

The quantitative filter technique for measuring phytoplankton absorption: Interference by MAAs in the UV waveband

Isabelle Laurion¹, Frédéric Blouin, and Suzanne Roy

Institut des Sciences de la Mer, Université du Québec à Rimouski, 310 Allée des Ursulines, Rimouski, Québec G5L 3A1, Canada.

Abstract

The absorption of suspended particulate material is commonly estimated with the filter pad technique, which requires a correction for pathlength amplification. The pathlength amplification factor (β) varies among different studies and phytoplankton species or communities. It remains the largest source of uncertainty in estimated absorption coefficients. Recently, several empirical models estimating this correction were developed but mostly for visible range. In this study, β was calculated empirically from the ratio of filtered to suspension absorption between 280 and 850 nm for cultures of two dinoflagellates and one diatom. Results show that in the visible waveband, β values are relatively constant and fall within the published range (averages between 2.4 and 2.8). These values remain flat over the UV waveband for the diatom tested. However, below 400 nm, the presence of mycosporine-like amino acids (MAAs) strongly influences the absorbance measurements. For the dinoflagellates studied, the ultraviolet (UV) absorbance measured on frozen filters (stored in liquid nitrogen) reveals a large peak (Sosik 1999) caused by high concentrations of MAAs, which is much smaller on absorbance scans of suspended cells. This amplified UV peak adds to the true amplification effect caused by the extended pathlength of light in filter pads. This artifact, caused by the extracellular release of the water-soluble MAAs during freezing, also was observed to a lower degree with measurements performed on fresh filters (immediate scanning), precluding the use of this method to estimate UV absorption in the species tested.

Phytoplankton absorption is inferred most often from measurements on cells retained on glass fiber filters (Mitchell and Kiefer 1988) because the concentration of phytoplankton in the water column often is too low to quantify absorption in suspension. The estimation of spectral absorption coefficients from the absorption of particles retained on a filter requires a correction for pathlength amplification in the glass fiber filter (Butler 1962; Kiefer and SooHoo 1982). This amplification occurs due to multiple

scattering of light through the filter (increased pathlength). The pathlength amplification factor (β) can be estimated from the following relationship when multiple scattering is negligible in the cell suspension:

$$\beta = a_f(\lambda)/a_s(\lambda) \quad (1)$$

with $a_f(\lambda)$ and $a_s(\lambda)$ being the spectral absorption coefficients measured on the filter and in suspension, respectively.

The major mechanisms determining the amplitude of β remain poorly understood. β has sometimes been assumed constant (Kiefer and SooHoo 1982; Lewis et al. 1985) and often shows little or no spectral dependence (Kishino et al. 1985; Mitchell 1990; Cleveland and Weidemann 1993). Others have found an inverse relationship between β and the particle load on the filter (Mitchell and Kiefer 1988; Bricaud and Stramski 1990; Arbones et al. 1996; Lohrenz 2000). The current practice is to estimate the optical density of particles in suspension from a quadratic or a power function of the optical density of filtered particles (Bricaud and Stramski 1990; Hoepffner and Sathyendranath 1992; Cleveland and Weidemann 1993; Moore et al. 1995; Arbones et al. 1996; Finkel and Irwin 2001). Arbones et al. (1996) and

¹Present address: Institut National de la Recherche Scientifique, Centre Eau, Terre et Environnement, 2800 rue Einstein, C.P. 7500, Sainte-Foy, Québec, G1V 4C7, Canada. E-mail: isabelle_laurion@inrs-ete.quebec.ca

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Cleveland and Weidemann (1993) suggested that a unique empirical correction might exist for a wide range of phytoplankton. On the other hand, others have found that the β -correction varied for different phytoplankton species (Moore et al. 1995; Allali et al. 1997; Finkel and Irwin 2001), suggesting that the size and physical properties of cells may also influence the light behavior and the scattering on filters. On the basis of a theoretical approach, Roesler (1998) proposed a constant value of 2 for β (for GF/F filters), regardless of the concentration or composition of absorbing particles on the filter. This author argues that multiple scattering and self-shading in the cuvette or methodological problems (e.g., blank filter preparation, instrument sensitivity for low particle loads) can explain the apparent disagreement among published β factors empirically derived. Tassan and Ferrari (1995, 1998) propose another method that accounts for backscattering losses and presents lower variability for the evaluation of β .

In none of these cases do the models extend below 400 nm, with the exception of the work of Allali et al. (1997), which extends to 350 nm. Hence, warranted or not, correction for pathlength amplification determined for the visible range (described above) has been applied to estimate the absorption of phytoplankton in the ultraviolet (UV) region (e.g., Neale et al. 1998 applied the model of Cleveland and Weidemann 1993). The objective of the present study was to verify if the previously published correction models for pathlength amplification in the visible waveband also apply in the UV. In addition, Sosik (1999) has shown that freezing storage of filters causes a variable increase in UV absorption, which depends on the type of plankton community sampled. This suggests that algae containing UV-absorbing compounds might be more susceptible to freezing. Artificial changes in phytoplankton absorption upon filtration or storage also have been observed in the visible range and were related to cell rupture (Barber et al. 1969; Stramski 1990). The absorption of particles in suspension and on fresh and frozen filters was measured in this work to estimate β in the visible (400 to 700 nm) and the UV (280 to 400 nm) wavebands for three species of phytoplankton, including two mycosporine-like amino acid (MAA)-rich dinoflagellates. Several tests were performed to understand the phenomenon described by Sosik (1999) and its methodological implications.

Materials and procedures

Phytoplankton cultures—Mono-specific cultures of two dinoflagellates, *Alexandrium tamarense* (CCMP1771; diameter ca. 38 μm) and *Heterocapsa triquetra* (CCMP449; diameter ca. 18 μm), and one diatom, *Thalassiosira pseudonana* (CCMP1335; diameter ca. 6 μm), were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton. The cultures were grown under similar conditions (batch cultures in borosilicate Erlenmeyer flasks,

15°C, 14:10 h light:dark cycle at ca. 300 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ of incident photosynthetic available radiation [PAR]). The culture media consisted of filtered seawater adjusted to a salinity of 32‰ and enriched with L1 nutrients (Guillard and Hargraves 1993). For *T. pseudonana*, the L1 media was enriched with silicate.

Absorption measurement—The absorption spectra of phytoplankton suspensions were obtained using a Perkin-Elmer Lambda 2 spectrophotometer equipped with an integrating sphere (Labsphere RSA-PE-20). The samples were placed in a 1-cm quartz cuvette, and the cuvette was positioned at the entrance of the integrating sphere. Scans were conducted between 280 and 850 nm at a speed of 240 nm min^{-1} with a slit width of 2 nm. Scans of filtered fresh medium (0.2 μm) were used as a baseline and the baseline stability was checked regularly. The optical density (or absorbance) values, OD_λ , were adjusted with OD_λ averaged over 790 to 800 nm to correct for residual offsets and scattering losses within the measurement system (null point correction; Mitchell et al. 2000; Babin and Stramski 2002). Correction for backscattering losses (Tassan and Ferrari 1998) was not applied to the absorbance spectra. The spectra were run in triplicates (each time using a new suspension aliquot). The absorption of the dissolved organic matter naturally released in the media during growth also was measured (except for *T. pseudonana*). Cells were removed by low-pressure filtration, OD_{filtr} and also by gentle centrifugation (2×3 min at $5000 \times g$), $\text{OD}_{\text{centrif}}$.

For measurements of particle absorption from filter pads, 1.86-mL aliquots were filtered onto 25-mm Whatman GF/F glass fiber filters. To improve uniform cell distribution on the filter, 10 mL filtered seawater was mixed with the sample before filtration. One set of triplicate filters was kept fully hydrated in petri dishes at 4°C for fresh measurements (performed within 2 to 7 h), OD_{fresh} . A second set of triplicate filters was frozen immediately in liquid nitrogen (in Fisher Histoprep™ tissue capsules) and stored at -80°C for 2 d, $\text{OD}_{\text{frozen}}$. A third set of triplicate filters was stored frozen as above but upon thawing, the filters were rinsed with 5 mL filtered seawater (0.2 μm) before they were scanned, $\text{OD}_{\text{frozen rinsed}}$. This step collected the water-soluble material that might have exuded from the algal cells or been released upon cell breakage. The filtrate of this rinsing step was kept for further analysis, $\text{OD}_{\text{rinsing}}$. Fresh and frozen filters were scanned after they reached ambient temperature (ca. 2 to 3 min) and were hydrated up to the point that a small drop remained on the petri dish when lifted. The filters were placed at the entrance of the integrating sphere with the cells facing the incident beam, and scans were conducted with the same specifications as above (baseline using air). The absorbance values were adjusted with OD_λ averaged over 790 to 800 nm as above. Several blank filters were scanned, averaged, and subtracted from each spectrum prior to the null adjustment. The filtration volume of 1.86 mL was chosen so that the geometrical pathlength of filtered

samples (volume filtered divided by the clearance area of the filter) matched the 1-cm pathlength of the cuvette (Arbones et al. 1996; Finkel and Irwin 2001), thus $OD_f(\lambda)$ differed from $OD_s(\lambda)$ by only the magnitude of the pathlength amplification factor.

Mitchell et al. (2000) recommended keeping OD_f values in the range 0.05 to 0.4 (in the visible range) for best performance of empirical algorithms used for pathlength amplification correction, most likely to avoid possible bias caused by instrument noise and baseline correction errors at low OD_f and by self-shading at high OD_f . In this study, the OD measured on filters was always below 0.1 in the visible waveband and below 0.6 in the UV. However, OD reached values below 0.05 in the visible range after the blank filter was subtracted. On the other hand, OD of cells in suspension was generally below 0.05 in the visible range, with reduced chance of significant multiple scattering effects (optically thin solution; Sathyendranath et al. 1987; Lohrenz 2000), but increased chance that the instrument noise and other artifacts linked to blank adjustment and null point normalization become significant. The coefficients of variation for absorbance values of triplicate measurements (suspensions and filtered cells) were lower than 10% throughout the visible and UV region. Part of the variability between replicate OD_s spectra may be caused by the capacity of dinoflagellates to swim during scanning (which lasts 2.4 min). The variability in replicate OD_f might have been caused by the small volume pipetted for the filtrations (1.86 mL). Additionally, the use of low filter loadings may be a significant source of variability in the estimation of β .

MAAs and cell counts—For the measurement of the concentration of MAAs, 8 to 25 mL of the cultures were filtered onto glass fiber filters (Whatman GF/F), immediately frozen in liquid nitrogen, and kept at -80°C until subsequent extraction. A two-step extraction procedure was used whereby labile MAAs were extracted first and kept aside, and the filters were then re-extracted for less labile MAAs, a method developed to optimize the extraction efficiency of the diverse MAA assemblages. For instance, mycosporine-glycine and shinorine were best extracted at 45°C , whereas a combination of palythine and an unknown MAA absorbing at 333 nm (not separated by the elution used in this test) was best extracted at 4°C (unpubl. data). The filters were placed first in 2 mL of 25% aqueous methanol (vol:vol), sonicated, and left in the dark at 4°C for ca. 18 to 20 h. Samples were then centrifuged at $5000 \times g$ for 5 min, and the supernatants were kept cold and dark. Another 2 mL fresh solvent were added to the filters, and they were placed in a water bath at 45°C for 2 h (Sommaruga and Garcia-Pichel 1999). The second extracts were thereafter centrifuged, added to the first, and clarified using a $0.22 \mu\text{m}$ pore size Acrodisc filter (Gelman). The air was replaced with argon and the samples were stored at -80°C for further high-performance liquid chromatography (HPLC) analysis. MAAs were separated by

reverse-phase isocratic HPLC, injecting 50 μL aliquots in a Phenosphere C-8 column (Phenomenex, $250 \times 4.6 \text{ mm}$, $5 \mu\text{m}$) protected by a Brownlee RP-8 guard (Applied Biosystems, $30 \times 4.6 \text{ mm}$, $5 \mu\text{m}$), in a mobile phase of 0.1% acetic acid in 55% aqueous methanol (Shick et al. 1999) followed by a further passage with 0.1% acetic acid in 25% aqueous methanol for best separation of early peaks. The extracts were adjusted to 55% MeOH before the injection in 55% MeOH eluent, to obtain higher peak resolution. The column was maintained at 25°C , and the peaks were detected using a fast-scanning absorbance detector (Spectra Focus). The MAAs were identified by comparison of retention times and order of appearance with published results, from the absorption spectra of the various peaks and by co-chromatography with secondary standards prepared from invertebrate extracts (*Aplysia dactylomela* eggs obtained from D. Karentz; mycosporine-glycine, shinorine, porphyra-334, palythine, and asterina-330) and red macroalgae (porphyra-334, palythine, asterina-330, and palythinol). Further confirmation of MAA identification was done by liquid chromatography-mass spectrometry (LC-MS) (K. Whitehead, pers. comm.). Peak areas and published extinction coefficients were used to calculate the MAA concentration. An extinction coefficient of $137.1 \text{ L g}^{-1} \text{ cm}^{-1}$ was used for the unidentified MAAs (averaged from the identified MAAs). An additional filter was prepared to analyze pigments by HPLC following the method by Zapata et al. (2000; eluent B1).

A subsample of 20 mL of each culture was fixed with acidified Lugol's solution and cells were counted under an inverted microscope.

Assessment

Absorption spectra and MAA analysis—The absorbance spectra of suspensions of the different cultured algae suggested the presence of UV-absorbing compounds in the dinoflagellates (Fig. 1). The presence of UV-absorbing compounds was confirmed by the analysis of the MAA composition and

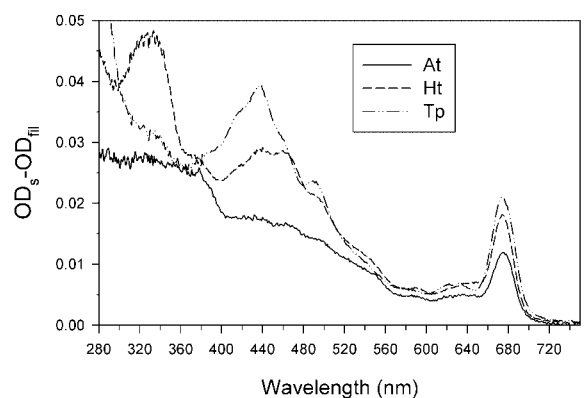


Fig. 1. Absorbance spectra of suspended cells for *A. tamarensis* (At), *H. triquetra* (Ht), and *T. pseudonana* (Tp). $OD_{f,i}$ was subtracted from OD_s except for *T. pseudonana*.

Table 1. The concentration of mycosporine-like amino acids (MAAs), with the percentage of individual MAAs (with wavelength at maximum absorption) as determined by high performance liquid chromatography

Species	MAAs		MG*	SH*	PR*	PA*	M333*	PI*	US*	PE*	Other MAAs (%)
	$\mu\text{g Chl } \alpha^{-1}$	$\mu\text{g MAAs cell}^{-1}$	310 nm (%)	334 nm (%)	334 nm (%)	336 nm (%)	333 nm (%)	320 nm (%)	357 nm (%)	360 nm (%)	
<i>A. tamarensis</i>	7.8	329.5	8.6	7.3	2.8	1.9	22.1	1.7	5.8	49.2	0.5
<i>H. triquetra</i> †	6.8	35	3	41.5	0.8	nd	39.7	2.5	0.5	6.5	5.5
<i>T. pseudonana</i>	—‡	—	—	—	—	—	—	—	—	—	—

* MG, mycosporine-glycine; SH, shinorine; PR, porphyra-334; PA, palythenic acid; M333, unidentified; PI, palythine; US, usujirene; PE, palythene.

† MAAs measured in a separate culture grown under the same conditions.

‡ —, not detected.

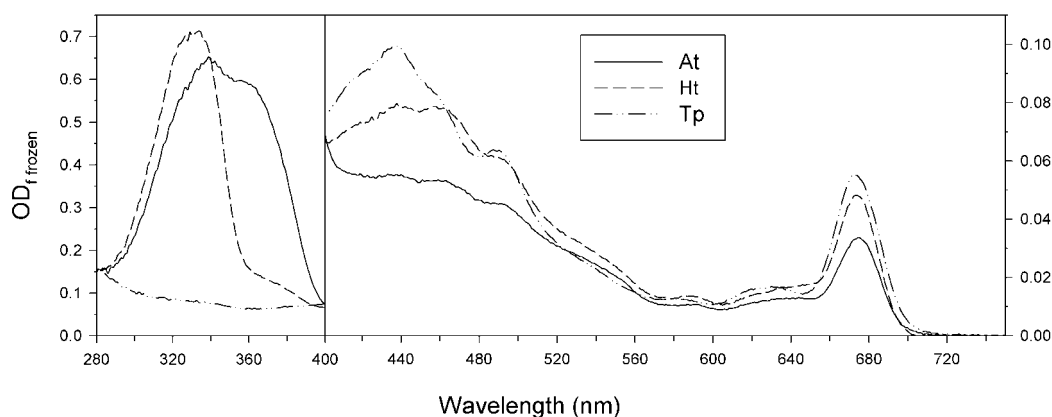
concentration found in these species (Table 1). In *A. tamarensis*, the dominant MAAs were M333 (unidentified MAAs; $\lambda_{\text{max}} = 333$ nm) and palythene ($\lambda_{\text{max}} = 360$ nm), with smaller contents of mycosporine-glycine ($\lambda_{\text{max}} = 310$ nm), shinorine ($\lambda_{\text{max}} = 334$ nm), porphyra-334 ($\lambda_{\text{max}} = 334$ nm), palythenic acid ($\lambda_{\text{max}} = 336$ nm), palythine ($\lambda_{\text{max}} = 320$ nm), and usujirene ($\lambda_{\text{max}} = 357$ nm), giving a wide peak of UV absorption (this can be seen on scans of frozen filters; Fig 2). The dominance of shinorine and M333 (absorbing at roughly the same wavelength) in *H. triquetra* gave a narrower absorption peak. The diatom did not have MAAs.

Filter freezing effect—The UV absorption peak observed in the spectra of frozen filters for the dinoflagellates was much larger than the one observed for cells in suspension (Figs. 1 and 2). The effect of freezing on the absorption spectra is presented in details for *A. tamarensis* and *H. triquetra* in Figs. 3 and 4, respectively. These figures show the increased UV peak caused by freezing which considerably decreased or disappeared when the frozen filter was rinsed prior to scanning (compare $\text{OD}_{\text{f frozen}}$ to $\text{OD}_{\text{f frozen rinsed}}$). The absorbance spectra of these rinses ($\text{OD}_{\text{rinsing}}$ in Figs. 3B and 4B) show that the UV-absorbing compounds came from the liquid associated with the moist filter. Indeed, the difference between $\text{OD}_{\text{f frozen}}$ and $\text{OD}_{\text{f frozen rinsed}}$ scales to $\text{OD}_{\text{rinsing}}$ once the dilution factor is

taken into account. The results were similar for *A. tamarensis* and *H. triquetra*, except that a small UV peak remained after the filters were rinsed in the case of *H. triquetra* (Fig. 4A, insert). Cultures of *A. tamarensis* showed no dissolved organic compounds in the filtrate of fresh cells (OD_{fil}) or in the supernatant of centrifuged fresh cells ($\text{OD}_{\text{centrif}}$), with no difference between the two methods for collecting the dissolved compounds (Fig. 3B). However, cultures of *A. tamarensis* grown under slightly different light conditions showed varying UV absorption in the filtrates (up to 42% of OD_{f} ; not shown). On the other hand, cultures of *H. triquetra* always had considerable amounts of dissolved organic compounds (especially high UV absorption peak caused by the presence of dissolved MAAs in the culture media; Fig. 4B).

Scan delay effects—The influence of the delay time between filtration and spectral scan was examined for *H. triquetra* (Fig. 4) by comparing fresh filters scanned immediately after filtration (scans completed within 3 min of filtration; red line) with filters scanned after 3 h and 24 min of dark and cold storage (refrigerator). The UV peak increased as the delay between filtration and spectrophotometric scan increased.

β factor—For species with no MAAs (*T. pseudonana*), β was relatively flat over the entire spectra (Fig. 5; similar results were observed for other small-sized species including an

**Fig. 2.** Absorbance spectra of frozen filters for *A. tamarensis* (At), *H. triquetra* (Ht), and *T. pseudonana* (Tp) in the UV and visible wavebands. Note the different scales.

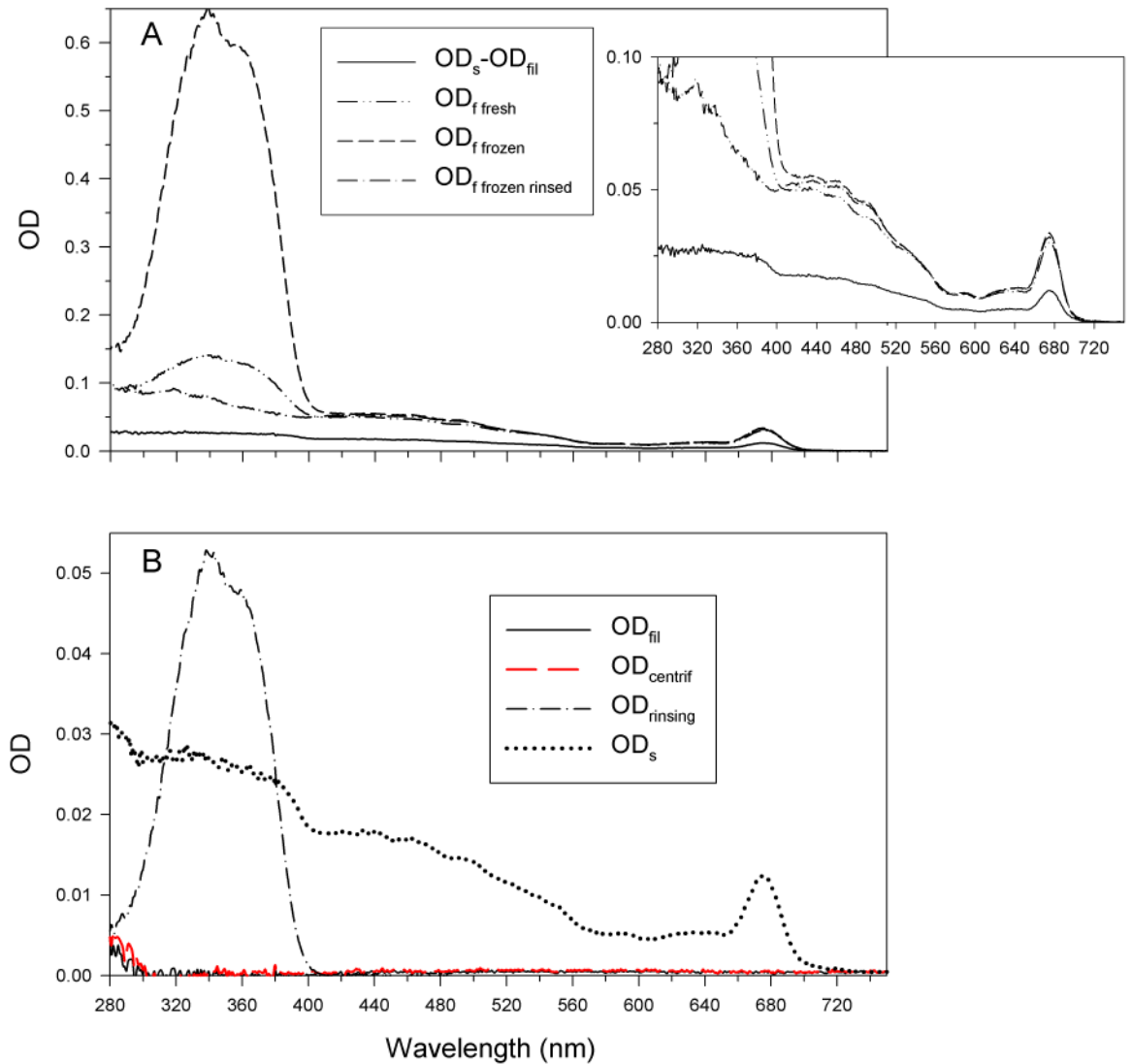


Fig. 3. The effect of freezing on the absorbance spectra of *A. tamarensis*. Optical density for A) cells in suspension ($OD_s - OD_{fil}$), cells on a fresh filter ($OD_{f\ fresh}$), on a frozen filter ($OD_{f\ frozen}$), on a frozen filter rinsed with 5 ml of filtered see water ($OD_{f\ frozen\ rinsed}$), and B) filtrate from the culture (OD_{fil}), the supernatant of centrifuged culture ($OD_{centrif}$), and the filtrate of rinsed frozen filter ($OD_{rinsing}$) as compared with OD_s .

eustigmatophyte, a diatom, and two prymnesiophytes; Laurion et al., unpubl. data), with a slight decrease below ca. 300 nm. In this case, OD_{fil} was not measured, hence not subtracted from OD_s , leading to an underestimation of β at the lower end of the spectrum. OD_{fil} was measured for separate cultures of *T. pseudonana* grown under similar conditions, revealing a minor contribution of dissolved organic matter to the absorption by suspended particles (not shown). During handling of species with MAAs, the MAAs were released into the liquid associated with the filter, so the ratio of absorption coefficients measured on the filter and in suspension (i.e., the β factor defined above) cannot be ascribed solely to pathlength amplification resulting from multiple scattering within the filter in the UV region of the spectra. However, the examination of this ratio in the UV waveband

and its comparison to values in the visible waveband can be used as a tool to verify under which conditions the artifact was present. The β factors were calculated using fresh (Fig. 5) and frozen filter absorbance. For filters scanned immediately after filtration, the spectral values of β still showed a UV-peak, but the peak was smaller (Fig. 5, red curve) than for filters scanned after a few hours of storage at 4°C. Averages of β over the visible waveband as calculated with fresh and frozen filter absorbance values are presented in Table 2.

Selected models used to describe the amplification of the absorbance signal caused by multiple scattering in the filter pads (i.e., the relationship between β or OD_s and OD_f) were compared with the results obtained for *A. tamarensis* and *T. pseudonana* (fresh filters used here for optimal fit) (Fig. 6). The models of Bricaud and Stramski (1990; i.e., $\beta(\lambda) =$

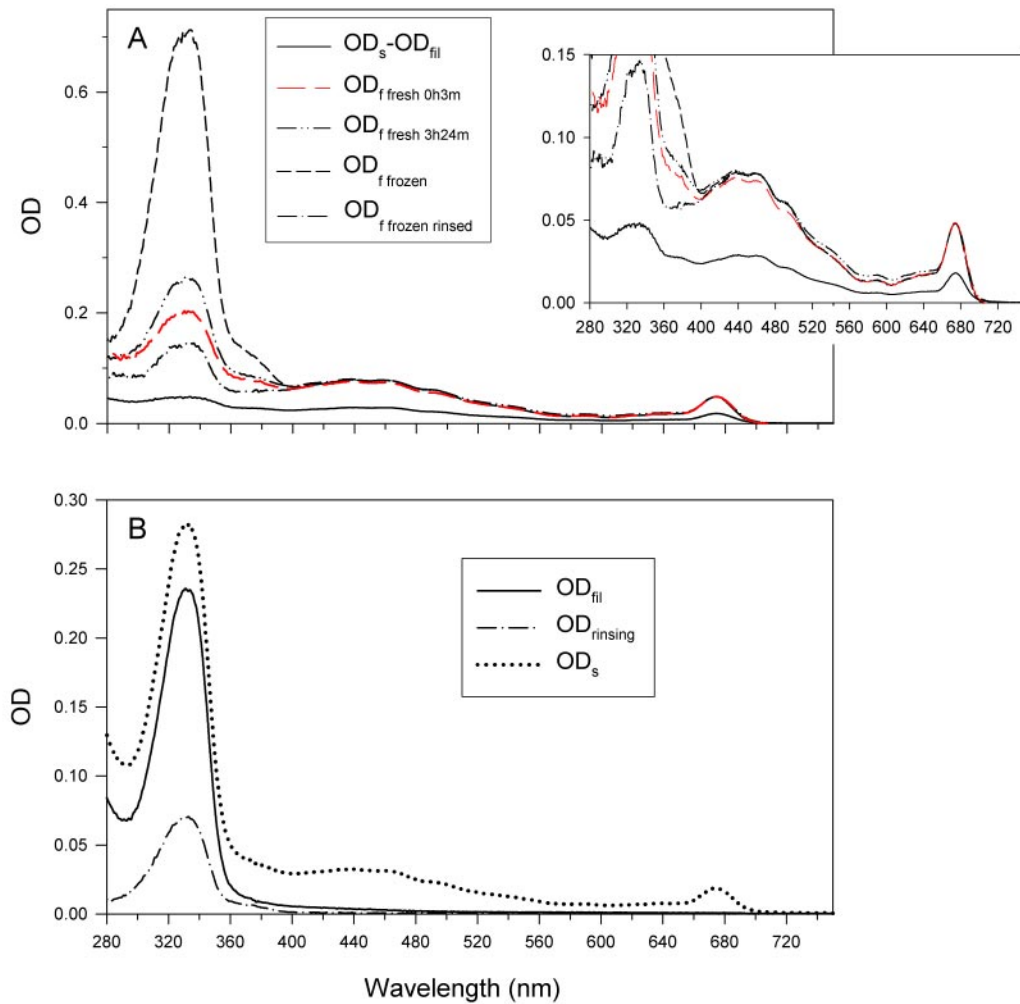


Fig. 4. The effect of time on the absorbance spectra of fresh filters left in the dark at 4°C compared with cell suspension and frozen filters for *H. triquetra*. See legend of Fig. 3 for abbreviations.

1.63 $OD_f (\beta)^{-0.22}$) and of Cleveland and Weidemann (1993; i.e., $OD_s (\lambda) = 0.378 OD_f (\lambda) + 0.523 OD_f (\lambda)^2$) applied to OD_f values of *A. tamarese* are also shown (solid lines). The relationship proposed by Bricaud and Stramski does not show a good fit to our data, i.e., there was no increase of β values at low OD_f (note that this divergence is not linked to the MAA artifact since it does not concern UV data). As seen in Fig. 6B, OD_s can be predicted with relative accuracy using the model of Cleveland and Weidemann, with a slight overestimation of OD_s , but its applicability is only valid in the visible waveband for the dinoflagellates (open symbols).

Discussion

Exudation of MAAs—The difference in spectral absorption between suspensions and filtered cells is particularly striking in the UV waveband. The presence of MAAs in the dinoflagellates can be detected by a large absorbance peak between ca. 285 and 405 nm for frozen filtered cells (Fig. 2). MAAs

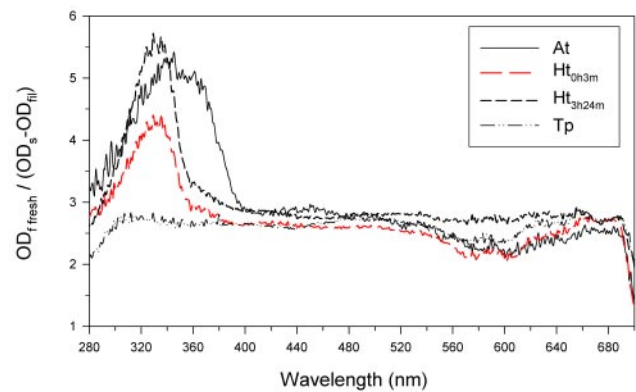


Fig. 5. The β factor calculated with fresh filters for *A. tamarese* (At), *H. triquetra* (Ht), and *T. pseudonana* (Tp), corresponding to the path-length amplification factor in the visible waveband, whereas the effect of MAA exudation during handling adds to the pathlength amplification in the UV waveband.

Table 2. Average β values over the visible waveband (400 to 700 nm) with standard deviation in parentheses

Species	Average β	
	$OD_{f \text{ fresh}}/OD_s$	$OD_{f \text{ frozen}}/OD_s$
<i>A. tamarensis</i>	2.6 (0.3)	2.8 (0.4)
<i>H. triquetra</i>	2.5* (0.2)	2.5 (0.3)
<i>T. pseudonana</i>	2.6 (0.1)	2.4 (0.2)
All species	2.5 (0.2)	2.6 (0.3)

* For fresh filters measured after 3 min

were indeed abundant in the dinoflagellates we tested in this study, with concentrations reaching up to 7.8 μg MAAs (μg chlorophyll a^{-1}) in *A. tamarensis* (Table 1). Sosik (1999) showed that freezing of filters (as standard storage practice) can cause an increase in UV absorption. Hence, she recommended the analysis of in vivo absorption immediately after sampling for reliable measurements in the UV. Our results confirm the effect of freezing in the MAA-rich dinoflagellates and demonstrate that MAAs are released out of the cells in the liquid associated with the moist filter during freezing/thawing (Figs. 3 and 4). However, the increasing UV absorbance with time after filtration (Fig. 4) and the presence of a UV peak on the spectra of β calculated with scans performed immediately after filtration (Fig. 5; in other words, $OD_{f \text{ fresh } 3\text{min}} > [OD_s \times \beta_{\text{PAR}}]$ in the UV range) indicate that mobilization of MAAs is not only caused by freezing but also by the stress imposed to the cells when they lay on a filter. The results suggest that MAAs are not released instantaneously upon filtration, but the release process starts immediately after or during filtration. The absorbance spectra in the visible waveband remained unaffected by handling even after the rinsing step (Figs. 3 and 4), indicating that pigments were not lost due to cell breakage. Hence, MAAs seem to be released out of the cells through intact membranes.

On the other hand, there are reports in the literature where suspensions of cultured phytoplankton showed UV absorption peaks up to 9 times the chlorophyll a absorbance at 676 nm (diatoms and colonial prymnesiophytes: Moisan and Mitchell 2001; Riegger and Robinson 1997), although comparisons with filters and/or subtraction of the filtrates were not always done. These results suggest that large UV absorbance peaks are not necessarily artifacts associated with the quantitative filter technique. Hence, UV absorbance peaks such as observed on frozen filters collected from natural communities of the Gulf of St. Lawrence (Fig. 7) cannot be confidently attributed to an artificial release of MAAs until more work is done to see how widespread this phenomenon is.

Cultures of *A. tamarensis* grown under slightly different light conditions showed varying UV absorption in the filtrates that may, at least partly, be explained by the shear stress to which the cells were exposed before measure-

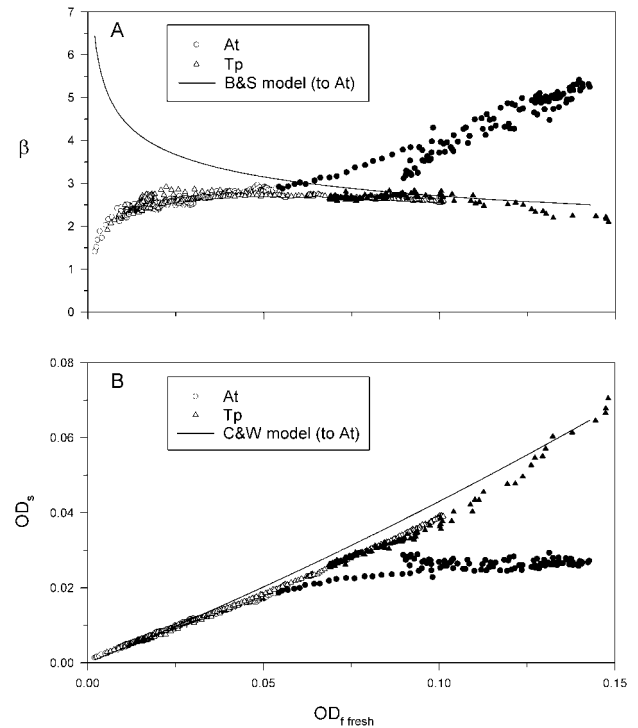


Fig. 6. Spectral values of A) β and B) OD_s as a function of OD_f (fresh) between 280 to 700 nm for *A. tamarensis* (At) and *T. pseudonana* (Tp) (closed symbols correspond to values in the UV waveband). The solid lines represent the application of models from (A) Bricaud and Stramski (1990) and (B) Cleveland and Weidemann (1993) to OD_f values of *A. tamarensis* between 400 to 700 nm.

ments, even though they were manipulated cautiously. The release of MAAs associated with handling (culture mixing, filtration, or centrifugation) may reflect the sensitivity of many dinoflagellates to fluid shear stress (Juhl et al. 2001), although not all dinoflagellates display the same sensitivity (Sullivan and Swift 2003). Cultures of the colonial cyanobacteria *Trichodesmium* subjected to variable stirring conditions also showed varying UV absorbance (Subramaniam et al. 1999). In this case however, the UV-absorbing compounds were likely extracellular and would be liberated from the disaggregated colonies.

β factor—The correction for pathlength amplification, β , was relatively constant over the visible waveband and varied little between species (range of 2.4 to 2.8, but differences between species were significant for most comparisons; Mann-Whitney rank sum test; $P < 0.05$) despite their different cell sizes (from 6 to 38 μm ; Table 2). The use of frozen filters instead of fresh ones had little effect on evaluating β in the visible range, suggesting that storage at -80°C is satisfactory to measure particle absorption in the visible waveband, as concluded by Sosik (1999).

In the UV waveband, the β values are highly variable and affected by the abundance of MAAs, the physical or physio-

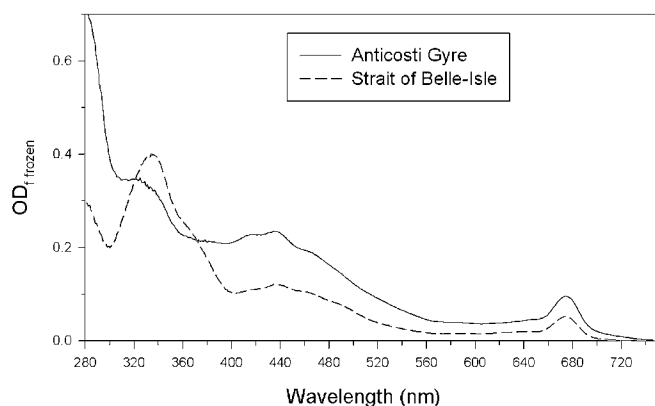


Fig. 7. Spectral absorbance (OD_{frozen}) of natural community from the St. Lawrence Anticosti Gyre and Strait of Belle-Isle sampled in May 2000.

logical response of cells during handling (i.e., the extent of extracellular release) and the different handling practices. Hence, the presence of MAAs precludes the application of empirical models to estimate the in vivo UV absorption from the absorption of filtered cells, at least for the dinoflagellates tested. However, for MAA-free species, β values in the UV (290 to 400 nm) were not significantly different from values in the visible range (for *T. pseudonana*, Mann-Whitney rank sum test, $P > 0.05$; Fig. 5). These results suggest that the pathlength amplification factor does not behave differently in the UV waveband (i.e., is independent of wavelength as in Cleveland and Weidemann 1993). Hence, empirical models of pathlength amplification correction developed for the visible waveband would most likely be applicable to absorption values in the UV waveband for cells that do not have MAAs or that do not release their MAAs during handling.

Comments and recommendations

The present study shows that common handling practices (filtration and freezing) for estimating the spectral in vivo absorption of phytoplankton might lead to large overestimations in the UV waveband. Such large UV-absorbing peaks were not only observed for the dinoflagellates tested here but also in frozen field-collected samples (Fig. 7 and Sosik 1999). Since UV absorption of particles cannot be evaluated properly with the filter pad technique when MAAs are present, the in vivo absorption should be measured on particle suspensions in these cases. We recognize however that suspension measurements are not possible or practical in many cases, especially in areas of low biomass. The elusive nature of these mobile water-soluble compounds also may have implications for alternative methods for measuring absorption of dissolved and particulate components of natural waters in the UV waveband, which most often require some

type of handling (e.g., collection of dissolved matter using filtration cartridges, D'Sa et al. 1999; absorption spectra for particles filtered then freeze-transferred to glass slides, Allali et al. 1995). Special care is thus recommended when handling samples containing MAAs to reduce contamination by, or losses of, these water-soluble compounds. Furthermore, the fact that spectral absorption of suspended dinoflagellates is relatively flat in the MAA-absorbing region despite high concentration of these compounds suggests that MAAs are highly packaged in intact cells. This phenomenon will be considered more closely in another paper.

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