

Comparison of different filter types on *chlorophyll-a* retention and nutrient measurements

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Abstract

In studies on the biochemical compounds in phytoplankton, water samples generally are (pre-) filtered to retain the organisms for extraction. Such filters can be used for further investigations in microscopic or chromatographic (for example High-Performance-Liquid-Chromatography, HPLC) methods, while the filtrates can be used for nutrient or fluorometric measurements as well as for microscopic examinations. Which filter is chosen for a study often depends on its pore size, the costs and, in particular for HPLC measurements, on its chemical compatibility. In our study we compared the *chlorophyll-a* retention on the filters by HPLC as well as the fluorescence before and after filtration, and nutrient content of the filtrates. The filters we tested were of different material and with various pore sizes. Although Whatman GF/C and GF/F filters are preferred in phytoplankton studies, we found that the Nylon Membrane filter of 0.2 μm pore size provided the most consistent results in *chlorophyll-a* retention and the one of 0.45 μm pore size in nutrient investigations.

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1. Introduction

The importance of picoplanktonic organisms in phytoplankton research has increased immensely over the last decades ever since the essential role of (pico-) phytoplankton organisms in the food webs was realized (Landry et al., 1996; Detmer and Bathmann, 1997; Zubkov et al., 2000; Guillou et al., 2001; Callieri and Stockner, 2002; Kawachi et al., 2002; Liu et al., 2002; Siokou-Frangou et al., 2002; Agawin et al., 2004; Not et al., 2004). The main methods of recognition of

picoplankters are microscopic examinations and genetic and biochemical analysis. The latter is usually less time consuming and places more of an emphasis on estimations of biomass parameters and cell-biochemical fingerprinting. An example of such biochemical fingerprinting and biomass evaluation is the High-Performance-Liquid-Chromatography (HPLC) determination of *chlorophyll-a* and other pigments. For nearly all biochemical measurements, size fractionations over different filters with decreasing pore widths are used to separate different size classes of phytoplankton preceding an analysis thereof. Such a filtration step is usually taken for granted as being a relatively conservative step in an extraction procedure and its influence on the results is often underestimated. Although some scientists like Marvin

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Table 1
Filter characteristics

Filter type	Pore size [μm]	Diameter [mm]	ID used in this study	Filtration volume [ml]
Nylon Membranes (Whatman)	0.45	47	Nylon 0.45	1000
Nylon Membranes (Whatman)	0.2	47	Nylon 0.2	750
Nuclepore Polycarbonate Track-Etch Membranes (Whatman)	0.4	47	Track-Etch 0.4	1000
Polycarbonate Track-Etch Membranes (Sartorius)	0.2	47	Track-Etch 0.2	350
Glas Microfibre GF/C (Whatman)	1.2	47	GF/C 1.2	1000
Glas Microfibre GF/F (Whatman)	0.7	47	GF/F 0.7	1000

et al. (1972), Lenz and Fritsche (1980), Prepas et al. (1988), Altabet (1990), Dickson and Wheeler (1993), Chavez and Buck (1995), Mantoura et al. (1997), Morán et al. (1999), Hashimoto and Shiomoto (2000) and Nayar and Chou (2003) made investigations on the effectiveness and influence on the results of different filters in phytoplankton research studies, these were only done to a small extent. Against the backdrop of our increasing interest in picophytoplankton organisms (0.2–3 μm) in aquatic systems, where filters often play a central role in the separation of the microalgae, the effectiveness of established filters in phytoplankton research studies must urgently be considered more detailed on a larger scale and the results circulated.

In this study we compared common filter types used in phytoplankton research, to evaluate how they differ with regard to *chlorophyll-a* retention (as an equivalent to phytoplankton biomass). Furthermore, because in our work we are interested in the nutrient composition of the water associated with the organisms, we analysed the

filtrates regarding to fluorescence (for escaped cells) and nutrient content. Thus, in comparison to previous studies, we examined more aspects in parallel using higher filtration volumes, more replicates, fluorometric measurements before and after filtration as well as nutrient investigations. With our study we give a complete and final comparison of the common filter types in phytoplankton research. The filters were: Whatman Nylon Membranes (Hexamethylenediamine; Nylon-66), Whatman and Sartorius Polycarbonate Track-Etch Membranes (4,4'-thydroxydiphenyl-2,2'-propane) and Whatman Glass Microfibres (GF/C and GF/F). Filter characteristics of the used pore size and the diameter are mentioned in Table 1. As you can see on the electron micrographs in Fig. 1, the material structure of the filters varies widely. Pictures were taken from the internet (Millipore.com, 2spi.com, Whatman.com).

2. Materials and methods

Water samples were taken at the end of the south pier of the North-East harbour of Helgoland, German Bight (54°11'02"N, 7°53'28"O), and were transferred into 5 and 10 l PE-bottles. Each 10 l sample was well mixed and used for ten replicates for each filter type. In synchrony five replicates were filtered with two water vacuum pumps. Because of the varying retentions (e.g. pore widths), the maximum filtration volume of the first filter of each type was used as a standard for the nine replicates (Table 1).

2.1. The extraction and analysis of chlorophyll-a after Wiltshire et al. (1998, 2000)

After filtration, each filter (60 in total) was transferred to a marked 15 mL polypropylene conical tube and 2 ml of 100% acetone (p.A. grade) were added for chemical extraction. Then the filters were stored

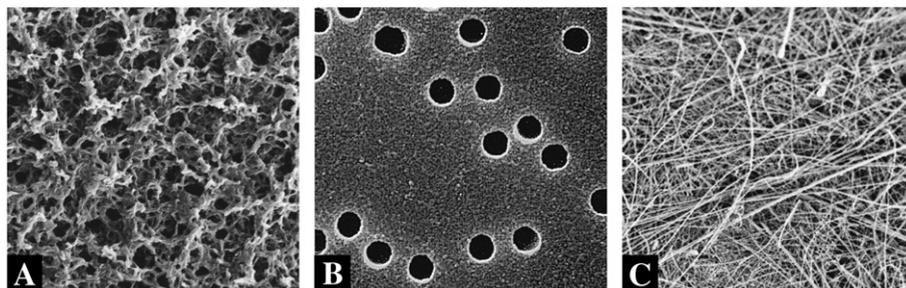


Fig. 1. Electron micrographs of the filters: A = Nylon Membrane (Millipore.com), B = Polycarbonate Track-Etch Membrane (2spi.com), C = Glass Microfibre (Whatman.com).

frozen ($-80\text{ }^{\circ}\text{C}$) and in the dark. Mechanical extraction was carried out after 48 h: a little bit of quartz-sand was added to all replicates of each filter and the samples were homogenized with a teflon-pestle. An ice-cooled ultrasound bath (*Bandelin Sonorex Super Rk 103/H*) for 90 min followed. With a syringe, each sample liquid was passed through a $0.2\text{ }\mu\text{m}$ filter (*Spartan 30/0,2 RC*) into a marked 1.5 mL HPLC vial.

In this study, a Waters 2695 Separation Module was used together with a Waters 996 Photodiode Array Detector, running with a Waters Millennium software programme. The C_{18} Nucleosil-column ($250\times 4\text{ mm}$) contained $5\text{ }\mu\text{m}$ packing material and was thermostated in a column oven at $15\text{ }^{\circ}\text{C}$. The autosampler was cooled to $4\text{ }^{\circ}\text{C}$. To obtain sharper peaks, $20\text{ }\mu\text{l}$ of distilled water (millipore) were injected before and after $60\text{ }\mu\text{l}$ of each sample (Wiltshire et al., 2000).

The following solvents were used: solvent “A” consisted of an ion-pairing reagent (1.5 g tetrabutylammonium acetate and 7.7 g ammonium acetate dissolved in 100 ml distilled water (millipore)), distilled water (millipore) and methanol (proportion 10:10:80, v/v). The second solvent (“B”) was an acetone:methanol mixture (10:90, v/v) and solvent “C” a propanol/methanol mix (7.7:10, v/v). The applied gradient of the different solvents is shown in Fig. 2 (for gradient-elution see Mantoura and Llewellyn, 1983). A flow rate of 1 ml/min, a pressure of 2900 psi and a run time of 40 min were chosen. Hence, the *chlorophyll-a* concentration is used as a marker for phytoplankton biomass, the instrument was calibrated using external *chlorophyll-a* standards in 100% acetone (0.2, 0.5, 1 and $4\text{ }\mu\text{g/l}$). For photometrical calibration to the exact concentration (Lambert–Beer–Law), the extinction coefficient of 88.15 (Jeffrey et al., 1997) was used. The chromatograms were detected at 430 nm and 668 nm and the *chlorophyll-a* concentration in each of the 60 samples

was determined by quantifying the peak areas at 430 nm (Peeken, 1997; Wiltshire et al., 1998).

2.2. Chlorophyll detection by fluorescence

The laboratory fluorometer of *bbe Moldaenke* measures the emission intensity at six wavelengths (370 nm, 450 nm, 525 nm, 570 nm, 590 nm and 610 nm) employing pulsed light-emitting diodes (LEDs). These wavelengths are characteristic for the norm spectra of the distinct spectral algal groups and yellow substances. Depending on the pigment composition in the water, a specific emission is measured by a detector. A software programme then calculates the concentrations of the different spectral groups and the total chlorophyll concentration (for further information see Beutler, 1998, 2003; Beutler et al., 2000, 2002).

In our study we carried out threefold fluorometric measurements for each well mixed initial 10 l sample (3 times 25 ml). These 10 l were then filtered over 10 replicates of each filter type (The exact filtration volumes are noted in Table 1). To see how much total chlorophyll went through the filters, each filtrate of all replicates was directly measured again threefold (3 times 25 ml). Afterwards we compared the results of the total chlorophyll content before and after the filtration step.

2.3. Nutrients

To see if the different filter types release chemicals, the nutrient content was measured threefold in each filtrate of all replicates. The methods used here were established by Grasshoff (1976). For a more detailed picture about the influence of each filter on the nutrient content in the associated filtrate, we carried out measurements with distilled water (millipore) in addition. We therefore calculated the nutrient content threefold in the unfiltered water and after filtration of 1 l over each filter type, we calculated the nutrient content threefold in each filtrate. After comparing the results we decided to carry out parallel analysis for the two filters that seemed most suitable for nutrient measurements in filtrates. We therefore took 25 seawater samples on changing dates and calculated the nutrient content in the filtrates.

3. Results

3.1. Chlorophyll-a detection by HPLC

The chromatographic results of the *chlorophyll-a* concentration on the different filter types (Table 1) differed widely (Fig. 3): the replicates of the Nylon 0.45

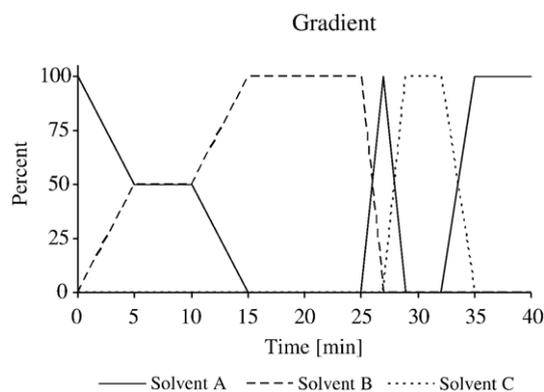


Fig. 2. Gradient of solvents A, B and C in the HPLC run.

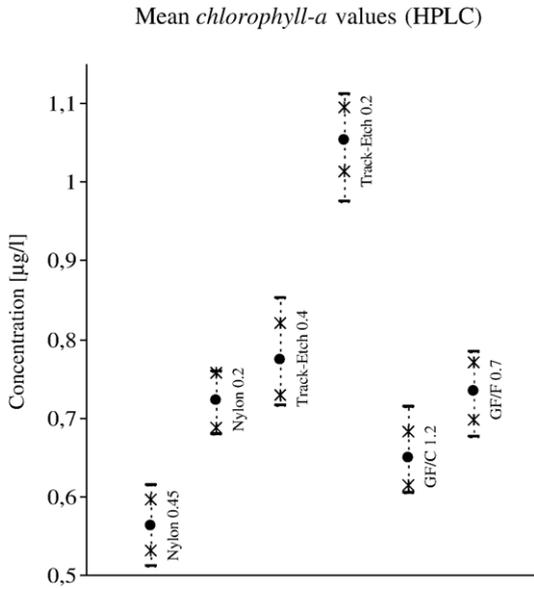


Fig. 3. Mean *chlorophyll-a* content on the replicates of the different filter types measured by HPLC. Dots show the mean values of the respective replicates, lines indicate the range between lowest and highest value and crosses the standard deviation.

filter showed the lowest mean *chlorophyll-a* retention with 0.56 µg/l and the lowest standard deviation of 0.032 µg/l. Its concentration was lower than that of the GF/F 0.7 (0.73 µg/l, standard deviation: 0.037 µg/l) and the GF/C 1.2 (0.65 µg/l, standard deviation: 0.034 µg/l) filter replicates, although the pore width was smaller and hence it must have retained more particles. The Track-Etch filters lay in an expected correlation to the two Glass Microfibre filters: the Track-Etch 0.4 filters led to a *chlorophyll-a* concentration of 0.78 µg/l with the highest standard deviation of 0.046 µg/l, while the Track-Etch 0.2 caused a concentration of 1.05 µg/l (standard

deviation: 0.041 µg/l). With the Nylon 0.2 replicates a high convergence around the mean concentration of 0.72 µg/l (standard deviation: 0.035 µg/l) was measured. This result is comparable to that of the GF/F 0.7 filters with 0.73 µg/l. It was suspected that filter types with a larger pore width would show lower *chlorophyll-a* concentrations, but there seemed to be no such correlation. However, when comparing filter types of the same material, the lower pore size always showed, as was expected, the higher *chlorophyll-a* concentration i.e. Nylon 0.2 with 0.72 µg/l and Nylon 0.45 with 0.56 µg/l.

According to the variations between the ten repetitive measurements of each filter (scale bars in Fig. 3), the Nylon 0.2 filter showed the most constant values. Its difference between smallest and highest value was only 0.08 µg/l (standard deviation: 0.035 µg/l). While the Nylon 0.45 and the Glass Microfibre filters lay in the region of 0.1 µg/l difference, the Track-Etch filters showed discrepancies of up to 0.13 µg/l.

3.2. Chlorophyll detection by fluorescence

Comparing the fluorescence of each sample before and after filtration, showed that the different filter types released varying amounts of phytoplanktonic organisms to the filtrate (Fig. 4). The only filter type whose replicates were very constant in the releasing percentage (7.7 to 13.8%) and showed the smallest loss of phytoplankton cells (10.5% in average), was the Nylon 0.2. Furthermore its standard deviation was at the lowest (1.3%). However, the GF/F 0.7 filters gave good results as well: they discharged only 9.9 to 20% (mean: 14.9%) chlorophyll and had a standard deviation of 2.6%. The Track-Etch 0.2 (12–27.1%, mean: 18.2%) and Track-Etch 0.4 filters (15.6–30.5%, mean: 22.2%)

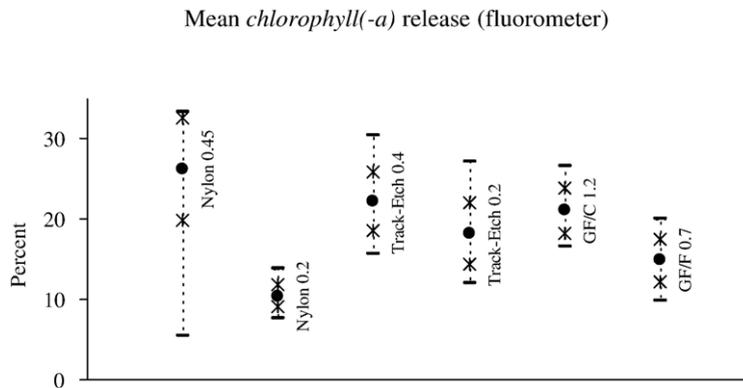


Fig. 4. Mean *chlorophyll-a* release measured by fluorescence before and after filtration. Dots show the mean values of the respective replicates, lines indicate the range between lowest and highest value and crosses the standard deviation.

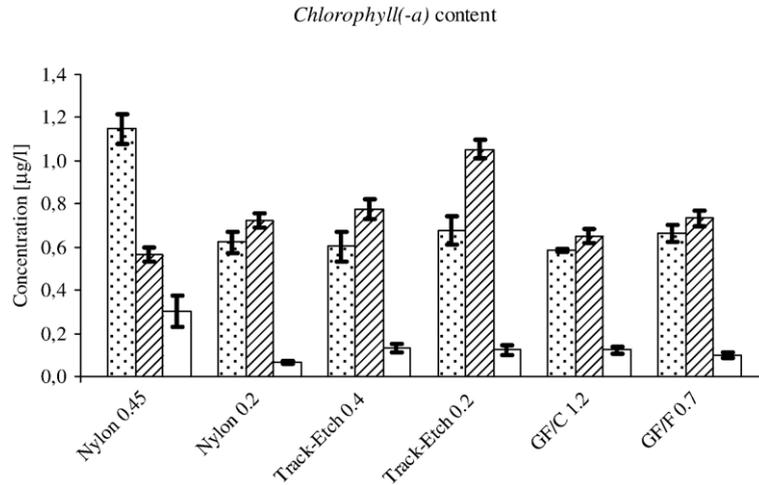


Fig. 5. Comparison of the *chlorophyll(-a)* concentration. Dots: fluorescence in initial samples, lines: absorption by HPLC, white: fluorescence in filtrates (bars = standard deviation).

had nearly the same standard deviation (~3.7%) and showed almost the same range and mean percentage of release as the GF/C 1.2 filters (16.5–26.7%, mean: 21.6%). But its standard deviation was lower: 2.7%. The worst result was given by the Nylon 0.45 filters because their average release was the highest of all filter types with 26.3%. Furthermore, the releasing values of the replicates differed between 5.4 and 33.4% and the standard deviation was at 6.4%. As for the *chlorophyll-a* concentrations measured by HPLC, there was no correlation between the different pore sizes in the fluorometric results as well.

A comparison of the chlorophyll concentration measured by fluorescence in the initial 10 l seawater samples, by absorption (HPLC) of the filter retentions and by fluorescence in the filtrates is shown in Fig. 5. The absorptions were, except in case of the Nylon 0.45 filters, higher than those measured by fluorescence in the analogical natural sample. For the Nylon 0.45 filter the initial fluorescence results (mean: 1.15 µg/l) were twice the absorption value (mean: 0.56 µg/l). Although the primary average fluorescence of the other filter types was nearly the same (Nylon 0.2: 0.62 µg/l, Track-Etch 0.4: 0.60 µg/l, Track-Etch 0.2: 0.68 µg/l, GF/C 1.2: 0.59 µg/l and GF/F 0.7 µg/l: 0.67 µg/l), the absorption results varied. The replicates of the Track-Etch 0.2 filter (1.05 µg/l) led to around 0.33 µg/l higher *chlorophyll-a* values than the other filter types: Nylon 0.2: 0.72 µg/l, Track-Etch 0.4: 0.78 µg/l, GF/C 1.2: 0.65 µg/l, GF/F 0.7: 0.73 µg/l. The filtrate fluorescence of the replicates did not show any correlation to the fluorescence in the initial water samples or to the divergence between initial fluorescence and HPLC values. Only the results of the

Nylon 0.45 filters are absolutely plausible: a high fluorescence in the initial seawater sample and a lower concentration on the filters results in a high fluorescence of the filtrates.

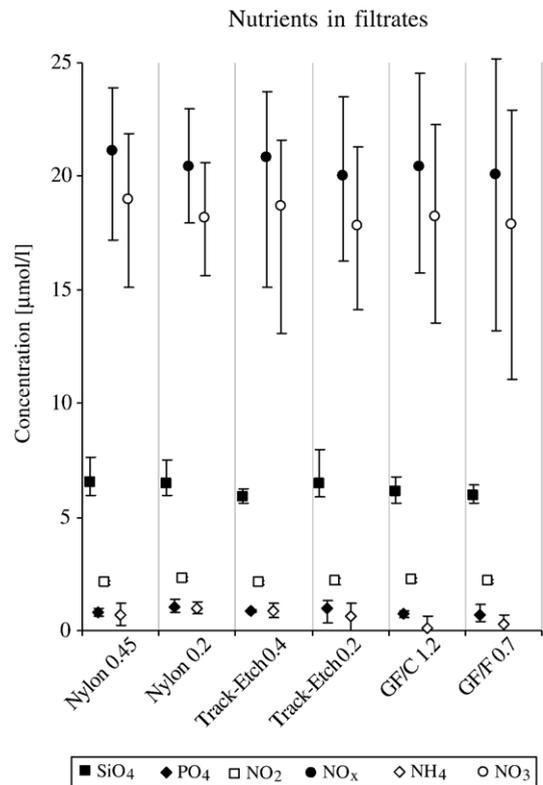


Fig. 6. Mean nutrient contents in the different seawater filtrates. Dots: mean values of the respective replicates, bars: range between lowest and highest value.

3.3. Nutrients

Fig. 6 shows that the nutrient contents in the different seawater filtrates did not vary significantly and that the mean values of each nutrient always lay in the range of the other filtrates (all filtrates of the 10 replicates for each filter type were measured threefold, calculation after Grasshoff 1976). Obvious to see is that the concentrations of NO_x and the calculated nitrate (NO_3) were most variable in the replicates. Because of these variations, which also result from different conditions of the cadmium column, it cannot be said if there is an influence of the filters on the nutrient content.

Because these results did not satisfy us in the clarity of different influences on the nutrients, we filtered 1 l of distilled water (millipore) over each filter type for a better comparison and used its initial nutrient content as blank value. The average concentrations of the threefold nutrient measurement of the different filtrates are shown in Table 2. While the Nylon 0.2 and Track-Etch 0.2 filter had a slight influence on the silicate (SiO_4) content (0.01 and 0.04 $\mu\text{mol/l}$), the other filters did not show an influence. The phosphate (PO_4) and nitrite (NO_2) concentrations never changed, independent of the used filter. The Nylon 0.2 filter manipulated the NO_x and therefore the nitrate (NO_3) concentration at the most (calculated after Grasshoff 1976): 0.13 $\mu\text{mol/l}$. While the Track-Etch 0.2 and the GF/C 1.2 filter had no effect on this nutrient, the other filters changed it somewhat: Nylon 0.45: 0.04 $\mu\text{mol/l}$, Track-Etch 0.4: 0.02 $\mu\text{mol/l}$ and GF/F 0.7: 0.01 $\mu\text{mol/l}$. Once more we need to mention, that the changes in the NO_x and nitrate concentrations vary as well because of the different

Table 2
Mean differences of nutrient contents in the different filtrates of distilled water

Filter type	SiO_4 [$\mu\text{mol/l}$]	PO_4 [$\mu\text{mol/l}$]	NO_2 [$\mu\text{mol/l}$]	NO_3 [$\mu\text{mol/l}$]	NO_x [$\mu\text{mol/l}$]	NH_4 [$\mu\text{mol/l}$]
Nylon 0.45	0	0	0	0.04	0.04	0
Nylon 0.2	0.01	0	0	0.13	0.13	0.56
Track-Etch 0.4	0	0	0	0.02	0.02	0.06
Track-Etch 0.2	0.04	0	0	0	0	-0.02
GF/C 1.2	0	0	0	-0.03	-0.03	-0.03
GF/F 0.7	0	0	0	0.01	0.01	0.03

Table 3
Asymptotic *t*-test, calculated after Osius (1999)

	SiO_4 [$\mu\text{mol/l}$]	PO_4 [$\mu\text{mol/l}$]	NO_2 [$\mu\text{mol/l}$]	NO_3 [$\mu\text{mol/l}$]	NO_x [$\mu\text{mol/l}$]	NH_4 [$\mu\text{mol/l}$]
Nylon 0.45	1.62	0.34	0.38	14.20	14.57	0.94
Track-Etch 0.4	1.67	0.43	0.16	14.64	14.80	1.03
<i>t</i>	7.27	20.44	-42.73	73.85	64.83	28.12

conditions of the cadmium column. On the ammonium (NH_4) concentration, the Nylon 0.2 filter had again the highest influence: 0.56 $\mu\text{mol/l}$ in average. While the Track-Etch 0.4 (0.06 $\mu\text{mol/l}$) and the GF/F filters (0.03 $\mu\text{mol/l}$) changed this nutrient slightly, the other two filters (Track-Etch 0.2 and GF/C) did not change its concentration.

The four filter types with the smallest influence on the nutrient content were: Nylon 0.45, Track-Etch 0.4, GF/C 1.2 and GF/F 0.7. Hence the flow rate of the GF/F 0.7 filter was very low (small pore size), and the pore size of the Nylon 0.45 and the Track-Etch 0.4 filters define the division point (0.45 μm) between dissolved and particulate matter (Hickel 1984; Horowitz et al. 1992) more precisely, we tested the last two filters further on.

With the mentioned filters, we carried out a comparison of 25 different seawater samples. The results were processed with a one-sided asymptotic *t*-test (Osius 1999). The 0-hypothesis was $H_0: \mu_{\text{Track-Etch}} \leq \mu_{\text{Nylon}}$ and the test level α was chosen to be 1%, therefore was $t_{m;\alpha} = 2403$. Table 3 shows the average values of the nutrient measurements and the results for *t*. Only for nitrite H_0 was accepted because $t < t_{m;\alpha}$. For the other nutrients H_0 was denied. This means that the Track-Etch 0.4 filter increased the content of silicate, phosphate, nitrate and ammonium in the natural samples in comparison to the Nylon 0.45 filter.

Table 4
Summary of absorption and fluorescence results

Filter type	<i>Chlorophyll-a</i> absorption [$\mu\text{g/l}$]	<i>Chlorophyll-a</i> release [%]
Nylon 0.45	0.51–0.61	05.4–33.4
Nylon 0.2	0.68–0.76	07.7–13.8
Track-Etch 0.4	0.72–0.85	15.6–30.5
Track-Etch 0.2	0.98–1.11	12.0–27.1
GF/C 1.2	0.60–0.71	16.6–26.7
GF/F 0.7	0.68–0.78	09.9–20.0

4. Discussions

Studying phytoplankton usually entails filtering a water sample either for use of the filtrate or the filters for further studies (Mantoura et al., 1997). Consequently, filtration is a main and important step. However, until today no comparing tests of the different filter types used in our study were carried out to see which one is the most suitable. Especially in the last years, now that the important role of picoplankton is accepted, filters with smaller pore sizes were chosen more often. But, regarding to the review in Mantoura et al. (1997), do they give better results in the *chlorophyll-a* retention? In our study we used natural seawater as well as distilled water and six different filters (Table 1) to see which one is the best for phytoplankton and nutrient measurements in oceanography. We compared three different filter materials with varying pore sizes. Our results show that there was no correlation between pore size and *chlorophyll-a* retention (Fig. 3, Table 4; compare Lenz and Fritsche, 1980) or pore size and releasing amount (Fig. 4, Table 4) of the different filter types. Only the results of each single filter type correlated to the pore widths: the smaller pore size resulted in higher absorption values and lower release. This inability to combine pore sizes of different filter types with the amount of released and/or retained particles was mentioned by other authors before. Sheldon (1972) recognized that the shape and the amount of particles in a water sample can influence the effectiveness of the special pore size. According to the Whatman® Glass Microfibre filters, Hickel (1984) mentioned, that the pore size is not well defined. Therefore these filters are supposed to be “unsuitable for size-fractionation” (Mantoura et al., 1997, compare Fig. 1). Our results

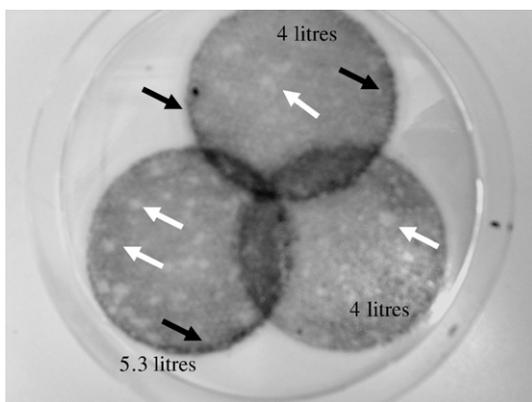


Fig. 7. Irregular distribution of phytoplanktonic organisms on 10 μm filters (diameter: 47 mm). White arrows: low organism amount, black arrows: high organism amount.

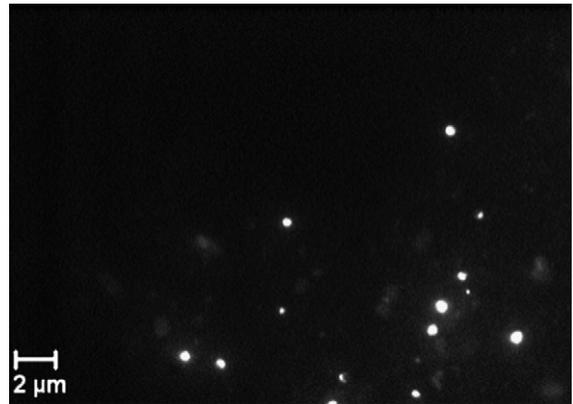


Fig. 8. Example of irregular distribution of organisms on a filter prepared for FISH analysis.

correlate with those of Morán et al. (1999). They found no significant differences between the *chlorophyll-a* retention of Glass Microfibre filters (GF/F, GF/C) and membrane filters (polycarbonate 0.2, mixed cellulose esters 0.22) as well. However, Mantoura et al. (1997) decided to use GF/F filters in their pigment experiments because they seemed to retain close to 100% of the pigments. With our study we checked the effectiveness of established filters in phytoplankton research studies to draw the attention on not enough considered problems.

The Nylon 0.45 filter showed the highest (26.3%) but also most dissimilar values (standard deviation: 6.4%) for the release of organisms and its replicates gave the lowest *chlorophyll-a* absorption (0.564 $\mu\text{g/l}$). These variable results make it unsuitable for phytoplankton studies. Both Track-Etch filters and the GF/C filter almost had the same releasing percentage to the original sample ($\sim 20.5\%$). By comparing these three filters, the *chlorophyll-a* retention arose, as suspected, with decreasing pore size. Although our results support Prepas et al. (1988), who claimed that GF/C 1.2 and GF/F 0.7 do not differ widely in their *chlorophyll-a* retention, in our study the GF/F 0.7 filter showed a more obvious similarity with the Nylon 0.2 filter. This fact has already been presented by Hashimoto and Shiomoto (2000): they found no significant difference between GF/F 0.7 and 0.2 μm nucleopore filters. On the other hand, Altabet (1990) has described a significant particle release through GF/F 0.7 filters, which in turn is held back by 0.2 μm filters. These unsteady results of the Glass Microfibre filters lead us to a comparison of the filters of 0.2 μm pore width used in our study. Although on the first view the Track-Etch 0.2 filters seemed to be the most suitable filter type in phytoplankton studies because of the high *chlorophyll-a* retention (1.054 $\mu\text{g/l}$), its high organism release (18.2%) and the high standard

deviation in the HPLC results (0.041 µg/l) put the authenticity of the high retention in question. In contrast, the Nylon 0.2 filter led to the most reliable and small ranged results in phytoplankton studies: its replicates led to the lowest release (10.5%) and to a low standard deviation in the absorption results (0.035 µg/l).

When we compare the fluorescence in the initial seawater samples and the absorption measurements (HPLC) of the filter replicates (Fig. 5), we need to remember that the two methods base on totally different aspects: 1) absorption is measured for all pigments present, while fluorescence only detects pigments in living organisms with intact photosystems, 2) absorption is only estimated in the filter fraction, whereas fluorescence is measured in the whole initial water sample and 3) the fluorometer measures the whole chlorophyll content, while with the HPLC the absorption of *chlorophyll-a* is detected. Consequently, these two methods cannot be compared in their *chlorophyll(-a)* results.

In our study the Nylon 0.45 filters did not resemble the high amount of fluorescent particles in the initial water sample in their absorption results. This can be explained by the small size of fluorescent particles (<0.45 µm). The other filters showed slightly higher levels in the absorption than in the fluorescence of the whole sample. There seemed to be no dependency between the initial fluorescence, the absorption of the filter retention and the fluorescence in the filtrates to the pore size (Fig. 5).

The phytoplankton studies, which generally preferred GF/C and GF/F filters, did not show convincing results in our study and in considering previous publications. Furthermore, their handling in the sample preparation for the HPLC method was very difficult because the glass microfibrils absorbed all the solution in the homogenization step. Hence it was not easy to get enough liquid into the syringe for the injection into the HPLC instrument. In contrast, the organisms were easily extracted from the Nylon and Track-Etch filters in the same step and their sample liquid was withdrawn into the syringe without difficulty because the filters nearly remained in one and did not absorb the added acetone. Hence the Polycarbonate Track-Etch filters are not recommended for use with acetone (Whatman.com) and the nuclepore ones “contain extractable yellow dye(s)” (Mantoura et al., 1997), their HPLC results should be handled with care. Consequently we conclude that the Nylon filters are most suitable for absorption measurements as we carried them out.

According to Horowitz et al. (1992) we tried to eliminate all factors that can influence the nutrient content in a filtrate. Furthermore, we worked out the samples in parallel and do not see any kind of storage

effect (Kattner, 1999). Hence, we only have different filter types with varying pore sizes as variable factors in our analysis. Next to the phosphate, also the nitrite concentration was not changed by any of the filters. In comparison, the nitrate and ammonium contents were most frequently influenced. This correlates with the results of Marvin et al. (1972) for the filtration of distilled water over unwashed filters. Our results of the different comparing tests bring us to the conclusion, that Nylon 0.45 filters are the most suitable ones in nutrient studies. They affect the nutrient content at the lowest and furthermore their pore size of 0.45 µm exactly defines the division point between dissolved and particulate matter (Hickel, 1984; Horowitz et al., 1992) and makes it possible to work with a higher flow rate.

5. Conclusions

We would like to add, that filtrations do not provide an homogeneous distribution of phytoplanktonic organisms on the filter. As you can see in Fig. 7 the organisms are dispersed very irregularly, although in this case we filtered large volumes of seawater with a maximum pressure of 200 mbar. The white arrows indicate areas with unusual low organism amounts that can be caused by soil particles, gelatinous colonies or irregular filtration. Black arrows indicate unusual high amounts that are mainly found at the filter periphery and are caused by the volume holder. Scientists should always keep this fact of irregular spreading of organisms on filters in mind when analysing them. Especially when only small parts of the filter are analysed, as in microscopic investigations (e.g. FISH, Fig. 8).

After this detailed comparison of the six different filters, we suppose that the Nylon 0.2 filter type is the most suitable one in phytoplankton studies particularly with regard to the retention of picophytoplankton. Its small pore size and therefore its high retention and low release of organisms make it ideal for investigations on the whole phytoplankton community. Furthermore, the Nylon 0.45 filter is the most suitable one for nutrient measurements in seawater samples because it influenced the nutrient content in the filtrate at the lowest: In tests with distilled water (millipore, Table 2) it changed only the NO_x and therefore nitrate (NO₃) results slightly. These variations may also occur from the different conditions of the cadmium column. In a more complex parallel measurement with the Track-Etch 0.4 filter, it only influenced the nitrite (NO₂) content. Furthermore it showed a high flow rate in the filtration step.

Hence the *chlorophyll(-a)* content in algae is influenced by many abiotic factors like light, temperature

and nutrients (Van Leeuwe and Stefels, 1998; Marañón et al., 2000; Barlow et al., 2002; Lalli and Parsons, 2002; Malinsky-Rushansky et al., 2002; Sosik and Olson, 2002), we recommend not to increase the probability of a wrong measurement by using an unsuitable filter. We were able to show how various the results of different filter types with same pore sizes can be. With this work we hope to help other scientists in choosing the best filters for phytoplankton and nutrient studies and to decrease possible errors.

References

- Agawin, N.S.R., Duarte, C.M., Agustí, S., Vaqué, D., 2004. Effect of N:P ratios on response of Mediterranean picophytoplankton to experimental nutrient inputs. *Aquat. Microb. Ecol.* 34, 57–67.
- Altabet, M.A., 1990. Organic C, N, and stable isotopic composition of particulate matter collected on glass-fiber and aluminium oxide filters. *Limnol. Oceanogr.* 35/4, 902–909.
- Barlow, R.G., Aiken, J., Holligan, P.M., Cummings, D.G., Maritorena, S., Hooker, S., 2002. Phytoplankton pigment and absorption characteristics along meridional transects in the Atlantic Ocean. *Deep-Sea Res., Part 1, Oceanogr. Res. Pap.* 47, 637–660.
- Beutler, M., 1998. Entwicklung eines Verfahrens zur quantitativen Bestimmung von Algengruppen mit Hilfe computergestützter Auswertung spektralaufgelöster Fluoreszenzanregungsspektren. Diplomarbeit der Mathematisch-Naturwissenschaftlichen Fakultät der Christian-Albrechts-Universität zu Kiel.
- Beutler, M., 2003. Spectral fluorescence of chlorophyll and phycobilins as an in-situ tool of phytoplankton analysis-models, algorithms and instruments; Dissertation zur Erlangung des Doktorgrades der Mathematisch-Naturwissenschaftlichen Fakultät der Christian-Albrechts-Universität zu Kiel.
- Beutler, M., Wiltshire, K.H., Lüring, C., Moldaenke, C., 2000. Fluorometric depth-profiling of chlorophyll corrected for yellow substances. Poster Presentation. ASLO.
- Beutler, M., Wiltshire, K.H., Meyer, B., Moldaenke, C., Lüring, C., Meyerhöfer, M., Hansen, U.-P., Dau, H., 2002. A fluorometric method for the differentiation of algal populations in vivo and in situ. *Photosynth. Res.* 72, 39–53.
- Callieri, C., Stockner, J.G., 2002. Freshwater autotrophic picoplankton: a review. *J. Limnol.* 61/1, 1–14.
- Chavez, F.P., Buck, K.R., 1995. On the chlorophyll a retention properties of glass-fiber GF/F filters. *Limnol. Oceanogr.* 40/2, 428–433.
- Detmer, A.E., Bathmann, U.V., 1997. Distribution patterns of autotrophic pico- and nanoplankton and their relative contribution to algal biomass during spring in the Atlantic sector of the Southern Ocean. *Deep-Sea Res., Part 2, Top. Stud. Oceanogr.* 44, 299–320.
- Dickson, M.-L., Wheeler, P.A., 1993. Chlorophyll *a* concentrations in the North Pacific: does a latitudinal gradient exist? *Limnol. Oceanogr.* 38/8, 1813–1818.
- Grasshoff, K., 1976. *Methods of Seawater Analysis*. Verlag Chemie, Weinheim.
- Guillou, L., Jacquet, S., Chrétiennot-Dinet, M.-J., Vaulot, D., 2001. Grazing impact of two small heterotrophic flagellates on *Prochlorococcus* and *Synechococcus*. *Aquat. Microb. Ecol.* 26, 201–207.
- Hashimoto, S., Shiimoto, A., 2000. Comparison of GF/F filters and 0.2 and 0.6 μm nuclepore filter on the chlorophyll a retention. *Bull. Natl. Res. Inst. Far Seas Fish. Shimizu* 37, 45–48.
- Hickel, W., 1984. Seston retention by Whatman GF/C glass-fiber filters. *Mar. Ecol. Prog. Ser.* 16, 185–191.
- Horowitz, A.J., Elrick, K.A., Colberg, M.R., 1992. The effect of membrane filtration artifacts on dissolved trace element concentrations. *Water Res.* 26/6, 753–763.
- Jeffrey, S.W., Mantoura, R.F.C., Bjørnland, T., 1997. Data for the identification of 47 key phytoplankton species. In: Jeffrey, S.W., Mantoura, R.F.C., Wright, S.W. (Eds.), *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods*. UNESCO, Paris, pp. 449–559.
- Kattner, G., 1999. Storage of dissolved inorganic nutrients in seawater: poisoning with mercuric chloride. *Mar. Chem.* 67, 61–66.
- Kawachi, M., Atsumi, M., Ikemoto, H., Miyachi, S., 2002. *Pinguicrhysis pyriformis* gen. et sp. nov. (Pinguicophyceae), a new picoplanktonic alga isolated from the Pacific Ocean. *Phycol. Res.* 50, 49–56.
- Lalli, C.M., Parsons, T.R., 2002. *Biological oceanography an introduction*, The Open University, Butterworth-Heinemann, An imprint of Elsevier Science, Great Britain, second edition.
- Landry, M.R., Kirshtein, J., Constantinou, J., 1996. Abundances and distributions of picoplankton populations in the central equatorial Pacific from 12°N to 12°S, 140°W. *Deep-Sea Res., Part 2, Top Stud. Oceanogr.* 43, 871–890.
- Lenz, J., Fritsche, P., 1980. The estimation of chlorophyll a in water samples: a comparative study on retention in a glass-fibre and membrane filter and on the reliability of two storage methods. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* 14, 46–51.
- Liu, H., Suzuki, K., Minami, C., Saino, T., Watanabe, M., 2002. Picoplankton community structure in the subarctic Pacific Ocean and the Bering Sea during summer 1999. *Mar. Ecol. Prog. Ser.* 237, 1–14.
- Malinsky-Rushansky, N., Berman, T., Berner, T., Yakobi, Y.Z., Dubinsky, Z., 2002. Physiological characteristics of picophytoplankton, isolated from Lake Kinneret: responses to light and temperature. *J. Plankton Res.* 24/11, 1173–1183.
- Mantoura, R.F.C., Llewellyn, C.A., 1983. The rapid determination of algal chlorophyll and carotenoids pigments and their breakdown products in natural waters by reverse-phase HPLC. *Anal. Chim. Acta* 151, 297–314.
- Mantoura, R.F.C., Wright, S.W., Jeffrey, S.W., Barlow, R.G., Cunnings, D.E., 1997. Filtration and storage of pigments from microalgae. In: Jeffrey, S.W., Mantoura, R.F.C., Wright, S.W. (Eds.), *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods*. UNESCO, Paris, pp. 283–305.
- Marañón, E., Holligan, P.M., Varela, M., Mourino, B., Bale, A.J., 2000. Basin-scale variability of phytoplankton biomass, production and growth in the Atlantic Ocean. *Deep-Sea Res., Part 1, Oceanogr. Res. Pap.* 47, 825–857.
- Marvin, K.T., Proctor Jr., R.R., Neal, R.A., 1972. Some effects of filtration on the determination of nutrients in fresh and salt water. *Limnol. Oceanogr.* 17/5, 777–784.
- Morán, X.A.G., Gasol, J.M., Arin, L., Estrada, M., 1999. A comparison between glass fibre and membrane filters for the estimation of phytoplankton POC and DOC production. *Mar. Ecol. Prog. Ser.* 187, 31–41.
- Nayar, S., Chou, L.M., 2003. Relative efficiencies of different filters in retaining phytoplankton for pigment and productivity studies. *Estuar. Coast. Shelf Sci.* 58, 241–248.
- Not, F., Latasa, M., Marie, D., Cariou, T., Vaulot, D., Simon, N., 2004. A single species, *Micromonas pusilla* (Prymnesiophyceae), dominates the eukaryotic picoplankton in the Western English Channel. *Appl. Environ. Microb.* 70, 4064–4072.

- Osius, G., 1999. Material zur Veranstaltung - Statistik in den Naturwissenschaften, Institut für Statistik, Fachbereich Mathematik/Informatik, Universität Bremen.
- Peeken, I., 1997. Photosynthetic pigment fingerprints as indicators of phytoplankton biomass and development in different water masses of the Southern Ocean during austral spring. *Deep-Sea Res., Part 2, Top Stud. Oceanogr.* 44, 261–282.
- Prepas, E.E., Dunnigan, M.E., Trimbee, A.M., 1988. Comparison of in situ estimates of chlorophyll a obtained with Whatman GF/F and GF/C glass-fiber filters in mesotrophic to hypertrophic lakes. *Can. J. Fish Aquat. Sci.* 45/5, 910–914.
- Sheldon, R.W., 1972. Size separation of marine seston by membrane and glass-fiber filters. *Limnol. Oceanogr.* 17/3, 494–498.
- Siokou-Frangou, I., Bianchi, M., Christaki, U., Christou, E.D., Giannakourou, A., Gotsis, O., Ignatiades, L., Pagou, K., Pitta, P., Psarra, S., Souvermezoglou, E., Van Wambeke, F., Zervakis, V., 2002. Carbon flow in the planktonic food web along a gradient of oligotrophy in the Aegean Sea (Mediterranean Sea). *J. Mar. Syst.* 33–34, 335–353.
- Sosik, H.M., Olson, R.J., 2002. Phytoplankton and iron limitation of photosynthetic efficiency in the Southern Ocean during late summer. *Deep-Sea Res., Part 1, Oceanogr. Res. Pap.* 49, 1195–1216.
- Van Leeuwe, M.A., Stefels, J., 1998. Effects of iron and light stress on the biochemical composition of Antarctic *Phaeocystis* sp. (Prymnesiophyceae). II. Pigment composition. *J. Phycol.* 34, 496–503.
- Wiltshire, K.H., Boersma, M., Möller, A., Buhtz, H., 2000. Extraction of pigments and fatty acids from the green alga *Scenedesmus obliquus* (Chlorophyceae). *Aquat. Ecol.* 34, 119–126.
- Wiltshire, K.H., Harsdorf, S., Smidt, B., Blöcker, G., Reuter, R., Schroeder, F., 1998. The determination of algal biomass (as chlorophyll) in suspended matter from the Elbe estuary and the German Bight: a comparison of high-performance liquid chromatography, delayed fluorescence and prompt fluorescence methods. *J. Exp. Mar. Biol. Ecol.* 222, 113–131.
- Zubkov, M.V., Sleight, M.A., Burkill, P.H., Leakey, R.J.G., 2000. Picoplankton community structure on the Atlantic Meridional Transect: a comparison between seasons. *Prog. Oceanogr.* 45, 369–386.