies 2000). In this study, Yu et al. (2007) determined sublethal levels of copper toxicity by combining measurements of cell autofluorescence with a number of cell probes, including FDA (described above) and 2′,7′-dichlorodihydro-fluorescein diacetate (H2DCFDA), which is used to assess intracellular reactive oxygen species.

Flow cytometry is also proving to be a valuable tool for marine aquaculture because of the speed with which samples can be analyzed. Within the dense collections of fish present in aquaculture facilities, the early detection of pathogenic bacteria is essential so that preventive measures can be taken. The traditional method for detecting bacteria is the colony-counting method, which requires an incubation period of at least 24 hours. Flow cytometry is a valuable alternative for a number of reasons: When combined with antibody labeling for specific types of pathogenic bacteria, the abundance of these specific types, relative to the larger bacterial population, can readily be determined; assays can be performed in a few hours; and it can distinguish between live and dead pathogenic cells (e.g., Endo et al. 2000).

Another area where rapid determination of harmful organisms is critical is in the treatment of ballast water. Ships with empty cargo holds take on ballast water in a given port for stability during transit. This ballast water is then released in a region near the next port when cargo is taken on. This practice has resulted in the dispersal of nonindigenous species around the world, with an estimated 15,000 species transported per week (Waite 2002). A number of methods have been proposed to treat ballast water to prevent this inadvertent transfer of organisms, but determining the efficiency of these treatments has been challenging. One approach is to monitor one or more indicator species. Dinoflagellate cysts are one potential indicator species because of their prevalence and the difficulty in killing them. The cysts of *Alexandrium catenella* are ideal indicators because *A. catenella* is easy to culture, and the cysts have short dormancy periods, are small in size, and have very thick cell walls that make them highly resistant to damage, ensuring easy identification. Binet et al. (2006) developed a rapid flow cytometry protocol to determine the viability of cysts of *A. catenella* using the viability stain SYTOX. This new method was successful at predicting the viability of cysts up to 60 weeks old in under 2 days, as opposed to the former approach, which took 4 weeks. Flow cytometry has also been used in a viability assay for nematode worms (Gill et al. 2003) and to enumerate bacteria in ballast water (e.g., Joachimsthal et al. 2003).

The ability of flow cytometers to provide analyses of the phytoplankton present and their size distribution, as well as additional aspects or characteristics beyond gross morphology, has made them useful in the study of harmful algal blooms. Although flow cytometers can determine the abundance of a given group, not all members may be toxic. Orellana et al. (2004) took advantage of the autofluorescence of *Pseudo-nitzschia multeries*, a toxic diatom known to produce domoic acid—the neurotoxin responsible for amnesic shellfish poisoning—to propose this as a method to selectively identify this diatom from co-occurring nontoxic species. Furthermore, the evolution of the first *Dinophysis* bloom observed in U.S. waters was studied with the use of a moored Imaging

FlowCytoBot (Campbell et al. 2010). *Dinophysis* is known to produce okadaic acid, which can be concentrated by filter-feeding bivalves and is known to cause diarrhetic shellfish poisoning in humans. The ability to monitor phytoplankton continuously and remotely allowed the researchers to determine the presence of *Dinophysis*, resulting in a rapid response and closure of shellfish beds.

ENVIRONMENTAL METAGENOMICS

Information about the diversity of marine microbes is being increasingly obtained by isolating DNA from water samples. The approach of describing anonymous DNA sequences from a mixed assemblage of microbes encountered in a given environment is known as metagenomics. As the cost of sequencing comes down, databases with sequences of genes, gene complexes, and regulatory cassettes are rapidly expanding. In early 2010, the complete genomes of over 200 species, mostly microbes, have already been described (Armbrust 2009, Worden et al. 2009). This number is growing at an exponential rate, increasing the chance that anonymous pieces of DNA isolated from environmental samples can be assigned to a taxonomic branch or can be related to sequence families with known biological functions.

The ability to identify the metabolic potential or physiology of an organism before it has been isolated is the power of metagenomics. However, there are inherent complications in the reassembly of diverse environmental samples following sequencing. Combining the sorting capabilities of the flow cytometer with the sequencing power of genomics will improve researchers' ability to target specific functional groups and nullify some of these complications. This will also facilitate the examination of the rare microbial biosphere, which may yet hold unique and unknown metabolisms. For example, Zehr et al. (2008) isolated a unique cyanobacterium using a laborious cell sorting strategy. This cyanobacterium contains a DNA cassette incorporating both photosynthesis genes and genes that are used in nitrogen fixation; these processes were considered incompatible in a single-cell organism. Genome sequencing, on flow-cytometrically isolated cells, revealed that the cyanobacterium has an incomplete photosynthetic apparatus and lacks the photosystem II genes.

Metagenomics can also be conducted in a directed fashion that searches for common gene functions across a large number of species or among ecotypes of the same species. Sorting ecotypes from the *Prochlorococcus* group, using phylogenetic probes, demonstrates genome differences that segregate according to depth, global location, and season (e.g., Johnson et al. 2006), providing further evidence that microbial ecotypes are specialized for different niches. Such studies have also discovered that marine viruses may carry host-derived genes expressed during the infection process, boosting the host's metabolic processes in favor of virus reproduction (Sullivan et al. 2009).

In a short time, the metagenomics approach has led to important discoveries. New, surprisingly abundant microbial life forms with unique metabolic processes have been identified by their DNA fingerprints in the environment. Cell sorting has been found to be an incredibly useful tool in mapping anonymous DNA sequences onto individual microbes. As databases cataloging DNA sequences expand, the metagenomic approach will become increasingly productive.

THE FUTURE OF FLOW CYTOMETRY IN AQUATIC SCIENCES

Since the discovery of *Prochlorococcus*—now recognized as the most abundant and one of the most biogeochemically important autotrophs in the ocean gyres—using flow cytometry (Chisholm et al. 1988), this technique has earned a firm place in phytoplankton analysis. Enumeration of *Prochlorococcus* and *Synechococcus* in fixed samples or in fresh samples during cruises has become a

routine activity. Flow cytometry methods for visualizing and identifying marine microbes will continue to improve. The availability and reduction in cost of easy-to-operate solid state lasers with new colors offer wavelengths that better excite the inherent pigments of different phytoplankton groups. New detection schemes employing scatter and fluorescence polarization are being used to identify specific autotrophic groups, such as the coccolithophores (e.g., Von Dassow et al. 2009). Staining protocols for heterotrophic microbes and viruses, which do not have innate pigments, are being optimized (e.g., Brussaard 2004a). The detection of accessory photosynthetic pigments by measuring action spectra rather than total chlorophyll fluorescence promises to parse complex phytoplankton mixtures into tight, well-separated clusters. We can expect that the number of groups of phytoplankton and heterotrophic microbes that can be identified with confidence in environmental samples will continue to increase and so too will our understanding of their biogeochemical role in the ocean.

Despite improvements in optical aspects of flow methodology for phytoplankton enumeration, cytometric identification must ultimately be validated with genome analysis. DNA/RNA-expression measurements will further broaden our insight into the makeup and activities of a microbial community (e.g., Stepanauskas & Sieracki 2007). Single-cell DNA amplification and single-cell RNA-to-DNA copying and amplification must be adapted for phytoplankton. Also, for identification and isolation of new microbial groups, cell sorting technology must be more efficiently coupled to downstream single-cell genome analysis. As these genomic tools become more reliable, cell sorters will be used to generate arrays of individual cells for hybridization with DNA probes or even direct DNA sequencing. For this purpose, new sort protocols that can deposit individual cells in complex patterns on a solid surface (e.g., glass slides) are being developed. The set of these technologies will make possible in a single experiment large-scale determination of the genetic variation and expression patterns in environmental samples.

In recent years, the manufacturers of flow instrumentation have started to specifically address the needs of marine microbiologists, resulting in a rapid increase in cytometers deployed by marine institutions. A number of such instruments has been installed in mobile laboratories and may be available for scientific cruises (e.g., University of Uppsala; Bigelow Laboratory; and University of Hawaii, National Oceanography Center at Southampton). Interest in expanding these seagoing capabilities remains high and will provide a strong cadre of researchers well positioned to move the field forward.

FUTURE ISSUES

1. New generation ecosystem models are using physiological submodels to describe phytoplankton "behavior," therefore future research needs include, where practical, cell quotas for elements of interest so that growth rates can be calculated from uptake rates and we can begin to understand the range, variability, and controls on elemental stoichiometry at the population level.
2. Future research should attempt to advance the use of proxies for intracellular biochemical pools (e.g., protein content, DNA, etc.) in phytoplankton to the same extent that one can for heterotrophic bacteria. This information, if routine, would help resolve some of the outstanding questions based on cell counts and/or bulk measurements (e.g., which group of bacteria, HNA or LNA, are growing faster).

3. Given increased difficulties with using radioisotopes in the field, from both a regulatory and a waste disposal perspective, additional nonradioactive techniques (e.g., BrdU) should be developed to assess microbial processes and expand the toolbox that is complementary to flow cytometry.
4. Future research efforts should consider the use of multiple tracers in experimental design as this would provide critical information on the coupling of elemental cycling at the single-cell level. Stable isotopes are particularly well suited to this technique because the combustion products often have distinct masses, whereas with radioisotopes, the energy windows of many β-emitters overlap.

DISCLOSURE STATEMENT

Michael Lomas and Debbie Bronk are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review. Ger van den Engh is employed by BD Biosciences, a manufacturer and seller of flow cytometers and cell sorters. G.vdE. stands to gain personal benefit from increased sales of such machines, in particular those of the BD brand.

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LITERATURE CITED

Alivisatos P. 2004. The use of nanocrystals in biological detection. *Nat. Biotechnol.* 22:47–52

Alonso-Sáez A, Gason JM, Lefort T, Hofer J, Sommaruga R. 2006. Effect of natural sunlight on bacterial activity and differential sensitivity of natural bacterioplankton groups in northwestern Mediterranean coastal waters. *Appl. Eniviron. Microbiol.* 70:5806–13

Alvain S, Moulin C, Dandonneau Y, Loisel H. 2008. Seasonal distribution and succession of dominant phytoplankton groups in the global ocean: a satellite view. *Glob. Biogeochem. Cycles* 22:GB3001

Amann RI, Binder JJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA. 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl. Eniviron. Microb.* 56:1919–25

Armbrust EV. 2009. The life of diatoms in the world's oceans. *Nature* 459:185–92

Barbesti S, Citterio S, Labra M, Baroini MD, Neri MG, Sgorbati S. 2000. Two and three-color fluorescence flow cytometric analysis of immunoidentified viable bacteria. *Cytometry A* 40:214–18

Beja O, Aravind L, Koonin EV, Suzuki MT, Hadd A, et al. 2000a. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science* 289:1902–6

Beja O, Suzuki M, Koonin E, Aravind L, Hadd A, et al. 2000b. Construction and analysis of bacterial artificial chromosome libraries from a marine microbial assemblage. *Environ. Microbiol.* 2:516–29

Binet M, Stauber J, Franklin N, Adams M. 2006. Applications of flow cytometry to marine ecotoxicology and ballast water studies. *Cytometry A* 69A:403–4

Bouvier T, del Giorgio PA, Gasol JM. 2007. A comparative study of the cytometric characteristics of high and low nucleic-acid bacterioplankton cells from different aquatic ecosystems. *Environ. Microbiol.* 9:2050–66

Boyanapalli R, Bullerjahn GS, Pohl C, Croot P, Boyd P, McKay R. 2007. Luminescent whole-cell cyanobacterial bioreporter for measuring Fe availability in diverse marine environments. *Appl. Environ. Microbiol.* 73:1019–24

Bradley P, Sanderson MP, Frischer ME, Brofft J, Booth MG, et al. 2010a. Inorganic and organic nitrogen uptake by phytoplankton and heterotrophic bacteria in the stratified Mid-Atlantic Bight. *Estuar. Coast. Shelf Sci.* 88:429–41

Bradley PB, Lomas MW, Bronk DA. 2010b. Inorganic and organic nitrogen use by phytoplankton along Chesapeake Bay, measured using a flow cytometric sorting approach. *Estuar. Coasts* 33:971–84

Bratbak G. 1985. Bacterial biovolume and biomass estimations. *Appl. Environ. Microbiol.* 49:1488–93

Brock TD. 1967. Micro-organisms adapted to high temperatures. *Nature* 214:882–85

Brussaard CPD. 2004a. Optimization of procedures for counting viruses by flow cytometry. *Appl. Environ. Microbiol.* 70:1506–13

Brussaard CPD. 2004b. Viral control of phytoplankton populations: a review. *J. Eukaryotic Microbiol.* 51:125–38

Brussaard CPD, Marie D, Thyrhaug R, Bratbak G. 2001. Flow cytometric analysis of phytoplankton viability following viral infection. *Aquat. Microb. Ecol.* 26:157–66

Button DK, Robertson BR. 2001. Determination of DNA content of aquatic bacteria by flow cytometry. *Appl. Environ. Microbiol.* 67:1636–45

Campbell L, Olson RJ, Sosik HM, Abraham A, Henriches DW, et al. 2010. First harmful *Dinophysis* (Dinophyceae, Dinophysiales) bloom in the U.S. is revealed by automated imagine flow cytometer. *J. Phycol.* 46:66–75

Casey J, Lomas MW, Michelou V, Dyhrman S, Orchard E, et al. 2009. Phytoplankton taxon-specific orthophosphate (Pi) and ATP utilization in the western subtropical North Atlantic. *Aquat. Microb. Ecol.* 58:31–44

Casey J, Lomas MW, Mandecki J, Walker D. 2007. *Prochlorococcus* contributes to new production in the Sargasso Sea deep chlorophyll maximum. *Geophys. Res. Lett.* 34:L10604

Charlson R, Lovelock J, Andreae M, Warren S. 1987. Oceanic phytoplankton, atmospheric sulfur, cloud albedo and climate. *Nature* 326:655–61

Chisholm SW, Olson RJ, Zettler ER, Goericke R, Waterbury JB, Welschmeyer NA. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature* 334:340–43

Collier JL. 2000. Flow cytometry and the single cell in phycology. *J. Phycol.* 36:628–44

Corzo A, Rodríquez-Gálvez S, Lubian L, Sobrino C, Sangrá P, Martínez A. 2005. Antarctic marine bacterioplankton subpopulations discriminated by their apparent content of nucleic acids differ in their response to ecological factors. *Polar Biol.* 29:27–39

Davey H, Kell DB. 1997. Fluorescent brighteners; novel stains for the flow cytometric analysis of microorganisms. *Cytometry* 28:311–15

del Giorgio PA, Bird DF, Prairie YT, Planas D. 1996. Flow cytometric determination of bacterial abundance in lake plankton with green nucleic acid statin SYTO 13. *Limnol. Oceanogr.* 41:783–89

del Giorgio PA, Bouvier T. 2002. Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. *Limnol. Oceanogr.* 47:471–86

del Giorgio PA, Prairie Y, Bird D. 1997. Coupling between rates of bacterial production and the abundance of metabolically active bacteria in lakes, enumerated using CTC reduction and flow cytometry. *Microb. Ecol.* 34:144–54

Dorsey J, Yentsch CM, Mayo S, McKenna C. 1989. Rapid analytical technique for the assessment of cell metabolic activty in marine microalgae. *Cytometry* 10:622–28

Dortch Q. 1990. The interaction between ammonium and nitrate uptake in phytoplankton. *Mar. Ecol. Prog. Ser.* 61:183–201

Dubelaar GBJ, Gerritzen PL. 2000. CytoBuoy: a step forward towards using flow cytometry in operational oceanography. *Sci. Mar.* 64:255–65

Dubelaar GBJ, Gerritzen PL, Beeker AER, Jonker RR, Tangen K. 1999. Design and first results of CytoBuoy: a wireless flow cytometer for in situ analysis of marine and fresh waters. *Cytometry* 37:247–54

Duhamel S, Gregori G, Van Wambeke F, Mauriac R, Nedoma J. 2008. A method for analyzing phosphatase activity in aquatic bacteria at the single cell level using flow cytometry. *J. Microbiol. Methods* 75:269–78

DuRand M, Olson R, Chisholm S. 2001. Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. *Deep-Sea Res.* II 48:1983–2003

Dyhrman S, Palenik B. 1999. Phosphate stress in cultures and field populations of the dinoflagellate *Prorocentrum minimum* detected by a single-cell alkaline phosphatase assay. *Appl. Environ. Microbiol.* 65:3205–12

Eglinton TI, Boon JJ, Minor EC, Olson RJ. 1996. Microscale characterization of algal and related particulate organic matter by direct temperature-resolved mass spectrometry. *Mar. Chem.* 52:27–54

Elsey D, Jameson D, Raleigh B, Cooney M. 2007. Fluorescent measurement of microalgal neutral lipids. *J. Microbiol. Methods* 68:639–42

Endo H, Nakayama J, Hayashi T. 2000. Application of flow cytometry to environmental control in marine aquaculture. *Mater. Sci. Eng. C* 12:83–88

Fawcett SE, Lomas MW, Casey JR, Ward BB, Sigman DM. 2010. Eukaryotes dominate new production in the Sargasso Sea. *Nature*. In review

Faber MJ, Smith LMJ, Boersmans HJ, Stephenson GR, Thompson DG, Solomon KR. 1997. Cryopreservation of fluorescent marker-labeled algae (*Selenastrum capricornutum*) for toxicity testing using flow cytometry. *Environ. Toxicol. Chem.* 16:1059–67

Falcioni T, Papa S, Gasol JA. 2008. Evaluating the flow-cytometric nucleic acid double-staining protocol in realistic situations of planktonic bacterial death. *Appl. Environ. Microbiol.* 74:1767–79

First MR, Hollibaugh JT. 2009. The model high molecular weight DOC compound, dextran, is ingested by the benthic ciliate *Uronema marinum* but does not supplement ciliate growth. *Aquat. Microb. Ecol.* 57:79–87

Flynn KJ. 2010. Ecological modelling in a sea of variable stoichiometry: dysfunctionality and the legacy of Redfield and Monod. *Prog. Oceanogr.* 84:52–65

Follows MJ, Dutkiewicz S, Grant S, Chisholm SW. 2007. Emergent biogeography of microbial communities in a model ocean. *Science* 315:1843–46

Fu YT, O'Kelly C, Sieracki M, Distel DL. 2003. Protistan grazing analysis by flow cytometry using prey labeled by in vivo expression of fluorescent proteins. *Appl. Environ. Microbiol.* 69:6848–55

Gill MS, Olsen A, Sampayo JN, Lithgow GL. 2003. An automated high-throughput assay for survival of the nematode *Caenorhabditis elegans*. *Free Radic. Biol. Med.* 35:558–65

Giovannoni S, Bibbs L, Cho J, Stapels M, Desiderio R, et al. 2005a. Proteorhodopsin in the ubiquitous marine bacterium SAR11. *Nature* 438:82–85

Giovannoni S, Tripp H, Givan S, Podar M, Vergin K, et al. 2005b. Genome streamlining in a cosmopolitan oceanic bacterium. *Science* 309:1242–45

Gonzales-Gil S, Keafer B, Jovine R, Anderson DM. 1998. Detection and quantification of alkaline phosphatase in single cells of phosphorus limited marine phytoplankton. *Mar. Ecol. Prog. Ser.* 164:21–35

Gregori G, Citterio S, Ghiani A, Labra M, Sgorbati S, et al. 2001. Resolution of viable and membrane-compromised bacteria in freshwater and marine waters based on analytical flow cytometry and nucleic acid double staining. *Appl. Environ. Microbiol.* 67:4662–70

Gundersen K, Heldal M, Norland S, Purdie DA, Knap AH. 2002. Elemental C, N, and P cell content of individual bacteria collected at the Bermuda Atlantic Time-series Study (BATS) site. *Limnol. Oceanogr.* 47:1525–30

Hampel M, Moreno-Garrido I, Sobrino C, Lubián LM, Blasco J. 2001. Acute toxicity of LAS homologues in marine microalgae: esterase activity and inhibition growth as endpoints of toxicity. *Ecotoxicol. Environ. Saf.* 48:287–92

Heldal M, Norland S, Tumyr O. 1985. X-ray microanalytic method for measurement of dry matter and elemental content of individual bacteria. *Appl. Environ. Microbiol.* 50:1251–57

Hutchinson G. 1961. The paradox of the plankton. *Am. Nat.* 95:137–45

Jacobsen C, Rasmussen J, Jakobsen M. 1997. Viability staining and flow cytometric detection of *Listeria* monocytogenes. *J. Microbiol. Methods* 28:35–43

Jardillier L, Zubkov M, Pearman J, Scanlan D. 2010. Significant CO_2 fixation by small prymnesiophytes in the subtropical and tropical northeast Atlantic Ocean. *ISME J.* doi:10.1038/ismej.2010.36

Jin D, Connally R, Piper J. 2007. Practical time-gated luminescence flow cytometry. II. Experimental evaluation using UV LED excitation. *Cytometry A* 71A:797–808

Joachimsthal EL, Ivanov V, Tay SL, Tay JH. 2003. Flow cytometry and conventional enumeration of microorganisms in ships' ballast water and marine samples. *Mar. Pollut. Bull.* 46:308–13
Jochem F, Smith G, Gao Y, Zimmerman R, Cabello-Pasini A, et al. 2000. Cytometric quantification of nitrate reductase by immunolabeling in the marine diatom skeletonema costatum. *Cytometry A* 39:173–78
Johnson ZI, Zinser ER, Coe A, McNulty NP, Woodward EMS, Chisholm SW. 2006. Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. *Science* 311:1737–40
Jorio H, Tran R, Meghrous J, Bourget L, Kamen A. 2006. Analysis of baculovirus aggregates using flow cytometry. *J. Virol. Methods* 134:8–14
Jost G, Zubkov MV, Yakushev E, Labrenz M, Jurgens K. 2008. High abundance and dark CO_2 fixation of chemolithoautotrophic prokaryotes in anoxic waters of the Baltic Sea. *Limnol. Oceanogr.* 53:14–22
Kaprelyants AS, Kell DB. 1993. The use of 5-cyano-2,3-ditolyl tetrazolium chloride and flow cytometry for the visualisation of respiratory activity in individual cells of *Micrococcus luteus*. *J. Microbiol. Methods* 17:115–22
Keller MD, Shapiro LP, Haugen EM, Cucci TL, Sherr EB, Sherr BF. 1994. Phagotrophy of fluorescently labeled bacteria by an oceanic phytoplankter. *Microb. Ecol.* 28:39–52
Kiene R, Linn L, Bruton J. 2000. New and important roles for DMSP in marine microbial communities. *J. Sea Res.* 43:209–24
Larsen A, Castberg T, Sandaa R-A, Brussaard CPD, Egge JK, et al. 2001. Population dynamics and diversity of phytoplankton, bacteria and viruses in a seawater enclosure. *Mar. Ecol. Prog. Ser.* 221:47–57
Lebaron P, Servais P, Agogue H, Courties C, Joux F. 2001. Does the high nucleic acid content of individual bacterial cells allow us to discriminate between active cells and inactive cells in aquatic systems? *Appl. Environ. Microbiol.* 67:1775–82
Lebaron P, Servais P, Baudoux AC, Bourrain M, Courties C, Parthuisot N. 2002. Variations of bacterial-specific activity with cell size and nucleic acid content assessed with flow cytometry. *Aquat. Microb. Ecol.* 28:131–40
Leblanc K, Hutchins DA. 2005. New applications of a biogenic silica deposition fluorophore in the study of oceanic diatoms. *Limnol. Oceanogr.: Methods* 3:462–76
Legendre L, Courties C, Troussellier M. 2001. Flow cytometry in oceanography 1989–1999: environmental challenges and research trends. *Cytometry* 44:164–72
Levy JL, Angel BM, Stauber JL, Poon WL, Simpson SL, et al. 2008. Uptake and internalisation of copper by three marine microalgae: comparison of copper-sensitive and copper-tolerant species. *Aquat. Toxicol.* 89:82–93
Leyval D, Debay F, Engasser J-M, Goergen J-L. 1997. Flow cytometry for the intracellular pH measurement of glutamate producing *Corynebacterium glutamicum*. *J. Microbiol. Methods* 29:121–27
Li WKW. 1993. Estimation of primary production by flow cytometry. *ICES Mar. Sci. Symp.* 197:79–91
Li WKW. 1994. Primary production of prochlorophytes, cyanobacteria, and eukaryotic ultraphytoplankton: measurements from flow cytometric sorting. *Limnol. Oceanogr.* 39:169–75
Li WKW. 2009. From cytometry to macroecology: a quarter century quest in microbial oceanography. *Aquat. Microb. Ecol.* 57:239–57
Lipschultz F. 1995. Nitrogen specific uptake rates of marine phytoplankton isolated from natural populations of particles by flow cytometry. *Mar. Ecol. Prog. Ser.* 123:245–58
Longnecker K, Sherr BF, Sherr EB. 2005. Activity and phylogenetic diversity of bacterial cells with high and low nucleic acid content and electron transport system activity in an upwelling ecosystem. *Appl. Environ. Microbiol.* 71:7737–49
Malits A, Weinbauer MG. 2009. Effect of turbulence and viruses on prokaryotic cell size, production and diversity. *Aquat. Microb. Ecol.* 54:243–54
Malmstrom RR, Kiene RP, Vila M, Kirchman DL. 2005. Dimethylsulfoniopropionate (DMSP) assimilation by *Synechococcus* in the Gulf of Mexico and northwest Atlantic Ocean. *Limnol. Oceanogr.* 50:1924–31
Mann EL, Chisholm SW. 2000. Iron limits the cell division rate of *Prochlorococcus* in the eastern equatorial Pacific. *Limnol. Oceanogr.* 45:1067–76
Marie D, Brussaard CPD, Thyrhaug R, Bratbak G, Vaulow D. 1999. Enumeration of marine viruses in culture and natural samples by flow cytometry. *Appl. Environ. Microbiol.* 65:45–52
Martiny AC, Kathuria S, Berube P. 2009. Widespread metabolic potential for nitrite and nitrate assimilation among *Prochlorococcus* ecotypes. *Proc. Acad. Nat. Sci. Phila.* 106:10787–92

Mary I, Heywood JL, Fuchs BM, Amann R, Tarran G, et al. 2006. SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an Atlantic meridional transect. *Aquat. Microb. Ecol.* 45:107–13

Mary I, Tarran GA, Warwick PE, Terry MJ, Scanlan DJ, et al. 2008. Light enhanced amino acid uptake by dominant bacterioplankton groups in surface waters of the Atlantic Ocean. *FEMS Microbiol. Ecol.* 63:36–45

Massana R, del Campo J, Dinter C, Sommaruga R. 2007. Crash of a population of the marine heterotrophic flagellate *Cafeteria roenbergensis* by viral infection. *Environ. Microbiol.* 9:2660–69

Matrai P, Keller M. 1994. Total organic sulfur and dimethylsulfoniopropionate in marine phytoplankton: intracellular variations. *Mar. Biol.* 119:61–68

Medintz I, Uyeda H, Goldman E, Mattoussi H. 2005. Quantum dot bioconjugates for imaging, labeling and sensing. *Nat. Mater.* 4:438–46

Miao F, Todd P, Kompala D. 1993. A single cell assay for ß-galactosidase in recombinant *E. coli* using flow cytometry. *Biotechnol. Bioeng.* 42:708–15

Michelou VK. 2009. *Uptake of dissolved organic matter and phosphate by phototrophic and heterotrophic bacteria*. PhD thesis. Univ. of Delaware. 133 pp.

Michelou VK, Cottrell MT, Kirchman DL. 2007. Light-stimulated bacterial production and amino acid assimilation by cyanobacteria and other microbes in the North Atlantic Ocean. *Appl. Environ. Microbiol.* 73:5539–46

Minor EC, Eglinton TI, Olson R, Boon JJ. 1998. The compositional heterogeneity of particulate organic matter from the surface ocean: an investigation using flow cytometry and DT-MS. *Org. Geochem.* 29:1561–82

Mioni C, Handy S, Ellwood M, Twiss M, McKay R, et al. 2005. Tracking changes in bioavailable Fe within high-nitrate low-chlorophyll oceanic waters: a first estimate using a heterotrophic bacterial bioreporter. *Glob. Biogeochem. Cycles* 19:GB4S25

Mizumoto H, Tomaru Y, Takao Y, Shirai Y, Nagasaki K. 2008. Diverse responses of the bivalve-killing dinoflagellate *Heterocapsa circularisquama* to infection by a single-stranded RNA virus. *Appl. Environ. Microb.* 74:3105–11

Moore JK, Doney SC, Lindsay K. 2004. Upper ocean ecosystem dynamics and iron cycling in a global three-dimensional model. *Glob. Biogeochem. Cycles* 18:GB4028

Nedoma J, Strojsova A, Vrba J, Komarkova J, Simek K. 2003. Extracellular phosphatase activity of natural phytoplankton studied with ELF97 phosphate: fluorescence quantification and labeling kinetics. *Environ. Microbiol.* 5:462–72

Obst U, Holzapfel-Pschorn A, Wiegand-Rosinus M. 1988. Application of enzyme assays for toxicological water testing. *Toxic. Assess.* 3:81–91

Olson RJ, Frankel SL, Chisholm SW, Shapiro H. 1983. An inexpensive flow cytometer for analysis of fluorescence signals in phytoplankton: chlorophyll and DNA distributions. *J. Exp. Mar. Biol. Ecol.* 68:129–44

Olson RJ, Shalapyonok A, Sosik HM. 2003. An automated submersible flow cytometer for analyzing pico- and nanophytoplankton: FlowCytobot. *Deep-Sea Res.* I 50:301–15

Olson RJ, Sosik HM. 2007. A submersible imaging in-flow instrument to analyze nano- and microplankton: imaging FlowCytobot. *Limnol. Oceanogr.: Methods* 5:195–203

Olson RJ, Zettler ER. 1995. Potential of flow cytometry for "pump and probe" fluorescence measurements of phytoplankton photosynthetic characteristics. *Limnol. Oceanogr.* 40:816–20

Olson RJ, Zettler ER, DuRand MD. 1993. Phytoplankton analysis using flow cytometry. In *Aquatic Microbial Ecology*, ed. P Kemp, B Sherr, E Sherr, J Cole, pp. 175–86. Boca Raton, FL: Lewis

Orcutt K, Gundersen K, Wells ML, Poulton N, Sieracki ME, Smith GJ. 2008. Lighting up phytoplankton cells with quantum dots. *Limnol. Oceanogr.: Methods* 6:653–58

Orellana MV, Petersen TW, Diercks AH, Donohoe S, Verdugo P, van den Engh G. 2007. Marine microgels: optical and proteomic fingerprints. *Mar. Chem.* 105:229–39

Orellana MV, Petersen TW, van den Engh G. 2004. UV-excited blue autofluorescence of *Pseudo-nitzschia* multiseries (Bacillariophyceae). *J. Phycol.* 40:705–10

Pabi S, Arrigo K. 2006. Satellite estimation of marine particulate organic carbon in waters dominated by different phytoplankton taxa. *J. Geophys. Res.* 111:C09003

Partensky F, Hess W, Vaulot D. 1999. *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Microbiol. Mol. Biol. Rev.* 63:106–27

Paul J, Scholin C, Van Den Engh G, Perry MJ. 2007. In situ instrumentation. *Oceanography* 20: 70–78

Pernthaler J, Amann R. 2005. Fate of heterotrophic microbes in pelagic habitats: focus on populations. *Microbiol. Mol. Biol. Rev.* 69:440–61

Rioboo C, O'Connor JE, Prado R, Herrero C, Cid A. 2009. Cell proliferation alterations in *Chlorella* cells under stress conditions. *Aquat. Toxicol.* 94:229–37

Rivers AR, Jakuba RW, Webb EA. 2009. Iron stress genes in marine *Synechococcus* and the development of a flow cytometric iron stress assay. *Environ. Microbiol.* 11:382–96

Rivkin RB, Phinney DA, Yentsch CM. 1986. Effects of flow cytometric analysis and cell sorting on photosynthetic carbon uptake by phytoplankton in cultures and from natural populations. *Appl. Environ. Microbiol.* 52:935–38

Robertson BR, Button DK, Koch A. 1998. Determination of the biomasses of small bacteria at low concentrations in a mixture of species with forward light scatter measurements by flow cytometry. *Appl. Environ. Microb.* 64:3900–9

Satoh M, Karaki E, Kakehashi M, Okazaki E, Gotoh T, Oyama Y. 1999. Heavy-metal induced chages in nonproteinaceous thiol levels and heavy-metal binding peptide in *Tetraselmis tetrathele* (Prasinophyceae). *J. Phycol.* 35:989–94

Schapira M, Buscot M-J, Leterme SC, Pollet T, Chapperon C, Seuront L. 2009. Distribution of heterotrophic bacteria and virus-like particles along a salinity gradient in a hypersaline coastal lagoon. *Aquat. Microb. Ecol.* 54:171–83

Scharek R, Latasa M. 2007. Growth, grazing and carbon flux of high and low nucleic acid bacteria differ in surface and deep chlorophyll maximum layers in the NW Mediterranean Sea. *Aquat. Microb. Ecol.* 46:153–61

Sekar R, Fuchs RM, Amann R, Pernthaler J. 2004. Flow sorting of marine bacterioplankton after fluorescence in situ hybridization. *Appl. Eniviron. Microb.* 70:6210–19

Servais P, Casamayor EO, Courties C, Catala P, Parthuisot N, Lebaron P. 2003. Activity and diversity of bacterial cells with high and low nucleic acid content. *Aquat. Microb. Ecol.* 33:41–51

Shapiro H. 2003. *Practical Flow Cytometry*. Hoboken, New Jersey: Wiley. 529 pp. 4th ed.

Sherr BF, del Giorgio P, Sherr EB. 1999. Estimating abundance and single-cell characteristics of respiring bacteria via the redox dye CTC. *Aquat. Microb. Ecol.* 18:117–31

Sherr E, Sherr B. 1994. Bacterivory and herbivory: key roles of phagotrophic protistis in pelagic food webs. *Microb. Ecol.* 28:223–35

Sherr E, Sherr B, Verity P. 2002. Distribution and relation of total bacteria, active bacteria, bacterivory, and volume of organic detritus in Atlantic continental shelf waters off Cape Hatteras NC, USA. *Deep Sea Res. II* 49:4571–85

Shimizu K, Del Amo Y, Brzezinski M, Stucky G, Morse D. 2001. A novel fluorescent silica tracer for biological silicification studies. *Chem. Biol.* 8:1051–60

Sieracki C, Sieracki M, Yentsch C. 1998. An imaging-in-flow system for automated analysis of marine microplankton. *Mar. Ecol. Prog. Ser.* 168:285–96

Silver M, Davoll P. 1978. Loss of ^{14}C activity after chemical fixation of phytoplankton: error source for autoradiography and other productivity measurements. *Limnol. Oceanogr.* 23:362–68

Sosik HM, Chisholm SW, Olson R. 1989. Chlorophyll fluorescence from single cells: interpretation of flow cytometric signals. *Limnol. Oceanogr.* 34:1749–61

Sosik HM, Olson RJ, Neubert MG, Shalapyonok A, Solow AR. 2003. Growth rates of coastal phytoplankton from time-series measurements with a submersible flow cytometer. *Limnol. Oceangor.* 48:1756–65

Stauber JL, Davies CM. 2000. Use and limitations of microbial bioassays for assessing copper bioavailability in the aquatic environment. *Environ. Rev.* 8:255–301

Stauber JL, Franklin NM, Adams MS. 2002. Applications of flow cytometry to ecotoxicity testing using microalgae. *Trends Biotechnol.* 20:141–43

Stepanauskas R, Sieracki ME. 2007. Matching phylogeny and metabolism in the uncultured marine bacteria, one cell at a time. *Proc. Natl Acad. Sci. USA* 104:9052–57

Sullivan M, Krastins B, Hughes J, Kelly L, Chase M, et al. 2009. The genome and structural proteome of an ocean siphovirus: a new window into the cyanobacterial 'mobilome'. *Environ. Microbiol.* 11:2935–51
Suttle CA. 2005. Viruses in the sea. *Nature* 437:356–61
Swalwell J, Petersen T, van den Engh G. 2009. Virtual-core flow cytometry. *Cytometry A* 75A:960–65
Telford W, Cox W, Singer V. 2001. Detection of endogenous and antibody-conjugated alkaline phosphatase with ELF-97 phosphate in multicolor flow cytometry applications. *Cytometry* 43:117–25
Tomaru Y, Nagasaki K. 2007. Flow cytometric detection and enumeration of DNA and RNA viruses infecting marine eukaryotic microalgae. *J. Oceanogr.* 63:215–21
Trask B, Van Den Engh G, Elgershuizen J. 1982. Analysis of phytoplankton by flow cytometry. *Cytometry A* 2:258–64
Urbach E, Vergin KL, Geovannoni SJ. 1999. Immuniochemical detection and isolation of DNA from metabolically active bacteria. *Appl. Environ. Microb.* 65:1207–13
Veldhuis MJW, Cucci TL, Sieracki ME. 1997. Cellular DNA content of marine phytoplankton using two new fluorochromes: taxonomic and ecological implications. *J. Phycol.* 33:527–41
Veldhuis MJW, Kraay GW. 2000. Application of flow cytometry in marine phytoplankton research: current applications and future perspectives. *Sci. Marina* 64:121–34
Vila-Costa M, Simo R, Harada H, Gasol J, Slezak D, Kiene RP. 2006. Dimethlysulfoniopropionate uptake by marine phytoplankton. *Science* 314:652–54
Von Dassow P, Ogata H, Pobert I, Wincker P, Da Silva C, et al. 2009. Transcriptome analysis of functional differentiation between haploid and diploid cells of *Emiliania huxleyi*, a globally significant photosynthetic calcifying cell. *Genome Biol.* 10:R114
Waite AM, Olson RJ, Dan HG, Passow U. 1995. Sugar-containing compounds on the cell surfaces of marine diatoms measured using concanavalin A and flow cytometry. *J. Phycol.* 31:925–33
Waite TD. 2002. Rationale for ballast water treatment standards to minimize translocation of unwanted species. *Mar. Technol. Soc. J.* 3:2–29
Wommack KE, Colwell RR. 2000. Virioplankton: viruses in aquatic ecosystems. *Microbiol. Mol. Biol. Rev.* 64:69–114
Worden AZ, Lee J-H, Mock T, Rouzé P, Simmons M, et al. 2009. Green evolution and dynamic adaptations revealed by genomes of the marine picoeukaryotes *Micromonas*. *Science* 324:268–72
Yentsch CM, Campbell JW. 1991. Phytoplankton growth: perspectives gained by flow cytometry. *J. Plankton Res.* 13:83–108
Yentsch CM, Horan P, Muirhead K, Dortch Q, Haugen EM, et al. 1983. Flow cytometry and cell sorting: a technique for analysis and sorting of aquatic particles. *Limnol. Oceanogr.* 28:1275–80
Yooseph S, Sutton G, Rusch D, Halpern A, Williamson S, et al. 2007. The *Sorcerer II* Global Ocean Sampling expedition: expanding the universe of protein families. *PLoS Biol.* 5:432–66
Yu Y, Kong FX, Wang ML, Qian LL, Shi XL. 2007. Determination of short-term copper toxicity in a multispecies microalgal population using flow cytometry. *Ecotoxicol. Environ. Safety* 66:49–56
Zehr J, Bench S, Carter B, Hewson I, Niazi F, et al. 2008. Globally distributed uncultivated oceanic N_2-fixing cyanobacteria lack oxygenic photosystem II. *Science* 322:1110–12
Zubkov MV, Fuchs BM, Archer D, Kiene RP, Amann R, Burkhill P. 2002. Rapid turnover of dissolved DMS and DMSP by defined bacterioplankton communities in the stratified euphotic zone of the North Sea. *Deep-Sea Res.* II 49:3017–38
Zubkov MV, Fuchs BM, Burkill P, Amann R. 2001. Comparison of cellular and biomass specific activities of dominant bacterioplankton groups in stratified waters of the Celtic Sea. *Appl. Environ. Microbiol.* 67:5210–18
Zubkov MV, Fuchs BM, Eilers H, Burkill PH, Amann R. 1999. Determination of total protein content of bacterial cells by SYPRO staining and flow cytometry. *Appl. Environ. Microbiol.* 65:3251–57
Zubkov MV, Fuchs BM, Tarran GA, Burkill PH, Amann R. 2003. High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. *Appl. Environ. Microbiol.* 69:1299–304
Zubkov MV, Mary I, Woodward EMS, Warwick PE, Fuchs BM, et al. 2007. Microbial control of phosphate in the nutrient-depleted North Atlantic subtropical gyre. *Environ. Microbiol.* 9:2079–89

Zubkov MV, Tarran GA. 2005. Amino acid uptake of *Prochlorococcus* spp. in surface waters across the South Atlantic Subtropical Front. *Aquat. Microb. Ecol.* 40:241–49

Zubkov MV, Tarran GA. 2008. High bacterivory by the smallest phytoplankton in the North Atlantic Ocean. *Nature* 455:224–26

Zubkov MV, Tarran GA, Burkill PH. 2006. Bacterioplankton of low and high DNA content in the suboxic waters of the Arabian Sea and the Gulf of Oman: abundance and amino acid uptake. *Aquat. Microb. Ecol.* 43:23–32

Zubkov MV, Tarran GA, Fuchs B. 2004. Depth related amino acid uptake by *Prochlorococcus* cyanobacteria in the Southern Atlantic tropical gyre. *FEMS Microbiol. Ecol.* 20:153–61

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Errata

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