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A slide preparation technique for light microscopy analysis of ciliates preserved in acid Lugol's fixative

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Abstract

A slide preparation method for seawater samples preserved in acid Lugol's is presented here as an alternative to the traditional Utermöhl settling chamber method for microplankton analysis. This preparation maintains the integrity of fragile cells, such as ciliates, resolves issues associated with the transience of samples prepared in settling chambers, and allows the use of automated image acquisition methods. Samples are filtered onto polycarbonate membranes and analyzed with transmitted light microscopy. The visibility of pore outlines is minimized by using mounting oil (Cargille Series A immersion oil, Certified Refractive Index, $n_D^{25°C}$ 1.5840 ± 0.0002) with a refractive index matching that of the membrane material. We assessed the efficacy of this new method by comparing abundance and biomass estimates for ciliates in settled and filtered samples. Acceptable results were found for the most delicate of samples stored long-term in acid Lugol's. Some cell shrinkage occurred during the filtration and brief drying steps. Therefore, corrections for ciliate length and width measurements in filtered samples were determined to counteract this effect on total cell biovolume. Overall, the method provides a simple and stable alternative to settling chamber analysis for ciliates preserved in acid Lugol's.

The Utermöhl settling chamber method is a standard and widely used inverted microscopy procedure for enumerating marine protistan microplankton (Lund et al. 1958; Müller et al. 1991; Sohrin et al. 2010; Utermöhl 1931). Major advantages of the technique are that cells are concentrated by gentle gravitational settling and remain in the liquid medium during analysis. The technique can therefore be used in conjunction with acid Lugol's preservative, which is optimal for delicate groups like ciliates (Leakey et al. 1994; Stoecker et al. 1994) but does not fix their cell walls rigidly. Disadvantages are that it requires a laborious set-up procedure, a long settling time in specialized columns (typically 24-48 h, but see Claessens and Prast [2008]), and does not result in a permanent preparation. In practice, once cells have been concentrated onto a coverslip in the lower chamber and most of the original sample water removed, a skilled technician will scan transects or the full area of the chamber. During this analysis, dimensions of representative organisms are taken with an ocular micrometer for biovolume estimations, data are recorded for abundance, cell shape, and taxa, for all cells of interest, and ultimately the sample is discarded. Besides being a very tedious process, questions that arise later about poorly resolved taxa, unmeasured cell properties, or specific taxa that were not enumerated originally, are difficult or impossible to answer without the original sample or an image record. Additionally, given the effort required for analysis and the transient nature of settling column preparations, samples intended for Utermöhl analysis are generally kept in bottles until they are used. This is not only a practical problem for storage of large numbers of samples, but also may lead to degradation of cells stored in the acidic media over time (Menden-Deuer et al. 2001; Sherr et al. 1993; Stoecker et al. 1994).

For the majority of microbial populations, alternate methods such as epifluorescence microscopy and flow cytometry have become increasingly popular for routine analysis of community abundance and biomass (e.g., Taylor et al. 2011). These techniques are especially suited for distinguishing autotrophic from heterotrophic cells based on chlorophyll *a* (Chl *a*) autofluorescence and for quantifying contributions of functional groups too small to be enumerated effectively in settling chambers. They are also convenient for sample concentration and analysis at sea, and various steps of the analyt-

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ical process can be automated for faster processing. For example, with a fully automated epifluorescence microscope, it is possible to program a prearranged pattern of sampling locations on a prepared slide, section the visual field at precise increments of vertical resolution at each location, acquire separate images at precisely the same slide location (x, y, z) with filters that optimize for different fluorescence signals, reassemble the images into one best-of-focus color image per location with minimal halo effect, resolve and quantify dimensions and fluorescence properties of each image, and capture the data in spreadsheets (e.g., Taylor et al. 2012). It remains later for human technicians to identify and place cells into appropriate functional categories, but the digital imaging can be done immediately after sample preparation, producing a permanent visual record of the slide contents in addition to the spreadsheet information. Such a process is efficient and robust to handle many hundreds of slides per cruise (Taylor et al. 2011, 2012), and accurate enough to resolve production contributions of phytoplankton functional groups from biomass and growth rate estimates (Landry et al. 2011). However, it clearly gives severe underestimates of abundance and biomass for ciliated protists (Taylor et al. 2011), whose fragility makes them vulnerable to significant loss from commonly used aldehyde preservatives and filtration procedures (Choi and Stoecker 1989; Leakey et al. 1994).

In search of a more efficient image-analysis approach for routine enumeration of acid Lugol's preserved marine microplankton, particularly ciliates, we first considered some published protocols for slide mounting: filter-transfer-freeze (Hewes and Holm-Hansen 1983), soluble methacrylic resin (Crumpton 1987), and Steedman's wax (Steedman 1957). The first is inherently nonquantitative, and the resin and wax techniques involve heating, drying, and strong adhesive steps that we found even preserved ciliates could not withstand. We also attempted to analyze standard Utermöhl chambers with automated inverted microscopy. However, the large number of motor-driven movements of the microscope stage caused the fluid-suspended cells to move and precluded reconstruction of focused images from multiple pictures at each location. We also eliminated Cyto-clear slides (Poretics) from consideration due to expense per slide and the fact that the frosted slides are incompatible with phase contrast. Nevertheless, according to their description and results of Logan et al. (1994), these slides should minimize membrane pore visibility in transmitted light microscopy in a similar manner to the method described in this article.

In the present study, we examine the effectiveness of an alternative slide preparation method for transmitted light microscopy that uses mounting oil matching the refractive index of polycarbonate membrane filters (Ocklind 1987). The oil is used to fill the membrane pores and visually reduce their outlines, which otherwise greatly detract from identifying cells on the slide. This simple solution is complicated by the fact that polycarbonate is a birefringent material, with one refractive index at 1.584 and one that varies with the exact chemical composition of the membrane and potential crystallization of the polycarbonate material. For pores to disappear, the refractive index of the mounting oil must match at least one of these two refractive indices. Cargille Immersion Liquid Index A, with refractive index 1.584 ($n_D^{25°C}$ 1.5840 ± 0.0002), is the only available material that has an appropriate refractive index and is stable. However, being an oil-based product, it is immiscible with the seawater remaining in the membrane pores after sample filtration, which must be wicked away before mounting the filter. Here, we describe procedures for making these slides and an assessment based on comparison to standard Utermöhl results, with an emphasis on ciliates.

Materials and procedures

Seawater samples available from different cruises were analyzed as part of the method development. Most samples were collected from 2008 to 2010 during quarterly cruises of the California Cooperative Oceanic Fisheries Investigations (CalCOFI) Program in the southern coastal region of the California Current Ecosystem. We also analyzed older samples collected during 1997-98 cruises of the Antarctic Environment and Southern Ocean Process Study (AESOPS) in the Southern Ocean to assess implications of longer storage on slide preparations with fragile cells (Table 1). Regardless of cruise and date, all samples were originally collected from 10-L Niskin bottles by gentle direct transfer to the sample bottle via a silicone tube, preserved in 5% acid Lugol's, and stored in dark polyethylene bottles.

Slide preparation

Based on preliminary observations, acid Lugol's preserved ciliates could be filtered onto polycarbonate membranes under low vacuum pressure (<50 mmHg) without the massive losses seen for slide preparations of aldehyde-preserved epifluorescence samples, or in the making of permanent slides with mounting resin (Crumpton 1987). To toughen the cell walls further for slide preparations, we added 37% formaldehyde to the 250 mL acid Lugol's samples (2% final concentration) and let them fix overnight before filtration. We used a glass filtration system to filter 100 mL sample onto 25-mm, 8-μm black polycarbonate filters with a 10-μm nylon backing filter (GE Water and Process Technologies) to promote even cell distribution. Filtrations were done under low pressure (<50 mmHg), and the vacuum pump was turned off during the final few milliliters to minimize cell damage from rapid pressure change (Crumpton 1987; Taylor et al. 2011).

After the samples were completely concentrated on the filters, both the backing and polycarbonate filters were placed together on plain paper to briefly wick away the residual water trapped in the pores of the membrane. It is important to remove as much water from the pores as possible to enhance visibility during later microscopy analysis, but at the same time, minimize dehydration of cells from air drying. We found that about 30 seconds or less was optimal for this part of the process.

Table 1. Ciliate and dinoflagellate abundance (cell L^{-1}) of both filtered and settled samples, as well as collection information including cruise, date, location, and depth for each sample. Replicate counts of ciliate abundance were available for samples 1, 2, 3, and 6.

						Ciliate abundance (cell L^{-1})				Dinoflagellate abundance (cell L^{-1})	
Sample	Cruise	Lat (°N)	Long (°W)	Depth (m)	Date	Filter	Replicate filter	Settling chamber	Replicate settling chamber	Filter	Settling chamber
1	AESOPS	–62.00	170.10	5	Jan 98	3239	2337	2903	2233	3553	6699
2	AESOPS	–59.30	170.00	30	Nov 97	1375	1141	1116	2030	5964	2664
3	CalCOFI	34.32	120.80	20	Aug 08	4717	5094	2791	5730	7242	10,658
4	CalCOFI	34.32	120.80	10	Oct 08	4826	—	4019	—	5845	3015
5	CalCOFI	34.27	120.03	20	Jan 10	3255	—	5981	—	2846	665
6	CalCOFI	34.27	120.03	15	Jan 09	2294	4246	5806	6364	2174	5471
7	CalCOFI	34.27	120.03	20	Oct 08	6586	—	7927	—	9185	3349
8	CalCOFI	33.18	118.39	30	Oct 08	3014	—	4354	—	12,107	2121
9	CalCOFI	32.65	119.48	20	Oct 08	2197	—	2568	—	9185	4578

A single drop of Cargille Series A immersion oil (Certified Refractive Index Liquids, $n_D^{25°C}$ 1.5840 ± 0.0002) was spread in a thin, even layer across the slide using the side of a glass pipette. The polycarbonate membrane with the sample was then carefully separated from the backing filter using 2 forceps and placed on top of the layer of oil, with a second drop of oil applied on top of the filtered membrane before adding the cover slip (No. 2 glass). Excess oil was removed from the edges of the coverslip, and it was sealed on all four sides to the glass slide with clear nail polish. An initial coat of quick dry polish followed by a coat of durable, long-lasting polish was most effective at completely sealing the coverslip to the slide, preventing the oil from leaking, which might compromise the slide over time. After a few months of storage, it was noted that the nail polish on a few slides had begun to peel, so a coat of acrylic paint varnish was applied to the edges of the coverslip on these slides as an additional sealant. Sealed slides were stored in slide boxes at –20°C with no apparent negative effect. These were brought to room temperature before microscopic analysis to prevent temperature effects on the refractive index.

Sample imaging and processing

Sample slides were analyzed on a Zeiss Axiovert 200M inverted compound microscope equipped with a fully motorized stage and controlled by Zeiss AxioVision software. Digital images were captured with a Zeiss AxioCam MRm black and white 8-bit CCD camera at 200× magnification using automated image acquisition. We imaged 200 visual fields at random positions on each slide, with 7 z-plane images taken at each position. The z-plane images were combined into a single 8-bit black and white image using an extended depth-of-field algorithm to produce a single, completely focused image (image dimensions 425.77 μm × 319.02 μm). Using a VBA script within the Image Pro software, a series of processing steps were made to the images for semi-automated counting and sizing (length, L, and width, W) of cells. A fast Fourier transform was applied to the images to remove background noise, followed by a Laplace filter to improve the definition of cell edges. Poorly resolved field images were discarded. Each cell in each image was manually outlined and identified, and the cell measurement data were exported for processing (Taylor et al. 2012)

Cells were manually identified and placed into one of 4 categories (ciliates, diatoms, dinoflagellates, and others). All of the 200 field images per sample, equivalent to about 10% of total filtered area, were analyzed for ciliates. The more abundant organisms (diatoms, dinoflagellates, others) were identified in random subsets of 50 images, sufficient to obtain 100 cells or more per category whenever possible. Finally, all cells were binned into three size-categories (10 to 20 μm, 20 to 40 μm, and >40 μm) based on measurement of the longest cell axis.

Settling chamber samples

To compare quantitatively against abundance and biomass estimates from the slide preparations, we analyzed 100 mL aliquots of each sample by the Utermöhl method. These sam-

ples were settled at least 20 hours, more than sufficient settling time according to ciliate sinking rates determined by Claessens and Prast (2008), and cells were enumerated in two transects across the diameter of the settling chamber. The area enumerated (about 9% of the total chamber area) was, therefore, roughly equivalent to the 200 microscope fields in the slide analyses. Unlike the automated image acquisition of the slide analyses, the settling chamber transects were analyzed manually, using fine adjustments to locate cells vertically in the chamber and to optimize image taking. Cell sizes for biovolume calculations and carbon biomass estimates were taken as described above for the slide samples.

Biovolume and carbon biomass estimations

Cell biovolumes for settled and filtered samples were calculated from measured dimensions and general cell shapes, assuming that the unmeasured cell height was equal to measured cell width. Equations for estimates of cell biovolumes (BV) followed Hillebrand et al. (1999), with diatoms, dinoflagellates and some ciliates, approximated as prolate spheroids (BV = 0.524×L×W×H). For ciliates, an additional shape option of a cone plus half-sphere was also used where more appropriate (BV = $0.262\times W^2\times[L+W]$). As discussed further in the "Assessment" section, filtered ciliate cell lengths and widths were corrected for shrinkage according to the equations in Fig. 4(*c-d*) before calculating biovolume and biomass.

Carbon biomass per cell was estimated from biovolume calculations using the following equations from Menden-Deuer and Lessard (2000): $C = 0.288\times BV^{0.811}$ for diatoms and $C = 0.216\times BV^{0.939}$ for non-diatoms and non-ciliates. We used the formula C = 0.19×BV from Putt and Stoecker (1989) for carbon estimates of aloricate ciliates. Carbon biomass (µg C L^{-1}) was calculated from carbon per cell and abundance data for each group.

Assessment

Qualitative visual assessment

Fig. 1 compares the visual quality of images taken manually in the settling chamber (*a-b*) to images from slides prepared with Cargille immersion oil Type DF with refractive index 1.515 (*c-d*), which is typically used in epifluorescence microscopy (Booth 1993), and to images from slides prepared with the oil proposed in this method, Cargille Series A with refractive index 1.584 (*e-h*). As a trade-off to minimizing cell dehydration and subsequent damage, we did not dry the filter membranes completely during slide preparation. Consequently, the Series A oil did not make the membrane pores disappear entirely into the background. Even so, the pores are dramatically less visible using Series A compared with standard Type DF immersion oil, greatly decreasing the distracting pore outlines that can obscure cell shapes and complicate the process of cell identification. It was also noted that Type DF immersion oil often spread unevenly across the slide, creating shadows in the transmitted light images (Fig. 1d). This was not apparent in slides prepared with the Series A oil, which spread evenly over the filters. Whereas the settling chamber still offers the least distracting image background for cell enumeration when manually focused prior to image capture, Series A oil sufficiently diminishes the membrane pores so that high quality images can be rapidly taken using automated microscope systems.

Abundance estimates and replication

Ciliate abundance estimates for filtered samples ranged from 40% to 169% of the corresponding abundance estimates for settling chambers (Table 1). On average, filter abundances were 9% lower than settling chamber estimates, but the results were not statistically different (non-parametric Mann-Whitney test, df = 8, $p = 0.453$). A strong positive relationship ($R^2 = 0.60$) was also found between estimates by the two methods (Fig. 2 inset). Results for dinoflagellates and diatoms are likely biased by insufficient settling times for these slower sinking cells. As determined by Claessens and Prast (2008), the sinking rates of acid Lugol's preserved ciliates in seawater are rapid enough to clear the water in 50 mL settling chambers in less than 3 hours. Thus, overnight settling times of at least 20 hours is sufficient for complete sinking of ciliates in our 100 mL columns, but not necessarily for smaller and less dense dinoflagellates and diatoms. Results for diatoms support this explanation, as diatom cells were significantly more abundant in the filtered samples than the settling chambers (Fig. 2, Mann-Whitney test, df = 8, $p = 0.047$). Dinoflagellate abundances were also notably higher in the filtered preparations, averaging more than double the corresponding estimates in settling chambers. This difference, however, was not statistically significant (Mann-Whitney test, df = 8. $p = 0.145$) for the number of samples enumerated. Clearly, the filter preparations minimize potential artifacts that could arise from large differences in sinking rates of different cells in settling chambers.

Because dinoflagellates and diatoms are believed to have been under-sampled in settling chamber counts, our remaining assessment focuses on ciliates only, which were the initial target group for the method development. For samples in which sufficient sample volumes were available (samples 1, 2, 3, 6), replicate subsamples were processed for ciliates by both the filtration and settling methods to determine the consistency of abundance estimates. Although methodological differences were larger than can be explained by replicate counts in one case (sample 6), error bars broadly overlap in the other three comparisons (Fig. 3). Differences between the ciliate abundance estimates in filtered and settled samples are therefore largely explained by counting variability within and between methods.

Biovolume estimates and shrinkage correction

Direct length and width measurements for ciliate biovolumes were conspicuously lower for filtered compared with settled samples, which prompted a closer look at the measurement distributions for each method (Fig. 4). Acid Lugol's fixative is known to shrink cells (Leakey et al. 1994; Stoecker

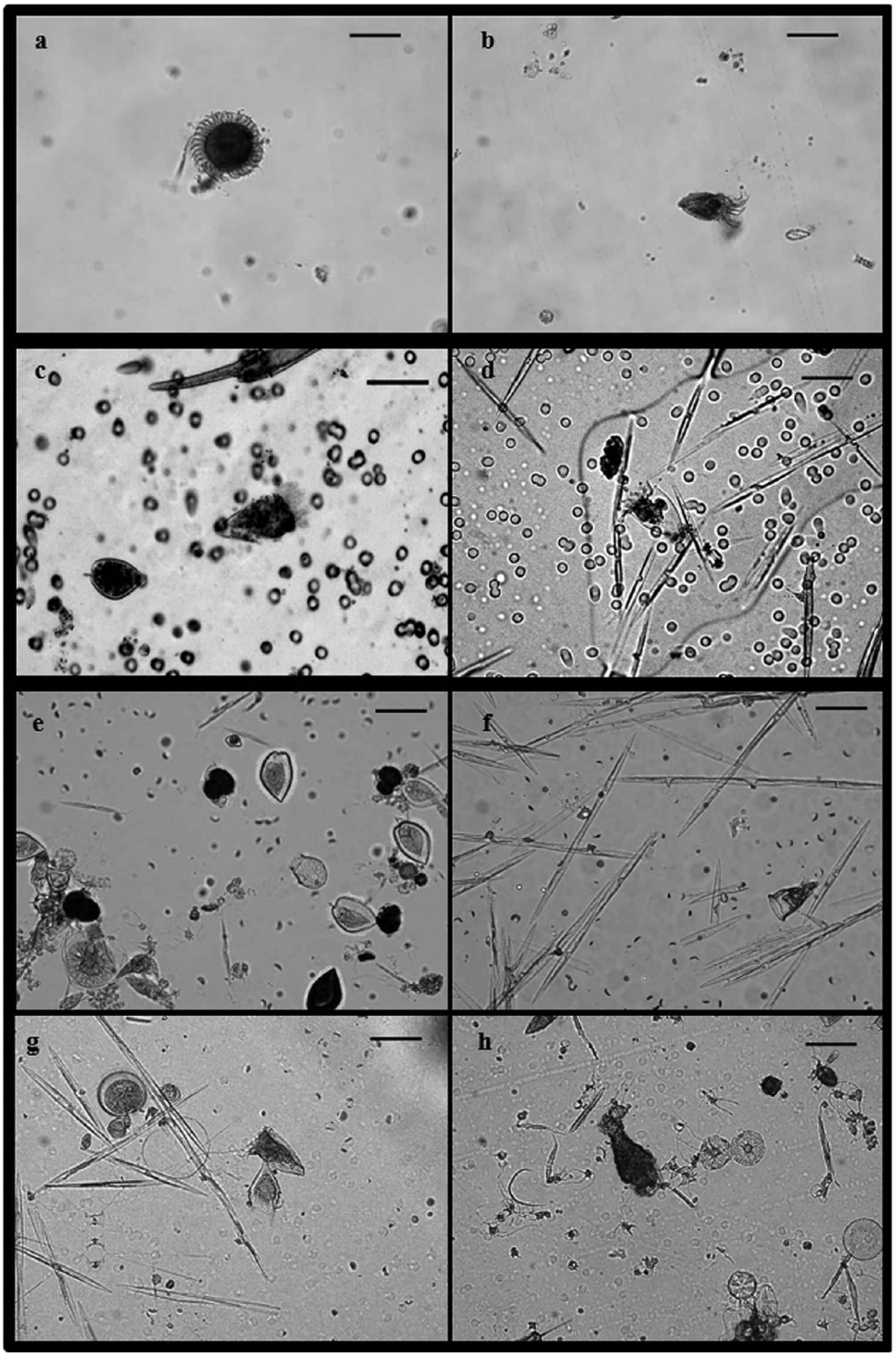

Fig. 1. Representative images of ciliates settled in a settling chamber (*a-b*), mounted on an 8 μm polycarbonate membrane filter with standard epifluorescence immersion oil (*c-d*, Cargille immersion oil Type DF), and mounted on a filter with the method described in this paper (*e-h*). All images are from samples collected in the southern California Current region and imaged as described in the methods section. All scale bars are 50 μm.

et al. 1994). However, since both filtered and settled subsamples had been stored in this fixative for the same amount of time before sample handling, their cell biovolumes should have been similar in the absence of other effects. We attribute the biovolume discrepancy to cell shrinkage during the filtration and brief drying process.

To quantify the mean measurement differences as rigorously as possible, we compared the size frequency distribu-

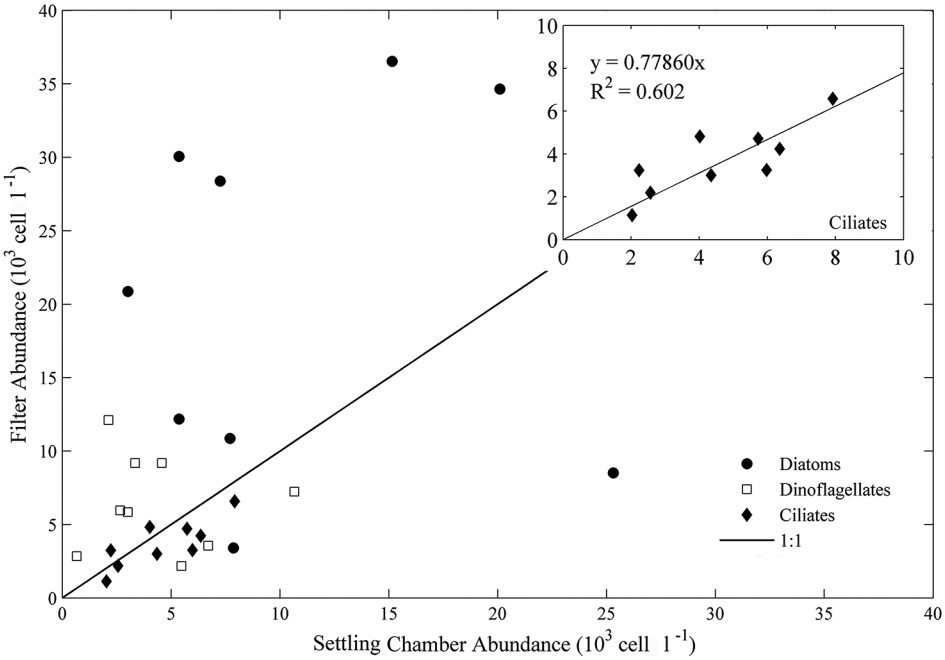

Fig. 2. Abundances (10^3 cell L^{-1}) of diatoms, dinoflagellates, and ciliates in settled versus filtered samples with a 1:1 line for reference. Inset graph is a linear regression for ciliates.

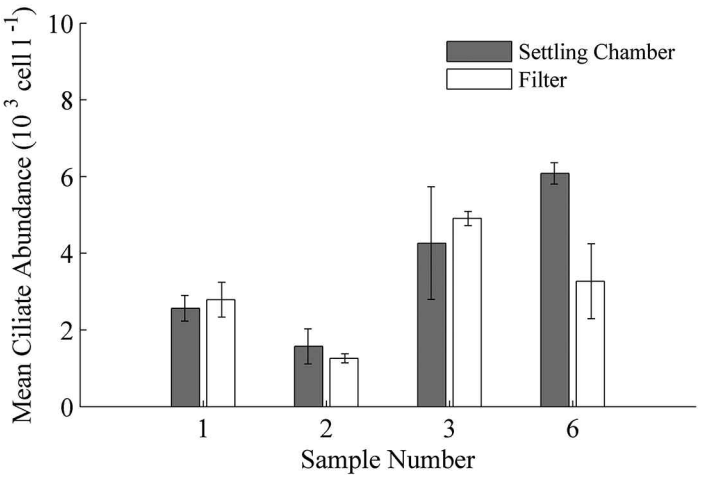

Fig. 3. Mean abundances (10^3 cell L^{-1}) with standard error bars estimated for settled and filtered samples calculated from replicate counts of 100 mL aliquots. Standard error was calculated as standard deviation divided by the square root of the sample size. Information for samples 1, 2, 3, and 6 given in Table 1.

tions of length and width measurements for ciliate cells analyzed by each method (>160 cells). The majority of cells fell into the 10-20 μm length category for both methods, but wider cells were more frequently found in settled (15-35 μm) rather than filtered (5-15 μm) samples (Fig. 4*a*-*b*). To account for the loss of cell volume during slide preparation, we chose randomly an equal number of ciliates from those analyzed by each method, ordered them by size, and then compared their length and width measurements by regression analysis (Fig. 5*c*-*d*). Strong exponential trends could be fit to both length (R^2 = 0.98) and width (R^2 = 0.99) data, and the resulting equations were used as correction factors for cells from the slide preparations. The curvilinear relationships indicate that cell dimensions shrink proportionately less with increasing cell size during slide preparation, perhaps reflecting greater resistance to dessication (reduced surface area to volume ratio) during the brief water-wicking process. Very large cells (>50 μm length, >35 μm width) were, however, very rare in the samples and therefore difficult to compare statistically from analysis by the two techniques. Individual length and width values measured from filtered cells were corrected according to these equations, and recalculated biovolumes were a much better approximation of settled biovolume values (Fig. 5*a*). Additionally, the biovolume corrections more than doubled the total biomass estimates from filtered samples (Fig. 5*b*), resulting in comparable total biomass estimates for settled and filtered samples (Fig. 5*b*-*c*).

Biomass comparisons

Ciliate biomass estimates for the six samples were pooled and compared across methods (Fig. 5*b*). Only six samples had sufficient measurement data from settling chambers to calculate ciliate biomass for comparison with filtered samples. Comparable results for total settling chamber ciliate biomass and filter biomass were found for all samples using the correction factor (Fig. 5*c*). A nonparametric Mann-Whitney test determined no significant difference between settling chamber and corrected filter biomass (df = 5, p = 1.00).

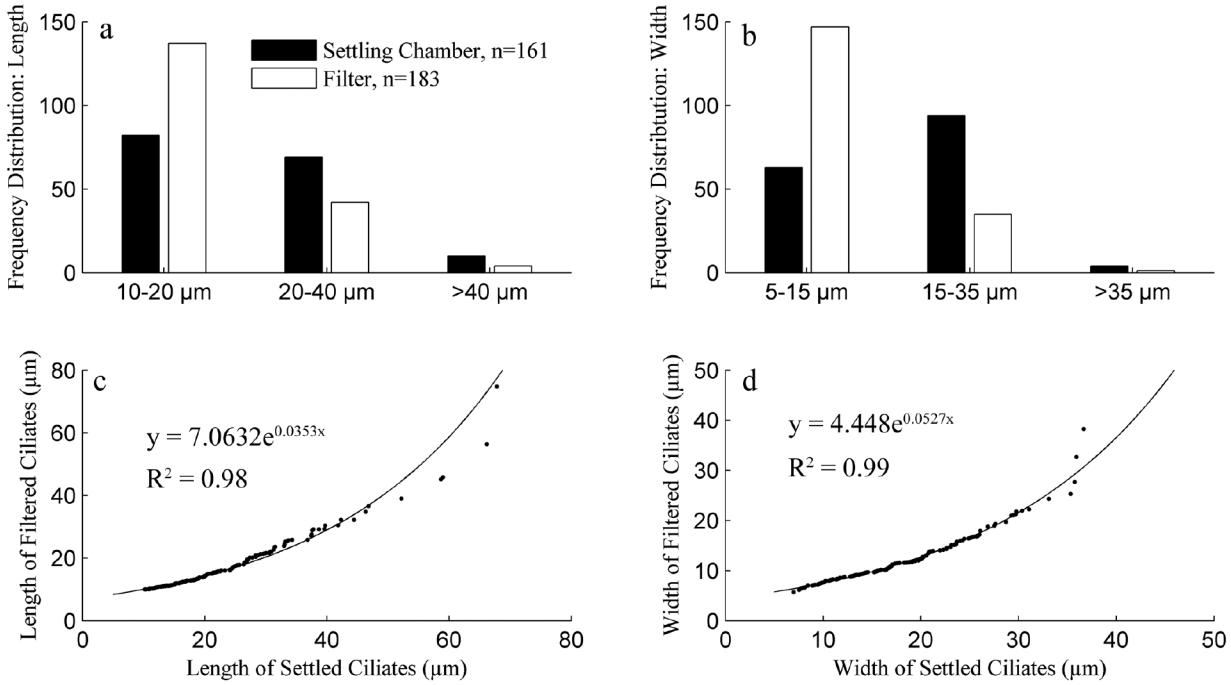

Fig. 4. Frequency distributions for measured lengths (*a*) and widths (*b*) of 161 ciliates in settled samples and 183 ciliates in filtered samples. Exponential regressions for the length ($y = 7.0632e^{0.0353x}$) and width ($y = 4.448e^{0.0527x}$) measurements in each method are shown in the bottom panels (*c-d*). Filtered cells were randomly chosen to create an equal number of measurements in both filtered and settled data sets for regression analysis.

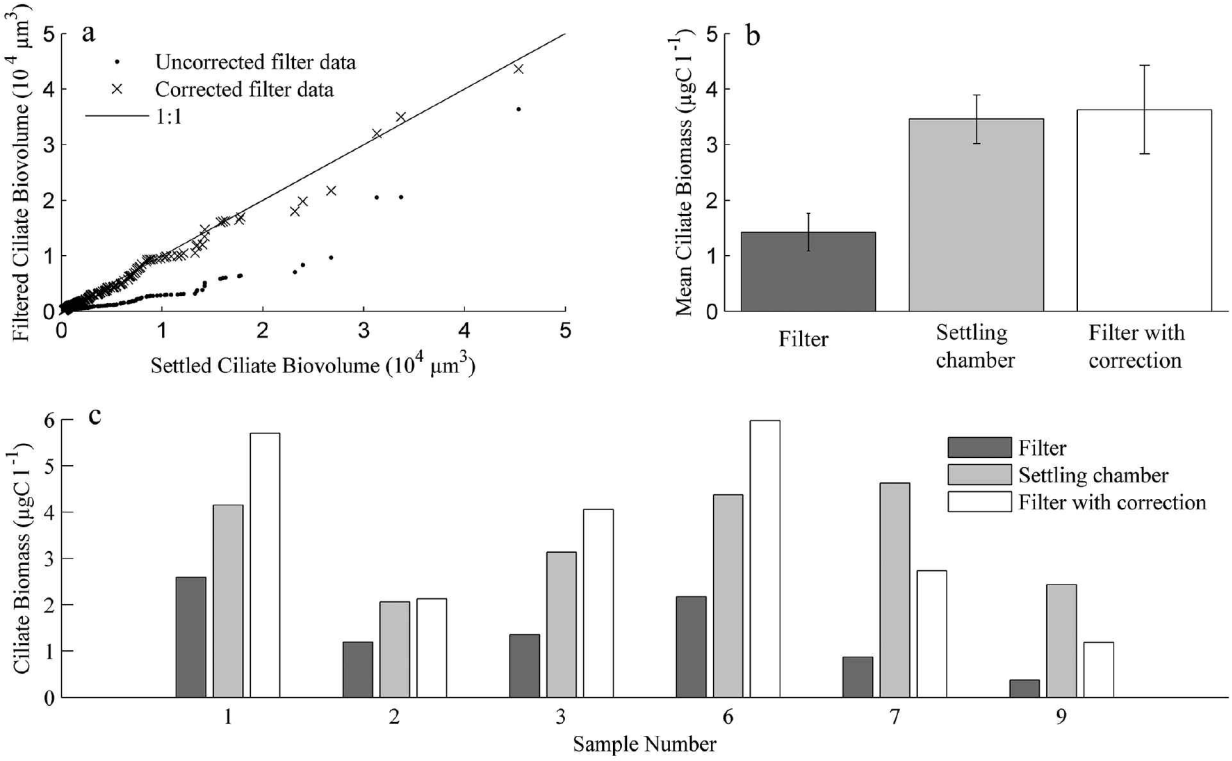

Fig. 5. Corrected and uncorrected biovolume values (10^4 µm^3) for 161 representative filtered ciliates compared with settled ciliate biovolumes across samples (*a*). Corrected filter biovolume values better approximate settled ciliate biovolumes than uncorrected values, indicated by a better fit to the 1:1 line. Total mean biomass (µg C L^{-1}) of all samples with standard error bars for filtered, settled, and corrected filter measurements (*b*). Total biomass per sample (µg C L^{-1}) for filter, settling chamber, and corrected filter data (*c*).

Discussion

In this study, we sought to develop a slide preparation procedure that would be an adequate alternative to the Utermöhl method for routine bright-field analyses of microplankton samples by automated image-analysis techniques. We emphasize ciliates in our assessment of this method because they are widely regarded to be the most delicate and fragile of the major microplankton functional groups, and due to incompatible preservation and handling methods, are often grossly underestimated in community assessments based on slides prepared for epifluorescence microscopy. As part of this process, we explored in depth a published resin-based method for permanent slide preparation (Crumpton 1987). While some thecate dinoflagellates fared relatively well with this technique, the heating and drying steps were much too damaging for ciliates. This limited further options for use of existing preparation methods that require similar steps.

In contrast, the slide method developed here is sufficiently gentle to retain these fragile cells on filters, thus allowing them to be readily visualized, counted, and sized by automated techniques that require rigid positioning of cells on an observational plane. Abundance and biomass estimates for ciliates enumerated on prepared slides compared favorably to those from the standard settling chamber method. Our data suggests a slight loss of cells, <10% on average, in the slide-making process, but this is far superior to results achieved in standard slide preparations for epifluorescence microscopy using aldehyde or alkaline Lugol's fixatives, which typically produce ciliate abundance estimates more than an order of magnitude lower than settling chambers (Taylor et al. 2011). Cell shrinkage, however, does need to be accounted for to achieve comparable estimates of size-composition and biomass relative to those for acid Lugol's preserved cells in settling columns alone.

This new slide-making technique is easy enough to be done at sea. It has the advantage that automated image acquisition systems can rapidly capture and retain permanent digital record of the slide contents for later analysis or reference, thereby reducing some of the tedium associated with traditional manual microscopic analysis. It also avoids sample degradation during bottle storage (Menden-Deuer et al. 2001), and reduces space requirements for long-term sample storage.

Comments and recommendations

Based on the present results, our new slide preparation method for seawater samples preserved in acid Lugol's can be an efficient and effective alternative to the traditional Utermöhl method for enumerating ciliates in environmental samples. Whereas this technique has advantages over the traditional approach in terms of preparation speed, automated imaging, shipboard use, and potentially less settling bias, it does not replace Utermöhl settling chambers as the standard for this kind of analysis, just as quantitative protargol stain (QPS) remains a superior alternative for taxonomic analysis and permanent storage (Montagnes and Lynn 1993). The cell volume shrinkage effects that we observed during the preparation process are likely to vary somewhat among locations and taxonomic groups (e.g., diatoms and dinoflagellates), and are especially critical to constrain assessments of biovolumes and biomass. It is therefore recommended that researchers intending to use this method determine appropriate correction factors for their study system and target organisms.

Whereas not tested rigorously, we expect that our method is well suited for dinoflagellates and diatoms, depending on the age and state of the sample being analyzed. Such organisms are typically more robust than ciliates and better able to stand up to filtration pressures and aldehyde fixatives. Menden-Deuer et al. (2001) have observed that dinoflagellates in acid Lugol's preservative can expand and lyse over time, whereas diatoms simply dissolve. If relative abundances of these taxa decline in long-term acid Lugol's storage, slide preparations with fresher samples, and digital image records, may reasonably provide more reliable estimates of plankton community composition and biomass than those from older bottled samples. However, whether the slide preparation stops the negative effects of long-term storage in acid Lugol's, or how long a prepared slide retains its original community composition and integrity cannot be answered here. At the time of this writing, initial slide preparations have been maintained for 9 months at –20°C as described with no detectable deterioration. However, this is a short time relative to the timescales at which samples comparisons may be relevant, for instance, to assess climate changes on decadal or longer timescales.

Advancing technologies, such as flow-through imaging cytometry of living cells (Olson and Sosik 2007), may eventually render community assessments from microscopy unnecessary. However, that is far from what is currently available to researchers around the world to study and compare ocean habitats. Ciliates are an important but frequently missing component in ocean ecosystem studies because they are relatively fragile and inadequately sampled by traditional methods for quantifying phytoplankton or net-collected zooplankton. We hope that the present technique provides a convenient way to include them more often in plankton community analyses.

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