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Technical Notes

Short-Term Physiologic Effects of Mechanical Flow Sorting and the Becton-Dickinson Cell Concentrator in Cultures of the Marine Phytoflagellata *Emiliania huxleyi* and *Micromonas pusilla*

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Background: In contrast to large, high-efficiency cytometers, mechanically sorting benchtop instruments provide a feasible alternative for shipboard cell sorting of oceanic microbial communities. However, sorting efficiency of these instruments is constrained by their maximum sorting rate of approximately 300 cells/s and by constant dilution of sorted samples by sheath flow. These factors often render too low sorted cell concentrations for postsorting experiments of oceanic phytoplankton populations of low natural abundance. A Cell Concentrator module has been marketed to overcome these dilution effects. Postsorting experiments also have to consider potential physiologic effects of cell sorting. Short-term physiologic effects on phytoplankton photosynthetic rates and esterase activities by mechanical flow sorting and cell concentration and on the efficiency of the Cell Concentrator module are evaluated.

Methods: Increasing numbers of the oceanic phytoflagellates *Micromonas pusilla* and *Emiliania huxleyi* were sorted and concentrated, and recovery in the concentrated samples was compared with the sorted-only samples (concentration rate) and the total number of sorted cells (recovery rate). Photosynthetic rates and metabolic activities of sorted and sorted/concentrated cells were compared with unsorted cells. Photosynthetic rates were estimated from $^{14}CO_2$ uptake experiments and metabolic activity quantified cytometrically after cleavage of fluorescein diacetate.

Results: Irrespective of the total number of sorted cells, concentration rates between concentrated and sorted cells remained mostly below 10-fold and did not increase with the number of concentrated cells. Recovery rates in the concentrated samples amounted to fewer than 10% of total sorted cells, except for forceful resuspension attempts in the Concentrator insert (25–44%), which might be unsuitable for delicate species. Cell sorting resulted in a 24–49% decrease in photosynthetic rates. Metabolic activity within metabolically active cells was not affected by cell sorting, but the share of metabolically active cells decreased by 32–37%. Cell concentration did not affect metabolic activity or the fraction of active cells but did increase photosynthetic rate several-fold compared with unsorted cells.

Conclusion: Low recovery of concentrated cells, probably due to cell adhesion to the filer bottom of the Concentrator insert, render the Cell Concentrator of limited use to overcome dilution problems of mechanical flow sorting, particularly when results are extrapolated to natural, low-abundance populations. Severe changes in photosynthetic rates also render concentrated cells suspicious for subsequent physiologic experiments. Mechanical sorting alone also exhibited significant physiologic effects on sorted cells, some of which might not be temporary. Comparable effects between mechanical sorting and droplet sorting as previously reported confirm that physiologic effects might be caused predominantly by shear stress and laser exposure during cytometric analysis rather than the sorting process. Sufficient recovery time must be allowed before postsorting experiments, but potential changes in cell physiology from the natural conditions during postsorting recovery must be considered. © 2005 Wiley-Liss, Inc.

Key terms: mechanical flow sorting; phytoplankton; photosynthetic rate; metabolic activity; cell concentrator

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Flow cytometry has significantly advanced the study and understanding of oceanic phytoplankton communities. It has helped in surveying the distribution and abundance of the smallest aquatic phytoplankton (1–3) and discovering the most abundant phytoplankton of the oligotrophic ocean, *Prochlorococcus* spp. (4), leading to the recognition of the significance of microbial food webs in pelagic ecosystems (5). Cytometric flow sorting enabled an assessment of the contribution of phytoplankton and bacteria subpopulations to community productivity, which could not be separated by traditional size fractionation or other methods (6–10). Flow sorting also allowed the unraveling of phylogenetic differences of *Prochlorococcus* populations co-occurring at different depth horizons of the open ocean (11) and the establishment of monospecific cultures of aquatic microbes (12–15).

Although large flow cytometers with electromagnetic droplet sorting can achieve high-efficiency cell sorting at rates up to 1,000 cells/s, their use is principally land based because these instruments do not lend themselves for ocean-going shipboard work. However, field samples returning to shore for later flow sorting pose severe problems for marine microbial communities. The population structure of microbial communities may change within as little as 24 h of storage/cultivation (16–19), and culturing and storage can alter cells' physiologic characteristics (20). Benchtop instruments with mechanical, piezo-driven flow sorting might provide an alternative for shipboard use, and such instruments have been employed successfully for shipboard cytometry of marine plankton (6,21,22). However, benchtop, mechanically sorting instruments encounter two potential problems for cell sorting in the oceanic environment: potential physiologic effects on sorted cells and low sorting efficiencies combined with the dilution of sorted cells.

Potential effects of flow sorting on physiologic measurements can be avoided when proxies of targeted rates can be labeled fluorescently or radioactively and populations are sorted after incubating the communities. This approach proved successful to distinguish cell-specific photosynthesis rates among oceanic picophytoplankton by $^{14}CO_2$ uptake (6) and for cell-specific ^{3}H-thymidine and -methionine uptake rates of bacteria subpopulations with different apparent DNA contents (7–10). Physiologic processes that cannot be permanently labeled in this way, e.g., species-specific growth rate responses to altered nutrient concentrations or ratios, which might be masked by intraspecific resource competition in mixed populations, or species-specific contribution to community respiration rates, would require preincubation sorting. Early reports have indicated short-term physiologic effects of electromagnetic droplet sorting in phytoplankton cells (23,24), but potential effects of mechanical flow sorting have not been addressed adequately.

Another drawback of mechanically sorting benchtop instruments is their low sorting efficiency. Their maximum sort rate is limited to approximately 300 cells/s. However, higher sorting rates are rarely achieved with oceanic phytoplankton communities due to the low abundance of target cells in these waters. The major problem arises from the permanent dilution of sorted cells by the constant flow of sheath fluid even during periods when no cells are sorted. Resulting sorted samples often exhibit too low cell concentrations for further processing or physiologic measurements because detection limits of analyses cannot be met with these low cell concentrations. To overcome this constraint of mechanical flow sorting, a postsorting Cell Concentrator module has been marketed by Becton-Dickinson.

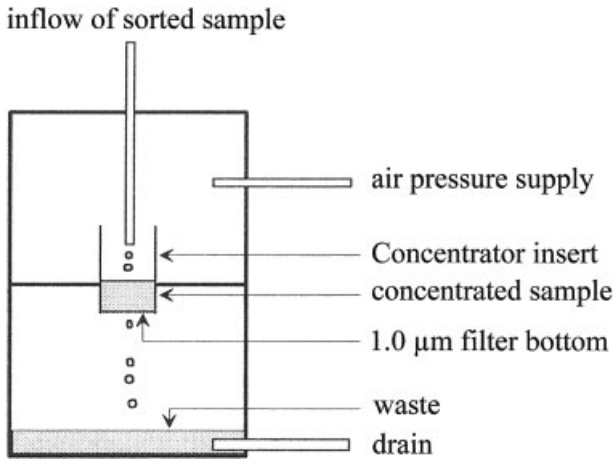

FIG. 1. Schematic view of the Becton-Dickinson postsorting Cell Concentrator.

The present study addresses potential short-term effects of mechanical flow sorting on phytoplankton physiology as reflected by cellular photosynthetic rates and metabolic activity measured as intracellular esterase activity. The efficiency of the Cell Concentrator module to overcome dilution problems of sorted samples and potential additional physiologic effects of cell concentration are evaluated.

MATERIALS AND METHODS

The oceanic phytoflagellates *Emiliania huxleyi* (CCMP374) and *Micromonas pusilla* (CCMP487) were grown in batch cultures of 33‰ filtered seawater amended with f/2 nutrients (25) at 18°C and 180 $\mu E/m^2/s$ light intensity under a 14-h/10-h light/dark cycle. Sorting experiments were performed with cultures in late exponential phase except for the *E. huxleyi* primary production experiment 2, which was performed with stationary phase cells.

Cultures were sorted on a FACSort flow cytometer (Becton-Dickinson, San José, CA, USA) equipped with a mechanical, piezo-driven cell sorter and a postsorting Cell Concentrator. Samples were sorted at a flow rate of 1 μl/s (setting High of the FACSort) and in recovery mode to achieve sorting rates close to the instrument's maximum of 300/s to ensure as densely sorted samples as technically possible. Sorted cells were diverted into 50-ml Falcon tubes. For cell concentration, one of the three Falcon tube slots was replaced by a silicon tubing line that fed into the external Cell Concentrator (Fig. 1). The upper compart-

ment of the Cell Concentrator is pressurized by the air system of the FACSort, which results in filtration of the sorted sample stream through the filter bottom of the Concentrator insert. Air pressure in the upper compartment is adjusted (by an electronic control panel added to the FACSort) so that the incoming sorted sample stream is filtered sufficiently fast to avoid overflow but slow enough for the sample not to run dry.

For all experiments, 33‰ seawater pre-filtered through a Millipore pressure filtration with 0.2-μm cellulose mixed ester filter, refiltered through 47-mm 0.2-μm cellulose mixed ester membrane filters, and enriched with f/2 nutrients was used as sheath fluid. To assess the feasibility and efficiency of the Cell Concentrator, increasing numbers of cells (100,000 to 500,000) were sorted from the original cultures and concentrated by 1.0-μm pore polycarbonate filter Concentrator inserts. Each number of cells was sorted and concentrated in three to nine replicates, and recovery in the concentrated samples was compared with cell concentrations in the sorted samples without concentration (concentration rate) and with the total number of sorted and concentrated cells (recovery rate). Samples were retrieved from the Concentrator inserts by gentle but rigorous resuspension with 1,000-μl pipet tips; recovery volume after cell concentration was 1.3 ml. To quantify cell abundance, samples were run at a flow rate of 1 μl/s, target cells gated on their forward-angle light scatter and chlorophyll fluorescence (FSC vs. FL3), and cell abundance was calculated from measurement times based on weight calibration of the flow rate.

Cellular primary production (photosynthesis) was assessed by ^{14}C incubations. Triplicate original culture (10 ml), sorted (10 ml), and concentrated (3.3 ml) samples were amended with 1 μCi/ml $NaH^{14}CO_3$ and incubated under culture conditions as described above for 2–3 h; for each sample set (original, sorted, concentrated), one dark control incubation was added. Cells were filtered onto 0.2-μm cellulose mixed ester membrane filters. Filters were washed three times with 10 ml of filtered seawater, placed in a Lumagel (Baker, Phillipsburg, NJ, USA) scintillation cocktail, and measured in a Packard Tri-Carb scintillation counter (Perkin-Elmer, Boston, MA, USA) after 24 h. Total ^{14}C uptake was converted to cellular production rates by cell abundances that were estimated cytometrically before ^{14}C additions.

Catabolic cellular metabolic activity was assessed by cytometric quantification of the cleavage of fluorescein diacetate (FDA) by unspecific intracellular esterases according to a previously published protocol (26). Samples were amended with 1.7 μg/ml FDA (F-7378, Sigma Chemical Co., St. Louis, MO, USA), and green fluorescein fluorescence (FL1; 535 ± 15 nm) was recorded on a 4-decade log scale after 10 min of incubation at room temperature.

RESULTS

Cell Concentrator Efficiency

In normal sorting mode (i.e., without the Cell Concentrator), sheath fluid is continually fed into the sorting tube even when no cells are sorted, and sorting can be continued until the sorting tube volume is exhausted (typically 43 ml on the used machine). Therefore, cell concentration in the sorted sample is independent of the total number of sorted cells as long as target cells remain sorted at a more or less constant rate throughout the sorting process. In the concentrated samples, however, final cell abundance should be dependent on the total number of sorted cells because, ideally, all sorted cells should be kept in and recovered from the 1.3-ml Concentrator insert volume.

Table 1 compares cell concentrations in sorted and concentrated samples. Although final abundances in concentrated samples tended to increase with larger numbers of sorted cells, this increase was not proportional to the total number of sorted cells. In fact, some concentrated samples from runs with larger total numbers of sorted cells resulted in lower final abundances than did samples with fewer sorted cells. Consequently, concentration rates did not increase with total number of sorted cells. For *M. pusilla*, concentrated samples were not more enriched than up to 15 times, but mostly less than 10 times, compared with sorted-only samples, and concentration rates remained below 3 times for *E. huxleyi*. These low concentration rates illustrate the major problem of the Cell Concentrator, i.e., resuspension and recovery of cells from the Concentrator inserts. An inherent problem to the Concentrator concept turned out to be cell adhesion to the insert filter bottoms, which is reflected by total sorted cells recovery rates of only 4–10%. In a second experiment with *M. pusilla*, concentrated cells were recovered from Concentrator inserts by more forceful resuspension, which increased recovery rates to 25–44% but still left concentration rates in the 4- to 14-fold range compared with sorted only samples.

Physiologic Effects of Sorting and Concentrating

Neither cell sorting nor cell concentration affected cellular light scatter or chlorophyll autofluorescence signals (Table 1), except in one concentrating experiment with *M. pusilla*, for which the decrease in chlorophyll fluorescence remained unclear and no effect on light scatter was observed. Cell sorting and concentration also had no clear effect on the average cellular fluorescein fluorescence after FDA cleavage (metabolic activity) of FDA-positive (i.e., metabolically active) *E. huxleyi* cells (Table 2). Sorted, metabolically active cells exhibited, on average, a 35% increase in FDA-mediated fluorescence, but sorting also increased the standard error of cellular FDA fluorescence (i.e., heterogeneity of metabolic activity within the population), rendering this difference to the unsorted culture statistically insignificant (t test, $P > 0.1$, df = 4). Mean cellular FDA fluorescence of concentrated, metabolically active cells also did not differ from unsorted cells (t test, $P > 0.1$, df = 4).

However, sorting and concentrating had a significant effect on the number of metabolically active (FDA-positive) cells, which decreased from 92% in the stock culture to 55–60% in the sorted and sorted/concentrated samples

Table 1
Efficiency Test of the Becton-Dickinson Postsort Cell Concentrator With Cultures of Oceanic Flagellates Micromonas pusilla *and* Emiliania huxelyi*

Species	No. of cells sorted and concentrated	Sorted/ concentrated sample (cells/ml)	FSC	FL3	Concentration rate	Recovery rate (%)	No. of experiments
M. pusilla	Sorted only	1,728 ± 69	125 ± 2	401 ± 6			3
	1.0×10^5	7,183 ± 48	129 ± 1	412 ± 1	4.2 ± 0.1	9.3 ± 0.1	3
	1.5×10^5	8,653 ± 125	163 ± 1	516 ± 5	5.0 ± 0.1	7.5 ± 0.1	3
	2.0×10^5	6,769 ± 193	119 ± 3	277 ± 10	3.9 ± 0.1	4.4 ± 0.1	3
	2.5×10^5	15,942 ± 119	132 ± 1	431 ± 5	9.2 ± 0.1	8.3 ± 0.1	3
	3.0×10^5	27,225 ± 348	166 ± 1	523 ± 1	15.8 ± 0.2	11.8 ± 0.2	3
	5.0×10^5	16,642 ± 597	131 ± 2	429 ± 6	9.6 ± 0.4	4.3 ± 0.2	3
M. pusilla	Sorted only	7,125	190	79			1
	1.5×10^5	28,858	168	77	4.1	25.0	1
	2.0×10^5	60,992 ± 15,633	161 ± 1	74 ± 0	8.6 ± 2.2	39.6 ± 10.2	2
	2.5×10^5	57,125	162	74	8.0	29.7	1
	3.0×10^5	100,938 ± 313	162 ± 3	77 ± 1	14.2 ± 0.1	43.7 ± 0.1	2
E. huxleyi	Sorted only	4,986 ± 123	468 ± 11	191 ± 5			3
	1.0×10^5	6,101 ± 162	462 ± 2	198 ± 1	1.2 ± 0.0	7.9 ± 0.2	6
	2.0×10^5	11,976 ± 227	457 ± 4	196 ± 1	2.4 ± 0.1	7.8 ± 0.2	9
	3.0×10^5	10,797 ± 966	446 ± 5	194 ± 1	2.2 ± 0.2	4.7 ± 0.4	6
	4.0×10^5	14,267 ± 529	444 ± 4	187 ± 2	2.9 ± 0.1	4.6 ± 0.2	3

*Number of sorted and concentrated cells reflects the preset amount of cells to sort when the Cell Concentrator was used; sorted only samples were not treated with the Cell Concentrator. Cell concentrations (cells per milliliter, mean ± standard error) reflect abundance in the sorted or concentrated samples. Concentration rate is the ratio of cell abundances in the concentrated samples to the sorted-only samples; recovery rate (in percentage units) is the ratio of cell abundances in the concentrated samples to the total number of sorted cells reported by the cytometer software during the sorting process.

(Table 2; *t* test, $P < 0.01$, df = 4). The share of metabolically active cells was similar in sorted and concentrated samples (*t* test, $P > 0.6$, df = 4), indicating that the loss of metabolic activity was induced by the cytometric measurement and subsequent sorting rather than cell concentration.

Flow sorting and concentrating showed similar effects on primary production in *E. huxleyi* and *M. pusilla* (Table 3). Flow sorting alone resulted in decreases in cellular carbon uptake by 24% and 49% for *M. pusilla* and *E. huxleyi* exponentially growing cells, respectively. Stationary phase *E. huxleyi* cells exhibited extremely low cellular carbon uptake rates in the stock culture, and no further decrease was detected after flow sorting. Concentrating of sorted cells resulted in a 3- to 10-fold increase in cellular carbon uptake rates compared with untreated cells. Effects were strongest in stationary phase *E. huxleyi* cells.

DISCUSSION

Unchanged light scatter and chlorophyll autofluorescence signals of sorted cells confirm that phytoplankton endure mechanical flow sorting without physical cell damage, as has been shown previously for electromagnetic droplet sorting (14,24). However, a decrease in cellular primary production of 24–49% compared with unsorted cells points toward significant physiologic effects of mechanical flow sorting. Observed decreases in primary production compare with a 39–68% decrease in cellular carbon uptake rates in five dinoflagellate species and a 24–37% decrease in the (presumably more robust) diatom *Ditylum brightwellii* after electromagnetic droplet sorting (23). The same study concluded that the majority of cellular production decrease was mediated by the cells' exposure to the cytometer laser beam, which seems to be confirmed by similar effects of droplet and mechanical sorting on cellular carbon uptake rates.

Significant short-term decreases in cellular carbon uptake rates might explain previously reported delays in cell growth and cell division of phytoplankton after cell sorting (24) because phytoplankton growth rates are usually coupled to photosynthesis as their primary carbon and energy source. Postdroplet sorting growth in several strains of phytoplankton of different taxonomic affiliation (24) and in aquatic ciliates (15) were delayed for 12–48 h, depending on the studied species, but resumed growth rates equivalent to those of unsorted cells thereafter. Sorting of X and Y chromosome bovine sperm also resulted in a 0.5- to 1-day delay in embryonic development but showed no significant decrease in egg fertilization rates, long-term developmental damage, or health effects on the resulting calves (27,28). These results confirm ubiquitous physiologic effects of cell sorting in various cell types but suggest that these physiologic effects are of short-term nature and mediated after 1 to 2 days.

Table 2
Effects of Mechanical Flow Sorting and the Becton-Dickinson Cell Concentrator on the Metabolic Activity of Emiliania huxleyi *as Assessed by FDA (n = 3)**

Treatment	Cellular FDA fluorescence	% FDA positive
Unsorted	425.3 ± 5.8	92.3 ± 0.1
Sorted	575.8 ± 149.5	55.6 ± 7.9
Concentrated	447.8 ± 12.3	60.1 ± 3.1

*Values are presented as mean ± standard error. FDA, fluorescein diacetate.

Table 3
Effects of Mechanical Flow Sorting and the Becton-Dickinson Cell Concentrator on ^{14}C Primary Production of Emiliania huxleyi *and* Micromonas pusilla *(n = 3)*

Species	Treatment	Primary production[a]	% Unsorted culture
E. huxleyi 1	Unsorted	241 ± 17	
	Sorted	123 ± 4	51
	Concentrated	773 ± 82	321
E. huxleyi 2	Unsorted	8 ± 1.6	
	Sorted	8 ± 1.5	100
	Concentrated	83 ± 11	1037
M. pusilla	Unsorted	33 ± 6	
	Sorted	25 ± 3	76
	Concentrated	171 ± 46	518

[a]Picograms of carbon per cell per hour; mean ± standard error.

Recovery periods of cellular carbon uptake rates in mechanically sorted phytoplankton were not assessed in this study. However, a roughly 40% decrease in the number of metabolically active, sorted *E. huxleyi* cells might suggest that not all physiologic effects of flow sorting are temporary. Similarly, only 40% of metabolically active (positive staining with carboxy-FDA) *Bifidobacterium lactis* with uncompromised cell membranes (propidium iodide negative) grew in culture after mechanical flow sorting (29). Droplet sorting increased the share of cell membrane-compromised tumor cells from less than 10% in unsorted cells to 20–25% and increased cell membrane asymmetry, an early indicator of apoptosis, by 15–19% (30). Long-term and/or ultimate cell damage might also explain "difficulties" (14) and low success rates (20–30%) (13) in establishing monoclonal cultures from sorted phytoplankton.

The long-term cell damage reflected by the 40% decrease in metabolically active cells also warrants revisiting the decreased postsorting photosynthetic rates. Assuming that only the remaining metabolically active cells incorporated ^{14}C, cellular photosynthetic rates in sorted, metabolically active cells were comparable to rates in unsorted cells. Thus, the observed decrease in photosynthetic rates might not be linked to a general physiologic effect on all cells but rather the decrease in metabolically active cells, rendering sorted samples a mixture of highly affected, metabolically inactive and virtually unaffected cells. Because previous studies (14,23,24) did not account for a potential change in metabolically active cells, their reported decrease in photosynthetic activity might also be linked to similar losses in active cells rather than to a physiologic effect on all cells.

This dichotomy between strongly affected, metabolically inactive and almost unaffected cells in sorted samples might also explain the basically unchanged catabolic activity in the remaining metabolically active cells as reflected by the FDA assay. The increased heterogeneity in FDA activity in postsorting *E. huxleyi* suggests, however, that sorting did exert some physiologic stress on some of the active cells, which might have initiated cellular repair mechanisms requiring increased catabolic activity.

Postsorting cell concentration resulted repeatedly in astonishing, several-fold increases of cellular photosynthetic rates, not only compared with sorted but also with unsorted cells. Because concentrated cells demonstrated the same physical effects as decreased carbon uptake rates in sorted cells, additional effects must be responsible for the dramatic increase in photosynthetic rates after cell concentration. Loss of dissolved free amino acids and other dissolved organic carbon compounds from algal and bacterial cells after pressure filtration even under weak pressure differential has been proposed (31,32). Such loss of cellular dissolved organic carbon after pressure filtration occurring in the Cell Concentrator might have led to a severe deprivation of intracellular organic carbon pools that could have triggered overactivity of the photosystem. However, other studies have concluded that prefiltration does not alter photosynthetic rates (1,33). It cannot be excluded that differences in photosynthetic rates, even when normalized to cell counts, might also be an artifact of different incubation conditions (10-ml vs. 3.3-ml incubations). Although the reason for increased cellular carbon uptake rates after cell concentration remains unresolved, these results render concentrated cells suspicious for subsequent physiologic analysis. Indifferent metabolic activity between concentrated and sorted or unsorted cells might indicate deprivation of organic carbon compounds after sorting and concentrating limited catabolic processes as discussed for sorted cells.

A major constraint of mechanical flow sorting is the permanent and severe dilution of sorted cells in the sorting tube. Cell concentrations in the initial stock cultures were not quantified but ranged in the order of 10^5 cells/ml (sorting rates of 200–300 cells/s at a flow rate of 1 μl/s; thus, stock culture cell concentrations were $2–3 \times 10^5$ cells/ml). Sorted samples exhibited cell concentrations in the range of 10^3/ml (Table 1). Although this dilution renders still usable samples after sorting of dense laboratory cultures, oceanic field samples with typical nanophytoplankton cell abundances of 10^3 to 10^4 cells/ml (34–36) often produce sorted samples too dilute for further processing. The Becton-Dickinson Cell Concentrator was offered to overcome the dilution constraints on mechanical sorting cytometers such as the FACSort and the Calibur.

The performance of the Cell Concentrator remained disappointing. Operation of the Concentrator was not straightforward because fine-tuning the pressure differential within the Concentrator module required permanent attention. One pressure setting (observed by the digital display of the control panel) was not applicable between subsequent sorting and concentrating runs of the same culture. In addition, concentrating larger cell numbers required repeated readjustment of the pressure differential to avoid overflow of the sample in the insert, probably due to increasing clogging of the Concentrator insert filter bottom by concentrated cells.

The major problem of the Concentrator concept turned out to be cell recovery from the inserts. An overwhelming number of concentrated cells remained attached to the filter bottom of the insert even after careful but rigorous

resuspension attempts, leaving concentration rates compared with sorted-only samples mostly below 10% and recovery rates of sorted cells below 10%, irrespective of the total number of sorted cells. Forceful resuspension increased recovery rates for *M. pusilla* to 25–44% and provided concentrated samples of 10^4 to 10^5 cells/ml, but only after sorting more than 10^5 cells (equivalent to ~20-min sorting time). Recovery rates remained unpredictable from the number of total sorted cells, and such forceful resuspension may only be applicable to robust cells such as *M. pusilla* but might cause cell damage in more delicate species.

These results suggest limited use of the Cell Concentrator to overcome dilution problems of mechanical flow sorting of natural aquatic samples. Even with higher recovery rates as achieved for *M. pusilla*, starting with natural nanophytoplankton populations of 10^4 cells/ml would require 2.7 h of sorting and concentrating to provide a 10^4 cells/ml concentrated sample of 1.3-ml volume. Applications that allow filtration of sorted cells for further analysis, such as for population-specific $^{14}CO_2$ or ^{3}H-substrate uptake by postincubation sorting (6–10,23) or DNA extraction for phylogenetic analysis (11), appear to remain the best use of ocean-going benchtop cytometry. The Cell Concentrator also offers the option to replace the concentrator insert with a 25-mm filter holder. Although this option might be valuable for sorting harmful cells, such as in a clinical setting, similar results can be obtained for planktonic cells by normal sorting into 50-ml Falcon tubes and subsequent filtration by conventional filtration units. In this case, a 2.7-h sorting run would provide 10^5 cells/filfilter, approximately 10 times more than to be recovered from the Concentrator cell insert.

For experiments that do not allow for presorting labeling, postsorting physiologic experiments must be evaluated with care. Presented results confirm short-term and potential long-term physiologic effects of mechanical flow sorting. Comparable effects on cellular photosynthetic rates of phytoplankton and on the share of metabolically active cells compared with survival rates of various cell types (phytoplankton, bacteria, sperm, and tumor cells) between electromagnetic droplet and mechanical sorting also confirm that physiologic effects are related to shear stress and laser exposure during cytometric analysis rather than to cell sorting (23). Sufficient recovery time between cell sorting and physiologic experiments should be ensured, although care must be given to potential change in physiologic properties of the cells to be characterized during the recovery incubation.

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