Cytometry 44:164–172 (2001)

Original Articles

Flow Cytometry in Oceanography 1989–1999: Environmental Challenges and Research Trends

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Received 7 November 2000; Revision Received 25 April 2001; Accepted 29 April 2001

Background: The present review is based on the identification of four major environmental crises that have been approached from a biological oceanographic viewpoint. These crises are the release of contaminants in nearshore marine waters, the collapse of marine resources that were renewable until recently, the loss of biodiversity, and global climate change
Methods: The review examines the contribution of cytometry-based biological oceanography to the resolution of the four environmental crises. Using a database of 302 papers, flow cytometric (FCM) studies in biological oceanography over the 1989–1999 decade are examined. Future biological oceanographic applications of FCM are discussed.
Results: Most of the published FCM oceanographic studies focus on phytoplankton and bacterioplankton. Analysis of our 1989–1999 database shows the predominance of studies dedicated to phytoplankton (77%), followed by heterotrophic bacteria (21%). The latter progressively increased over the last decade, together with the improved understanding of the biogeochemical and trophic roles of marine bacteria. Most studies on these two microorganisms were conducted in vitro until 1996, after which the trend reversed in favor of in situ research. The most investigated areas were those with major international sampling efforts, related to the changing climate. Concerning environmental topics, 62% of papers on phytoplankton and bacterioplankton focused on the structure of microbial communities and fluxes (e.g., production, grazing); this provides the basis for biological oceanographic studies on resources and climate change.
Conclusions: Future progress in the biological oceanographic use of FCM will likely fall into two categories, i.e., applications where FCM will be combined with the development of other methods and those where FCM will be the main analytical tool. It is expected that FCM and other cytometric approaches will improve the ability of biological oceanography to address the major environmental challenges that are confronting human societies. Cytometry 44: 164–172, 2001. © 2001 Wiley-Liss, Inc.

Key terms: biological oceanography; environmental research; flow cytometry; plankton

Since the overview of flow cytometry (FCM) in biological oceanography published by Legendre and Yentsch in 1989 (1), the environment of planet Earth and the context of environmental research have both changed quite drastically. At the end of the 1980s, biological oceanographers had started to use FCM to address fundamental ecological questions such as the abundance, distribution, and fate of microscopic algae (phytoplankton) in oceans. During the following decade, there were major advances in our understanding of the structure and functioning of marine pelagic ecosystems but, simultaneously, several global environmental crises developed. As a consequence, biological oceanography is becoming progressively involved in programs that address some of the major socioecological problems that threaten human societies. These include the release of contaminants in nearshore marine waters, the collapse of marine resources that were renewable until recently, the loss of biodiversity, and global climate change.

This shift in focus occurred not only in biological oceanography, but in all environmental sciences. The new approach requires both in-depth disciplinary studies and

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multidisciplinary international programs, because the existing disciplinary knowledge is often too narrow to answer the broad, system-level questions that confront researchers. The new context requires broader and deeper studies than before, to which FCM-based biological oceanography can contribute original results.

FCM has been used to study the cell characteristics of large marine organisms such as sponges (2), kelps (3), macroalgae (4), oysters (5), sea squirts (6), periwinkles (7), sea bass (8), and mink and sea otters (9). The present review focuses on the use of FCM to study microorganisms. These are defined here as <200 μm equivalent spherical diameter (ESD). In the water column of oceans, organisms <200 μm include a variety of taxa: free viruses (<0.1 μm), autotrophic bacteria (cyanobacteria, which include the group known formerly as prochlorophytes, <1.0 μm), heterotrophic bacteria (<1.0 μm), unicellular algae (>1.0 μm), protozoa (flagellates and ciliates, >2 μm), and small metazoans (generally >20 μm). The water-column organisms 0.02–0.2 μm, 0.2–2.0 μm, 2–20 μm, and 20–200 μm are called femtoplankton, picoplankton, nanoplankton, and microplankton, respectively.

FCM has been used to study most types of marine microorganisms. Review papers on methodological applications of FCM to aquatic microorganisms were published recently (10–13). The smallest organisms detected to date have been free viruses (14), followed by heterotrophic bacteria (15) and, more generally, prokaryotic and eukaryotic picoplankton (16–18), autotrophic nanoplankton and microplankton (19), and heterotrophic nanoplankton and microplankton (20).

For routine studies, FCM is mostly used on cells 0.3 to 30–40 μm ESD. Even though instruments able to analyze cells and chains of cells >100 μm have been developed (21–25), most studies conducted offshore with commercially available flow cytometers have focused on autotrophic and heterotrophic picoplankton. This is because these organisms are abundant in the sea (typically 10^4 to 10^7 cells ml^{-1}) and are difficult (because of weak fluorescence) or almost impossible (e.g., fragile *Prochlorococcus* cyanobacteria) to observe with conventional microscopy. Another reason is that the larger cells are generally not abundant offshore.

We discuss some of the main environmental challenges encountered in biological oceanography and give examples of related FCM contributions. We also analyze the trends of FCM applications in biological oceanography, using a literature data base of 302 papers (1989–1999) that we assembled for this study.

ENVIRONMENTAL CHALLENGES: CONTRIBUTIONS OF CYTOMETRY-BASED BIOLOGICAL OCEANOGRAPHY

Biological oceanographers are involved in studies that concern at least four major environmental crises. These are briefly reviewed in the following paragraphs, as are the biological oceanographic approaches that involve FCM.

Contaminants

Human activities affect the marine coastal environment in several ways that include the release of chemical and biological contaminants in nearshore waters. Chemical contaminants may be either toxic or nontoxic. The fate of toxic contaminants in the marine environment, and their effects on the organisms that live in water and those that feed upon them (including people), is a public health concern. Nontoxic contaminants, such as inorganic and organic nutrients (coastal eutrophication; from municipal effluents or fertilized fields), often lead to the development of nearshore phytoplankton blooms. These may decrease environmental quality (when the algae decompose in shallow waters and cause oxygen depletion) or compromise public health (when the blooms include microscopic toxic algae that contaminate exploited resources). The dispersal of biological contaminants in the coastal environment, such as pathogenic viruses and bacteria, often create health hazards.

The development and use of fluorescent probes allow researchers to determine the presence of various marine organisms and/or to assess changes in their physiological status. These changes include the responses of pathogenic organisms as they disperse in the marine environment (26) and those of autochthonous organisms exposed to chemical contaminants [e.g., diatoms (27), sole hepatocytes (28), mussel hemocytes (29)] or subjected to increased ultraviolet (UV) radiation (30). Similarly, the effects of coastal eutrophication on phytoplankton were studied using FCM (31). Finally, immunofluorescence approaches have been combined with FCM to monitor the presence of toxic phytoplankton species, as well as to enumerate them (32–34).

Biodiversity

An additional effect of human activities is the loss of species (biodiversity). This is caused by the destruction of environments that are essential to their survival and by the destruction of the species themselves (e.g., by hunting and fishing). The early diversification of life occurred in the ocean. Therefore, the oceans contain more phyla than the terrestrial environment (using the animals as example, there are 23 phyla in oceans, 13 in fresh waters, and 8 on the continents). The continents offer a broader range of physical and chemical conditions than the oceans. Consequently, the phyla that adapted to land life have much more species than those in the sea. The decreasing biodiversity on continents affects mostly species or genera. The loss of biodiversity in oceans threatens higher taxonomic levels including low-diversified phyla (e.g., 7 of the 10 animal phyla represented by one or two classes are found exclusively in the oceans: Ctenophora, Placozoa, Mesozoa, Gnathostomulida, Kamptozoa, Sipuncula, and Chaetognatha).

Much less is known about marine biodiversity than about terrestrial biodiversity. One reason for this may be that marine organisms are generally small. The terrestrial biomass is dominated by slow-growing, long-lived, large

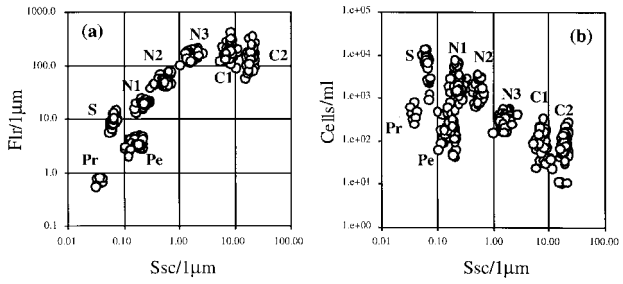

FIG. 1. Ataxonomic composition of a natural phytoplankton community from the Northwestern Mediterranean Sea, near the mouth of the Rhone River, April 1988. Each group of cells is characterized by **a:** its red fluorescence (proportional to the intracellular concentration of chlorophyll *a*) or **b:** the concentration of cells and its side scatter (related to cell size). The red fluorescence and side scatter are normalized to 1-μm beads. There are eight groups of cells: cyanobacteria belonging to the genera *Prochlorococcus* (Pr) and *Synechococcus* (S), picoeukaryotes (Pe), three groups of increasingly larger eukaryotes (N1, N2, and N3), and two groups of cryptophytes (C1 and C2). Cytometric data were obtained with a FACSCalibur cytometer (Becton-Dickinson, San Jose, CA).

plants that turn over between twice a year and once every second year. In contrast, the marine biomass is dominated by fast-growing, short-lived, picoplankton organisms that turn over about once per day. Although autotrophic and heterotrophic picoplankton account for much of the living carbon in oceans, their taxonomic description progresses slowly. Because the diversity of marine life is not well known and because environmental changes are more difficult to assess in the sea than on land, the future prospects of marine biodiversity have been little studied.

Studies on marine biodiversity include the discovery of new taxa, the identification of taxonomic groups, and the enumeration of organisms. Most discoveries of new marine pelagic taxa in the last decades concerned microorganisms. Cytometric approaches were often involved in finding these new organisms and assessing their abundances and distributions in oceans. These taxa include free viruses (0.1 μm ESD; 35,36), small cyanobacteria belonging to the genus *Prochlorococcus* (0.6–0.7 μm ESD; 17), slightly larger single-celled cyanobacteria (1 μm ESD; 37,38), and a bacterial-sized photosynthetic eukaryote (1 μm ESD; 18).

More than 95% of all algal taxa described during the last two decades come from the marine environment (39). The discovery of *Prochlorococcus* a decade ago (17) transformed drastically our understanding of the oceanic (offshore) environment. The more recent data on picoeukaryotes in oceanic and coastal waters revealed a rich reservoir of unknown species, as shown by the description of new genera, orders, and classes of autotrophic and heterotrophic picoeukaryotes (40–45) .

Figure 1 provides an example from the Northwestern Mediterranean Sea, near the mouth of the Rhone River. In contrast to the analysis of offshore waters, it is easy to discriminate among several size classes and taxonomic categories of phytoplankton (e.g., cyanobacteria, picoeukaryotes, nanoplankton, and cryptophytes) in routine analyses of coastal samples. In Figure 1a, each discriminated phytoplankton population is characterized by its mean red fluorescence (proportional to the intracellular concentration of chlorophyll *a*) and its mean side scatter (related to cell size), both normalized to internal standards (1-μm beads). Cells that are smaller than 2 μm are represented by two groups of prokaryotes (*Prochlorococcus*, Pr, and *Synechococcus*, S) and picoeucaryotes (Pe); there also are three groups of increasingly larger eukaryotes (N1, N2, and N3) and two groups of cryptophytes (C1 and C2; discriminated on the basis of their green fluorescence, not shown). In Figure 1b, the same assemblage is characterized by the concentration of cells (ordinate): overall, the abundance increases with decreasing size, but *Prochlorococcus* is represented by lower numbers than observed in oligotrophic oceanic waters (0.5–7 10^5 cells ml^{-1}), as usual in both the Western Mediterranean and coastal waters. Information from different stations or times can be used to assess the responses of phytoplankton to environmental changes.

The combination of immunological and nucleic acid probes with FCM helped to identify and enumerate these small cells. It also enabled understanding of their roles in marine waters (46–52).

Collapse of Marine Resources

Almost every month, newspapers report the collapse of marine stocks that were once sustainable. One of the fundamental reasons for the failure of traditional fisheries management is the lack of proper consideration of the physical and biological environments of exploited stocks (53). In many countries, there has been a shift from the traditional management approach to a new discipline, called fisheries oceanography. With this approach, the environmental conditions that influence the survival of young stages are considered to be the main determinant of exploited stocks (54).

Essential data on the biological environment of exploited stocks are provided by studies on the structure of marine pelagic ecosystems. Pelagic ecosystems are dominated by either the herbivorous food web or the microbial food web or loop (the two expressions are often used as synonyms in the literature). In the herbivorous food web, large phytoplankton (>5 μm) are efficiently grazed by large zooplankton (e.g., copepods), which in turn become the food of fish. In the microbial food web or loop, small phytoplankton and heterotrophic bacteria are consumed by small zooplankton, resulting in high respiration and little export to the large metazoans. Legendre and Rassoulzadegan (55) proposed to assess the trophic pathway that dominates a pelagic ecosystem (and, therefore, its potential in supporting renewable resources) by determining six interconnected ecological ratios: concentration of NH_4 to that of NO_3, phytoplankton uptake of NH_4 to that of NO_3, production of small to that of large phytoplankton, microbial to herbivorous grazing, bacterial utilization of dissolved organic carbon (DOC) to that of dissolved organic nitrogen (DON), and bacterial uptake to release of NH_4. The six ratios were defined to be minimum for the microbial loop and maximum for the herbivorous food

web. No single ratio provides unambiguous information on the dominant trophic pathway, but a combination of several ratios can identify the pathway that dominates a pelagic ecosystem.

Cytometry-based approaches can contribute to estimating some of the above ratios. FCM can be used to count and determine the sizes of microorganisms, including phytoplankton (56–58). It is also possible to use FCM for determining variables related to the production of phytoplankton (59–63) or bacteria (64). In addition, cell sorting was used to determine size-specific production of phytoplankton (65) or bacteria (66). Finally, the grazing rates of various microorganisms can also be determined using FCM (67–71). Hence, FCM provides approaches to facilitate the assessment of the trophic pathway that dominates the water column.

Climate Change

The oceans contain about 50 times as much CO_2 as the atmosphere, so that small changes in the ocean carbon cycle can have large effects on the atmospheric concentration of greenhouse-gas CO_2. The photosynthetic and calcifying activities of the marine biota mediate most of the transfer of carbon from the ocean surface to its depth (72), where it is stored for long periods (i.e., from centuries, as CO_2dissolved in deep waters, to millions of years, as biogenic carbon, BC, buried in sediments). Because phytoplankton photosynthesis (PP) does not increase in response to increased concentrations of dissolved CO_2, the marine biota do not sequester the additional CO_2resulting from human activities (called anthropogenic CO_2). In oceans, the sequestering of anthropogenic CO_2occurs by its dissolution in surface waters. These sink or are mixed vertically to depth (models indicate that oceans take up at least one third of the anthropogenic CO_2; 73). Future models concerning the fate of anthropogenic CO_2 must take into account biological processes because biological feedbacks have the potential to either offset or amplify chemical and physical effects, and thus to influence future climate (74). One such feedback is the production of dimethylsulfide (DMS) by the marine biota. Its release into the atmosphere favors the formation of clouds, which in turn affect atmospheric temperatures (75).

The exchanges of several climate-related gases between the lower atmosphere and the surface ocean are constrained by the functioning of the marine pelagic food web. This is because the pelagic biota determines the fluxes of matter into, within, and out of the upper ocean. The most important greenhouse gas, CO_2, is used as example. The local balance between PP and respiration by the pelagic community (CR) sets the direction of the physiologically controlled exchange of CO_2between the atmosphere and the ocean, i.e., PP $>$ CR drives a CO_2flux into the ocean (net autotrophy), whereas CR $>$ PP creates an efflux (net heterotrophy). When PP $>$ CR, there is downward export (E) of BC from the surface ocean (E = PP − CR), which may lead to BC sequestration at depth. Because heterotrophic bacteria are responsible for much of the CR in the water column, it follows that bacterial respiration (BR) is often almost equivalent to CR.

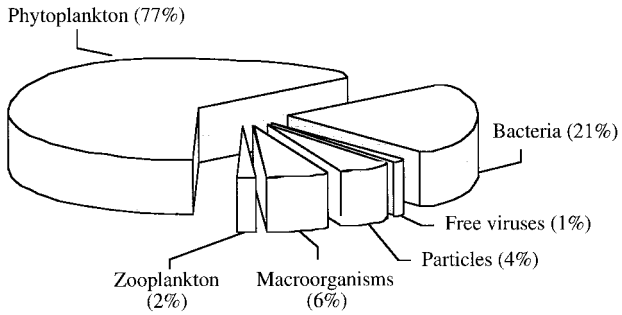

FIG. 2. FCM-based marine papers published between 1989 and 1999. Distribution among the main groups of marine organisms.

Cytometry-based approaches can be used in the above context to determine phytoplankton production. FCM thus contributes to the assessment of carbon fluxes of into, within, and out of the upper ocean.

Another effect of human activity that is publicized widely is the reduction of stratospheric ozone, especially over the polar regions. This has led to increased ultraviolet radiation at ground level. The higher UV radiation may have negative effects on terrestrial and marine biota. FCM approaches were used to assess the effects of UV radiation on the physiology of marine microorganisms (76–78) and on the structure of marine microbial communities (30,79). FCM has not been used to study the effects of UV on cell cycles at sea.

FLOW CYTOMETRY IN BIOLOGICAL OCEANOGRAPHY: 1989–1999 TRENDS

In order to assess numerically the main trends of FCM studies in biological oceanography that occurred since the review of Legendre and Yentsch (1), we assembled a data base of 302 papers dealing with FCM studies of marine organisms (80). References concerning the use of FCM in fresh waters or of image cytometry were not included. The data base is analyzed from different angles, including the four environmental problems discussed above.

Figure 2 illustrates the distribution of FCM-based papers published during the last decade. Phytoplankton were the main target of FCM studies (77%), followed by heterotrophic bacteria (21%). The other categories of organisms each accounted for less than 6%. There has been a steady increase in the total number of papers published and the proportion of studies devoted to heterotrophic bacteria (Fig. 3). The latter reflects methodological developments and the progressive understanding of the role of heterotrophic bacteria in marine biogeochemical cycles.

Table 1 provides the distributions (percentages and total numbers) of in situ and in vitro studies of phytoplankton and heterotrophic bacteria and lists papers according to focus. The first four topics (environment, biodiversity, structure, and flux) correspond to the four environmental problems (contaminants, biodiversity, re-

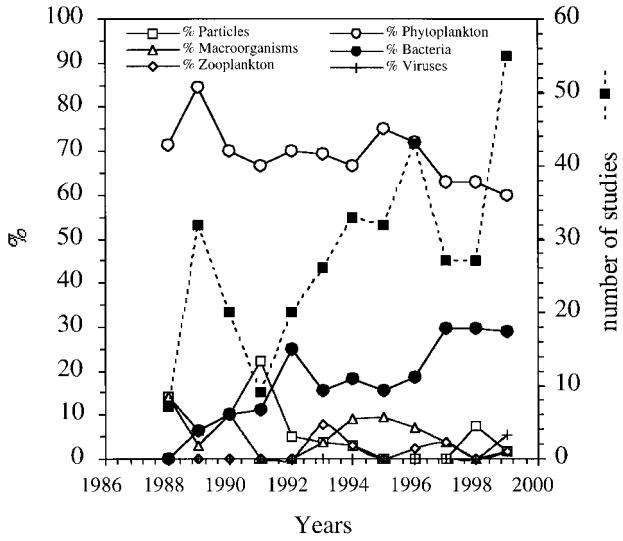

FIG. 3. FCM-based marine papers published between 1989 and 1999. Total number of papers (dashed line) and temporal changes in distribution among the main groups of marine organisms.

sources, and climate), respectively. For the two groups of organisms, studies with an environmental or biodiversity focus were mostly conducted in vitro. In the first case, toxicity tests and experimental studies on the biological effects of environmental variables are done under controlled conditions. The second case is somewhat more surprising, i.e., how could microbial biodiversity be studied in vitro? The explanation is that most studies with a biodiversity focus have been of methodological nature, their application to the field being for the future. Papers on the structure of microbial communities and fluxes (e.g., production and grazing) are evenly distributed between the laboratory and the field. As expected, most of the studies with methodological and physiological focuses were conducted in vitro.

The overall proportion of in vitro versus in situ studies on phytoplankton and heterotrophic bacteria has changed (data not shown). There were more in vitro than in situ papers until 1996, after which the trend reversed. Recent field studies on bacteria (1997–1999) were conducted mostly in coastal waters (10 of 16), as a response to coastal eutrophication (see above). For phytoplankton, there was a drastic change over the same period, e.g., from 14 of 31 (1996) to 27 of 34 (1999). A significant proportion of recent FCM field studies on phytoplankton were conducted in the Equatorial Pacific (13 of 27 studies), where there was a major international sampling effort related to the changing global climate (see above).

Among the 302 FCM-based studies published between 1989 and 1999, 130 were conducted at sea. Of these, 46 were in the North Atlantic Ocean, 36 in the Pacific Ocean, 20 in the Mediterranean Sea, 14 in the Indian Ocean and only 7 in sub-polar and polar waters. The studies in the North Atlantic, Pacific, and Indian Oceans were conducted within the framework of the international Joint Global Ocean Flux Study (JGOFS).

Phytoplankton and heterotrophic bacteria are the two main targets of FCM-based studies. Overall, 62% of the papers on phytoplankton and bacteria published between 1989 and 1999 focused on community structure and fluxes (Table 1).

In Table 1, studies on the structure of phytoplankton communities concerned the abundance (number of cells) of different taxa. Few studies addressed the use of FCM variables to obtain cell size (e.g., light scatter; 81), so that phytoplankton biomass is obtained by multiplication of cell numbers by a conversion factor. The phytoplankton taxa most studied belonged to prokaryotic picophytoplankton, more specifically, the cyanobacteria *Synechococcus* and *Prochlorococcus*. The number of papers published on picophytoplankton has increased steadily. In 1999, almost 80% of papers on the structure and fluxes of phytoplankton communities were devoted to these organisms (Fig. 4). Of the 77 studies on phytoplankton fluxes, 31 concerned the estimation of production and growth, 27 the estimation of grazing by various organisms, and 22 the cell cycles. In these flux studies, FCM is either an essential tool (cell cycles) or an alternative or complementary technique to the more usual approaches (growth production, grazing).

Table 1
*FCM-Based Papers on Marine Phytoplankton and Heterotrophic Bacteria Published Between 1989 and 1999**

	Phytoplankton			Heterotrophic bacteria		
Focus	In vitro	In situ	Total	In vitro	In situ	Total
Environment	83%	17%	24	100%	0%	12%
Biodiversity	83%	17%	23	—	—	0%
Structure	50%	50%	50	41%	59%	22%
Fluxes	57%	43%	77	50%	50%	16%
Methodology	86%	14%	58	79%	21%	14%
Physiology	77%	23%	31	94%	6%	17%
Total no.	182	81	263	56	25	81%

*Percentages and total numbers of in situ and in vitro studies, according to the focus of the paper. It must be noted that the total number of studies in the Table (344) is higher than the total number of papers published (302), because some papers address both phytoplankton and bacteria or different focus.

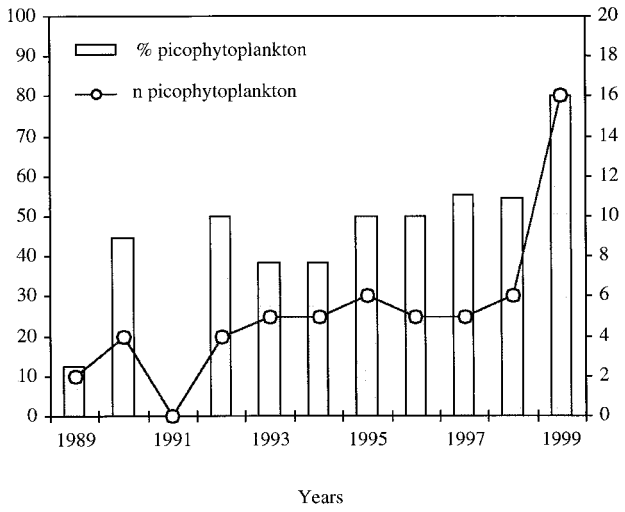

FIG. 4. FCM-based papers on marine picophytoplankton published between 1989 and 1999. Numbers and percentages of all studies on phytoplankton.

Studies on the structure of bacterial communities were often conducted simultaneously for phytoplankton (7 of 22 studies) . Most of the papers dealt with the total number of bacteria, but a few studies discriminated among bacterial populations on the basis of light scatter and concentration of nucleic acids (fluorescent probes; 82–86). This approach does not resolve taxa, but it identifies ecologically functional populations. Some FCM variables were also used to estimate components of bacterial fluxes, e.g., the proportion of metabolically active cells (5 of 16 studies) and predation on bacteria (6 of 16 studies).

There were no bacterial papers on field biodiversity and there were more bacterial than phytoplankton studies on environmental quality and physiology. For the latter two topics, most of the references dealing with heterotrophic bacteria concerned the survival of continental bacteria in marine waters. FCM provides a very efficient tool in this respect, being the survival of pathogenic bacteria in coastal waters is a major public health concern.

CONCLUSIONS: FUTURE CONTRIBUTIONS OF CYTOMETRY TO BIOLOGICAL OCEANOGRAPHY

Future progress in the biological oceanographic use of FCM could fall into two categories, i.e., applications where FCM will be combined with the development of other methods and those where FCM will be the main analytical tool.

Concerning the first category of possible applications (combination of FCM with the development of other methods), the main advantage of FCM is its ability to determine simultaneously and rapidly several cell properties on a large number of cells. For the environmental aspect, one major development would be the detection of organisms that are rare in natural assemblages, but are important because of their effects on public health, e.g., pathogenic bacteria (continental origin) and toxic phytoplankton (coastal eutrophication may increase the occurrence of toxic algae). In that context, an alternative to FCM may be scanner cytometry, which was applied to the parasitic protist, *Cryptosporidium*, by Anguish and Ghiorse (87) and Reynolds et al. (88), but could be used with other microorganisms. For the biodiversity aspect, the development of taxonomic probes (51,52,89,90) should allow FCM analysis of natural microbial assemblages, perhaps in combination with scanner cytometry (rare species), even if the approach still has some problems (13,91).

The second category of possible applications (FCM as the main analytical tool) covers at least four topics, i.e., the monitoring of phytoplankton, the study of cell cycles, marine food web and BC fluxes, and the sorting of natural organisms.

Oceanography is moving fast toward the instrumented observation of the ocean. Sensors mounted on moored buoys or drifting floats already record data on a number of variables, which are transmitted by satellite to land stations. The basic oceanographic data so monitored include water temperature, salinity, and in vivo fluorescence (as an index of in situ chlorophyll *a* concentration, and therefore of phytoplankton photosynthetic biomass). Other variables acquired routinely include horizontal and vertical current velocities, light-beam transmission (as an index of particle concentration), pH, dissolved O_2, and dissolved inorganic nutrients. The next step in global ocean observation is the Argo Network, which consists of the deployment of 3,000 floats in all oceans. The floats will drift at a typical depth of 2,000 m and rise to the surface every 10 days. As they rise, they will record the temperature and salinity of the water column. At the surface, each float will radio its data and position to an orbiting satellite, before returning to depth for another 10-day cycle. The coming years and decades will see the increased deployment of automatic buoys and floats and the development of oceanographic sensors. The latter could allow the in situ cytometric determination of phytoplankton abundance, e.g., the in situ flow cytometer for automatic and autonomous operation (CytoBuoy; 92).

FCM provides a unique tool for studying cell cycles at sea. Progress will likely be in two directions. First, the information on cell cycles will be used to estimate growth rates (59–63) for taxa with distinct cytometric signatures (e.g., *Prochlorococcus marinus*) and for well-defined cell cycles. Second, changes in cell cycles will be used to assess detrimental environmental effects caused by chemicals (e.g., for cells of metazoans, 9; no application yet to phytoplankton) or UV radiation (79).

Cytometric and imaging systems could be combined to assess the overall effects of marine food webs on BC fluxes in oceans. Following Legendre and Le Fèvre (93) and Legendre and Michaud (94), the potential effect of pelagic organisms on BC fluxes can be assessed from their sizes and those of their prey items. This approach requires knowledge of the numbers and sizes of pelagic organisms in a given environment and of their taxonomic categories in order to determine (from the literature) the sizes of

their prey items. Cytometry could be used for the smallest organisms (e.g., 0.5–20 μm) and it could be combined with other systems to extend the range of recognized and counted organisms up to 200 μm and above (e.g., the imaging-in-flow system of Sieracki et al., 25). In order to achieve the above objective, the approach must be able to detect the (autofluorescent) autotrophic and mixotrophic organisms, as well as the (nonfluorescent) heterotrophs. The rapid development of cytometric and imaging systems could lead to the automatic assessment of food-web effects on the water-column BC flux.

The combination of FCM and automatic sorting of natural organisms, followed by analyses on the sorted fractions, may develop in different directions. One approach may be sorting based on cell size followed by characterization of activity (e.g., uptake of radioactive substrates), in order to study allometric relationships. This approach has been applied to heterotrophic bacteria (66) and phytoplankton (19), but seldom has been used until now (65). Another approach could be sorting based on both cell size and autofluorescence (phytoplankton) followed by characterization of biochemical composition (95), in order to study the responses of organisms to environmental conditions. Finally, there could be sorting based on physiological characteristics (e.g., metabolically active versus inactive cells) followed by characterization of taxa (96–98), in order to determine the physiological activity or status of taxa in the natural environment.

Overall, it is expected that FCM and other cytometric approaches will improve the ability of biological oceanography to address the major environmental challenges that are confronting human societies.

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